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Provenance and integration of *Photobacterium phosphoreum* and *Photobacterium carnosum* into the spoilage consortium of modified atmosphere packaged meat

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„Es ist paradox, aber wahr, wenn man sagt, dass je mehr wir wissen, desto unwissender werden wir in einem absoluten Sinn. Nur durch Erleuchtung werden wir unserer Grenzen bewusst.“

Nikola Tesla

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1 Abstract

Much attention has been paid to the activity of common meat spoiling bacteria and effective methods to control their growth. Still, the development of the spoilage microbiota and concomitant onset of perceptible spoilage is prone to unpredictability in some cases. The contribution of bacteria that are not recorded by established surveillance strategies but reach high cell numbers on meat can be relevant in this context. Occurrence of members of the genus *Photobacterium* (*P.*) on spoiling meat has long been overlooked due to their particular growth requirements. The cultivation of photobacteria requires sodium chloride and low temperatures, which is in accordance with their distribution also in multiple marine habitats. First, this work dealt with the occurrence of *P. phosphoreum* and *P. iliopiscarium* on meats, in relation to their already demonstrated association with marine fish/seafood and marine environment. Contamination of multiple types of meats and independency of the contamination of packaging atmosphere, producer and retailer was detected in this context. Second, this work examined the growth and interaction of *P. phosphoreum* and *P. carnosum* in meat spoilage, with respect to their frequent detection on meat. To explore environmental adaptation correlated with respective isolation sources, isolates from meat and marine environments/modified atmosphere packaged (MAP) salmon were compared regarding their tolerance to hydrostatic pressure and sodium chloride. *P. carnosum* strains thereby exhibited lower tolerance to both, but shared genetic elements of adaptation towards marine environments with *P. phosphoreum* strains. Tolerance was in general lower for strains isolated from meat than for strains isolated from marine environment/MAP salmon. It suggests a general marine origin of both species, but proceeding adaptation towards the meat system and advanced adaptation especially of *P. carnosum*. This was confirmed by differences of strains of both species in their response to the presence of other meat spoilers. Growth of *P. carnosum* TMW2.2149 was improved by co-contamination with both, *Pseudomonas (Ps.) fragi* or *Brochothrix (B.) thermosphacta*, under MAP. Consequently, evaluation of its transcriptomic profile predicted enhanced metabolic activity in accordance. *Ps. fragi* and *B. thermosphacta* are assumed to contribute proteolytic and lipolytic functions. In contrast, growth of *P. phosphoreum* TMW2.2103 was impaired by the presence of these bacteria under modified atmosphere. Analysis of its transcriptomic profile predicted enhanced substrate competition for this strain, resulting from higher substrate demand during fermentative metabolism. All observed growth effects were reversed under air atmosphere. Reduced growth of *P. carnosum* TMW2.2149 in this case was predicted to result from the higher growth rates of the co-contaminants and the concomitant incapability of *P. carnosum* TMW2.2149 to compete with them. In contrast, growth of *P. phosphoreum* TMW2.2103 was enhanced in this case. The strain is assumed to benefit from the higher proteolytic and lipolytic activity of the co-contaminants under air atmosphere and resulting improved availability of extracellular fatty acids and amino acids. Consequently,

interaction with common meat spoilers can be advantageous for strains of both species, however under different conditions, which proves successful integration into the meat spoilage community by the use of different strategies. Application of CO_2 in the packaging gas showed little effect on the growth *P. phosphoreum* and *P. carnosum* strains on meat, whereas combined application of 70% O_2/30% CO_2 resulted in growth reduction. However, MAP meat from retail under the latter atmosphere can harbor high cell numbers of photobacteria. The impact of 70% O_2/30% CO_2 is therefore predicted to depend on an additional factor. *P. carnosum* and *P. phosphoreum* respond differently to co-contaminants and the initial contamination by all of these species varies from batch to batch. Hence, little effectiveness of MAP to restrict photobacteria on meat can be assumed in dependency of the spoilage consortium and the initial cell number. The ability of photobacteria to withstand MAP, to reach high cell numbers in the spoilage community and to produce biogenic amines thus form the basis for their relevance to meat spoilage.

2 Zusammenfassung

Bakterieller Fleischverderb ist ein gut untersuchter Prozess, der sich heutzutage durch diverse Möglichkeiten beeinflussen lässt. Trotzdem treten immer wieder unvorhergesehene Entwicklungen auf, die mit dem aktuellen Wissensstand nicht zu erklären sind. Bakterienarten, die zwar nicht mit etablierten Methoden erfasst werden, aber trotzdem hohe Zellzahlen auf verderbendem Fleisch erreichen, können dabei eine wesentliche Rolle spielen. In diesem Zusammenhang wurde das Vorkommen von *Photobacterium (P.)* auf verderbendem Fleisch lange übersehen, da eine Kultivierung dieser Gattung ungewöhnlich niedrige Temperaturen und ein salzhaltiges Medium voraussetzt. Diese Arbeit untersucht im ersten Schritt das Vorkommen der beiden auch von Meeresfisch bekannten Arten *P. phosphoreum* und *P. iliopiscarium* auf Fleischprodukten aus dem Handel. Kontaminationen wurden auf verschiedenen Fleischprodukten gefunden und konnten dabei weder auf eine bestimmte Verpackungsart, noch auf einen Erzeuger oder Händler zurückgeführt werden. Im zweiten Schritt befasst sich diese Arbeit mit Wachstum und Interaktion von *P. phosphoreum* und *P. carnosum*, den in diesem Zusammenhang am häufigsten nachgewiesene Arten, mit anderen fleischverderbenden Bakterienarten im System Fleisch. Um spezifische Anpassungen an die Umweltbedingungen mariner Habitate bzw. des Fleischsystems aufzudecken, wurden *P. phosphoreum* und *P. carnosum* Isolate aus beiden Habitaten hinsichtlich ihrer Druck- und Salztoleranz untersucht. Dabei waren *P. carnosum* Stämme weniger tolerant als *P. phosphoreum* Stämme und Isolate von Fleisch weniger tolerant als Isolate aus marinen Lebensräumen/von Schutzgasverpacktem Fisch. Da Stämme beider Arten trotzdem genetisch sehr ähnlich waren, lässt sich ein gemeinsamer mariner Ursprung mit fortschreitender Anpassung an das Fleischsystem vermuten. Die stärkere Anpassung von *P. carnosum* an das Habitat Fleisch spiegelte sich auch in den Unterschieden von *P. phosphoreum* und *P. carnosum* bei der Interaktion mit anderen Fleischverderbern wieder. *P. carnosum* TMW2.2149 zeigte verstärktes Wachstum in Anwesenheit sowohl von *Pseudomonas (Ps.) fragi,* als auch von *Brochothrix (B.) thermosphacta* unter Schutzgasatmosphäre. Eine Transkriptom-Analyse konnte dies auf eine allgemein verstärkte Stoffwechselaktivität des Stammes zurückführen. *Ps. fragi* bzw. *B. thermosphacta* steuern vermutlich proteolytische und lipolytische Aktivität bei. Das Wachstum von *P. phosphoreum* TMW2.2103 verringerte sich dagegen unter diesen Bedingungen, vermutlich aufgrund von verstärkter Nährstoffkonkurrenz durch den höheren Substratverbrauch beim Gärungsstoffwechsel. Gegenteilige Tendenzen wurden beim Wachstum unter Luft-Atmosphäre beobachtet. P*. carnosum* TMW2.2149 wuchs schlechter, da der Stamm vermutlich aufgrund der höheren Wachstumsrate der Co-Kontaminanten nicht konkurrenzfähig war. *P. phosphoreum* TMW2.2103 profitierte dagegen von der Anwesenheit der Konkurrenten und wuchs besser. Es kann angenommen werden, dass in Anwesenheit von *Ps. fragi* bzw. *B. thermosphacta* unter Luft-Atmosphäre mehr freie Fettsäuren und Aminosäuren und damit mehr Substrate für das Wachstum von *P. phosphoreum* TMW2.2103 verfügbar sind. Damit

existieren für beide *Photobacterium* Arten unterschiedliche Situationen, in denen sie jeweils von der Interaktion mit anderen Fleischverderbern profitieren. Es kann eine erfolgreiche Integration in das Fleischverderbs-Mikrobiom angenommen werden, die unterschiedlichen Strategien folgt. Dabei hat auch die Gasatmosphäre der Verpackung einen Einfluss. Anwendung von Schutzgasatmosphäre mit hohem CO_2 Gehalt reduzierte das Wachstum von Stämmen beider Arten auf Fleisch nur wenig, während die Kombination aus 70% O_2/30% CO_2 zu einer deutlichen Wachstumsreduktion führte. Da sich auf verpacktem Fleisch aus dem Handel aber Photobakterien in hoher Zellzahl finden, wird vermutet, dass der Effekt von 70% O_2/30% CO_2 zusätzlich von der Interaktion mit der Verdersbsmikrobiota abhängt. *P. carnosum* und *P. phosphoreum* reagieren unterschiedlich auf Co-Kontamination und die initiale *Photobacterium*-Zellzahl ist abhängig von der Fleischcharge. Damit kann der Einsatz von Schutzgasverpackung gegen das Wachstum von Photobakterien auf Fleisch generell als ineffektiv bewertet werden, in Abhängigkeit von initialer Zellzahl und Verderbsmikrobiota. Die Toleranz dieser Bakterien gegen Schutzgasverpackung, ihre erfolgreiche Integration und Fähigkeit zur Durchsetzung im Fleischverderbs-Mikrobiom und ihre Fähigkeit zur Produktion von biogenen Aminen bilden daher die Basis für ihre Bedeutung im Fleischverderb.

3 Introduction and methodology

3.1 Nutritional and economical importance of meat

Meat has played a decisive role in the human diet for a long time and its particular mix of nutrients remains challenging to replace by plant-based diets. Since it provides high amounts of multiple minerals, such as zinc, selenium, phosphorus or heme-iron, and several essential amino acids (Biesalski, 2005; Pereira and Vicente, 2013; Williams, 2007), its exclusion from the diet requires careful monitoring (Higgs, 2000). However, also excessive consumption of meat as it takes place in many Western countries today comes along with multiple health concerns (Pereira and Vicente, 2013). The worldwide meat production had a volume of 325 million metric tonnes (Mt) in 2019 and best prospects to further increase in the future (OECD/FAO, 2020). Every inhabitant of Germany has consumed an average of 59.5 kg thereof in 2020 (Deutscher-Fleischerverband, 2020). Over the next ten years, recent estimations have predicted an increase of the worldwide beef consumption to 76 Mt, of the pork consumption to 127 Mt and of the poultry consumption to 145 Mt. The latter has been assumed to become consequently the major source of meat by 2029 (OECD/FAO, 2020). Predictions on the development by the middle of this century even expect an increase of up to 50% of the total meat consumption (Barbut, 2020), thus emphasizing the importance of efficient and sustainable meat production chains.

The modern meat industry deals with a broad spectrum of processed- and non-processed meat products that can differ considerably regarding ingredients and storage. Products that are termed ‘processed’ may be enriched with additional proteins, carbohydrates, salts or other additives to improve taste, texture and shelf life (reviewed e.g. in (Feiner, 2006d)). The raw meat itself contains mainly water (up to 75%) and, depending on the meat type, approximately 22% protein and 4% fat. The latter thereby consists of a mixed tissue of 80-85% triacylglycerol, 10% connective tissue (mainly collagen) and up to 10% water. Meat proteins originate mainly from myofibrillar structures (up to 60%), as well as sarcoplasmic sources (up to 30%) and to a minor extent of structural tissue (e.g. collagen, up to 10%) (Feiner, 2006e). Yet, the exact composition of each piece of meat is subject to the type of cut - so its original place in the carcass - as well as to the type of livestock. In this context, skinless chicken breast contains e.g. comparably little fat and heme-iron when compared to lean beef- or pork loin (Clark *et al.*, 1997; Pegg and Shahidi, 2012; Pereira and Vicente, 2013).

Consequently, meat can be evaluated as very nutrient rich product - not only for humans but also for microorganisms (Reid *et al.*, 2017). It provides suitable environmental conditions for fast microbial growth due to the easy accessibility of substrates and minerals (Feiner, 2006e; Narasimha Rao *et al.*, 1998). Bacteria benefit especially of the presence of glucose, ribose, fructose, mannose and other

carbohydrates in this context that can account for up to 1.2% of the muscle wet weight after *rigor mortis* (Aliani and Farmer, 2005; Lawrie and Ledward, 2006; Meinert *et al.*, 2009; Mottram and Nobrega, 1998). Additionally, the high water availability of A_w = 0.98 of fresh meat (Feiner, 2006b) and its pH value of approximately pH 7 right after slaughter and pH 5.4-5.8 subsequently (Odeyemi *et al.*, 2020) generate favorable growth conditions. Fast growth of microorganisms will consequently affect meat structure, odor and color, thus forming the basis of microbial meat spoilage.

3.2 The issue of meat spoilage

The continuous process of meat spoilage sets in right after slaughter (Min and Ahn, 2005) and terminates finally with sensory rejection of the product. Spoilage causes relevant economical losses of up to 25% of all food products every year (Chaillou *et al.*, 2015; Gram and Huss, 2000). Losses during storage, distribution and consumption in the meat industry that can be assumed to result mainly from spoilage, sum up to >9-16% of the processed meats in this context (lower border: sub-Saharan Africa, upper border: North America and Oceania, (Gustavsson *et al.*, 2011)). Meat appearance can already change due to chemical spoilage prior to any detectable impact of the growing microbial spoilage community. Thereby, intrinsic factors, e.g. enzymatic activity, and extrinsic factors, e.g. presence of O_2 or irradiation, cause chemical changes of the meat compounds. Fatty acids are prone to peroxidation in that respect, resulting mainly from radical chain reactions initiated by reactive oxygen species (ROS) (Min and Ahn, 2005). ROS can develop from O_2 in presence of a suitable catalyst, such as metal ions (especially iron), UV light or heat (Warriss, 2000), as well as by microbial activity (e.g. (Vihavainen and Björkroth, 2007)). However, oxidation by enzymatic activity is also possible and it is mainly the proportion of unsaturated fatty acid that determines the rate of oxidation in both cases (Erkmen and Bozoglu, 2016b; Feiner, 2006e). Products of lipid peroxidation cause multiple off-odors and result in an overall impression of rancidity (Gray *et al.*, 1996; Warriss, 2000). Meat proteins are rather subject to degradation. In case of low pH, low water activity, high temperature or increased salt concentration, proteins will lose their original structure (Feiner, 2006e), thus influencing the meat texture (Erkmen and Bozoglu, 2016b). Both processes, oxidation and degradation, can additionally affect the meat color. Myoglobin as main pigment of muscle tissue contains a central iron atom. Based on the oxidative state of this atom and the pigment structure itself, meat can either have a red color, or greenish and brownish shades that will be associated with spoilage by the consumer (Erkmen and Bozoglu, 2016b).

Nevertheless, it is mainly microbial growth that causes meat spoilage and consequently reduced shelf life of meat products (Lambert *et al.*, 1991; Nollet, 2012). The time at which meat is rejected due to distinct signs of microbial spoilage thereby mostly depends on its initial microbial load and storage conditions. Microbial cell counts on the meat surface range between 2-4 $\log_{10}(CFU/cm^2)$ right after

slaughter and processing (Holzapfel, 1998). The initial spoilage community consists of a broad spectrum of different species from multiple contamination sources (Benson *et al.*, 2014; Cauchie *et al.*, 2020). Contaminating bacteria frequently origin from the animal itself, but also from the soil (De Filippis *et al.*, 2013), from work surfaces and tools (Gill, 2005) and from the air (Pearce *et al.*, 2006). Bacterial growth will thereby be limited to the first layers of the meat surface, if the tissue is not injured by processing steps (Nollet, 2012), and generation of fresh substrate will depend of the activity of extracellular enzymes for degradation in accordance. However, processing raises the possibility of increasing bacterial cell counts also in middle and bottom product layers, even if the surface cell counts are low (Dhananjayan *et al.*, 2006). The aqueous phase on the meat surface contains a mixture of solute low molecular compounds, such as glucose, lactate, amino acids, proteins and nucleotides that are readily available for metabolic exploitation (Nychas *et al.*, 1998). Consequently, also the intrinsic factors of the meat itself determine the impact of the microbial load on shelf life. Beside of the solute composition in the aqueous phase, pH value and fat content can be of relevance in this context (Blixt and Borch, 2002). Considerable signs of microbial spoilage will arise the latest at 7-8 $\log_{10}$ CFU per cm^2 or g of meat (Dainty and Mackey, 1992; Limbo *et al.*, 2010; Nychas *et al.*, 2008; Stoops *et al.*, 2015). Development of slime and strong off-odors thereby indicate beginning degradation of amino acids and other nitrogenous- or sulfuric compounds that follows the full exhaustion of available glucose, glucose-6-phosphate and lactate (Gill and Newton, 1978; Nychas *et al.*, 2008; Nychas and Skandamis, 2005). Even though off-odors may occur already during the initial exploitation of carbohydrates, they are considered less severe and evaluated rather fruity or sweetish in this context (Dainty, 1996).

Only approximately 10% of the initial contaminants on meat will start to grow under the conditions of meat storage at low temperatures (Nychas *et al.*, 1988), and of those only few species will become dominant (De Filippis *et al.*, 2013). This results in highly diverse species compositions during the early stages of spoilage, while the diversity decreases with the ongoing spoilage process (Säde *et al.*, 2017). The selective growth conditions applied by storage and product properties thereby decide upon the dominance of single species (Chaillou *et al.*, 2015). Species with efficient nutrient exploitation or advantageous competition strategies at given conditions will grow fast and contribute primarily to spoilage. These species have been designated specific spoilage organisms (SSO) or ephemeral spoilage organisms due to their predictable occurrence (Gram and Dalgaard, 2002; Nychas *et al.*, 2008). In contrast, species with poor metabolic activity will remain inferior and will be detected at most in low cell numbers (Labadie, 1999).

Still, some cases of evident spoilage exist that could not be associated with the occurrence and spoilage activity of SSO or with presence of other meat spoilers in high cell number at all (Brightwell *et al.*, 2007; Pothakos *et al.*, 2014). It demonstrates existence of relevant contributors to meat spoilage that are so far unexplored. In this context, some species without formerly known association to meat have been

detected in several recent studies on meat spoilage (Hilgarth *et al.*, 2018a; Lauritsen *et al.*, 2019). They are characterized by fastidious growth requirements and have been consequently overlooked by examinations based on established detection methods. One of them, *Photobacterium* sp., has already been isolated and successfully cultivated (Hilgarth *et al.*, 2018a) and is subject of this work.

3.3 Methods for the control of meat spoilage

Given the economic impact of spoilage, the meat industry applies manifold strategies to counteract microbial growth and minimize chemical changes. Thereby, good manufacturing practice during meat processing can be considered as most fundamental strategy and will reduce surface contaminations already in the processing plant to less than 2.5 CFU/cm^2 (Griffith, 2005; Møretrø and Langsrud, 2017). Immediate chilling after slaughter to 0-4°C represents another common strategy to limit growth of mesophilic bacteria that may harbor possible health hazards (Palumbo, 1986; Reid *et al.*, 2017). Chilling can extend the shelf life of fresh meat essentially. The shelf life of fresh meat is limited to approximately one day at 20-30°C (Lambert *et al.*, 1991), whereas literature reports a temperature-mediated extension to >7 days at 4°C for poultry (e.g.(Ghollasi-Mood *et al.*, 2017)) and increasing effectiveness of chilling when getting closer to 0°C (Ayres, 1960; Feiner, 2006c). While chilling extends the shelf life of meat it also selects for psychrotrophic species among the initial spoilage community. These species will grow despite of refrigeration, since they tolerate temperatures close to 0°C or even below (Feiner, 2006c; Labadie, 1999). In this context, positive selectivity has been reported e.g. for *Pseudomonas* sp., psychrotrophic lactic acid bacteria (LAB) and Enterobacterales, but also for *Vibrionaceae,* including the genus *Photobacterium* (Dainty and Mackey, 1992; Garcia-Lopez *et al.*, 1998).

Application of sealed packages with defined gas atmosphere (modified atmosphere packaging, MAP) has obtained increasing importance in meat preservation since approximately 70 years (Nychas and Skandamis, 2005). The method is based on the idea to shape the spoilage-associated microbiota by using species-specific differences in O_2 requirement and CO_2 tolerance and to simultaneously minimize oxidative changes of the meat. Simple air-wrap packaging can extend the shelf life of fresh beef already to more than 2 months, the one of fresh pork to approximately 6 weeks (Taylor, 1985) and the one of fresh chicken to approximately 2-3 weeks (Parry, 1993; Rossaint *et al.*, 2015). Even though air-wrap packaging creates an enclosed packaging space, it does not maintain a specific gas atmosphere. In comparison, MAP generates a comparably permanent gas atmosphere of defined composition and increases the shelf life of meat another 3-5 times accordingly (Bhat and Bhat, 2011; Xiong, 2017). Common modified atmospheres either combine the effects of CO_2 and O_2 on microbial growth and meat appearance (high CO_2, high O_2 packaging), utilize only the former as effective agent (high CO_2

packaging) or make use of an exclusion of both gases (N_2 packaging, vacuum packaging) (Nychas and Skandamis, 2005). While addition of other gases such as carbon monoxide has been investigated, its application remains negligible due to questionable side effects (Cenci-Goga *et al.*, 2020). Today, the most common gas mixture of modified atmospheres for fresh meat combines 70-80% O_2 and 20-30% CO_2 (Eilert, 2005; Feiner, 2006a; Rossaint *et al.*, 2015). Presence of O_2 thereby stabilizes an appealing red meat color and inhibits facultative anaerobic species (Farber, 1991; Young *et al.*, 1988). However, it can also stimulate growth of aerobic spoilers and cause lipid- and protein oxidation (Ezraty *et al.*, 2017). Utilization of CO_2 aims at the general growth reducing effect of this gas on bacteria that manifests in an extended lag phase and increased generation time (Stanbridge and Davies, 1998; Stier *et al.*, 1981; Sutherland *et al.*, 1977). Its exact mode of action is still not fully understood. However, it has been suggested to affect membrane function, enzyme activity and cellular pH, and consequently respiration, energy production and nutrient uptake of spoilage bacteria (Farber, 1991; Garcia-Gonzalez *et al.*, 2007; Gill and Tan, 1980; Tan and Gill, 1982). Application of high levels of CO_2 can also cause changes in meat color and pH, resulting in reduced meat quality (Daniels *et al.*, 1985; Leeson, 1987). In contrast, no direct impact on microbial growth and meat quality has been reported for the inert gas N_2. Its main function is confined to maintaining the desired packaging volume in presence- as well as in absence of O_2 and CO_2 (Church, 1993). Absence of O_2 and CO_2 in relevant concentration also characterizes vacuum packaging (VP). In this case, remaining O_2 will be consumed quickly by cellular respiration of the meat cells and its final concentration will decrease to less than 1%. Concomitantly, the CO_2 concentration will increase from a low level to approximately 20% of the total volume with proceeding spoilage of VP meat (Dainty and Mackey, 1992; Garcia-Lopez *et al.*, 1998). VP beef can have a shelf life of approximately 3 months, VP pork of approximately 9 weeks and VP poultry of approximately 5 weeks, however, statements on the maximum length of storage vary considerably (Blixt and Borch, 2002; Dainty, 1989; Holley *et al.*, 2004; Narasimha Rao and Sachindra, 2002; Nollet, 2012; Patterson *et al.*, 1984).

Good manufacturing practice, chilling and MAP/VP can be considered as major aspects with relevance to microbial growth on fresh meats. However, applications with minor importance exist and are mostly employed in addition to the former mentioned methods. Treatment with low-dose irradiation has been shown to efficiently suppress bacterial growth and represents an accepted strategy in many countries (Aymerich *et al.*, 2008). Advantages lie in its low impact on meat quality, the possibility for treatment after packaging and its independency of the product dimensions (Ahn *et al.*, 2017; Borsa, 2006). However, irradiation may influence meat color and odor and requires expensive equipment (Ahn *et al.*, 2017; Aymerich *et al.*, 2008). Treatment with high hydrostatic pressure (HHP) up to 600 MPa could also inactivate microorganisms (Sehrawat *et al.*, 2021) and has been approved for application in several countries (Aymerich *et al.*, 2008). It preserves the most relevant nutritional

ingredients of meat (Cheftel and Culioli, 1997), but the pressure that is required for efficient reduction of meat contaminants (at least 300-400 MPa) can have a negative impact especially on the meat color (reviewed e.g. by (Bermúdez-Aguirre and Barbosa-Cánovas, 2011)). Additionally, its efficiency can decrease in presence of resistant microorganisms or spores (Aymerich *et al.*, 2008). Treatment with salts, organic acids or other substances with antimicrobial effect complements many strategies for meat protection (reviewed e.g. in (Zhang *et al.*, 2010)). However, these applications touch the border of fresh meat to processed meat and will not be further considered here.

With this, storage at low temperatures and especially MAP/VP exhibit challenging growth conditions for bacteria in the context of meat spoilage. Tolerance of extreme O_2 and CO_2 concentrations can therefore constitute a significant factor for the prevalence of single species on spoiled meat. Information on the existence of adaptation towards the effect of the packaging gases can be considered essential for the understanding of the role of specific species in meat spoilage.

3.4 Common meat spoiling bacteria

Meat spoilage communities comprise a variety of different species, of which only few dominate the spoilage process (compare chapter 3.2). These SSO as main drivers of spoilage are characterized by adaptation towards the specific environmental conditions on chilled MAP/VP-stabilized meat. Well-known SSO on meat include e.g. *Pseudomonas* spp., especially *Ps. fragi,* and several enterobacteria (Gram-negatives), as well as *B. thermosphacta* and LAB (Gram-positives) (reviewed in (Casaburi *et al.*, 2015; Doulgeraki *et al.*, 2012). Cell counts of all aforementioned species can reach levels of 8 $\log_{10}$ CFUs per g of meat during spoilage (Chaillou *et al.*, 2015).

Pseudomonas is characterized by its high growth rate in presence of O_2 and can predominate under these conditions (Dainty and Mackey, 1992; Gill and Newton, 1977; Lambert *et al.*, 1991). Occurrence of several *Pseudomonas* species has been reported on meat, however, *Ps. fragi* has been demonstrated to become the most dominant one with proceeding spoilage (Ercolini *et al.*, 2010a; Wang *et al.*, 2018a). Species of the genus prefer to metabolize glucose and lactate before switching to amino acid metabolism (Nychas *et al.*, 2008; Thomas, 2012). Its metabolic activity includes extracellular conversion of glucose to gluconate, and since the ability to utilize gluconate is almost confined to pseudomonads (Nychas *et al.*, 1988), this forms the basis for several growth advantages. Many *Pseudomonas* species improve their competitiveness further by producing highly efficient siderophores. In this context, their growth has been reported to be directly subject to the given iron availability in the environment (Ercolini *et al.*, 2010a; Gram, 1993). Additionally, growth improvement by presence of other meat spoilers, such as *Carnobacterium* (*C.) maltaromaticum* and *Serratia*

proteamaculans, has been reported for meat spoiling *Pseudomonas* species (Ercolini *et al.*, 2009). Pseudomonads on meat have been observed to be inhibited by high levels of CO_2. The inhibition has been suggested to result from the impact of this gas on substrate transport and the respiratory electron transport chain (Gill and Tan, 1980; Tan and Gill, 1982; Wang *et al.*, 2017; Wang *et al.*, 2018a). However, absence of high levels of CO_2 can allow for anaerobic growth of *Pseudomonas* species on meat by arginine fermentation, as it has recently been demonstrated by (Kolbeck *et al.*, 2021a).

Similar to pseudomonads, *B. thermosphacta* demonstrates competitive growth in presence of O_2 and can dominate the aerobic spoilage community on meat accordingly (De Filippis *et al.*, 2013; Kolbeck *et al.*, 2019). Likewise, it is inhibited by high CO_2 concentrations and will be dominated itself in this case by less sensitive species (Devlieghere and Debevere, 2000; Erichsen and Molin, 1981; Labadie, 1999). *B. thermosphacta* is facultative anaerobic and tolerates decreasing O_2 availability, although it is outcompeted by LAB in case of exhaustion of this gas (Gribble and Brightwell, 2013; Sakala *et al.*, 2002). Its metabolic activity is mainly confined to several carbohydrates as substrate, especially glucose, and includes exploitation of few amino acids, such as glutamate (Casaburi *et al.*, 2014; Gill and Newton, 1977). Presence of O_2 as well as of high amounts of CO_2 decide, if *B. thermosphacta* performs an aerobic or anaerobic metabolism. Increased availability of O_2 enhances its aerobic growth to a certain extent (Kolbeck *et al.*, 2021b; Pin *et al.*, 2002), however, additional presence of CO_2 in high amounts induces anaerobic metabolism (Pin *et al.*, 2002). Aerobic growth has been associated with considerable production of acetoin and other volatiles in this context (Casaburi *et al.*, 2014). Dominance of *B. thermosphacta* over *Ps. fragi* has been demonstrated in mixed cultures on agar plates and interpreted to result from acetic acid production of the former (Drosinos and Board, 1995; Russo *et al.*, 2006). In case of mixed cultures of several Gram-positive meat spoilers, i.e. *B. thermosphacta* and several LAB have been suggested to grow in co-existence based on different substrate preferences. *B. thermosphacta* has thereby been predicted to exploit myo-inositol and glycerol under oxic conditions and ethanolamine under anoxic conditions to avoid substrate competition (Kolbeck *et al.*, 2020).

The order of LAB comprises multiple species with relevance in meat spoilage that can become dominant under MAP and VP (Jääskeläinen *et al.*, 2016). LAB contribute to a different extent to spoilage by e.g. formation of volatiles and changes in meat color (e.g. (Doulgeraki *et al.*, 2010)), but are also subject to research on bioprotective cultures on meat (e.g. (Chaillou *et al.*, 2014; Hilgarth *et al.*, 2018c)). Species with relevance in meat spoilage include *C. maltaromaticum* and *C. divergens*, *Leuconostoc* sp., *Lactococcus* sp. and *Lactobacillus* sp. (Pothakos *et al.*, 2015; Vihavainen *et al.*, 2007). The spoilage potential has been evaluated higher for *Leuconostoc* sp. than for *Lactococcus* sp. and *Lactobacillus* sp. In this context, whereas carnobacteria have been attributed to be less competitive and to disappear with proceeding spoilage (Jääskeläinen *et al.*, 2016). However, the contribution of LAB to spoilage is known to vary significantly even at strain level (Pothakos *et al.*, 2014; Schirmer *et al.*,

2009) and these strain-specific differences form consequently the basis for the utilization of LAB as protective cultures. Competitive advantages of LAB in meat spoilage communities result from its higher tolerance to CO_2 when compared e.g. to *Pseudomonas* sp. and *B. thermosphacta* (Erichsen and Molin, 1981; Garcia-Lopez *et al.*, 1998; Pothakos *et al.*, 2014). Beside of this, its particular influence on the extracellular pH can impair the growth conditions for competitors. The fermentative metabolism of LAB results mainly in acid production (Pothakos *et al.*, 2015), thus lowering the pH in this context. Predominance of LAB has been shown e.g. in co-culture with *B. thermosphacta* (Russo *et al.*, 2006). However, a recent proteomic study has predicted co-existence of several LAB and *B. thermosphacta* from meat, based on the ability of the former also for citrate fermentation, allantoin degradation and β-oxidation (Kolbeck *et al.*, 2020). Regarding the impact of MAP, some LAB have also been reported to be sensitive to high O_2 atmosphere (e.g. (Säde *et al.*, 2017)).

Of the enterobacteria, common species on meat include *Serratia* sp., *Hafnia liquefaciens*, *Enterobacter agglomerans* and *Shewanella putrefaciens* (Casaburi *et al*., 2015). Enterobacteria can become relevant for spoilage especially in case of high meat pH (>pH 6) and comparably high temperature ≥ 4°C (Blickstad and Molin, 1983; Dainty and Mackey, 1992). They prefer to grow on fatty tissue, but utilize available glucose first (Dainty and Mackey, 1992; Gill, 1986; Gill and Newton, 1977). Growth of enterobacteria correlates with pronounced off-odor production, including sulfuric and putrid volatiles (Dainty and Mackey, 1992; Garcia-Lopez *et al.*, 1998). Thereby, significant off-odors can already occur at low cell numbers of 4 $\log_{10}$ (CFU/cm^2)(Dainty and Mackey, 1992), demonstrating high spoilage potential. While enterobacteria grow facultative anaerobic, inhibition by high CO_2 concentrations has been reported, as well as by dropping pH and decreasing temperature (Berruga *et al.*, 2005; Carrizosa *et al.*, 2017; Jiménez *et al.*, 1999).

Meat spoilage can be considered to result from the overall metabolic activity of spoilage consortia rather than single species. First mechanistic insight in the co-existence of Gram-positive meat spoilers has been provided in a proteomic study depicting their metabolic complementarity and adaptation to different gas atmospheres (Kolbeck *et al.*, 2021b; Kolbeck *et al.*, 2020). First insight of *in situ* interaction of meat spoiling bacteria is provided in metatranscriptomic studies (Höll *et al.*, 2019; Höll *et al.*, 2020). However, a detailed view on mechanisms of *Photobacterium* integration into this network has not been elaborated yet.

3.5 Distribution and detection of photobacteria

Research in the field of meat spoilage often focuses on only few species that are considered to be major contributors. Isolation and handling of these species is well established and the collected knowledge on their spoilage contribution assembles to a complex network of correlation and interaction. Still, some spoilage events remain unexplained (Pothakos *et al.*, 2014). This can result from limited knowledge on the mutual influence of spoilage contributors on each other, but also from the existence of so far underestimated spoilage contributors besides of the well-known. Comprehension of the topic has been improved by the development of culture-independent methods (Van Reckem *et al.*, 2021) that allow for unspecific detection of present microorganisms, such as metatranscriptomics and metagenomics. Based on these methods it has been shown that established isolation methods underestimate the occurrence of species with unusual growth requirements and consequently their contribution to spoilage (e.g. (Pothakos *et al.*, 2012; Schirmer *et al.*, 2009).

Species of the genus *Photobacterium* have been overlooked until Hilgarth *et al*. evaluated their occurrence on meat in 2018 and developed a specific cultivation method in accordance (Hilgarth *et al.*, 2018a). The genus comprises five clades (Labella *et al.*, 2017) with in total 36 valid species to date (Parte *et al.*, 2020). Of those, only *P. phosphoreum*, *P. iliopiscarium* and *P. carnosum* have been isolated from meat so far (Hilgarth *et al.*, 2018a), and some studies report culture- independent detection also of *P. kishitanii* (Cauchie *et al.*, 2020; Delhalle *et al.*, 2016). Though, the latter might have been mistaken for *P. carnosum* in studies prior to the species description of *P. carnosum* in 2018, due to their close phylogenetic relationship (Hilgarth *et al.*, 2018b). Species of *Vibrio* sp. as closely related genus of *Photobacterium* sp, have been reported to occur also sporadically on meat and poultry (Casaburi *et al.*, 2015; Nychas *et al.*, 2008). The taxon *Photobacterium* has been framed in 1889 by Beijerinck and has been named with respect to the ability for strain-specific bioluminescence of some of its species (Beijerinck, 1889). Arising application of 16S rRNA based methods nearly ten years later allowed for the current classification of *Photobacterium* as distinct clade within the *Vibrionaceae* family (reviewed in (Urbanczyk *et al.*, 2010)). The genus itself has been proposed to exhibit the clades Phosphoreum, Profundum, Damselae, Leiognathi and Ganghwense (Labella *et al.*, 2018).

Nowadays, detection of photobacteria aside from well-known meat spoilers on chilled meats has been reported frequently. Reports demonstrate their dominance on meat and occurrence in all common types of packaging. This includes MAP meat with high O_2/high CO_2 atmosphere (60-80% O_2/20-30% CO_2; (Cauchie *et al.*, 2020; Höll *et al.*, 2019; Nieminen *et al.*, 2016; Stoops *et al.*, 2015)) or high CO_2 atmosphere (70% N_2, 30% CO_2, (Chen *et al.*, 2020; Duthoo *et al.*, 2021; Höll *et al.*, 2019)), as well as VP meat (Jääskeläinen *et al.*, 2016; Juszczuk-Kubiak *et al.*, 2021; Pennacchia *et al.*, 2011), air-sealed meat (Aladhadh *et al.*, 2018; Dourou *et al.*, 2021; Pennacchia *et al.*, 2011) and food wrap film stored meat

(Cauchie *et al.*, 2020). Also, photobacteria have been found to occur on all major types of fresh meat, on several processed meat products (Table 1), as well as during the ripening process of some of these products (Settanni *et al.*, 2020). Pennacchia and co-workers have provided data on increasing numbers of *Photobacterium*-positive samples within a batch of meat products over 20 days of storage (Pennacchia *et al.*, 2011), showing the ability of photobacteria to become relevant in the issue of meat spoilage. Studies on their detection also in meat processing facilities (Table 1, (Stellato *et al.*, 2016)) and on the skin of chicken carcasses (Yu *et al.*, 2019) thereby allow for speculations on the relevance either of the processing environment or the livestock itself in proliferation.

Photobacteria occur on spoiled as well as on unspoiled meats and thereby reach cell counts of >7 $\log_{10}$(CFU/g) (Juszczuk-Kubiak *et al.*, 2021; Nieminen *et al.*, 2016). Significant cell counts have been found during the early stage of spoilage prior to expiry e.g. on minced pork (Cauchie *et al.*, 2020) or on cooked ham/cooked chicken products (Duthoo *et al.*, 2021). In contrast, relevant amounts of photobacteria have been reported to be confined to the end of shelf life or later stages of spoilage on beef (Pennacchia *et al.*, 2011), minced meat (Stoops *et al.*, 2015), lamb meat (Aladhadh *et al.*, 2018), chicken meat (Dourou *et al.*, 2021) and ostrich meat (Juszczuk-Kubiak *et al.*, 2021). Consequently, reports demonstrate the possibility for decreasing abundancy as well as for increasing abundancy of this genus on spoiling meat. Consistent increase of the cell numbers with proceeding meat spoilage and dominance under these conditions has been stated only by Juszczuk-Kubiak and co-workers (Juszczuk-Kubiak *et al.*, 2021). Literature therefore provides hints on a persistent presence of this genus on spoiling meat and dependency of its growth from so far unexplored circumstances.

Occurrence of photobacteria on meat from retail has been shown to fluctuate considerably. Highly variable initial abundancy of 0-95% has been reported for different batches of cooked ham/cooked chicken slices (Duthoo *et al.*, 2021) and also for minced meat (Cauchie *et al.*, 2020; Stoops *et al.*, 2015). It questions the role of the contamination process for the development of high cell numbers of this genus on meat. Still, reports on the detection of photobacteria on meats are occasional and do not represent a systematic recording of their distribution on meat from retail. Most are random detections, arising as minor observations in culture-independent studies of spoilage communities. Thus, conclusions on growth and viability of the detected cells are impossible to draw. To date, no comprehensive isolation of photobacteria with focus on specific isolation sites exist, although this would allow an estimation on their relevance to the meat industry.

Table 1 Occurrence of Photobacterium *species on meat, on meat products and in the meat processing environment. Occurrence was demonstrated by culture-dependent and culture-independent methods.*

Sample type	Sample	Reference
fresh meat	chicken	(Dourou *et al.*, 2021; Hilgarth *et al.*, 2018a; Höll *et al.*, 2019; Yu *et al.*, 2019)
	pork	(Bassey *et al.*, 2021; Hilgarth *et al.*, 2018a; Kuang *et al.*, 2012; Li *et al.*, 2019b; Nieminen *et al.*, 2016; Stellato *et al.*, 2016)
	beef	(Ercolini *et al.*, 2010b; Hilgarth *et al.*, 2018a; Jääskeläinen *et al.*, 2016; Pennacchia *et al.*, 2011; Stellato *et al.*, 2016)
	lamb	(Aladhadh *et al.*, 2018)
	ostrich	(Juszczuk-Kubiak *et al.*, 2021)
	donkey	(Wei *et al.*, 2021)
minced meat	pork	(Cauchie *et al.*, 2020; Koo *et al.*, 2016)
	beef	(Stoops *et al.*, 2015)
processed meat	steak tartare (beef)	(Delhalle *et al.*, 2016)
	fresh sausages (pork)	(Bouju-Albert *et al.*, 2018)
	fresh sausages (beef)	(Poirier *et al.*, 2020)
	fresh sausages (poultry)	(Poirier *et al.*, 2020)
	naturally fermented salami (boar)	(Settanni *et al.*, 2020)
	naturally fermented salami (horse)	(Settanni *et al.*, 2020)
	naturally fermented salami (pork)	(Greppi *et al.*, 2015; Settanni *et al.*, 2020)
	dry-fermented sausage (pork)	(Pini *et al.*, 2020)
	cooked pork sausages	(Efenberger-Szmechtyk *et al.*, 2021)
	Chinese dry-cured sausage (pork)	(Wang *et al.*, 2018b)
	cooked ham slices	(Duthoo *et al.*, 2021)
	cooked chicken slices	(Duthoo *et al.*, 2021)
	roasted duck meat	(Chen *et al.*, 2020)
meat processing plant	knifes	(Stellato *et al.*, 2016)
	chopping boards	(Stellato *et al.*, 2016)
	operator's hands	(Stellato *et al.*, 2016)

Apart from the recent discovery of the genus *Photobacterium* on meats, it is mainly associated with marine habitats or habitats with marine background. Indeed, comparably few reports on the detection of photobacteria in non-marine habitats are available and even less without evident link to the sea. *P. phosphoreum* has been detected e.g. on fresh water salmon, however, the salmon migrated from marine to fresh water environment (Budsberg *et al.*, 2003). Mathew *et al.* have reported isolation of *P. halotolerans/P. rosenbergii* from the rhizosphere of terrestrial weed (Mathew *et al.*, 2015) and Li *et al*. isolation of *P. salinisoli* from a mixture of saline- and herbicide-contaminated soil (Li *et al.*, 2019a). Yet, *P. carnosum* is the only species that has never been associated with marine environment so far and reports on its isolation are limited to meat associated habitats (Hilgarth *et al.*, 2018b).

Photobacteria in marine habitats colonize diverse ecological niches including open water- and animal-related ones (Lo *et al.*, 2014; Nealson, 1978; Urbanczyk *et al.*, 2010). It has been suggested to consider them as in general free-living, possessing the additional ability to settle marine animals (Labella *et al.*, 2017; Reichelt and Baumann, 1973). The interaction of photobacteria with marine animals can be nonspecific, e.g. parasitic or commensalistic, but also highly specific, as in the mutualistic symbiosis of

luminous photobacteria and fish (reviewed e.g. by (Urbanczyk *et al.*, 2010)). Switching between free-living and animal-associated lifestyle comes along with switching between nutrient-poor and nutrient-rich environment in this context (Urbanczyk *et al.*, 2010). Concomitant with their diversity regarding lifestyles, photobacteria colonize marine niches with differences in geographic localization and water depth. Consequently, ambient temperature and hydrostatic pressure of respective niches can differ considerably. Species of the genus occur in general more frequently in the top water layers of up to 200 m deep, however, they also grow in niches of greater depth (Machado, 2017). Their distribution is thereby subject to environmental preferences as well as to the extent of adaptation to extreme environmental conditions. This forms the basis e.g. for the occurrence of different symbiotic *Photobacterium* species in fish from different water depth (Ast *et al.*, 2007; Dunlap, 2009). However, it is the saprophytic lifestyle of photobacteria and their participation in elemental 'spoilage' processes of the food chains (Bjornsdottir-Butler *et al.*, 2018; Urbanczyk *et al.*, 2010) that determine their role as spoilage organism. Photobacteria enter the global food markets as part of the microbiota on fish and seafood and can become dominant members of the core spoilage community of these products (Chaillou *et al.*, 2015). They reach cell counts of >7 $\log_{10}$ CFU/g on different types of marine fish and can thereby constitute more than 90% of the total viable cell counts (e.g. (Dalgaard *et al.*, 1997; Gornik *et al.*, 2013)).

The occurrence of photobacteria in marine habitats raises the general issue of its occurrence also on meats. To date, no information is available concerning the provenance of *Photobacterium* contaminants and possible relationships of its occurrence on meat and marine fish/seafood. Likewise, the correlation of *Photobacterium* species isolated from marine environment and species isolated from meat remains unexplored.

3.6 Physiology and metabolism of photobacteria

Photobacteria are Gram-negative, facultative anaerobic organisms and possess respiratory- as well as fermentative metabolic pathways (Thyssen and Ollevier, 2007). Their particular growth requirements stand out from the ones of many meat spoilers and resulted in an underestimation of their role in meat spoilage so far (compare chapter 3.5; (Dalgaard *et al.*, 1997)). In this context, it is mainly their dependency of the presence of NaCl that differentiates *P. phosphoreum*, *P. carnosum* and *P. iliopiscarium* from well-known meat spoilers. The whole genus *Photobacterium* is characterized by a general and specific sodium requirement (Gram and Huss, 1996; Reichelt and Baumann, 1973), which is in accordance with its occurrence in marine environment. Marine bacteria are in general weakly halophilic and need 0.2-0.5 M NaCl for growth (Unemoto and Hayashi, 1993). Since established cultivation media contain only 0.5-1% NaCl, detection of photobacteria will be poor (Farmer III *et al.*,

2007). *Photobacterium* species are mesophilic or psychrophilic (Chimetto *et al.*, 2010; Torido *et al.*, 2014), however, the three species isolated from meat so far, *P. phosphoreum*, *P. carnosum* and *P. iliopiscarium*, prefer low temperatures of approximately 15°C for growth (Hilgarth *et al.*, 2018b). Growth has also been reported below 5°C (Hilgarth *et al.*, 2018b; Nealson, 1978), which is in contrast to the majority of the described *Photobacterium* species (Baumann and Baumann, 1977; Chimetto *et al.*, 2010; Reichelt and Baumann, 1973). A distinct sensitivity to elevated temperature has been reported for photobacteria in general (Hansen, 1996; Kanki *et al.*, 2004) that additionally limits the success of cultivation of *P. phosphoreum*, *P. carnosum* and *P. iliopiscarium*, if standard incubation temperatures ≥ 23°C are applied (Dalgaard *et al.*, 1997).

Occurrence of some *Photobacterium* strains not only in shallow water but also in deep sea habitats (Al Ali *et al.*, 2010; Bartlett *et al.*, 1989) has drawn attention to its adaptation to hydrostatic pressure. Piezophilic photobacteria, such as *P. profundum*, *P. frigidiphilum* and *P. kishitanii,* prefer hyperbaric conditions for growth (Bartlett, 2002; Machado, 2017; Martini *et al.*, 2013; Seo *et al.*, 2005) and even upregulate their stress response at atmospheric pressure (Vezzi *et al.*, 2005). High pressure adaptation of microorganisms includes changes in several essential processes of the cell. Effects have been reported e.g. on gene expression, membrane fatty acid- and protein composition, cell division, energy metabolism and motility (reviewed in (Bartlett, 2002; Zhang *et al.*, 2016)). Since hydrostatic pressure specifically characterizes marine- and deep-sea habitats, it may be utilized to separate strains with respect to their terrestrial- or marine origin. However, of the three species detected on meat only *P. phosphoreum* has been mentioned in the context of high pressure adaptation so far. Martini *et al*. have reported improved growth of a *P. phosphoreum* strain at 10 MPa pressure (Martini *et al.*, 2013), but this strain has been reclassified as *P. kishitanii* later (e.g. (Bjornsdottir-Butler *et al.*, 2016)). Consequently, the impact of hydrostatic pressure as characteristic trait of marine environment on *Photobacterium* species from meat is confined to speculations to date. Existing studies on the pressure adaptation of photobacteria focus mainly on deep-sea isolates of *P. profundum*. For this species, up-regulation of glycolysis and gluconeogenesis during hydrostatic pressure conditions has been shown, whereas atmospheric pressure has resulted in enhanced oxidative phosphorylation (Le Bihan *et al.*, 2013). A pressure-adapted strain of *P. profundum* has been shown to possess a particular flagellation system, maintaining motility also at increased pressure (Eloe *et al.*, 2008). Additionally, increased amounts of unsaturated fatty acids, smaller cell size and increased cell aggregation at high pressure conditions have been detected for *P. kishitanii* (Martini *et al.*, 2013). This species also up-regulates expression of some genes encoding trimethylamine n-oxide (TMAO) reductases in response to hydrostatic pressure (Zhang *et al.*, 2016). The organic osmolyte TMAO can serve as alternative electron acceptor during anoxic respiration (reviewed in (Barrett and Kwan, 1985)). General up-regulation of TMAO reductases can therefore enable *P. kishitanii* to react to sudden availability of nutrients in deep

sea habitats (Zhang *et al.*, 2016). However, accumulation of organic osmolytes such as TMAO also preserves protein functionality at (osmotic) pressure conditions (Yancey *et al.*, 1982). In this context, *Photobacterium* species are known to counteract osmotic pressure by production e.g. of ectoine and betaine as compatible solutes (Schmitz and Galinski, 1996).

Likewise, bioluminescence represents a widely distributed trait in marine bacteria, but is comparably rare in terrestrial- and freshwater-associated species (Dunlap and Kita-Tsukamoto, 2006). It has been suggested that light emission by marine bacterial communities on fecal pellets or small particles enhances the possibility to attract phototactic organisms and consequently to re-colonize the nutrient-rich niches on marine animals (Brodl *et al.*, 2018; Dunlap and Kita-Tsukamoto, 2006; Nealson and Hastings, 1979; Tanet *et al.*, 2019). Several *Photobacterium* species are known for bioluminescent strains, including *P. phosphoreum* (Urbanczyk *et al.*, 2010). Occurrence of this trait is independent of the accepted clades of the genus (Labella *et al.*, 2018; Machado, 2017), however, based on the classification of these clades it can be assumed that *P. iliopiscarium* has lost the genes for bioluminescence (Machado, 2017; Urbanczyk *et al.*, 2010). Likewise, no genes for bioluminescence have been detected in *P. carnosum* strains (Hilgarth *et al.*, 2018b), while its classification suggests a loss of the respective cluster. The *Photobacterium* bioluminescence gene cluster includes *lux*C, *lux*D and *lux*E, coding for a fatty acid reductase that provides the long chain aldehyde substrate, *lux*A and *lux*B, specifying a luciferase that catalyzes the oxidation reactions, and *lux*G, coding for a flavin reductase (Brodl *et al.*, 2018; Urbanczyk *et al.*, 2010). The *lux* genes are followed by several genes for riboflavin synthesis (Lee *et al.*, 1994) that together constitute the *lux-rib* operon. The autoinducers that control expression of this operon in many species have thereby been suggested to be involved also in control of other metabolic pathways (Meighen, 1993).

The *lux-rib* operon is part of an additional small chromosome detected in photobacteria, which is characterized by high genetic variability (Machado, 2017). Presence of this small chromosome has been suggested to be a main reason for the observed high diversity of the genus regarding not only lifestyle but also bioluminescence, histamine production (compare chapter 3.7) and motility (Machado, 2017). Regarding motility, many photobacteria possess 1-3 polar flagella (Moi *et al.*, 2017), however, varying presence of motility can be observed already on strain level (Reichelt and Baumann, 1973). The small chromosome together with a more conserved large one and several plasmids form a genome of 4.2-6.4 Mb with 38.7-50.9% GC content. Thereby, *Photobacterium* exhibits a core genome twice as large as the one of the closely related genus *Vibrio* (Machado, 2017).

Despite of its larger genome, photobacteria are characterized by narrow substrate versatility, especially when compared to *Vibrio* (Bamann and Baumann, 1984; Baumann and Baumann, 1981). They accept only 7-22 different substrates as main carbon- and energy source, including hexoses, some

pentoses, disaccharides, intermediates of the tricarboxylic acid (TCA) cycle, sugar acids, monocarboxylic fatty acids and amino acids (Thyssen and Ollevier, 2007). Few substrates can be fermented (Baumann and Baumann, 1981). Even if their substrate versatility is limited, photobacteria possess multiple metabolic pathways for substrate exploitation. They exhibit the Embden-Meyerhof pathway (Reichelt and Baumann, 1973), as well as the pentose phosphate pathway and full TCA cycle (Hilgarth, 2018). Literature on the presence of the Entner-Doudoroff pathway is contradicting. Reichelt has shown increased activity of its key enzymes -6-P-gluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase- during growth with gluconate (Reichelt and Baumann, 1973). In contrast, Hilgarth has reported absence of the genes of the former enzyme in several photobacteria (Hilgarth, 2018). The respiratory chain of photobacteria is based on cytochromes of b and c type (Reichelt and Baumann, 1973; Reichelt *et al.*, 1976) and ubiquinone Q8 as dominant respiratory quinone (Lo *et al.*, 2014; Nogi *et al.*, 1998). Presence of cytochrome c oxidase has been confirmed, however, successful detection can depend on the growth conditions (Baumann and Baumann, 1977). Photobacteria utilize different alternative electron acceptors for anaerobic respiration. Thereby, reduction of fumarate, nitrate and TMAO for respiration has been confirmed (Höll *et al.*, 2019; Proctor and Gunsalus, 2000; Zhang *et al.*, 2016) and reduction of succinate, nitrite and Fe(III) has been taken into consideration (Höll *et al.*, 2019). Of those, TMAO has been suggested as preferred electron acceptor (Zhang *et al.*, 2016), although suppression of TMAO- and fumarate reductase by presence of nitrate has been shown before (Proctor and Gunsalus, 2000). Levels of reductase activity in *Photobacterium* remain comparably stable when changing from oxic- to anoxic conditions, which is in contrast to species of *Vibrio* (Proctor and Gunsalus, 2000). In addition to alternative electron acceptors, anaerobic respiration can also involve alternative electron donors. Literature reports utilization of formate via formate dehydrogenase by photobacteria in this context (Zhang *et al.*, 2016). Beside of anaerobic respiration photobacteria also employ a fermentative metabolism. Presence of fermentative pathways similar to the 'mixed acid fermentation' of enterobacteria has been suggested (Doudoroff, 1942). Thereby, different carbohydrates are fermented via the glycolytic pathway, with two enzymes, -pyruvate formate lyase and lactate dehydrogenase-, initiating the entry into this fermentation type (Ciani *et al.*, 2008). Final products generated by photobacteria include especially acetic acid and formic acid, but also lactic acid, succinic acid, ethanol, CO_2 and sometimes H_2, e.g. in case of some strains of *P. phosphoreum* (Doudoroff, 1942). Additionally, production of diacetyl, acetoin and 2,3-butanediol as volatiles with relevance in spoilage has been reported (Nieminen *et al.*, 2016).

Presence of all necessary elements for aerobic and anaerobic β-oxidation and glycerol degradation has been demonstrated in photobacteria from meat (Hilgarth, 2018; Höll *et al.*, 2019). Some *Photobacterium* strains also excrete lipases for lipid hydrolysis (Baumann and Baumann, 1977; Hilgarth, 2018), however, contradicting information is available concerning details of the glycerol

metabolism. Hilgarth *et al*. have reported acid production from glycerol at anoxic conditions in *P. carnosum* (Hilgarth *et al.*, 2018b). In contrast, other authors have stated the opposite for other species, but have reported glycerol degradation at oxic conditions and in presence of methionine instead (Doudoroff, 1942; Nealson and Hastings, 1979). Regarding fatty acid metabolism, photobacteria can utilize monocarboxylic fatty acids with 2-10 carbon atoms (Farmer III and Hickman-Brenner, 2006). While they are not able to grow with CO_2 as sole carbon source, exclusive utilization of amino acids as substrate has been confirmed (Doudoroff, 1942; Reichelt and Baumann, 1973). Photobacteria possess the arginine deiminase (ADI) pathway and decarboxylate several amino acids, including tyrosine, lysine, arginine, ornithine and glutamate (Hilgarth, 2018). In some cases, even a specific amino acid requirement has been observed, e.g. for some strains of *P. phosphoreum* that need methionine for growth (Doudoroff, 1942; Reichelt and Baumann, 1973). Additionally, oxidation of amino acids linked to concomitant fumarate reduction can take place (Doudoroff, 1942). Expression of the full Stickland reaction has not been confirmed yet, however, all necessary genes have been detected in a deep sea strain of *P. profundum* (Vezzi *et al.*, 2005).

As outlined, general information on the metabolic abilities of photobacteria is available in literature, depicting them as metabolically flexible organisms with pretentious demands to growth temperature and NaCl availability. However, most of this information has been derived from experiments in simplified laboratory systems or from observations on spoiling fish. Information on the metabolism of photobacteria on meat is limited to one metatranscriptomic study, reporting on the general metabolic activity of this genus *in situ* on MAP chicken (Höll *et al.*, 2019). Investigation of the metabolic activity of single *Photobacterium* species on meat and the influence of meat-related factors on metabolic regulation remain to be conducted. *Photobacterium* species occurring frequently in association with meat will for example be subject to competitive interaction and the selective pressure resulting thereof. Detailed knowledge on this issue will be important to the further understanding of the role of this genus in meat spoilage.

3.7 Relevance of photobacteria in meat spoilage

Concomitant with the limited knowledge on the metabolism of photobacteria on meat, information on their contribution to meat spoilage is rare. However, the genus has been extensively studied regarding its contribution to fish spoilage and some observations are transferable to the meat system. *Photobacterium* species are considered to be SSO of chilled fish and seafood and reach frequently high cell numbers on these products (e.g. (Kuuliala *et al.*, 2018)). Of the three species detected on meat so far, *P. phosphoreum* and *P. iliopiscarium* occur also on spoiled fish and seafood (e.g. (Olofsson *et al.*, 2007)), whereas detection of *P. carnosum* is confined to the meat system. *P. phosphoreum* has been

shown to dominate the spoilage community on several types of fresh and processed fish under various storage conditions (e.g. (Dalgaard, 1995b)). Its dominance thereby correlated with high initial cell counts (Gram and Huss, 1996), as well as with specific packaging conditions. Especially utilization of MAP with ≥ 60% CO_2 content or VP has been reported to form the basis for fast growth and dominance of photobacteria in this context (Emborg *et al.*, 2002; Gornik *et al.*, 2013). In modified atmosphere with lower CO_2 content or on air wrapped fish products, high cell numbers of *P. phosphoreum* have been shown to rather emerge during the late stages of spoilage (Reynisson *et al.*, 2009).

MAP is also of importance in the meat industry and applied gas mixtures constitute a relevant growth factor for meat spoiling bacteria. Consequences for the growth and dominance of *Photobacterium* species on meat can be expected. However, it is hitherto unknown if the packaging atmosphere directly influences the growth of photobacteria on meat or if it has rather an indirect impact, e.g. by affecting its competitors. Support for a limited direct impact of the packaging gases has been obtained from a metatranscriptomic analysis on naturally contaminated chicken meat. It has demonstrated comparable effects of different modified atmospheres on the metabolism of photobacteria (Höll *et al.*, 2019). Also, a distinct tolerance of photobacteria to CO_2 has been stated (Dalgaard, 1995a). Literature reports enhanced growth rates of *P. phosphoreum* at anoxic conditions on fish, pointing out a certain extent of sensitivity to the effect of O_2 (Dalgaard, 1995c). However, experimental data concerning the impact of the packaging atmosphere on photobacteria from meat and therefore its potential to restrict the contribution of this genus to meat spoilage is not available.

Sensory defects have been revealed when *P. phosphoreum* cell counts reached approximately 7 $\log_{10}$ (CFU/g) on fish products and these products could consequently be regarded as microbiologically spoiled (Dalgaard *et al.*, 1997; Jorgensen *et al.*, 2000). *P. phosphoreum* produces several volatiles during fish spoilage that have an acidic or aminic smell, e.g. acetic acid, acetoin, diacetyl, 3-methyl-1-butanol or multiple biogenic amines (Nieminen *et al.*, 2016; Jorgensen *et al.*, 2000). Most attention has been paid to the production of TMA in this context. TMA results from TMAO reduction (compare chapter 3.6) and has a strong fishy off-odor (Gram and Dalgaard, 2002). *P. phosphoreum* cells produce up to 100 times more TMA than other spoilers, e.g. *Shewanella putrefaciens,* and have been attributed to possess a greater spoilage potential in accordance (Dalgaard, 1995b). Support has been obtained from Hansen, who has demonstrated higher biochemical activity of *P. phosphoreum* on fish when compared to several LAB and enterobacteria (Hansen, 1996). However, the TMAO content of meat is much lower than the one of (marine) fish (Bekhit *et al.*, 2021; Cho *et al.*, 2017). Dependent of the species, marine fish can contain up to 267 mg/100g wet weight of TMAO (Dyer, 2011), while 650 times lower concentrations have been measured for beef (Cho *et al.*, 2017). Consequently, volatiles associated with growth of photobacteria on meat can be expected to differ to the ones described for

fish. For *P. phosphoreum*, production of acetic acid, ethyl acetate and other esters has been reported on pork so far, together with sweetish and putrid off-odors (Nieminen *et al.*, 2016).

Besides of its contribution to sensory changes during spoilage, presence of *Photobacterium* contaminants also harbors the potential for food poisoning. Some species of the genus are known to produce histamine that can cause multiple symptoms of poisoning after ingestion, e.g. headache, nausea or diarrhea (Kanki *et al.*, 2004). Since the histamine content of a product cannot be reduced by processing methods (Etkind *et al.*, 1987), presence of histamine constitutes a relevant issue and prevention of histamine poisoning is confined to the prevention of its accumulation. In this context, histamine concentrations of ≥500 ppm (≥50 mg/g) are considered to compromise food safety (Lehane and Olley, 2000; Taylor, 1986). Histamine poisoning is mainly related to marine fish, however, hints on its occurrence linked to ingestion of meat also exist (Ruiz-Capillas and Jiménez-Colmenero, 2004; Taylor, 1985; Taylor, 1986). The extent of bacterial histamine production is directly dependent on the availability of free histidine. Thereby, histidine is converted into histamine by decarboxylation, as reviewed by (Lehane and Olley, 2000). The histidine content of meat ranges from 0.7-0.8% of fresh beef loin, 0.9-1.0% of fresh pork loin and 1.0-1.2% of fresh chicken breast (Lyman *et al.*, 1947; Whole-Food-Catalog, 2010) and is consequently comparable to the one reported for fish (Hungerford, 2010; Lehane and Olley, 2000). Additionally, the histidine availability on meat can increase by every process that causes proteolysis (e.g. extreme temperature, Lehane and Olley, 2000). *P. phosphoreum* and *P. iliopiscarium* strains have already been associated with histamine production on fish (Bjornsdottir-Butler *et al.*, 2018; Lehane and Olley, 2000), but only the former one with hazardous concentrations on fish itself (Torido *et al.*, 2014; Kanki *et al.*, 2004). Available literature on the histamine production by *P. phosphoreum* strains is equivocal in this context, since some strains have been misidentified and re-classified e.g. as strains of *P. kishitanii* later (Bjornsdottir-Butler *et al.*, 2018). Cells of *P. phosphoreum* have been described to exhibit particularly high histamine production rates when compared to other histamine producers (Kanki *et al.*, 2004). It is also the only species for which high histamine production rates and concomitant ability to grow below 10°C have been reported (Jorgensen *et al.*, 2000), thus raising its importance in the context of food poisoning in general. However, comparability of its role in histamine fish poisoning and its contribution to meat spoilage remains to be elucidated. Histamine production by *P. iliopiscarium* has been shown to exceed the critical border of ≥500 ppm in liquid culture (Takahashi *et al.*, 2015), whereas *P. carnosum*, has not been examined regarding histamine production so far.

The toxicity of histamine increases in presence of other biogenic amines, especially in interaction with putrescine and cadaverine (Bjornsdottir-Butler *et al.*, 2018; Lehane and Olley, 2000). Efficient production mainly of cadaverine has already been demonstrated for *P. phosphoreum* on fish, but also production of putrescine, agmatine, tyramine and β-phenylethylamine. The general extent of biogenic

amine production can thereby be transferable from the fish- to the meat system, depending on the species (Jorgensen *et al.*, 2000). In accordance, a transcriptomic analysis has provided support for production and excretion of several of the mentioned amines by photobacteria also on meat (Höll *et al.*, 2019). Even though this analysis has not revealed presence also of the gene for histidine decarboxylase that is required for histamine formation, recent literature reports on presence of a novel type of this enzyme in *P. phosphoreum* strains (Bjornsdottir-Butler *et al.*, 2020). Analysis of the production of biogenic amines by photobacteria on meat is confined to general presence of this genus and not attributed to specific species to date. Additionally, the available data are not suitable to identify correlations of the contribution of photobacteria to spoilage on the one hand and common environmental conditions of the meat system on the other hand. Involvement e.g. of the applied packaging atmosphere or of the presence of specific co-contaminants can only be speculated so far.

Many spoilage species affect human health at most by production of hazardous compounds, such as histamine, and this ability can be considered as most relevant safety issue. Still, some species also with pathogenic potential exist and may cause infection. In this context, cases of necrotizing fasciitis and multiple organ failure have been reported in association with presence of *P. damselae* (Clarridge and Zighelboim-Daum, 1985; Yamane *et al.*, 2004). In most cases, infection has been triggered by open wounds, but in some cases also by ingestion (Kim *et al.*, 2009). However, none of the three *Photobacterium* species detected on meat has been reported to infect humans to date.

3.8 Classification of photobacteria within the spoilage microbiota

Microbial spoilage results from the activity of a mixed, interacting spoilage community (Nychas *et al.*, 2007). Likewise, the contribution of photobacteria to meat spoilage can be expected to be subject to their performance within the spoilage community. It is therefore essential to correlate the occurrence of photobacteria on meat with the presence of other already known contaminants.

Interaction within a bacterial community can be in general either cooperative or antagonistic or can be limited to indifferent co-existence. While cooperative interaction will result in promoted growth, antagonistic interaction will cause growth inhibition of at least one partner (reviewed e.g. by (Fredrickson, 1977)). A specific species can thereby trigger cooperative as well as antagonistic interaction in dependence of its co-contaminant. Presence of *Pseudomonas* sp., for example, has been reported to cause growth impairment of *Shewanella putrefaciens* but growth improvement of *Listeria monocytogenes* on meat (Nychas *et al.*, 2007). Cooperative behavior mostly relies on the provisioning of required substances by at least one partner. Beside of this, also removal of substances that cause otherwise growth impairment of the interacting partner has been speculated (Fredrickson, 1977). If

only one partner benefits, the interaction is called commensalistic and if both partners benefit, mutualistic. Thereby, direct cooperative interaction with advantages for both partners but also mutual dependency corresponds to the definition of symbiosis. Parasitism as opposite strategy of symbiosis is confined to the direct invasion of one species by another for its own benefit (James *et al.*, 1995).
Antagonistic relations result either from the removal of required substances by at least one partner or from the production of harmful compounds that affect only the opponent (Fredrickson, 1977). Competitive interaction thereby describes rivalry for substrates or other essential elements, resulting in growth impairment for all involved partners. Amensalism refers to an indirect inhibition of one partner by another, e.g. by production of bacteriocins (James *et al.*, 1995). Interaction can be in general highly specific in case of direct correlation (e.g. symbiosis or parasitism), but also very unspecific in case of an indirect mutual influence (e.g. competition) (Fredrickson, 1977).

Antagonistic interaction has been shown for *P. phosphoreum* and several LAB on MAP/VP salmon (Table 2). Thereby, authors have suggested nutrient competition and acid production as main causes for the reduced growth of *P. phosphoreum* (Joffraud *et al.*, 2006). In contrast, coexistence of LAB and *P. phosphoreum* in high cell numbers of 4-5 $\log_{10}$(CFU/g) on chilled salmon has been reported by Hansen (Hansen, 1996). In any case, possible interactions have been exclusively proposed for the LAB, whereas concomitant strategies of *P. phosphoreum* to subsist in the spoilage community have rarely been considered. Additionally, its transfer to the meat system as logical consequence has not been carried out yet. *P. carnosum* and *P. iliopiscarium* have not been mentioned in this context at all to date. Considerations to interactions of photobacteria on meat are therefore merely speculative.
In general, competitive strategies could be based on the inhibitory effect e.g. of production of bacteriocins, H_2O_2 or CO_2, as it has been reported for other meat- and fish spoilers (Gram *et al.*, 2002; Holzapfel, 1998). Also, growth advantages by highly efficient uptake of essential compounds may exist. In this context, strains can for example gain advantages by production of siderophores in the competition for iron (Gram *et al.*, 2002) or by high substrate affinity in the competition for nutrients (Nychas *et al.*, 1998). However, flexible substrate utilization or production of antimicrobial compounds can still allow for high competitiveness in case of comparably low substrate affinity (Nychas *et al.*, 1998). Verification of the presence of such traits in photobacteria from meat can give explanations for their occasional dominance within the spoilage community. In this context, literature reports ubiquitous presence of photobacteria at the beginning of spoilage, but strongly varying abundancy at the end (compare chapter 3.5). Duthoo and co-workers, for example, have observed presence of up to 83% photobacteria in the community of spoiled meat products, but also < 5% and in many cases absence of this genus (Duthoo *et al.*, 2021). Similar observations have been reported on beef with up to 83% abundance on single samples in the late stage of spoilage (Jääskeläinen *et al.*, 2016). Differences

on species-level or even on strain-level regarding the competitive success of photobacteria on meat can consequently be expected.

Table 2 Confirmed interaction of P. phosphoreum *with other spoilers and resulting effects on its growth and spoilage activity. Fields are empty, if no information on that issue was available.*

Competitor	Isolation source	Effect on growth of *P. phosphoreum*	Effect on spoilage activity of *P. phosphoreum*	Reference
Latilactobacillus sakei (Lactobacillus sakei)	VP salmon	strong inhibition	none on production of total nitrogenous volatiles; reduced production of TMA	(Joffraud *et al.*, 2006)
Latilactobacillus sakei (Lactobacillus sakei)	MAP salmon (100% N_2)	inhibition	none	(Jorgensen *et al.*, 2000)
Latilactobacillus curvatus (Lactobacillus curvatus)	VP salmon	no influence/weak inhibition	reduced off-odors	(Leroi *et al.*, 2015)
C. maltaromaticum	VP salmon	weak inhibition	none	(Joffraud *et al.*, 2006)
C. maltaromaticum	MAP salmon (50% CO_2, 50% N_2)	no influence	none	(Macé *et al.*, 2014)
C. divergens	MAP salmon (100% N_2)	inhibition	none	(Jorgensen *et al.*, 2000)
Leuconostoc mesenteroides	MAP salmon (100% N_2)	inhibition	none	(Jorgensen *et al.*, 2000)
Leuconostoc gelidum	VP salmon	no influence/weak inhibition	reduced off-odors	(Leroi *et al.*, 2015)
Lactococcus piscium	VP salmon	no influence/weak inhibition	none	(Leroi *et al.*, 2015)
Staphylococcus equorum	VP salmon	no influence/weak inhibition	reduced off-odors	(Leroi *et al.*, 2015)
Carnobacterium	ostrich meat	decrease of abundance	-	(Juszczuk-Kubiak *et al.*, 2021)

Besides of competition for nutrients, competition for available O_2 can also take place and will be of relevance even at oxic conditions (Nychas *et al.*, 1998). Availability of gases can be considered to represent a decisive factor with respect to the frequent application of MAP and VP in the meat industry. In cases with limited O_2 availability, dominance of a species can therefore also be subject to its O_2 affinity, as suggested e.g. for *Pseudomonas* on meat (Gill and Newton, 1977). Competition for available O_2 has also been assumed for mixed cultures of *B. thermosphacta* and *Pseudomonas* ssp. (Newton and Rigg, 1979). To date, involvement of specific (packaging) gases in the competitive success of photobacteria on meat has not been considered.

Interaction within a spoilage community includes participation of signaling- and communication systems. Of those, the quorum sensing system is well-known for its role in regulating gene expression in dependence of the cell density and its frequent occurrence in Gram-negative bacteria has been confirmed (Ling *et al.*, 2019). Quorum sensing requires small-sized autoinducer molecules for signaling that can easily diffuse through membranes, such as acyl-homoserine lactones (Waters and Bassler, 2005). Production of these compounds by several Gram-negative food spoiling bacteria, including *P. phosphoreum*, has been reported, however, its relevance in meat and fish spoilage is not confirmed

yet (Gram and Dalgaard, 2002; Gram *et al.*, 2002). With this, it remains open how photobacteria deal and communicate with other spoilage species on fish and especially on meat. While presence of interaction has been demonstrated at least on fish, current knowledge proves to be insufficient for understanding the underlying mechanisms. The available information allows not more than a rough estimation on how photobacteria deal with the presence of other spoilers on meat and how interactions could possibly look like. However, detailed examination of the performance of photobacteria within the meat spoilage community requires preceding knowledge on its occurrence on meats and its adaptations to general issues of the meat system in the first step.

3.9 General methodology

A detailed material and methods section is provided in appendix section 12.6. Species and strains were selected for each experiment according to their isolation source or growth performance (Table 3). Selection was thereby dependent of the respective aim of the experiment.

Table 3. Summary of the selected strains and respective criteria for selection assigned to the performed experiments. The criteria for selection refer to information obtained from literature, as well as to observations on growth and occurrence obtained within the scope of this work.

Experiment	Species and strains	Selective criteria
General diversity of photobacteria on meats	*P. phosphoreum*: DSM15556^T, TMW2.2103, TMW2.2033, TMW2.2034, TMW2.2125, TMW2.2126, TMW2.2127, TMW2.2128, TMW2.2129, TMW2.2130, TMW2.2131, TMW2.2132, TMW2.2133, TMW2.2134, TMW2.2135, TMW2.2136, TMW2.2137, TMW2.2138, TMW2.2139, TMW2.2140, TMW2.2141, TMW2.2142, TMW2.2143, TMW2.2144, TMW2.2145 *P. iliopiscarium*: DSM9896^T, TMW2.2035, TMW2.2104, TMW2.2172	confirmed association also with marine environment
Metabolic diversity of photobacteria on meats	*P. phosphoreum*: DSM15556^T, TMW2.2103, TMW2.2033, TMW2.2034, TMW2.2125, TMW2.2126, TMW2.2129, TMW2.2130, TMW2.2132, TMW2.2134, TMW2.2140, TMW2.2141, TMW2.2142, TMW2.2143, TMW2.2144, TMW2.2145 *P. iliopiscarium*: DSM9896^T, TMW2.2035, TMW2.2104, TMW2.2172	confirmed association also with marine environment and coverage of all identified groups
Growth at hydrostatic pressure	*P. phosphoreum*: TMW2.2134, TMW2.2125, DSM15556^T *P. carnosum*: TMW2.2021^T, TMW2.2148, TMW2.2098, TMW2.2186	Common occurrence on meat; different isolation sources: meat and marine habitats/MAP salmon
Growth at different NaCl concentrations	*P. phosphoreum*: TMW2.2134, TMW2.2125, DSM15556^T *P. carnosum*: TMW2.2021^T, TMW2.2148, TMW2.2098, TMW2.2186	Common occurrence on meat; different isolation sources: meat and marine habitats/MAP salmon
Effect of hydrostatic pressure on the osmotolerance	*P. phosphoreum*: TMW2.2134, DSM15556^T *P. carnosum*: TMW2.2021^T, TMW2.2098	Common occurrence on meat; representative for different isolation sources

Growth on different meat types	*P. phosphoreum*: TMW2.2103, TMW2.2143, TMW2.2140 *P. carnosum*: TMW2.2148, TMW2.2169, TMW2.2149	Common occurrence on meat; isolation from different meat types
Growth within a mixed spoilage community	*P. phosphoreum*: TMW2.2134, TMW2.2103, TMW2.2140 *P. carnosum*: TMW2.2021^{T}, TMW2.2149, TMW2.2169	Common occurrence on meat; appropriate growth performance and recovery
Growth in different modified atmospheres	*P. phosphoreum*: TMW2.2103 *P. carnosum*: TMW2.2149	Common occurrence on meat; intraspecific competitiveness
Interaction with selected co-contaminants	*P. phosphoreum*: TMW2.2103 *P. carnosum*: TMW2.2149	Common occurrence on meat; intraspecific competitiveness

The experimental setup for examination of the tolerance of *P. phosphoreum* and *P. carnosum* strains to hydrostatic pressure involved preparation of pre-cultures, subsequent application of constant or short-term pressure and final cultivation on suitable medium for evaluation. Examination of the growth at 40 MPa hydrostatic pressure was performed with low initial OD_{600}, whereas examination of the tolerance to ≤125 MPa hydrostatic pressure was performed with cultures that reached the exponential phase (Figure 1, appendix section 12.6.5; (Hauschild *et al.*, 2021a)).

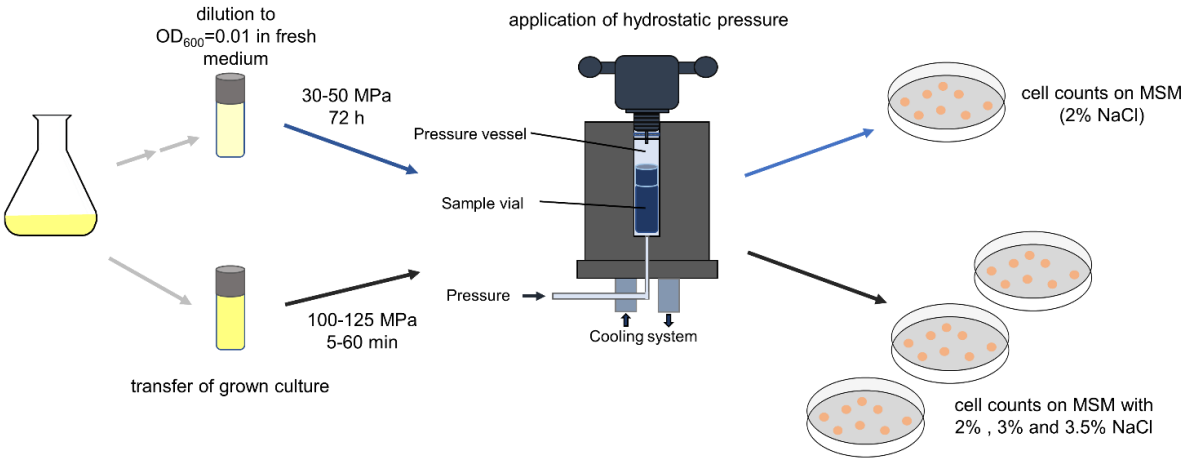

Figure 1 Workflow of the high pressure experiments. Blue arrows depict the work flow for determining the growth during application of hydrostatic pressure, black arrows indicate experiments on survival at hydrostatic pressure and development of sublethal injury.

Experiments concerning growth and interaction on meat were in general performed with MAP skinless chicken breast fillets obtained from a local supermarket of the area of Freising, Germany. If experiments involved other meat types, MAP pork and MAP beef steaks were utilized. The meat was carefully chilled during transport, cut in the required shape in the laboratory, inoculated with the selected bacteria and finally packaged with defined gas atmosphere (Figure 2, appendix section 12.6.6, (Hauschild *et al.*, 2021b)).

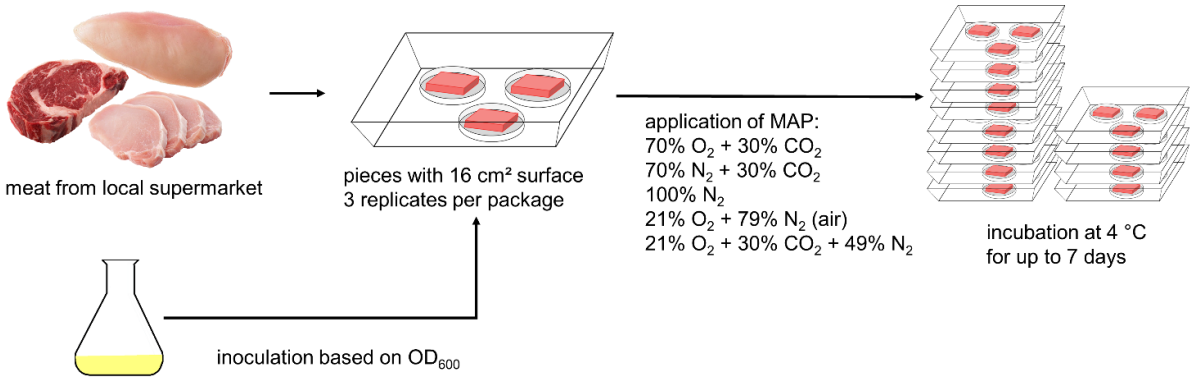

Figure 2 Workflow of the inoculation process for fresh meats. In case of experiments with co-contaminating species, bacteria were cultivated separately and mixed right before inoculation of the meat. Meat packages were carefully chilled before and after application of MAP.

The sealed packages were incubated at 4°C until the end of the experiment. Before opening a sealed package for analysis the composition of its gas atmosphere was checked (Figure 3, upper work-line) Cells were then isolated from the meat by homogenization and cultivation on selective medium to evaluate growth and persistence (Figure 3, middle work line, compare appendix section 12.6.1 for details). Species were identified by MALDI-TOF MS and strains were further separated by RAPD-PCR. For the analysis of the gene expression on the level of transcription strains were cultivated on entire chicken breast fillets. Afterwards, fillets were washed with RNA-stabilizing solution, cells were harvested and RNA was isolated following the RNeasy Mini Kit (Qiagen, Hilden, Germany) with some modifications (Figure 3, bottom work line, appendix section 12.6.8, (Hauschild *et al.*, 2021c)).

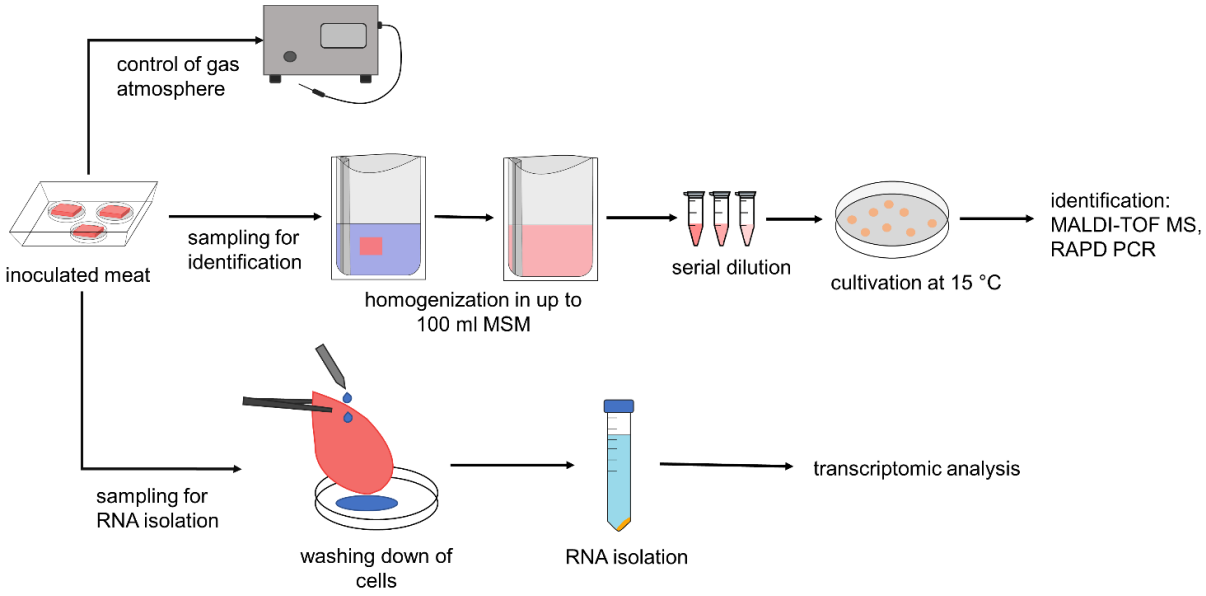

Figure 3 Workflow of the performed analysis steps following inoculation and incubation of meats. Growth experiments and studies on the interaction behavior were performed with meat pieces of 16 cm² (central work line), whereas interaction studies based on RNA isolation were performed with entire chicken breast fillets (bottom work line).

4 Aims of this work and hypotheses

The association of *Photobacterium* species not only with marine environments but also with spoiled meat represents a recent and so far poorly studied discovery. Consequently, occurrence, prevalence and role of photobacteria in the meat microbiota as well as their relevance to meat spoilage remains to be explored. Aims of this study were therefore

(i) to elucidate the occurrence of photobacteria on meat in the first step, by recording their distribution and examining the logical context of their occurrence on meats and in marine environments.

(ii) to subsequently verify their ability to grow and persist in the meat spoilage system in the context of relevant environmental parameters.

(iii) to finally assess their participation within the well-characterized interacting meat spoilage community.

Following working hypotheses thereby constituted the guiding principles of this work and structured it into four consecutive chapters:

Chapter 1 postulate: *Photobacterium* contaminants on meat originate from the livestock farming, where they colonize appropriate habitats on the animal itself or in its environment.

- Occurrence of *Photobacterium* contaminations on food with focus on meat and meat products should be evaluated. Food samples should cover different producers, retailers and packaging methods.
- Diversity of the *Photobacterium* isolates from different food products should be explored. Thereby, presence of sub-populations correlated with specific packaging conditions or producers should be evaluated.

This postulate was explored within the scope of the publication 'Fuertes-Perez, S.*, Hauschild, P.*, Hilgarth, M., Vogel, R.F., 2019. Biodiversity of *Photobacterium* spp. isolated from meats. Front. Microbiol. 10, 2399. https://doi.org/ 10.3389/fmicb.2019.02399.'

*shared first-authorship

Chapter 2 postulate: Photobacteria from meat and photobacteria from marine environments are adapted to different lifestyles.

- Representative *Photobacterium* isolates from meat should be compared to isolates from marine niches. Comparison should thereby focus on adaptation to and tolerance of distinguishing traits of the marine environment *versus* terrestrial habitats.
- Detected differences should be assessed regarding a possible association with the environmental conditions of the respective isolation source.
- Obtained information should be utilized to review a possible correlation of the occurrence of photobacteria on meat and in marine niches.

This postulate was explored within the scope of the publication 'Hauschild, P., Hilgarth, M., Vogel, R.F., 2021. Hydrostatic pressure- and halotolerance of *Photobacterium phosphoreum* and *P. carnosum* isolated from spoiled meat and salmon. Food Microbiol. 99, 103679. https://doi.org/10.1016/j.fm.2020.103679'.

Chapter 3a postulate: Growth of photobacteria on meat is influenced by the applied packaging atmosphere.

- Growth performance of relevant *Photobacterium* species on meat should be evaluated in different packaging atmospheres.
- Obtained data should be utilized to evaluate the general impact of CO_2 and O_2 on photobacteria on meat.

Chapter 3b postulate: Photobacteria interact with other members of the meat spoilage community.

- Impact of the presence of representative Gram-positive and Gram-negative meat spoilers on the growth of relevant *Photobacterium* species on meat should be recorded in different packaging atmospheres.
- Growth of relevant *Photobacterium* species in presence of a diverse meat spoilage community should be determined.

These postulates were explored within the scope of the publication 'Hauschild, P., Vogel, R.F., Hilgarth, M., 2021. Influence of the packaging atmosphere and presence of co-contaminants on the growth of photobacteria on chicken meat. Int J Food Microbiol. 351, 109264. https://doi.org/10.1016/j.ijfoodmicro.2021.109264'.

Chapter 4 postulate: The metabolism of photobacteria is influenced by competing meat spoilers.

- Regulation of metabolic pathways by photobacteria in presence of Gram-positive and Gram-negative competitors on meat should be compared to respective processes in absence of competitors. Metabolic activity should thereby be analyzed based on transcriptomic data.

This postulate was explored within the scope of the submitted manuscript 'Hauschild, P., Vogel, R.F., Hilgarth, M., 2021. Transcriptomic analysis of the response of *Photobacterium phosphoreum* and *Photobacterium carnosum* to co-contaminants on chicken meat'.

5 Results (publications)

5.1 Biodiversity of *Photobacterium* spp. isolated from meats

To examine the provenance of *Photobacterium* contaminants on meat, occurrence and diversity of *P. phosphoreum*, *P. iliopiscarium* and *P. carnosum* was studied on food products. Several types of food samples were obtained from local shops and retailers and tested for contamination by selective cultivation. *Photobacterium* contaminations were thereby detected on multiple meats and meat products from large retailers but also from local facilities. Positive samples mainly belonged to fresh beef, pork and chicken meat that were retailed in all commonly applied packaging types (modified atmosphere, vacuum, air stored). Occurrence of *Photobacterium*-positive samples exhibited strong variability and did not lead back to a specific brand, producer or processing type. If photobacteria were detected, they frequently occurred in high relative abundancy of the present spoilage community. *P. phosphoreum* strains reached a relative abundancy of up to 100% on marinated MAP beef and air-packed pork, while *P. iliopiscarium* constituted up to 69% on MAP pork. However, the latter was detected on comparably few samples. *P. carnosum* reached up to 100% relative abundancy on air-packed chicken/beef and >90% on vacuum beef/pork and marinated MAP chicken. The total cell number of photobacteria on meat samples exceeded 8 $\log_{10}$(CFU/g) in several cases and was not below 4 $\log_{10}$(CFU/g) after reaching the expiry date on positive samples. This suggests relevant participation of *P. phosphoreum* and *P. carnosum* in the meat spoilage process. Obtained isolates were then analyzed regarding their diversity and metabolic abilities. The analysis showed great biodiversity within the obtained 24 strains of *P. phosphoreum*, 3 strains of *P. iliopiscarium* and 31 strains of *P. carnosum*. Neither specific genotypes (RAPD-PCR fingerprints) nor specific growth properties (lag phase, growth rate, influence on the pH) or metabolic abilities (API 50CH, API ZYM, antibiotic resistance) could be correlated with a specific isolation source. *P. phosphoreum*/*P. iliopiscarium* differ from the third species *P. carnosum* by their association also with marine environment. In accordance, species differed regarding growth and metabolic activity, with *P. phosphoreum* showing fastest growth and strongest alkalization. Strains of *P. phosphoreum* and *P. iliopiscarium* were clearly distinguishable with respect to their isolation from meat/marine habitats, whereas strains of *P. carnosum* were not. Thus, differences in the provenance of *P. phosphoreum*/*P. iliopiscarium* contaminations on the one hand and *P. carnosum* contaminations on the other hand on meat can be assumed.

Author contributions: In this joint first authorship communication Philippa Frederieke Hauschild conducted all experiments dealing with *P. phosphoreum*- and *P. iliopiscarium* strains. She contributed to the experimental design, and visualized and evaluated all data concerning *P. phosphoreum*- and *P. iliopiscarium* strains. She wrote the original draft of introduction, results, materials and methods and

conclusions, and contributed to the original draft of the other parts of the manuscript and to establish the reviewed final version.

Sandra Fuertes Perez conducted all experiments dealing with *P. carnosum* strains. She contributed to the experimental design, and visualized and evaluated all data concerning *P. carnosum* strains. She wrote the original draft of the discussion, designed the layout of figures and tables and contributed to the original draft of the other parts of the manuscript. Maik Hilgarth performed the diversity index analysis and supervised the work of Philippa Frederieke Hauschild and Sandra Fuertes Perez and contributed to corrections that arose during the reviewing process. Rudi F. Vogel initiated the corresponding project, supervised the work of Philippa Frederieke Hauschild and Sandra Fuertes Perez and reviewed this manuscript.

Supplementary files of this publication are provided in appendix section 12.1.

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Biodiversity of *Photobacterium* spp. Isolated From Meats

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Photobacteria are common psychrophilic bacteria found in marine environments. Recently, several studies revealed high numbers of *Photobacterium* (*P.*) spp. on packaged fresh meat. Their occurrence appears relevant for the spoilage of meat, since species of the genus are already known as potent fish spoilage organisms. Here we report on distribution, biodiversity, and specific traits of *P. carnosum* (n = 31), *P. phosphoreum* (n = 24), and *P. iliopiscarium* (n = 3) strains from different foods. Biodiversity was assessed by genomic fingerprinting, diversity index analysis, growth dynamics, comparison of metabolic activities, and antibiotic resistance. We observed a ubiquitous occurrence of the species on all common meats independent of packaging conditions and producer, suggesting contamination during an established processing or packaging step. Regarding biodiversity, the three species differed clearly in their growth properties and metabolic characteristics, with *P. phosphoreum* growing the fastest and showing the strongest alkalization of the media. On strain level we also recorded variations in enzymatic reactions, acid production, and antibiotic resistances not restricted to specific meat types. This depicts high biodiversity on species and strain level on each contaminated meat sample. Our analysis showed that meat-borne strains of *P. phosphoreum* and *P. iliopiscarium* clearly differ from their type strains from a marine habitat. Additionally, we report for the first time isolation of *P. carnosum* strains from packaged fish, which in contrast showed comparable phenotypic properties to meat-borne strains. This hints at different initial origins of *P. phosphoreum/P. iliopiscarium* (marine background) and *P. carnosum* (no demonstrated marine background) contaminations on fish and meat, respectively.

Keywords: *Photobacterium carnosum*, *Photobacterium phosphoreum*, *Photobacterium iliopiscarium*, meat spoilage, psychrophilic spoilers, modified atmosphere packaging

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INTRODUCTION

Photobacteria are Gram-negative, facultatively aerobic members of the Vibrionaceae family and well known as marine-related species (Lo et al., 2014; Li et al., 2017; Wang et al., 2017). First described in 1889 (Beijerinck, 1889), the genus currently comprises 30 valid species, and 2 subspecies (Parte, 2018). Photobacteria occur free-living in seawater and sediments or in interaction with marine animals (Urbanczyk et al., 2011; Labella et al., 2017), e.g., the symbiosis of bioluminescent strains within the light organs of deep sea fish (Hendrie et al., 1970). However, photobacteria are also known as effective saprotrophs in marine habitats (Urbanczyk et al., 2011). In this context, certain species, i.e., *Photobacterium* (*P.*) *phosphoreum* and *P. iliopiscarium* constitute a

considerable problem in the food industry, representing potent spoilers of chilled fish and seafood products (Okuzumi et al., 1994; Dalgaard et al., 1997). The spoilage processes involve production of biogenic amines such as histamine (Okuzumi et al., 1994; Jorgensen et al., 2000; Emborg et al., 2002; Torido et al., 2012; Takahashi et al., 2015; Bjornsdottir-Butler et al., 2018) that can lead to scombroid fish poisoning (Lehane and Olley, 2000).

Previous studies based on culture-independent approaches have revealed presence of photobacteria gene sequences on pork (Nieminen et al., 2016), pork sausages (Bouju-Albert et al., 2018), beef (Pennacchia et al., 2011), and minced meat (Stoops et al., 2015; Nieminen et al., 2016). In only one of these studies very few isolates of *P. phosphoreum* were recovered (Nieminen et al., 2016) since common control methods rely on standard agars and cultivation at temperatures between 25 and 30°C, which do not allow isolation of fastidious and psychrophilic photobacteria. Highly frequent isolation was recently demonstrated by Hilgarth et al. (2018a) employing a novel, targeted selective isolation procedure for recovery of photobacteria from foods. *P. phosphoreum* and *P. iliopiscarium* were isolated from modified atmosphere packaged (MAP) poultry, pork, and beef (only *P. phosphoreum*) (Hilgarth et al., 2018a). *P. phosphoreum* was firstly described in 1878 (Cohn, 1878) and re-evaluated in 1889 (Beijerinck, 1889) as a luminous isolate from the sea. It is adapted to high-pressure (Labella et al., 2017), grows optimally at 15–20°C, and occurs frequently as predominant spoiler on fish products (Gram and Huss, 1996; Reynisson et al., 2009). *P. iliopiscarium* was described by Onarheim et al. (1994) as *Vibrio iliopiscarium* and later reassigned to *Photobacterium* by Urakawa et al. (1999). There are several studies reporting *P. iliopiscarium* isolates from marine fish (Dunlap and Ast, 2005; Olofsson et al., 2007; Thyssen and Ollevier, 2015; Hilgarth et al., 2018a) but only few that describe them as predominant (Olofsson et al., 2007). Just as *P. phosphoreum*, it prefers 15–20°C for growth (Onarheim et al., 1994; Hilgarth et al., 2018b). In addition, a new psychrophilic species, *P. carnosum*, was recently discovered on meat. It also prefers 10–15°C and was described as the first species of the genus that is unrelated to marine habitats (Hilgarth et al., 2018b). This new species was reported as the major representative of the *Photobacterium* genus on poultry and beef, while it was less abundant on pork.

Not only do these psychrophilic bacteria occur in high numbers on meat, but they also exhibit spoilage potential. A recent metatranscriptomic study has predicted its potential for production of several biogenic amines, such as putrescine, cadaverine, agmatine, tyramine, and gamma-amino-butyric acid as well as various other spoilage compounds that are known for other potent meat spoilers (Höll et al., 2019).

Until now, knowledge on the origin and biodiversity of *P. carnosum*, *P. phosphoreum*, and *P. iliopiscarium* on food products and especially meats is very limited. This study aimed at elucidation of their distribution and diversity in order to identify specific traits of the species and possible correlations between the source of isolation, genotypes, or physiotypes. For this, we surveyed and reviewed the occurrence of photobacteria on meat samples from local butchers and supermarkets. Selected isolates from different samples were then used to thoroughly study biodiversity.

MATERIALS AND METHODS

Isolation and Identification of Photobacteria

Isolation was carried out as described in the isolation protocol from Hilgarth et al. (2018a). Samples purchased and kept at 4°C were cut and homogenized in marine broth (DIFCO). Samples were plated on marine agar [marine broth, 1.6% agar-agar (w/v)] supplemented with 3 g/L meat extract and 7 mg/L vancomycin, and incubated at 15°C for 72 h. Composition of the base marine broth media includes: peptone 5 g/L, yeast extract 1 g/L, sodium chloride 19.45 g/L, ferric citrate 0.1 g/L, magnesium chloride 5.9 g/L, magnesium sulfate 3.24 g/L, calcium chloride 1.8 g/L, potassium chloride 0.55 g/L, sodium bicarbonate 0.16 g/L, potassium bromide 0.08 g/L, strontium chloride 34 mg/L, boric acid 22 mg/L, sodium silicate 4 mg/L, sodium fluoride 2.4 mg/L, ammonium nitrate 1.6 mg/L, and disodium phosphate 8 mg/L. Isolates were identified based on their low-molecular subproteome with MALDI-TOF MS on a Microflex LT Spectrometer (Bruker Corporation, Billerica, MA, United States) by direct transfer method and on-target extraction (Usbeck et al., 2013; Hilgarth et al., 2018a). An in-house database containing mass spectrometry profiles of various photobacteria species was established by sequencing of housekeeping genes in order to guarantee reliable identification. In total at least three packages per meat type were analyzed for abundance of photobacteria. Type strains *P. phosphoreum* DSM15556^T and *P. iliopiscarium* DSM9896^T, obtained from the German Strain Collection (DSMZ), were also part of the selected strains. Additionally, the type strain *P. carnosum* TMW2.2021^T and some already described strains of the species (TMW2.2022, TMW2.2029, and TMW2.2030) were included (Hilgarth et al., 2018b).

Genomic Fingerprinting

Randomly amplified polymorphic DNA (RAPD)-PCR fingerprinting was used to assess the number of different strains within all isolates and select them for subsequent characterization. RAPD-PCR was performed with the primer M13V (5′-GTT TTC CCA GTC ACG AC-3′) (Ehrmann et al., 2003). Bands were separated by electrophoresis in agarose gel (1.4% w/v, 150 V, 2.5 h). Lambda DNA/*Eco*RI plus *Hin*dIII Marker (Thermo Scientific, Hampshire, United Kingdom) was used as molecular weight marker and for normalization/standardization of the gel pattern for comparison. Similarities in fingerprint pattern were analyzed with Bionumerics V7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium). Hierarchical clustering analysis was carried out by unweighted pair group method with arithmetic mean (UPGMA) method and Dice similarity coefficient with 1% tolerance. After the initial strain delineation by RAPD-PCR for all isolates, the RAPD approach was again performed twice for all strains of the three species to assess the reproducibility of the observed

patterns and ensure the fidelity of the clustering. Furthermore, the similarity of triplicates of all strains was compared to the triplicates of the closest related strain in order to further validate the strain delineation and distinctness.

Randomly amplified polymorphic DNA PCR protocol was additionally carried out with primer M14V (5′-CTG TCC AGT CAC GTC-3′) with all selected strains in order to confirm their distinctness and diversity within the species. Protocol and standardization was performed as described for M13V primer.

Diversity Index Analysis

Individual rarefaction analysis and calculation of diversity indices for evenness (Simpson, 1949), entropy (Shannon and Weaver, 1949), and richness (Chao, 1984) was performed using PAST software 3.25 (Hammer et al., 2001) with operational taxonomic units [OTUs (Schloss and Handelsman, 2005)] defined as distinct/unique RAPD genomic fingerprinting representing distinct genotypes on strain level. A p-value < 0.05 was defined as significantly different. Coverage (%) of genotypes was calculated using Good's coverage estimator as described by Good (1953) with the equation:

$$C = \left(1 - \frac{N_1}{n}\right) * 100, \quad (1)$$

with N_1 representing OTUs only found once (singletons) and n as the total number of individuals (strains).

Growth Analysis in Meat Simulation Medium

Growth curves were performed with all isolated strains of the three species of photobacteria used in this study, a total of 31 strains of *P. carnosum*, 24 strains of *P. phosphoreum*, and 3 strains of *P. iliopiscarium*, in addition to the marine type strains of *P. phosphoreum* (DSM15556^T) and *P. iliopiscarium* (DSM9896^T). Inoculum was prepared from an overnight culture in marine broth at 15°C, by centrifuging the cells (4000 × *g*, 10 min), washing them with NaCl 2% (w/v), and resuspending on meat-simulation media. Growth curves were started by inoculating meat-simulation media (20 g/L meat extract, 20 g/L NaCl, pH 5.8) in 50 mL Erlenmeyer flasks at an initial OD_{600} of 0.05. Cultures were incubated at 4°C with constant agitation, and samples were taken regularly for OD_{600} measurement. The pH of the culture was measured at maximum OD_{600}. Growth curves were adjusted to parametric models with RStudio v1.1.463 and grofit package v.1.1.1-1 (Kahm et al., 2010) to determine lag phase (lag), maximum growth rate (U), and maximum OD_{600}. Growth curves were performed in triplicates and data were further analyzed in IBM SPSS Statistics v23.0.0.0. Tests for normality (Shapiro–Wilk) and homogeneity of variances (Levene test) were carried out for each set of data. One-way ANOVA followed by HSD Tukey *post hoc* test determined significant differences between the strains of each species. Welch-ANOVA and Games-Howell *post hoc* tests were used in case of heterogeneity of variances. Significance level was determined by $p < 0.05$.

Motility Test

Motility for all strains was determined by the soft agar stab method. Meat-simulation media supplemented with 3 g/L agar was poured into tubes. Motility was measured based on the turbidity of the soft agar around the stabbing zone.

Bioluminescence of *P. phosphoreum* Strains

Bioluminescence in darkness was scored by visual comparison of the intensity on marine agar plates for all *P. phosphoreum* strains. Suspensions with the same OD_{600} were prepared for all strains, plated on marine agar plates, and incubated at 15°C for 72 h.

Antibiotic Resistance Test

Antibiotic resistance of all strains of the three species of photobacteria was assessed by disc diffusion assay. All discs were purchased from Oxoid (Thermo Scientific, Hampshire, United Kingdom).

Metabolic Characterization

Metabolic characterization was assessed for a representative group of all the strains of the three species of photobacteria. A total of 14 strains of *P. phosphoreum*, 16 strains of *P. carnosum*, and 3 strains of *P. iliopiscarium* were assessed for carbohydrate acid production and enzymatic activities. Production of acid from different carbon sources was assessed by the API 50CH test (bioMérieux, Marcy-l'Étoile, France). Several enzymatic activities were tested with the API ZYM test (bioMérieux, Marcy-l'Étoile, France) according to the instructions from the manufacturer. Both procedures were performed according to the methodology followed by Hilgarth et al. (2018b) and data for *P. carnosum* TMW2.2021/2.2022/2.2029/2.2030, *P. phosphoreum* DSM15556^T, and *P. iliopiscarium* DSM9896^T were taken from this study.

Hierarchical Cluster Analysis

Hierarchical cluster based on the results for physiological tests of selected strains was carried out by a Heatmapper tool[1] with average linkage criteria and Euclidean distance.

RESULTS

Occurrence of Photobacteria on Selected Food Products

Various food samples were obtained from local retailers and butchers and screened on the presence of photobacteria. We detected them on several meat types and on marine fish (**Table 1**), on MAP packaged, vacuum packaged, and air stored samples and also on marinated meats. The contaminated samples originated thereby from large supermarket chains as well as from small local shops. However, not all samples contained photobacteria, even if they originated from the same producer. We also found different species compositions that were dependent on the meat type. In

[1] www2.heatmapper.ca/expression/

TABLE 1 | Detection of *Photobacterium* spp. on different meats.

Packaging atmosphere	Meat type	Origin	Detected *Photobacterium* spp.	Relative abundance of *Photobacterium* spp. (%)	CFU photobacteria [log_{10}(CFU/g)]	CFU bacteria [log_{10}(CFU/g)]
Air	Chicken	Local butchery	*P. carnosum*	100	6.29	7.67
Air	Beef	Local butchery	*P. carnosum*	100	7.54	9.22
Air	Pork	Local butchery	*P. phosphoreum*	100	8.57	9.34
Air	Codfish	Local fish shop	*P. phosphoreum*	100	NA	NA
Air	Marinated turkey	Supermarket	*P. carnosum* *P. phosphoreum*	25 75	7.17	8.28
MAP	Marinated chicken	Supermarket	*P. carnosum* *P. phosphoreum*	96 4	4.54	4.63
MAP	Marinated beef	Supermarket	*P. phosphoreum*	100	8.76	9.66
MAP	Chicken*	Supermarket	*P. carnosum* *P. phosphoreum* *P. iliopiscarium*	71 27 2	6.56	6.57
MAP	Beef*	Supermarket	*P. carnosum* *P. phosphoreum*	90 9	3.55	4.19
MAP	Pork*	Supermarket	*P. carnosum* *P. phosphoreum* *P. iliopiscarium*	5 26 69	7.07	7.13
MAP	Salmon	Supermarket	*P. carnosum* *P. phosphoreum* *P. iliopiscarium* *Photobacterium* sp.	7 58 22 13	6.77	6.8
Vacuum	Beef	Supermarket	*P. carnosum* *Photobacterium* sp.	96 4	6.72	6.72
Vacuum	Pork	Supermarket	*P. carnosum* *Photobacterium* sp.	99 1	7.15	7.15

*Representative types of spoiled meat samples where photobacteria were detected, and the common distribution of photobacteria found on them. The meat samples were bought in different supermarkets and shops and then incubated at 4°C until they were expired. Its spoilage community on selective medium was then analyzed with MALDI-TOF MS. CFU was determined on the base of the selective media consisting on marine broth supplemented with 3 g/L meat extract and 7 mg/L vancomycin. *Information obtained from Hilgarth et al. (2018a) and appended for comparison. NA, quantification was not possible due to overgrow of bacteria on plates, but photobacteria were recovered by observing bioluminescent colonies.*

addition to our previously published data, we identified only two species – *P. carnosum* and *P. phosphoreum* – on beef and turkey. On chicken and pork, and additionally on salmon, we detected *P. carnosum*, *P. phosphoreum*, and *P. iliopiscarium* (**Table 1**). Besides different meats, we analyzed a variety of additional food products to determine the distribution of photobacteria in the food industry. We did not detect photobacteria in algae (dried and salad), ready-to-eat salad (MAP, 2 samples), and sprouts (MAP); raw milk (12 samples), mozzarella cheese (3 samples), and eggs (3 samples); scallops (defrosted), trout, shrimps (cooked, defrosted) and sea salt; and minced meat (beef and mixed, 5 samples), bacon (2 samples), cooked ham, raw ham, and dried meat (pork).

Genetic Differentiation

In total, we recovered 163 *P. carnosum*, 113 *P. phosphoreum*, and 3 *P. iliopiscarium* isolates from chicken, turkey, pork beef, and salmon (total $n = 279$). Based on differences in their RAPD pattern obtained with primer M13V, we were able to discriminate 31 strains of *P. carnosum*, 24 of *P. phosphoreum*, and 3 strains of *P. iliopiscarium* within all isolates for further investigations on biodiversity. Genotypic distinctness of the strains were further validated with a RAPD approach using primer M14V. Isolates of *P. phosphoreum* from MAP farmed salmon showed no distinct or unique genotypes and were therefore considered as redundant strains. However, we recovered two strains of *P. carnosum* from salmon that were not abundant on other meats. Additional detailed information regarding the sample of origin of every strain used in this study can be found in **Supplementary Table S1**.

Calculation of diversity indices (**Table 2**) and an individual rarefaction analysis (**Supplementary Figure S1**) were carried out for all strains of each species with OTUs based on distinct genomic fingerprinting patterns. The analysis demonstrated that biodiversity of *P. phosphoreum* and *P. carnosum* was completely or almost completely covered by the strains isolated in this study, respectively. This was indicated by saturated rarefaction curves, a high calculated coverage value (>99%, >96%) and an identical or very similar richness of the expressed Chao-1 value to the actual number of genotypes. Additionally, both species were not significantly different regarding their ecological evenness and entropy ($p > 0.05$). Regarding *P. iliopiscarium*, calculation of diversity indices and comparison to the other two species were not expedient since only three isolates with three different genotypes could be recovered.

Chromosomal RAPD fingerprints of the strains of the three species were subjected to hierarchical cluster analysis and could be affiliated to several separate groups (**Figure 1**). In rare cases, RAPD pattern was highly similar and had a 100% dice similarity

TABLE 2 | Diversity indices of photobacteria species using genotyping OTUs.

Species	*P. phosphoreum*	*P. carnosum*	*P. iliopiscarium*
Individuals (isolates)	113	163	3
OTUs (strains)	24	31	3
Simpson (evenness)	0.9526	0.9406	–
Shannon (entropy)	3.106	3.081	–
Chao-1 (richness)	24	34.75	–
Good's coverage estimator (%)	99.12	96.32	0

in one RAPD approach using primer M13V with selected isolates. However, in the other two RAPD-PCR approaches with primer M13V, they exhibited different patterns indicating highly similar, but different strains (**Supplementary Figure S2**). Furthermore, patterns obtained with additional primer M14V validated their distinctness (**Supplementary Figure S3**).

Additional analysis of triplicates of all strains confirmed that they cluster together and apart from triplicates of the closest related strains in each case, indicating that respective replicates of one strain were more similar to each other than to other strains. The cluster similarity (dice coefficient) of the triplicates of each strain was at least 3.7% (*P. phosphoreum*), 3.9% (*P. carnosum*), and 22.7% (*P. iliopiscarium*) different from the cluster similarity of triplicates of the respective closest related strain.

Both *P. phosphoreum* and *P. carnosum* strains separated in 10 groups with a threshold of 76 and 79.5% similarity, respectively. Compared to this, the three strains of *P. iliopiscarium* clustered with lower similarity (≤66.7%). Strains from the same meat type did not form coherent cluster, except of *P. phosphoreum* strains from pork and the *P. carnosum* strains from fish. We additionally performed a cluster analysis of all strains of the three species with both primers M13V and M14V (**Supplementary Figures S4**, **S5**). All strains from one species cluster together and apart from strains of the other two species thus validating our approach.

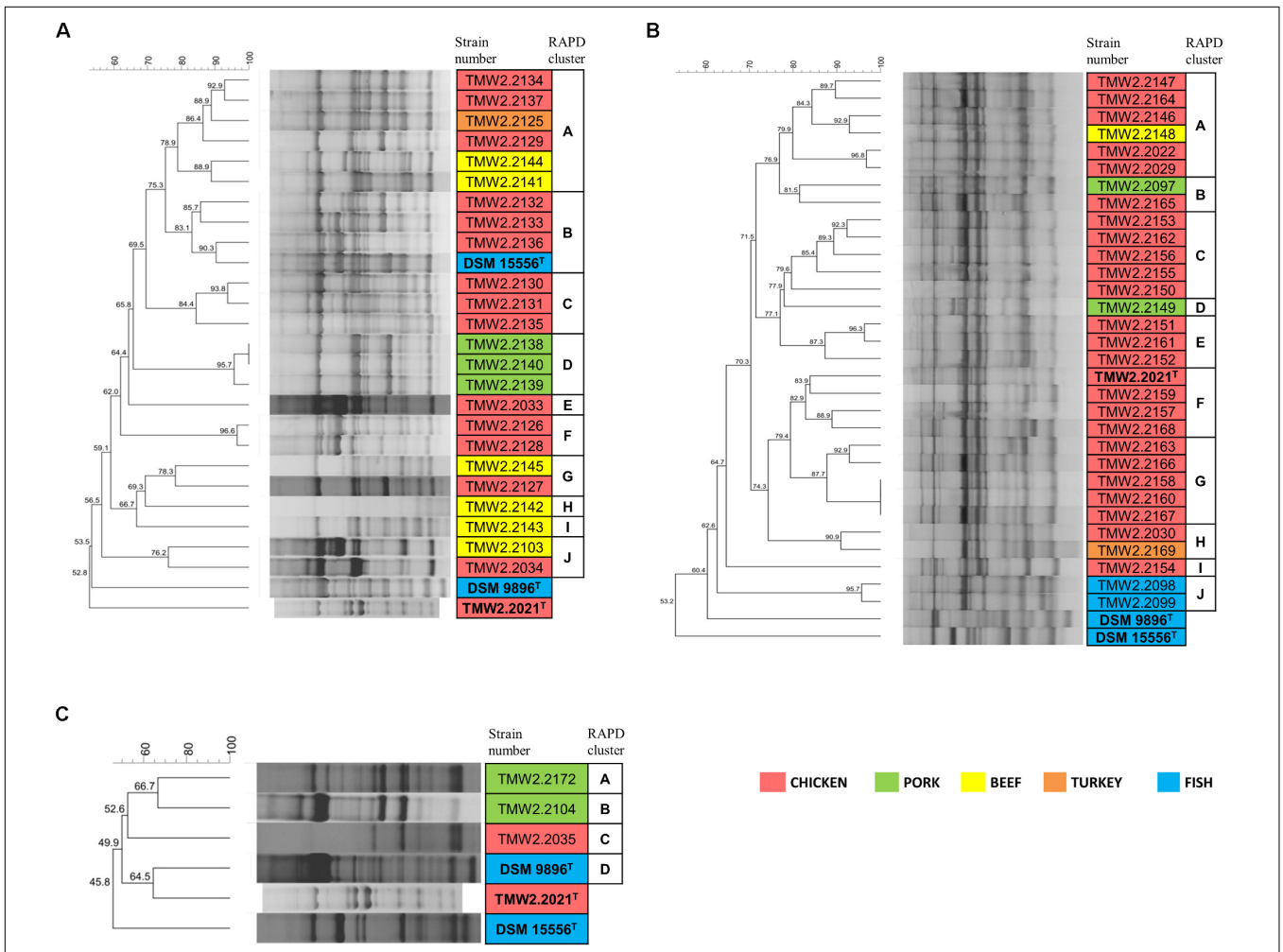

FIGURE 1 | RAPD-clustering of the selected strains. Hierarchical clustering was calculated with the unweighted pair group method with arithmetic mean (UPGMA), Dice similarity coefficient, and 1% tolerance. The similarity values are shown at the nodes of the tree. Different colors specify the isolation sources and bold letters the type strains. Further resolution of the most similar *P. phosphoreum* and *P. carnosum* strains RAPD-clustering is shown in **Supplementary Figure S2**.
(A) *P. phosphoreum*, type strain DSM 15556^T, **(B)** *P. carnosum*, type strain TMW2.2021^T, **(C)** *P. iliopiscarium*, type strain DSM 9896^T.

Physiological Differentiation

We furthermore performed physiotyping experiments to correlate the identified genome-based diversity in relation to phenotypic traits. For that, we monitored the maximum OD_{600}, maximum growth rate (U), and lag phase (lag) at 4°C in meat simulation medium at pH 5.8 to mimic cold storage of meats (**Table 3**). For both – lag phase and maximum growth rate – we could classify the strains in three statistically ($p < 0.05$) different groups within each of the species, and scores were assigned to each of them: short (3), medium (2), and long (0) lag phase and fast (3), medium (2), and slow (0) maximum growth rate. In the case of the pH, since its change is closely related to the production of spoilage substances like biogenic amines, the strains were classified in four groups as they decrease the pH (≤5.7, score 0), leave it unchanged (5.7–5.9, score 1), increase it up to 1 unit (5.9–6.8, score 2), or highly increase it (≥6.8, score 3). The behavior in the medium indicates highly diverse physiotypes that were independent of the isolation source (**Figure 2**).

Photobacterium phosphoreum strains reached the highest maximum OD_{600} (up to 4.99), had significantly higher growth rates than *P. carnosum* and *P. iliopiscarium* (p-values < 0.05), and tended to increase the pH to a considerable extent (up to pH 7.47). In contrast, *P. carnosum* strains grew up to comparatively low maximum OD_{600} (up to 1.7), had 10 times lower growth rates, and tended to decrease or keep the initial pH value. The only exception was strain TMW2.2169 that alkalized the medium to 7.08. The different influence of the species on the pH was statistically confirmed (p-values < 0.05); however, both species included strains that alkalized or acidified the medium at maximum OD_{600}. Regarding the lag phase, *P. carnosum* strains adapted to the media approximately half as fast as *P. phosphoreum* strains. Its average lag phase of 47–101 h was significantly longer than the one of both *P. phosphoreum* (21–55 h) and *P. iliopiscarium* (33–42 h, p-values < 0.05). The average lag phase of *P. iliopiscarium* was comparable to *P. phosphoreum* (p-value 0.767) whereas its maximum growth rate was comparable to *P. carnosum* (p-value 0.189). However, the tendency of *P. iliopiscarium* strains to increase the pH only slightly at its maximum OD_{600} (pH 6.32–6.56) was significantly different from the other two species (p-values < 0.05).

We observed no general correlation of the growth parameters with the RAPD fingerprint and the isolation source (**Figure 2**). Nevertheless, *P. phosphoreum* and *P. iliopiscarium* type strains from marine habitats were one of the slowest growing strains of each species, respectively.

Furthermore, *P. iliopiscarium* type strain and additional four *P. phosphoreum* strains from chicken (TMW2.2127, TMW2.2129, TMW2.2130, and TMW2.2134) showed motility after 3 days incubation. The rest of the strains, together with all strains from *P. carnosum*, were non-motile after 3 days. Bioluminescence was a frequent trait of the selected *P. phosphoreum* strains and several meat-borne strains exhibited much higher luminescence than the type strain. Only three *P. phosphoreum* strains from chicken (TMW2.2137, TMW2.2129, and TMW2.2134) did not show bioluminescence at all.

TABLE 3 | Growth parameters of *Photobacterium* spp. in meat-simulation media at 4°C.

Species	Maximum OD_{600}	Maximum growth rate	Lag phase (h)	pH
P. phosphoreum	3.10–4.99	0.168–0.468	21.17–55.08	5.62–7.47
P. iliopiscarium	1.38–2.05	0.033–0.144	32.76–41.83	6.32–6.56
P. carnosum	1.36–1.71	0.019–0.061	46.97–101.14	5.43–7.08

Summary of values obtained for the maximum OD_{600}, maximum growth rate (U), lag phase, and pH at maximum OD_{600} during growth of the three species of photobacteria in meat-simulation media at 4°C.

Resistance to Antibiotics

We recorded the tolerance of the strains for 15 antibiotics by measuring their inhibition zones (**Table 4** and **Figure 3**) to evaluate possible correlations between genotypes, isolation sources, and antibiotic resistances. In general, we observed high resistance in almost all strains to clindamycin, apramycin, penicillin G, and sulfonamides but sensitivity to chloramphenicol and norfloxacin. However, a few strains of *P. phosphoreum* exhibited resistance against chloramphenicol and norfloxacin (**Figure 3A** and **Supplementary Table S2**). In case of antibiotics with various extent of inhibition, the strains tended to be distributed to either low/high (*P. phosphoreum*) or low/medium/high resistance (*P. carnosum* and *P. iliopiscarium*). *P. carnosum* appeared to be the most sensitive species comprising the highest number of sensitive strains, especially regarding rifampicin, ampicillin, and tetracycline (**Supplementary Table S3** and **Figure 3B**). *P. iliopiscarium* strains appeared to be more similar to the *P. phosphoreum* group than to the *P. carnosum* group regarding resistance to antibiotics (**Figure 3C** and **Supplementary Table S4**). Within the species, we did not observe an explicit correlation of antibiotic resistance and isolation source or RAPD clustering. The same applied to the remarkable resistance of some *P. phosphoreum* strains for chloramphenicol and norfloxacin. Furthermore, the type strains revealed no clear differentiation compared to the other strains of the species.

Metabolic Properties of Representative Strains

Biochemical API 50CH and API ZYM tests were conducted with 20 strains of *P. carnosum*, 15 strains of *P. phosphoreum*, and 3 strains of *P. iliopiscarium* in order to study metabolic versatility (**Figure 4**). All three species produced acid from glucose, mannose, fructose, ribose, and *n*-acetylglucosamine. Additionally, they all responded positively in the tests for alkaline phosphatase, acid phosphatase, and leucine arylamidase. None of the strains produced acid from erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl-bD-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, amygdalin, arbutin, salicin, D-trehalose, inulin, D-melezitose, D-raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, and L-arabitol. None of the strains responded positively in the tests

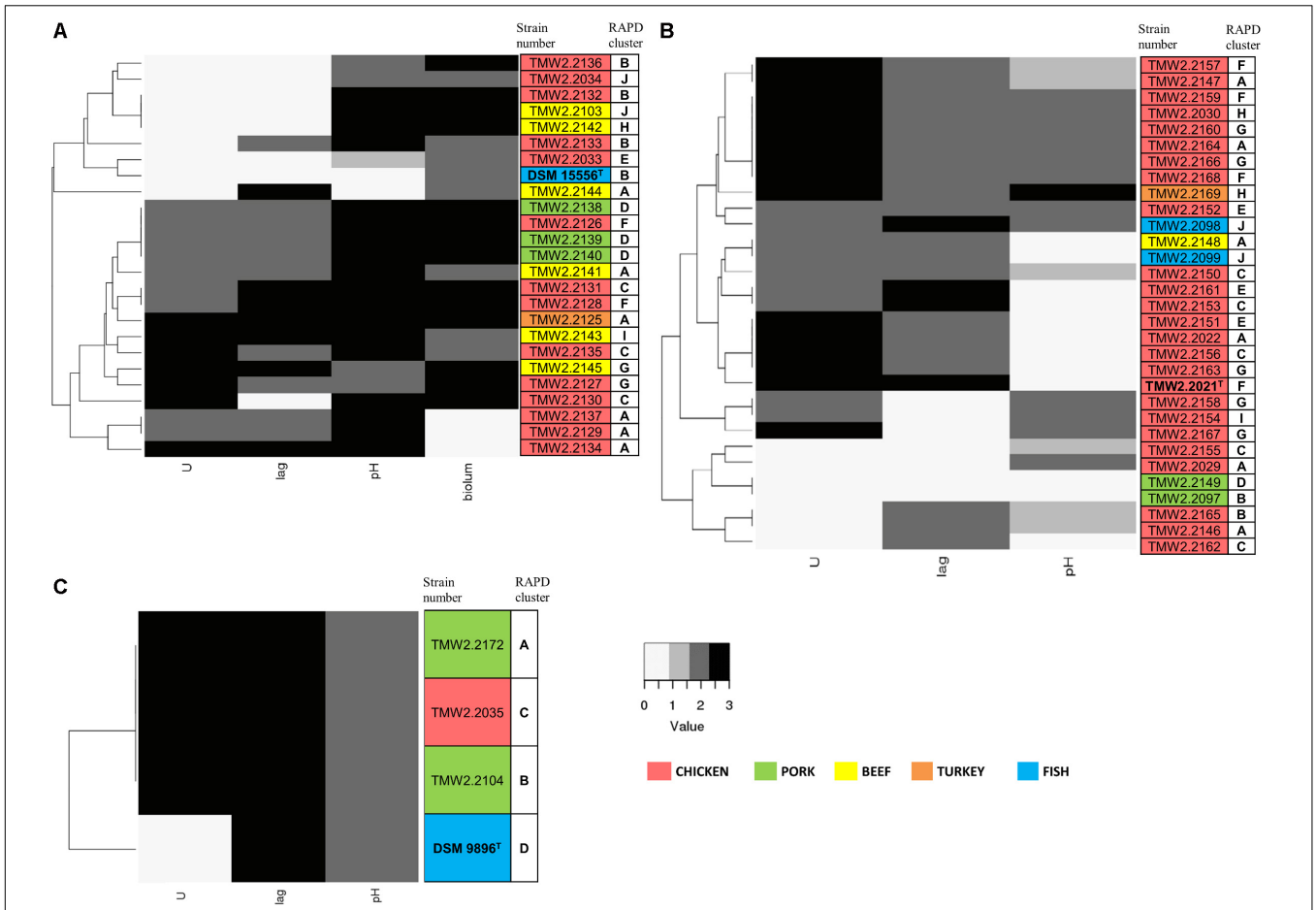

FIGURE 2 | Clustering of the strains by their growth rate (U), lag phase (lag), influence on pH, and bioluminescence (biolum, if existing). Growth rate and lag phase were determined in meat simulation medium at 4°C. Mean values were statistically grouped as low/medium/high and scored from 0 to 3. The highest score was given to the highest growth rates and lowest lag phases, respectively, and a score of 2 was given to medium values. The pH was measured when the cultures reached their highest OD_{600} and scored as follows: 0 – pH ≤5.7, 1 – pH 5.7–5.9, 2 – pH 5.9–6.8, 3 – pH >6.8. Bioluminescence of *P. phosphoreum* was evaluated manually on agar plates. The highest score was given to the strongest bioluminescence. The different isolation sources are displayed in colors and type strains are marked with bolt letters. **(A)** *P. phosphoreum*, type strain DSM 15556^T, **(B)** *P. carnosum*, type strain TMW2.2021, **(C)** *P. iliopiscarium*, type strain DSM 9896^T.

TABLE 4 | Range diameter of the inhibition zones (mm) as summary of all isolates per species.

Species	DA	NOR	NA	AMP	S3	W	P	S	APR	RD	CN	K	C	E	TE
P. carnosum	6	24–46	18–40	6–32	6–32	16–38	6–10	6–28	6–18	16–32	12–30	10–30	36–50	6–26	6–26
P. iliopiscarium	6	20–24	18–19	6–15	6	6–20	6–15	9–13	6–10	11–18	11–16	10–16	33–35	6–10	6–10
P. phosphoreum	6	14–34	8–25	6–12	6–22	6–26	6–12	6–18	6–11	9–22	7–23	6–22	6–38	7–25	6–21

A diameter of 6 mm was regarded as no inhibition at all. C, chloramphenicol 30 μg; NOR, norfloxacin 10 μg; S3, sulfonamides 300 μg; APR, apramycin 25 μg; P, penicillin G 5 μg; DA, clindamycin 2 μg; NA, nalidixic acid 30 μg; W, trimethoprim 5 μg; AMP, ampicillin 10 μg; S, streptomycin 25 μg; RD, rifampicin 5 μg; CN, gentamycin 10 μg; K, kanamycin 30 μg; E, erythromycin 15 μg; TE, tetracycline 30 μg.

for lipase C14, chymotrypsin, α-galactosidase, β-glucosidase, α-mannosidase, and α-fucosidase.

Still, we identified some traits that differed between the species (**Supplementary Table S5**). Several *P. carnosum* strains produced acid from methyl-α-D-glucopyranoside, cellobiose, saccharose, glycogen, gentiobiose, turanose, and L-fucose in contrast to *P. phosphoreum* and *P. iliopiscarium* strains. *P. carnosum* was additionally the only species with positive or weak positive reactions in the test for α-glucosidase but without acid production from potassium 5-ketogluconate. Strains of *P. phosphoreum* were the only ones being positive for cystine arylamidase and β-glucuronidase and also the only ones that did not produce acid from starch. In contrast, *P. iliopiscarium* strains did not show any unique spectrum of acid production from carbohydrates or enzymatic reactions within the tests. Overall, *P. carnosum* strains covered the broadest carbohydrate utilization spectrum and *P. phosphoreum* strains the most positive enzymatic reactions of all three species.

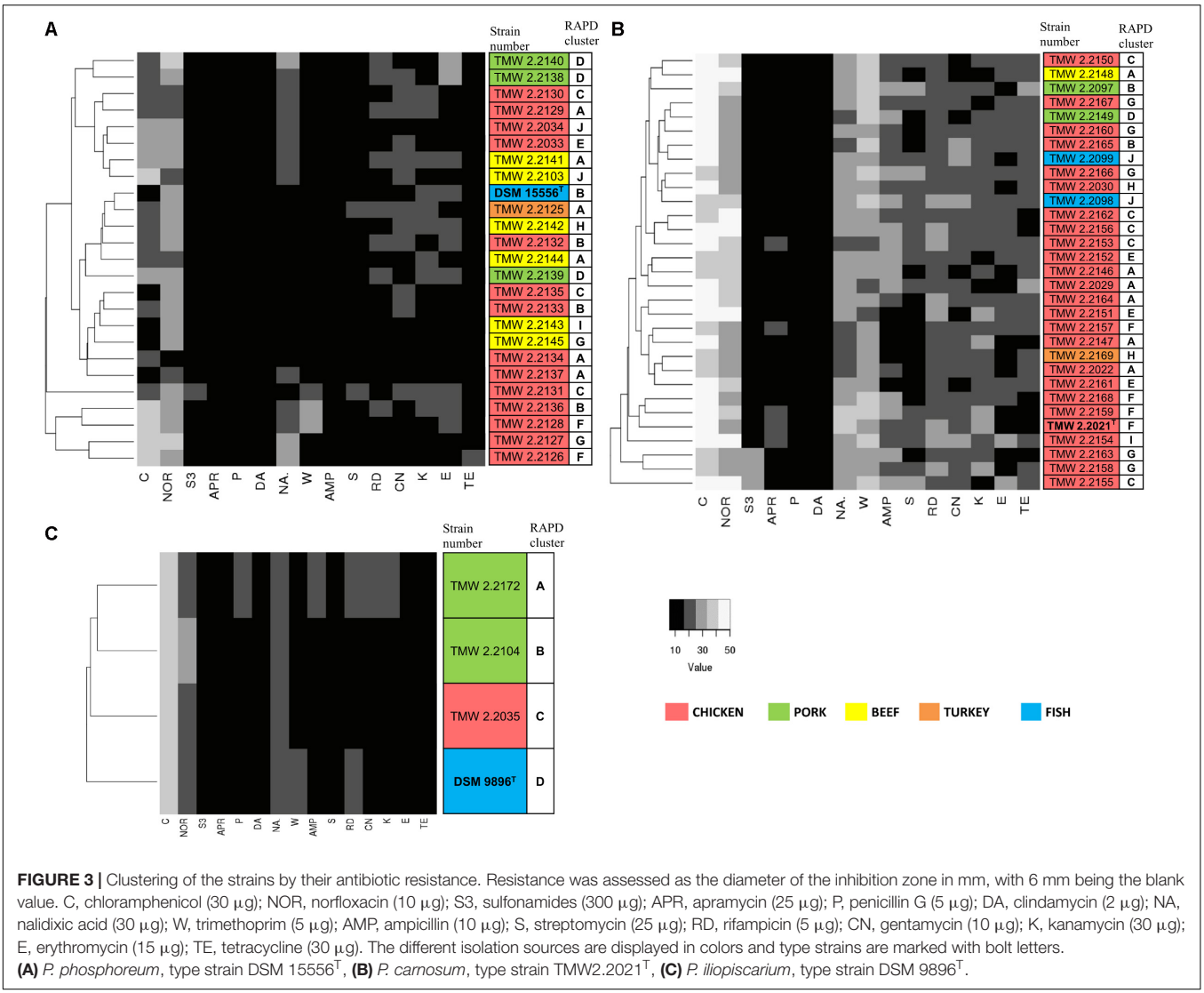

FIGURE 3 | Clustering of the strains by their antibiotic resistance. Resistance was assessed as the diameter of the inhibition zone in mm, with 6 mm being the blank value. C, chloramphenicol (30 μg); NOR, norfloxacin (10 μg); S3, sulfonamides (300 μg); APR, apramycin (25 μg); P, penicillin G (5 μg); DA, clindamycin (2 μg); NA, nalidixic acid (30 μg); W, trimethoprim (5 μg); AMP, ampicillin (10 μg); S, streptomycin (25 μg); RD, rifampicin (5 μg); CN, gentamycin (10 μg); K, kanamycin (30 μg); E, erythromycin (15 μg); TE, tetracycline (30 μg). The different isolation sources are displayed in colors and type strains are marked with bolt letters.
(A) *P. phosphoreum*, type strain DSM 15556^T, **(B)** *P. carnosum*, type strain TMW2.2021^T, **(C)** *P. iliopiscarium*, type strain DSM 9896^T.

Within the species, the differences of the marine type strains *P. phosphoreum* DSM15556^T and *P. iliopiscarium* DSM9896^T to the meat-borne strains were particularly notable. We observed three enzymatic tests that were negative in *P. phosphoreum* DSM15556^T but at least weakly positive in all the other *P. phosphoreum* strains (C4 esterase, C8 esterase–lipase, naphthol-AS-BI-phosphohydrolase; **Supplementary Table S5**). On the other hand, three carbohydrates were exclusively used by the type strain for acid production (glycerol, D-lactose, and D-melibiose). We saw also three reactions that were different for *P. iliopiscarium* DSM9896^T compared to meat-borne *P. iliopiscarium* strains (C8 esterase–lipase, valine arylamidase, and starch metabolism).

Furthermore, we identified a correlation of isolation source and metabolic properties that was depicted by the clustering of almost all *P. phosphoreum* strains from beef (**Figure 4A**). However, we could not identify clear differences of *P. carnosum* strains from meat and *P. carnosum* strains from fish (**Supplementary Table S5**). The test results of the *P. carnosum* type strain TMW2.2021^T were also not clearly different when compared to the other meat-borne strains. Nevertheless, both *P. carnosum* strains from salmon cluster together and both strains from pork cluster apart from the rest (**Figure 4B**). In each species we observed some reactions that were solely positive in single strains. *P. carnosum* TMW2.2163 was the only strain producing acid from saccharose, cellobiose, gentiobiose, turanose, and L-fucose (**Figure 4B**). *P. phosphoreum* TMW2.2130 was conspicuous by β-glucuronidase activity and *P. iliopiscarium* TMW2.2035 by acid production from potassium 2-ketogluconate (**Figures 4A,C**).

DISCUSSION

This is the first study that investigated biodiversity of meat-borne isolates of *Photobacterium* spp., isolated a wide variety of strains and explored strain- as well as species-specific traits. The data obtained from our study give further evidence that photobacteria,

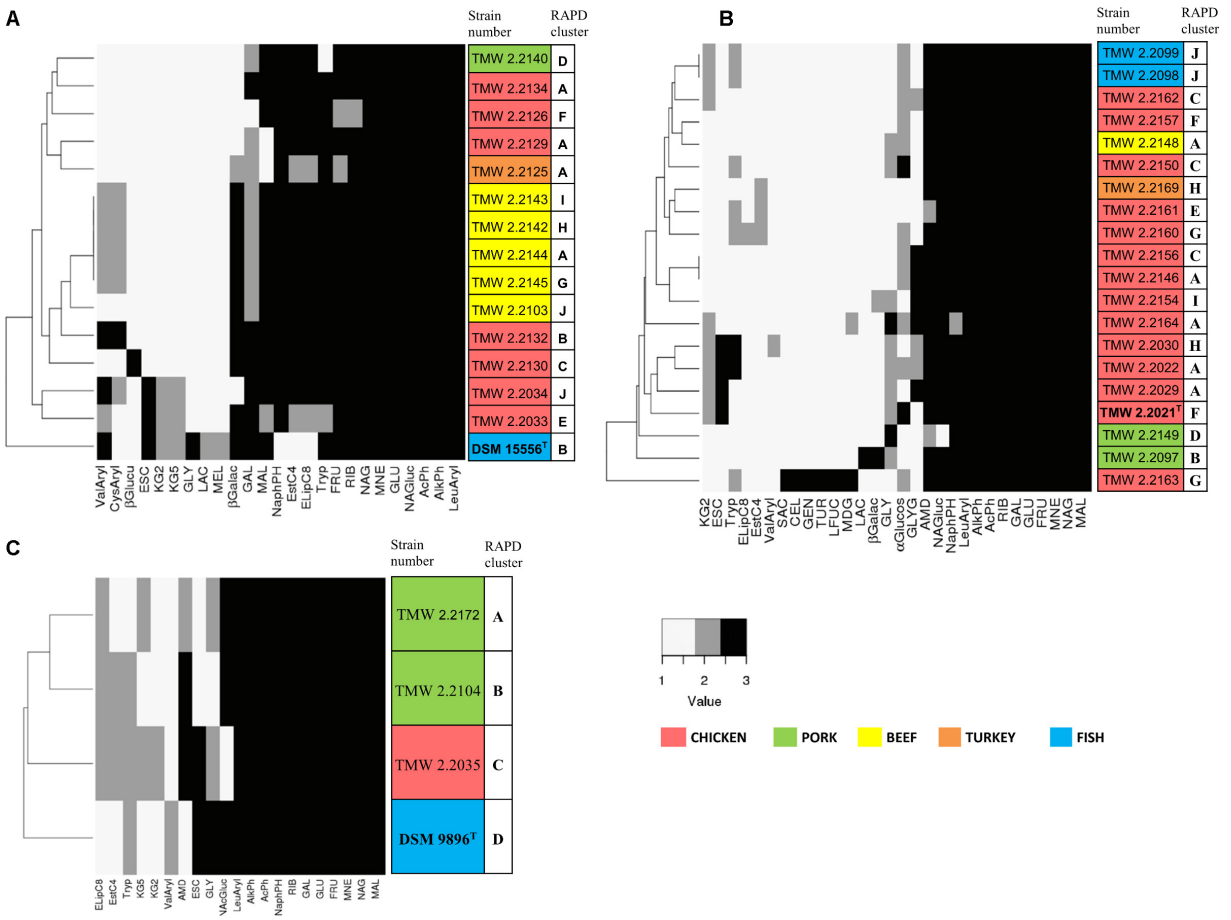

FIGURE 4 | Clustering of selected strains by metabolic properties based on API 50CH and API ZYM. The reactions prove acid production from single carbohydrates (API 50CH) and presence of selected enzymes (API ZYM). Each clustering shows only reactions that were positive at least in one isolate. Reactions were scored as 1 (negative reaction), 2 (weak positive reaction), and 3 (strong positive reaction). The different isolation sources are displayed in colors and type strains are marked with bolt letters. **(A)** *P. phosphoreum*, type strain DSM 15556^T, **(B)** *P. carnosum*, type strain TMW2.2021^T, **(C)** *P. iliopiscarium*, type strain DSM 9896^T. API50ch: GLY, glycerol; RIB, D-ribose; GAL, D-galactose; GLU, D-glucose; FRU, D-fructose; MNE, D-mannose; MDG, methyl-αD-glucopyranoside; NAG, *N*-acetylglucosamine; ESC, esculin; CEL, D-cellobiose; MAL, D-maltose; LAC, D-lactose; MEL, D-melibiose; SAC, D-saccharose; AMD, starch; GLYG, glycogen; GEN, gentiobiose; TUR, D-turanose; LFUC, L-fucose; KG2, potassium 2-ketogluconate; KG5, potassium 5-ketogluconate. APIzym: AlkPh, alkaline phosphatase; EstC4, esterase (C4); ELipC8, esterase lipase (C8); LeuAryl, leucine arylamidase; ValAryl, valine arylamidase; CysAryl, cystine arylamidase; Tryp, trypsin; AcPh, acid phosphatase; NaphPH, naphthol-AS-BI-phosphohydrolase; βGalac, β-galactosidase; βGlucu, β-glucuronidase; αGlucos, α-glucosidase; NAG, *N*-acetyl-β-glucosaminidase.

specifically *P. phosphoreum*, *P. carnosum*, and *P. iliopiscarium*, are widespread contaminants of different meats, as previously stated in Hilgarth et al. (2018a).

Distribution of *Photobacterium* spp. Contaminants

Recently, reports on the presence of photobacteria have emerged, mostly in culture-independent studies without actual isolation. All these reports were widespread over different countries, i.e., Germany (Hilgarth et al., 2018a), Belgium (Stoops et al., 2015), Italy (Pennacchia et al., 2011), Denmark (Nieminen et al., 2016), France (Bouju-Albert et al., 2018), and China (Li et al., 2019), demonstrating the global relevance of photobacteria to meat spoilage. Together with this, the data of our study confirm that contamination of meat with *Photobacterium* spp. is not sporadic, but rather a general issue associated with the meat industry. They also suggest that the contamination source might be similar in all types of meat, and therefore should be located in a common part of the slaughtering, processing, or packaging of the meat. This would also allow speculation on the presence of photobacteria associated with livestock, prior to the slaughtering process. However, given the psychrophilic nature of these organisms, and the inability of *P. carnosum* to grow at temperatures >20°C, or *P. phosphoreum* and *P. iliopiscarium* >25°C (Hilgarth et al., 2018b), it appears unlikely that these bacteria are autochthonous members of the animal gut-microbiome. Furthermore, we did not recover any photobacteria from other animal-derived products besides meat, nor from MAP packed-, protein- rich-, or sea-related vegetables. This suggests that, in relationship to food contamination and spoilage, photobacteria seem to only be able to reach detectable

numbers on meat (and fish). We also did not detect photobacteria on two types of seafood (scallops and shrimps). However, these products had been deep-frozen before sampling and it has been reported that deep-freezing reduces photobacteria below detection limits for culture-dependent methods (Emborg et al., 2002; Dalgaard et al., 2006).

Occurrence and Diversity of *Photobacterium* spp. on Packaged Meats

Calculated rarefaction and diversity indices revealed that the large quantity of isolates analyzed in this study reflects expected abundances. It therefore allows representative assessment of diversity within and between the species *P. carnosum* (31 strains from 163 isolates) and *P. phosphoreum* (24 strains from 113 isolates). The high evenness of *P. phosphoreum* and *P. carnosum* strains demonstrate the absence of dominant genotypes and suggest a rather general adaptation of the strains. However, even strains from the same meat sample showed clear genotypic and phenotypic variability, which suggests an initial contamination that is already considerably diverse. Furthermore, ecological entropy of both species was not significantly different meaning the same degree of overall biodiversity also on species level. Regarding *P. iliopiscarium*, the low number of recovered isolates (three isolates with three genotypes) suggests that there may be more diversity within the meat-borne strains than the ones recovered in this study.

We did not isolate any photobacteria from either minced beef- or mixed minced meat in this study. However, culture-independent reports of *Photobacterium* spp. (Pennacchia et al., 2011; Stoops et al., 2015) indicate that the genus can be present on minced meat, even if they do not grow to detectable numbers. It may be speculated that other meat spoilers dominate on minced meat and simply overgrow photobacteria due to shorter doubling time. Recently, presence of *Pseudomonas* spp. has been reported on MAP minced meat (Hilgarth et al., 2019) that might act as possible fast growing competitor of *Photobacterium* spp.

We also observed that not all samples of meat cuts are contaminated with photobacteria, even if they come from the same producer. This could indicate a low level of initial contamination and distribution by chance (Höll et al., 2019). A low initial contamination may also explain the different distribution of the three *Photobacterium* species on different meat types (Hilgarth et al., 2018a).

The growth of photobacteria appears also be independent of the packaging method since photobacteria occur independently of the employment of modified atmosphere, vacuum, or air packaging (Hilgarth et al., 2018a). This is supported by Höll et al. (2019) who predicted that there is little to no effect of the choice of atmosphere on the growth of photobacteria, based on similar gene expression under different MAP conditions. This suggests that the current modified atmosphere composition and vacuum packages, commonly used to extend the shelf-life and optimum qualities of meat and fish (McKee, 2007; McMillin, 2008; Bingol and Ergun, 2011; Lorenzo and Gomez, 2012; Rossaint et al., 2014), are insufficient to reduce spoilage-associated photobacteria on meat. Furthermore, the detection of photobacteria on marinated meats demonstrated that marinating – a process to introduce antimicrobials (Björkroth, 2005; Kargiotou et al., 2011) – will also not prevent photobacterial spoilage.

Adaptation to Food as an Ecological Niche

Results from the carbon metabolism and enzymatic activities, together with distribution of growth rates and lag phase, suggest that *P. carnosum* strains are more homogeneous with lower variability than *P. phosphoreum* and *P. iliopiscarium* strains. While it was possible to clearly differentiate the marine type strain of the two latter from the meat-borne strains, *P. carnosum* seems to share common traits for all the strains, independently of the source of isolation. Additionally, our results for the growth and metabolic traits indicate adaptation of *P. carnosum* to meat or other nutrient rich environments, as stated before by Hilgarth et al. (2018b). *P. carnosum* also lacks bioluminescence and motility, two common traits of symbiotic or free-living marine photobacteria. This supports missing adaptation of the species to sea-related environments. Still, for the first time, *P. carnosum*, a species described as terrestrial and unrelated to sea environments, was detected on MAP salmon. However, our data on missing subpopulations referring to respective environments support the hypothesis that the isolates from (freshwater) farmed salmon do not originate from a marine environment, but rather from a contamination later in the processing and packaging. The fact that *P. phosphoreum* isolates originating from the same MAP farmed salmon showed no distinct genotypes, i.e., were also found on meats, further supports that hypothesis. In contrast, *P. phosphoreum* and *P. iliopiscarium* appear to have different marine as well as meat-borne subpopulations with specific adaptations to the respective environment as demonstrated by the differences of the meat borne strains to their marine type strains.

Reactions for lipase C14, esterase C4, and esterase–lipase C8 were negative or at most weakly positive for almost all strains of the three species. Additionally, all of the *P. phosphoreum* meat-borne strains and some from *P. iliopiscarium* and *P. carnosum* were negative for glycerol. However, Höll et al. (2019) confirmed the expression of lipase and genes encoding for enzymes involved in lipid and glycerol utilization in photobacteria. This suggests that the lipase was not expressed in API medium or that this type of lipase do not lead to a positive reaction within the API ZYM test and that utilization of glycerol does not result in acidification of the medium. However, almost all strains of the three species showed positive reactions for the main monomeric carbohydrates found in meat, i.e., glucose, fructose, mannose, ribose (Aliani and Farmer, 2005a,b; Koutsidis et al., 2008a,b; Meinert et al., 2009a,b). Furthermore, the species *P. carnosum* shows a wider metabolic capability in terms of carbohydrate utilization than the other two species. Many of the carbohydrates used exclusively by *P. carnosum* are plant (e.g., starch, cellobiose, gentiobiose, turanose) or meat related (e.g., glycogen). Regarding growth on

meat-simulation media, we observed that the species has the lowest maximum growth rates and longer adaptation times in the meat-simulation media used in this study. However, it is found in some meat types in larger amounts and cell counts than any of the other two species. This suggests that *P. carnosum* is adapted to more complex media and has specific growth requirements that the other two species do not have.

Safety Concerning Aspects of *Photobacterium* Species

The observed variable alkalization or acidification of the growth medium with up to two pH values difference demonstrates the great variety of strain physiotypes. This might also be of relevance for the respective potential as meat spoiler since alkalization indicates production of biogenic amines and ammonia from amino acid metabolism. The ability of *P. phosphoreum* to produce histamine and other biogenic amines in fish has been previously reported (Jorgensen et al., 2000; Stoops et al., 2015; Nieminen et al., 2016). The increase of pH in the media up to 7.5 might be an indicator for the potential of some of our isolates, i.e., certain strains of *P. phosphoreum* to produce higher amounts of biogenic amines, which is also predicted in the transcriptomic analysis of Höll et al. (2019).

Another important safety aspect deals with bacterial resistance to antibiotics. Administration of antibiotics to poultry, swine, and calves in the agricultural industry is known as disease treatment and control (Nisha, 2008; Muaz et al., 2018) and therefore possibly linked to resistance of meat spoiling bacteria. However, we did not observe a clear pattern that would allow to link the source of isolation to the antibiotic resistances determined in this study. Our results suggest that the species have intrinsic resistance to clindamycin, apramycin, penicillin G, and sulfonamides. However, resistance to the other antibiotics occurs differentially on strain level. The fact that closely related strains with similar chromosomal fingerprints did not exhibit similar antibiotic resistances suggests that these resistances may be located on mobile genetic elements and therefore possibly be transferable. This transferability might also occur for chloramphenicol and norfloxacin resistance in *P. phosphoreum*, as only few of its strains show complete resistance to them in contrast to the common tendency of the three species. The suggested transferability of the resistance to chloramphenicol, being one of the drugs of last resort [DoLR (World-Health-Organization, 2001)], harbors potential health concerns.

CONCLUSION

This study demonstrates that, even though the initial contamination is likely to be low, photobacteria strains from meat display a great diversity with specific genotypic, phenotypic, and physiotypic traits. Due to previous association with solely marine environments and lack of optimized detection methods, biodiversity of meat-borne *P. phosphoreum*, *P. iliopiscarium*, and *P. carnosum* was hitherto unexplored. On the basis of our results, we can assume that their entry route as meat contaminants might occur during slaughtering, derived from the exterior of the animal or environment, but not from the gut – following colonization of general processing and packaging facilities. Divergence of the meat-borne and the marine type strains of *P. phosphoreum* and *P. iliopiscarium* on the one hand and homogeneity of *P. carnosum* strains on the other hand suggests different environmental adaptation and possibly also separate origin of contamination. Additionally, diversity of metabolic capabilities and antibiotic resistances appear to be widespread and mostly not linked to a specific isolation source. This reveals the presence of a highly variable and rich community of photobacteria on each meat that combines multiple physio- and genotypes with potential relevance to food safety worldwide.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/**Supplementary Files**.

AUTHOR CONTRIBUTIONS

SF-P and PH performed the laboratory work and data evaluation, wrote the first draft of the manuscript, and designed the study. MH performed the diversity index analysis, helped to draft the study, and supervised the work of SF-P and PH. RV initiated the project and supervised the work of SF-P and PH. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.02399/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.2 Hydrostatic pressure- and halotolerance of *Photobacterium phosphoreum* and *P. carnosum* isolated from spoiled meat and salmon

P. phosphoreum and *P. carnosum* both occur frequently as contaminants on spoiling meat, however, only the former has been associated also with spoilage of marine fish/seafood and occurrence in marine habitats. To evaluate the comparability of strains and respective species with respect to their isolation source, adaptation of isolates from meat and from marine environment/MAP salmon to elevated NaCl concentration and hydrostatic pressure was investigated. In this context, tolerance and requirement of elevated NaCl availability and elevated hydrostatic pressure was supposed to be correlated with adaptation towards the environmental conditions of marine habitats. *P. phosphoreum* strains TMW2.2125, TMW2.2134 and DSM15556^T revealed comparably higher tolerance to 40 MPa hydrostatic pressure and >3% NaCl than *P. carnosum* strains. They were therefore suggested to be characterized by stronger adaptation towards marine environment. In contrast, *P. carnosum* strains TMW2.2021^T, TMW2.2148, TMW2.2098 and TMW2.2186 showed distinct growth reduction in presence of 40 MPa hydrostatic pressure or >3% NaCl. Strains of the species were therefore suggested to be less adapted to the environmental conditions of marine niches. In accordance, the genomes of the *P. carnosum* strains harbored only incomplete gene clusters of the flagellar-operon and the *lux*-operon that have been associated with growth in marine niches before. Both operons were found to be complete in the genomes of all investigated *P. phosphoreum* strains. However, presence of remaining genomic elements of the flagellar- and lux-operon, as well as the given NaCl requirement of *P. carnosum* strains allow for speculations on a shared marine provenance of *P. carnosum* and *P. phosphoreum*. Loss of traits associated with adaptation to marine environment can consequently be assumed for *P. carnosum*. It suggests that the occurrence of *P. phosphoreum* strains on meats and of *P. carnosum* strains on MAP salmon result from cross-contamination events during processing. Examination revealed also differences regarding adaptation and optimal growth conditions on strain level. Since *P. phosphoreum* strains from meat possessed reduced halo- and pressure tolerance when compared to the strain from marine environment and since *P. carnosum* strains from MAP salmon showed the opposite trend compared to strains from meat, progressive adaptation of the strains to their respective environment can be speculated.

Author contributions: Philippa Frederieke Hauschild performed all experiments and evaluated and visualized the resulting data. She wrote the original draft of the manuscript and realized corrections that arose during the reviewing process.

Maik Hilgarth supervised the work of Philippa Frederieke Hauschild and contributed to corrections that arose during the reviewing process. Rudi F. Vogel initiated the corresponding project, supervised the work of Philippa Frederieke Hauschild and reviewed this manuscript.

Supplementary files of this publication are provided in appendix section 12.2.

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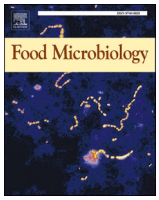

Hydrostatic pressure- and halotolerance of *Photobacterium phosphoreum* and *P. carnosum* isolated from spoiled meat and salmon

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ABSTRACT

Photobacterium spp. occur frequently in marine environments but have been recently also found as common spoilers on chilled meats. The environmental conditions in these ecological niches differ especially regarding salinity and ambient pressure. Linking the occurrence of photobacteria in different niches may elucidate its ecology and bring insights for the food industry. We investigated tolerance of *Photobacterium (P.) phosphoreum* and *P. carnosum* strains to high hydrostatic pressure and salinity and aligned our observations with presence of relevant genes. The strains were isolated from packaged meats and salmon (or the sea) to identify adaptations to marine and terrestrial habitats. Growth of all *P. carnosum* strains was reduced by 40 MPa hydrostatic pressure and >3% sodium chloride, suggesting loss of traits associated with marine habitats. In contrast, *P. phosphoreum* strains were only slightly affected, suggesting general adaptation to marine habitats. In accordance, these strains had gene clusters associated with marine niches, e.g. flagellar and *lux*-operons, being incomplete in *P. carnosum*. Occurrence of *P. carnosum* strains on packaged salmon and *P. phosphoreum* strains on meats therefore likely results from cross-contamination in meat and fish processing. Still, these strains showed intermediate traits regarding pressure- and halotolerance, suggesting developing adaptation to their respective environment.

1. Introduction

Photobacteria are common microorganisms in marine ecosystems (Sunagawa et al., 2015; Machado and Gram, 2017). Most of them live in association with marine organisms (Machado and Gram, 2017), as non-specific commensals and saprophytes, as pathogens or as bioluminescent symbionts in light organs of fish and squids (Urbanczyk et al., 2010). Photobacteria also colonize sediments and open water (Urbanczyk et al., 2010; Lo et al., 2014) and their distribution is not only limited to coastal areas but includes shallow and deep water of the pelagic zone (Machado and Gram, 2017; Nogi and MasuiNoriaki, 1998). Strains of *P. profundum* and *P. phosphoreum* are common deep sea isolates and occur in more than 2000 m depth, exhibiting piezophilic traits (Machado and Gram, 2017; Nogi and MasuiNoriaki, 1998; Al Ali et al., 2010). Life in deep sea is characterized by constant and mostly low temperature (Lauro et al., 2014), oligotrophic conditions resulting in low nutrient availability and high hydrostatic pressure (HHP) (Joint et al., 2010; Martini et al., 2013). HHP influences many processes in the bacterial cell including protein structure, regulation of the flagellar system, general gene expression and DNA synthesis as reviewed by Bartlett (2002) and therefore necessitates adaptation. This adaptation can result in physiological differences even on strain level (Campanaro et al., 2005; Eloe et al., 2008). Membrane structure and enzymatic processes are additionally influenced by the water temperature, which is very low in most marine environments with an average of 2–3 °C (Jebbar et al., 2015). In general, photobacteria are psychrotrophic or psychrophilic and prefer temperatures of 5–25 °C; however, they still grow below 0 °C (Campanaro et al., 2005; Moi et al., 2017). Marine environments also provide relatively constant but challenging ionic conditions for bacterial growth (Farmer and Hickman-Brenner, 2006). Seawater contains around 3.5% dissolved salts, most of it being sodium chloride (NaCl) (Lyman and Fleming, 1940). It has been proposed before that marine bacteria are characterized by high requirement of Na^+ ions for growth (Farmer and Hickman-Brenner, 2006; MacLeod, 1968). In concordance, photobacteria are termed halophilic to halotolerant and require salt rich medium for their cultivation and recovery (Moi et al., 2017; Hilgarth et al., 2018a). Certain species can grow with up to 8% NaCl but prefer mostly lower salt concentrations of 2–3% NaCl (Moi et al., 2017).

However, it has been recently proven that photobacteria do not only

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occur in marine habitats but also on chilled meats as anthropogenic niche (Fuertes-Perez et al., 2019). While these two niches share the low temperature, 100 g raw meat contains less than 100 mg NaCl and with this more than 30 times less salt than seawater (Ruusunen and Puolanne, 2005). Additionally, meat, in contrast to most marine habitats, provides a very nutrient rich environment with constantly changing living conditions. On meats, bacteria grow in competition with a broad spectrum of spoilage organisms that reach cell densities of >8 $\log_{10}$ colony forming units per gram (CFU/g (Nychas et al., 2008; Chaillou et al., 2015);). The microbial community thereby changes distinctly with ongoing spoilage. Protective packaging of meat, e.g. high- or low-oxygen modified atmosphere packaging (MAP), also influences the development of the spoilage community (Hilgarth et al., 2018a; Höll et al., 2019). Photobacteria are found in high numbers in MAP and vacuum packaging and appear to be not limited by any protective packaging method (Fuertes-Perez et al., 2019; Höll et al., 2019).

Photobacteria growing on meats or in deep sea environments should therefore display different adaptations e.g. of substrate metabolism, as well as piezo- and halotolerance. Ecotypes should be distinguishable, which are piezo- and halotolerant, or -sensitive, and are referred to as "marine" in contrast to "terrestrial" in this communication, respectively. Above this, the term 'halophilic' defines a general requirement of NaCl for growth and survival as established by MacLeod (Kushner, 1968; Macleod, 1965). In this sense, especially Gram-negative bacteria appear to be physiologically separated into marine and terrestrial species that cannot, or only under specific circumstances, shift their habitat (Farmer and Hickman-Brenner, 2006; Moebus, 1972). In this study we investigated presence of traits associated with adaptation to marine habitats in *P. phosphoreum* and *P. carnosum* strains to obtain insight in their respective lifestyles. Strains recovered from meat of both species were compared to strains isolated from the sea (type strain of *P. phosphoreum*) and MAP salmon (*P. carnosum*) in order to investigate differences in high pressure- and salt tolerance.

2. Materials and methods

2.1. Microorganisms and cultivation

P. phosphoreum and *P. carnosum* strains were isolated from different meat types, from MAP salmon and from marine habitats (Table 1). All strains were cultivated for 72 h (h) in high-salt meat-simulation media (HS-MSM, 20 g/L (g/l) meat extract (Merck, Darmstadt, Germany), 20 g/l NaCl (Roth, Karlsruhe, Germany)) at 15 °C and with constant agitation, unless otherwise stated. Plates were prepared by adding 1.6% (weight/volume (w/v)) agar-agar to the medium. Cells were stored in cryo stocks at - 80 °C and plated freshly for the experiments.

2.2. Growth analysis at HHP

Growth experiments were performed in 1.8 mL (ml) cryo vials (Nunc, Thermo Fisher Scientific) for 72 h under 30–50 Megapascal (MPa) HHP. Cultures were started with an optical density at 600 nm (OD_{600}) = 0.01 from pre-cultures. The pressure was built up with a manual pump linked to a pressure intensifier system (Unipress, Warsaw, Poland) that led to an 8 ml pressure vessel. Temperature was hold at 15 °C by a circulating oil bath (Witeg Labortechnik GmbH, Wertheim, Germany). Pressure was built up within 20 s (s) and released within 30 s to ensure as stable temperature conditions as possible. Reference cultures were incubated at 0.1 MPa (1 atm). All cultures were diluted with HS-MSM and plated on HS-MSM agar to count the CFUs after 72 h.

Table 1
Origin of the investigated strains of *P. phosphoreum* and *P. carnosum*.

Species	Strain number	Isolation source	Reference
P. carnosum	TMW2.2021^T	MAP chicken	Hilgarth et al. (2018b)
P. carnosum	TMW2.2148	Air-packed beef	Fuertes-Perez et al. (2019)
P. carnosum	TMW2.2098	MAP salmon	Fuertes-Perez et al. (2019)
P. carnosum	TMW2.2186	MAP salmon	This study
P. phosphoreum	TMW2.2134	MAP chicken	Fuertes-Perez et al. (2019)
P. phosphoreum	TMW2.2125	Air-packed turkey	Fuertes-Perez et al. (2019)
P. phosphoreum	DSM 15556^T	Marine habitat	German strain collection DSMZ

2.3. Salt tolerance assay

Influence of salt on the growth capacity was tested in liquid MSM with 2% (standard, HS-MSM), 3%, 4% or 5% (w/v) NaCl. Cells from pre-cultures were washed and used to inoculate 20 ml of fresh medium with OD_{600} = 0.02. The cultures were then incubated on a shaker at 15 °C. Growth was monitored by measuring the OD_{600} until cultures reached stationary phase. The maximum OD_{600} reached in each medium was then compared and used as a growth parameter to determine the highest possible yield in dependency of the salt concentration, thus indicating salt tolerance/sensitivity. Additional growth parameters (maximum growth rate, lag phase) were calculated with RStudio v1.1.463 and grofit package v1.1.1–1 (Kahm et al., 2010). Cell surfaces (π*length*-width) were determined for two representative strains (*P. phosphoreum* TMW2.2134 and *P. carnosum* 2.2021^T) under an Axiostar plus microscope (Zeiss, Jena, Germany) at 1000× magnification and at maximum OD in order to study the impact of salt concentration and verify the usage of OD as a valid growth parameter.

2.4. Tolerance to HHP-induced sublethal injury

Selected strains were cultivated until they reached the exponential phase in order to test survivability at HHP conditions. Strains were selected as representatives of their respective species and source of isolation. Cultures were put for 5–60 min (min) under constant hydrostatic pressure of 100 MPa/125 MPa. Temperature was set to 10 °C with a variance of ±5 °C during pressure building and release. Pressure was built up slowly within 20 s and released within 30 s to minimize adiabatic heating effects. After releasing the pressure, vials were carried on ice to avoid strong temperature changes. Cultures were diluted with HS-MSM and plated on MSM agar with 2%, 3% and 3.5% NaCl and colonies were counted after 72 h unless otherwise stated.

2.5. Genomic comparison of relevant genes

DNA of the strains was sequenced with whole genome shotgun sequencing using the MiSeq sequencing platform Illumina (Inc., San Diego, CA, USA). Genome annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP (Angiuoli et al., 2008)). First analysis was done using BlAst Diagnostic Gene findEr (BADGE (Behr et al., 2016)) with 85% MEGABLAST percent identity cut. DC-MEGABLAST percent identity cut was set to 70% and blastp percent identity cut to 50%. Output was compared between the strains and species to identify unique open reading frames (ORF) using the cut offs. Additionally, presence of relevant genes associated with high pressure- or osmotolerance was tested using BLAST (Basic Local Alignment Search Tool (Altschul et al., 1990),) for protein alignments. Unless otherwise stated, alignments were done using the sequences of *P. profundum* strain SS9 (Nogi and MasuiNoriaki, 1998).

2.6. Statistical analysis

All experiments were performed in triplicates and statistically evaluated with IBM SPSS Statistics v23.0.0.0. Significant differences were determined with one-way analysis of variances (ANOVA) and *post-hoc* Tukey test. If the variances were heterogeneous, Welch-ANOVA and Games-Howell *post-hoc* tests were performed instead. Significance was defined with $p < 0.05$.

3. Results

3.1. Growth under high pressure conditions

Growth experiments were performed under HHP conditions to investigate adaptation of *P. carnosum* and *P. phosphoreum* strains (Table 1) to deep sea environments. The optimal pressure for decisive growth investigations was tested in preliminary experiments as demonstrated with one selected strain and determined to 40 MPa, since its impairing effect on the growth of the strain was comparably strong (Supplementary Fig. S1).

Generally, *P. carnosum* and *P. phosphoreum* strains reached approx. 7 $\log_{10}$ CFUs after 72 h of cultivation at atmospheric pressure and their growth did not differ significantly (Fig. 1). Application of 40 MPa hydrostatic pressure also did not result in growth differences of *P. phosphoreum* strains isolated from meat and the marine type strain (Fig. 1A). In contrast, all *P. carnosum* strains reached cell counts 1–3 $\log_{10}$ fewer in magnitude at 40 MPa than at atmospheric pressure (Fig. 1B). The reduction was comparable for both *P. carnosum* strains isolated from meat and both strains isolated from MAP salmon, respectively (Supplementary Table S2). There was no significant difference between the cell counts of *P. carnosum* strains from MAP salmon and the *P. phosphoreum* type strain from marine environment after incubation under HHP (Supplementary Table S2).

3.2. Salt tolerance

Growth curves in liquid medium with different NaCl concentrations were recorded to investigate salt tolerance and requirement of the strains. The absolute effect of NaCl on growth was determined with the maximum OD_{600} reached over time compared to the OD_{600} in standard cultivation medium (2% NaCl). This method was chosen to deduce the influence of salt on the substrate exploitation of *P. phosphoreum* and *P. carnosum* and thus its salt tolerance.

Growth of *P. phosphoreum* DSM 15556^T was not reduced by increased NaCl concentration up to 5% (w/v) and was optimal at 4%, resulting in the highest maximum OD_{600} (Fig. 2C). In contrast, both *P. phosphoreum* strains from meat showed optimum growth at 2–3% NaCl and reduced maximum OD_{600} with 4–5% NaCl (Fig. 2A and B). Regarding the intraspecies relative growth, *P. phosphoreum* strains from meat were significantly less tolerant to ≥4% NaCl than the marine type strain but grew significantly better with 2% NaCl (Supplementary Table S3).

Strains of *P. carnosum* from meat showed optimum growth at 2% NaCl and reached a ≥40% lower maximum OD_{600} at 4% and 5% NaCl (Fig. 2D and E). Their salt tolerance did not vary significantly in most cases (Supplementary Table S3). However, salt tolerance of the strains from MAP salmon was more diverse. Strain TMW2.2098 was considerably sensitive resulting in a 15% lower maximum OD_{600} already with 3% NaCl (Fig. 2F) and significantly lower tolerance in comparison to all other strains (Supplementary Table S3). The second strain from MAP salmon, TMW2.2186, had the highest salt tolerance of all tested *P. carnosum* strains with optimum growth at 3% NaCl (Fig. 2G). Nevertheless, its sensitivity to 5% NaCl was comparable to the other strains of the species.

Regarding the interspecies relative salt tolerance, *P. phosphoreum* strains were in general more tolerant to >3% NaCl than *P. carnosum* strains. The reducing effect of 4% and 5% NaCl on the maximum OD_{600} was significantly higher for the latter (Supplementary Table S3). Only *P. carnosum* TMW2.2186 was not significantly different from *P. phosphoreum* TMW2.2125 from meat regarding growth at 4% NaCl (Supplementary Table S3). These described tendencies were similar for the influence of 2–5% NaCl on the maximum growth rate of *P. carnosum* and *P. phosphoreum* strains (Supplementary Fig. S4). Lag phases were determined to not be a decisive marker, therefore, this parameter was not chosen for comparison (data now shown). Cell size was not significantly affected by the increased salt concentration as demonstrated for a representative strain of *P. carnosum* and *P. phosphoreum* (Supplemental Table S5). Cell counts (CFU/ml) from growth experiments under 2–5% NaCl were also determined at maximum OD for these strains and higher/lower OD correlated with higher/lower CFU/ml, respectively (data not shown). Similar cell sizes as well as correlation of CFU/ml and OD verified the usage of OD comparison between strains and treatments.

3.3. Detection of sublethal injury resulting from the HHP treatment

Pressure-induced sublethal damages of the cells were investigated by plating high pressure treated cells on agar with elevated salt content. Preliminary experiments with HHP of 100–125 MPa and 5–60 min treatment revealed an appropriate test protocol of 125 MPa treatment for 5 min (data now shown).

Application of 125 MPa HHP did not influence the intrinsic viability of the strains and did not reduce the cell counts compared to untreated controls (Figs. 3 and 2 % NaCl). However, for *P. phosphoreum*, high pressure treated cells reached lower cell counts than non-treated cells on agar with increased NaCl concentration. The cell counts of treated and untreated cells differed with up to 1.5 $\log_{10}$ CFU/ml on both, 3% and 3.5% NaCl agar (Fig. 3), representing a significant reduction (Supplementary table S6 A). In contrast, cell counts of *P. carnosum* strain

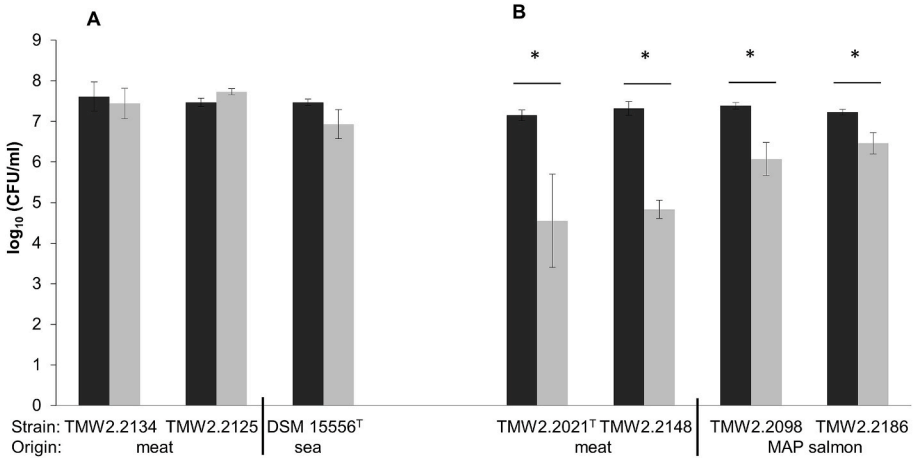


Fig. 1. Influence of HHP on the growth of *P. phosphoreum* and *P. carnosum* strains. Strains were plated after 72 h of cultivation at atmospheric pressure (negative control, (■) and 40 MPa (▩). **A** *P. phosphoreum*: TMW2.2134, TMW2.2125, DSM15556^T; **B** *P. carnosum*: TMW2.2021^T, TMW2.2148, TMW2.2098, TMW2.2186. Significant differences were accepted with $p < 0.05$. Error bars show the standard deviation of N = 3.

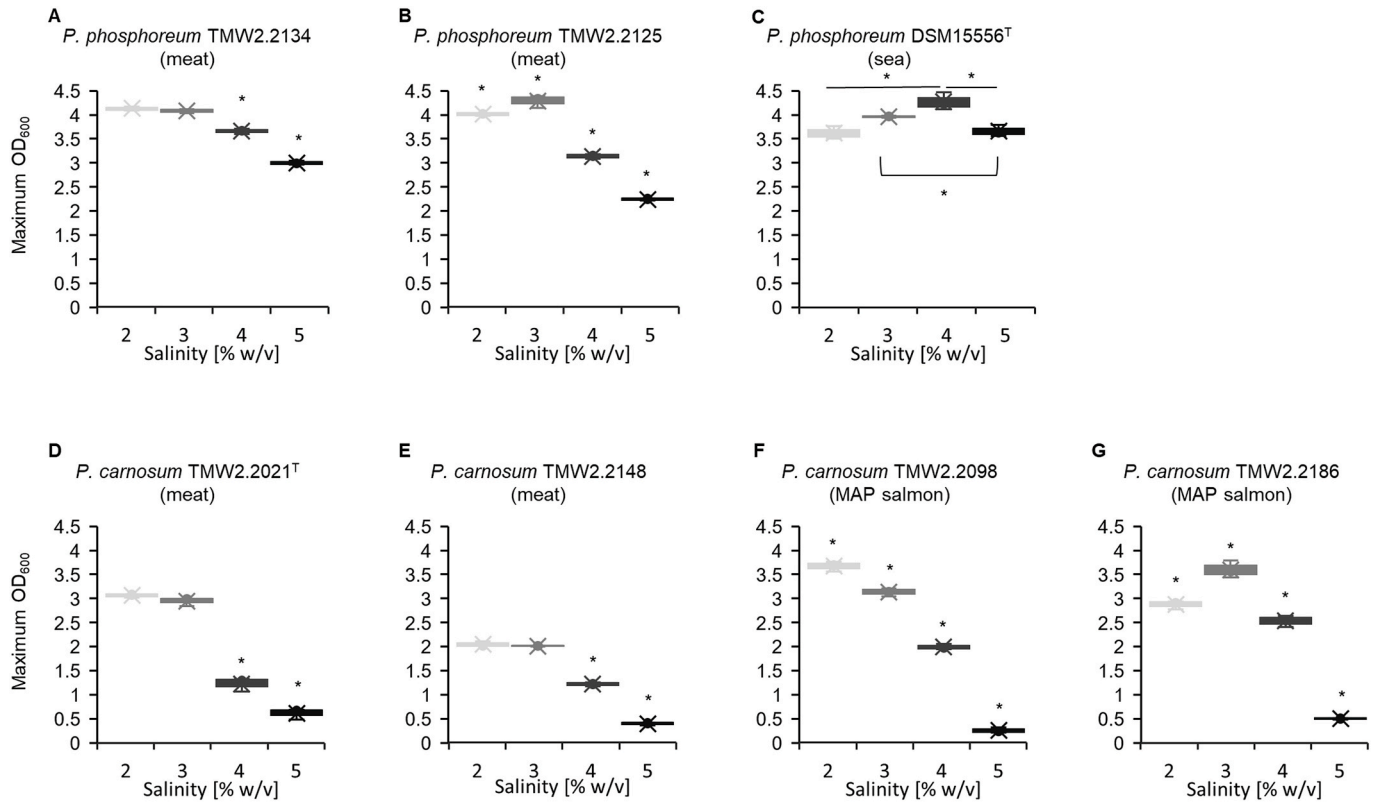


Fig. 2. Growth of *the P. phosphoreum* and *P. carnosum* strains in liquid medium with different salt content. Strains were cultivated in HS-MSM (2% NaCl (▨)), MSM with 3% NaCl (▩), MSM with 4% NaCl (■) and MSM with 5% NaCl (■). Tolerance is shown as maximum OD_{600} reached in the respective medium. **A - C** *P. phosphoreum*: TMW2.2134, TMW2.2125, DSM15556^T; **D – G** *P. carnosum*: TMW2.2021^T, TMW2.2148, TMW2.2098, TMW2.2186. Significance was accepted with $p < 0.05$. Error bars show the standard deviation of N = 3.

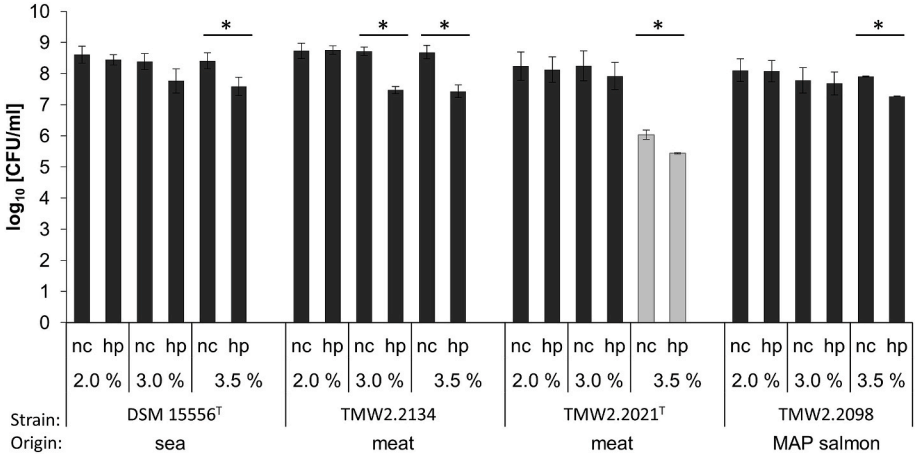


Fig. 3. Growth of high pressure treated cells of *P. carnosum* and *P. phosphoreum* on agar with different NaCl content. Cells were treated with 125 MPa for 5 min and plated on agar with 2%, 3% and 3.5% (w/v) NaCl (treated samples – hp). Controls were plated without high pressure treatment (negative control – nc). Colonies were counted after 72 h (■) or, if no colonies were observed, after 2 weeks of incubation (▨). *P. phosphoreum*: TMW2.2134, DSM15556^T; *P. carnosum*: TMW2.2021^T, TMW2.2098. Significance of differences of the cell counts were accepted with $p < 0.05$. Error bars show the standard deviation of N = 3.

TMW2.2098 from MAP salmon after pressure treatment were not reduced on 3% NaCl but were on 3.5% NaCl (Fig. 3). *P. carnosum* strain TMW2.2021^T from meat also did not show reduced cell counts on 3% NaCl agar but significant reduction of 1 $\log_{10}$ magnitude on 3.5% NaCl agar upon treatment (Fig. 3). Additionally, cell counts of treated and untreated cells were 2–2.5 $\log_{10}$ magnitudes lower compared to agar with <3.5% NaCl and the colonies did not grow within the given incubation time of three days. Tiny colonies were observed only after extended incubation of two weeks (Fig. 3). For both *P. carnosum* strains, differences of the cell counts after treatment on agar with 2%, 3% and 3.5% NaCl were significant (Supplementary Table S6A). In comparison of the species, sublethal effects of HHP treatment were stronger for *P. phosphoreum* strains than for *P. carnosum* strains (Supplementary Table S6B). The observed differences were significant for all strains on agar with 3% and 3.5% NaCl.

3.4. Presence of genomic traits associated with adaptation to HHP and halotolerance

Predictive genomic analyses were performed with an untargeted approach (BADGE comparison) as well as a targeted approach covering genes related to piezo-/halotolerance that were previously described for

Table 2
Presence of relevant genetic elements in *P. carnosum* and *P. phosphoreum* strains from meat and MAP salmon/marine environment. + all relevant ORFs of the trait present; (+) most ORFs of the trait present, relevant ORFs missing; - trait absent. For details see Supplementary Table S7.

Category	Genetic element	*P. carnosum*				*P. phosphoreum*		
		TMW2.2021^T	TMW2.2148	TMW2.2098	TMW2.2186	TMW2.2134	TMW2.2125	DSM 15556^T
		from meat		from MAP salmon		from meat		from the sea
Motility	Polar flagellar cluster	(+)	(+)	(+)	(+)	+	+	+
	Lateral flagellar cluster	–	–	–	–	–	–	–
Bioluminescence	Lux-operon	–	–	–	–	+	+	+
Pressure tolerance	General response	+	+	+	+	+	+	+
Salt tolerance	General response	+	+	+	+	+	+	+
	Number of exclusive sodium transporters	1				4		
	Additional transporters for organic osmolytes	–	–	–	–	+	+	+
Metabolism	Additional iron uptake systems	–	–	+	+	+	+	+
	Additional Fe–S transfer system	–	–	(+)	(+)	+	+	+
	Number of exclusive ORFs (transporters)	1109 (95) 43		122		1318 (120) 173		397
	Type IV secretion system	–	(+)	–	–	(+)	(+)	–

P. profundum.

Group-wise comparison of all analyzed strains revealed 1318 ORFs that were unique for *P. phosphoreum*. Of those, 120 were annotated as transporters with 4 of them being dependent of Na^+ and additionally 3 being associated with cation transport (Table 2, Supplementary Table S7). All analyzed *P. phosphoreum* genomes contained ORFs of the (glycine) betaine uptake system and ORFs of the *lux*-operon including luciferase subunits alpha and beta (Supplementary Table S7). Their genomes also contained relevant ORFs of high pressure tolerance and an additional copy of *rpoS*, coding for a sigma factor of the osmotic- and HHP-stress response. *P. phosphoreum* strains from meat had less unique ORFs than the type strain from marine environment (173 ORFs vs. 397 ORFs) but had additional genes of secretion system IV (Table 2, Supplementary Table S7). However, only minor genetic differences with relevance to piezo-/halotolerance were detected.

Strains of *P. carnosum* shared 1109 species-specific ORFs with 95 ORFs being transporters (Table 2). None of them was associated with sodium or cation transport (Supplementary Table S7). *P. carnosum* strains from MAP salmon had more unique ORFs than strains from meat (122 ORFs vs. 43 ORFs) and several of those coded for iron-sulfur cluster proteins or were genes for iron uptake (Supplementary Table S7). *P. phosphoreum* and *P. carnosum* strains shared most genes of the general stress response including osmotic and pressure induced stress, e.g. *torS*, *groEL* and *dnaK*. However, only strains of *P. phosphoreum* had a full flagellar cluster and all genes of the *lux*-operon (Table 2). Apart from this, *P. phosphoreum* strains and *P. carnosum* strains from MAP salmon shared several genes for iron uptake that were absent only in *P. carnosum* strains from meat.

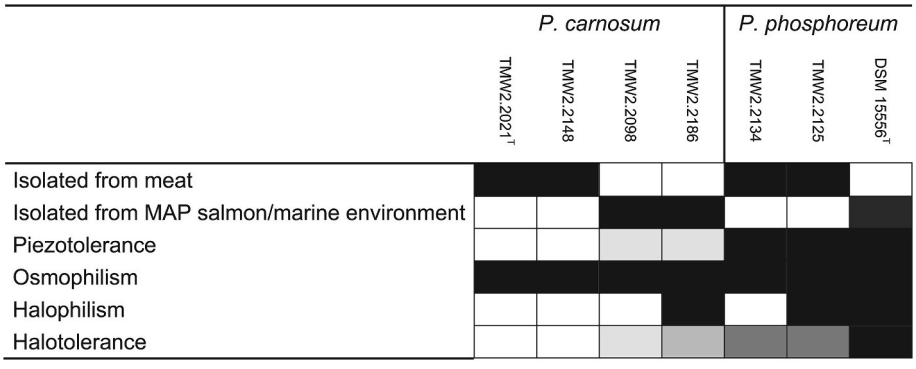
4. Discussion

Photobacteria are common isolates from marine habitats but have recently also been isolated from chilled meats as part of the spoilage microbiota. Still, information on possible links between physiological and genetic traits to their occurrence in these heterogeneous environments is very limited. Photobacteria in marine environments and especially in the deep sea experience high ambient pressure and high salinity and are consequently supposed to show piezo- and halotolerance (or even -philism). In this context, pressure- and osmotolerance are frequently related, as piezophilic organisms can accumulate compatible osmolytes to counteract high hydrostatic pressure (Martin et al., 2002). This study revealed physiological adaptations of *P. phosphoreum* and *P. carnosum* strains isolated from terrestrial and marine habitats to HHP and high salt concentrations, summarized in Table 3. These differences are also reflected in different genomic settings.

4.1. Adaptation of *P. phosphoreum* strains to hydrostatic pressure and high salinity

Application of 40 MPa hydrostatic pressure did not impair the growth of any of the tested *P. phosphoreum* strains, in contrast to strains of *P. carnosum*. These results correspond to reports in the literature on

Table 3
Comparison of the presence of physiological traits in relation to the isolation source. (■) Trait is present/statement is applicable; (■,▣, ▣) Trait is present but to a descending extent; (□) Trait is not present/statement is not applicable.



the isolation of *P. phosphoreum* strains from deep sea habitats and growth of those strains at 40 MPa (Al Ali et al., 2010; Martini et al., 2013). The observed homogeneous piezotolerance of *P. phosphoreum* strains suggests an adaptation to high pressure conditions and thus a deep sea adapted origin of this species, since pressure adaptation includes extensive changes in central mechanisms of the cell (Lauro et al., 2014; Bartlett, 2002). These changes include e.g. regulation of transcription and translation (Campanaro et al., 2005; Le Bihan et al., 2013), membrane structure, protein folding and energy production (Bartlett, 2002). As demonstrated for two representative strains, the salt concentration (2–5%) had no impact on cell size and consequently not on OD_{600} i.e. enabling to compare the growth yield by measuring max OD_{600}. All *P. phosphoreum* strains were also comparably tolerant to high salinity in liquid medium with up to 5% NaCl content. As expected, the marine type strain of *P. phosphoreum* reached its highest OD_{600} at a NaCl concentration similar to the one of sea water (3.5% NaCl). Salt requirement of marine bacteria concerns mainly the availability of Na^+ ions and is a well conserved and multigenic trait (Farmer and Hickman-Brenner, 2006; MacLeod, 1968; Baumann et al., 1972). Mg^{2+} and Ca^{2+} ions can replace Na^+, thus lowering the Na^+ requirement of marine bacteria to a certain extent and depending on the species (MacLeod, 1968; Reichelt and Baumann, 1974). This explains why the marine type strain of *P. phosphoreum* even reached its maximum OD_{600} with 4% instead of 3% NaCl here, since the medium contained only pure NaCl and not the mixed ionic composition of sea water. The two *P. phosphoreum* strains from meat reached their maximum OD_{600} at NaCl concentrations much lower than seawater, consistent with the lower NaCl content of meat. Still, the maximum OD_{600} of these strains was less affected by the NaCl content than the one of *P. carnosum* strains, also pointing at an adaptation to marine environment. Marine Gram negative bacteria are in general known to require higher NaCl concentrations than non-marine Gram negative (Farmer and Hickman-Brenner, 2006), concomitant with higher halotolerance. In contrast, terrestrial bacteria involved in food spoilage, e.g. many enterobacteria, rarely have specific NaCl requirements (Larsen, 1986) and are suggested to not survive in marine environments at all (Jannasch, 1968; Baumann and Baumann, 1977; Mitchell and Morris, 1969). Therefore, our results confirm our hypothesis that *P. phosphoreum* is originally a marine species that also occurs on meat, showing halophilism and piezotolerance. However, this hypothesis should be tested in further studies on additional isolates, since our results are based on data of a few representative strains from meat and marine environment.

4.2. Tolerance of P. carnosum to HHP and high salinity in comparison with P. phosphoreum

All tested *P. carnosum* strains showed reduced growth at 40 MPa hydrostatic pressure and differed with its piezo-sensitivity clearly from *P. phosphoreum* strains. This homogeneity suggests a shared origin of the strains unrelated to HHP, even though investigation of additional isolates from meat and fish is needed to confirm this assumption. In accordance, *P. carnosum* strains were also less halotolerant than *P. phosphoreum* strains. Increase of the salt concentration to 5% NaCl reduced the maximum OD_{600} reached by all *P. carnosum* strains more than fivefold. Strain TMW2.2186 that reached its maximum OD_{600} with 3% NaCl nevertheless showed distinct sensitivity to 5% NaCl. In this context, high pressure and high salinity may trigger correlated stress responses, e.g. accumulation of specific osmolytes (Martin et al., 2002; Smiddy et al., 2004, 2005), resulting in an energetic shift from cell division and biomass production to protective mechanisms or sparing energy rich substrates for usage as soluble osmolytes. It has been suggested before that high pressure stress response even consists of subsets of the cold shock and salinity stress responses (Hörmann et al., 2006). Altogether, this makes the occurrence of *P. carnosum* strains in deep sea environments appear very unlikely, corresponding to the fact that this species has never been isolated from marine environment so far. It is known that *P. carnosum* strains indeed require NaCl for growth under laboratory conditions (Fuertes-Perez et al., 2019) and could be termed halophilic in this sense. However, meat contains only minor amounts of NaCl (Ruusunen and Puolanne, 2005) and the requirement of Na^+ ions and other ions like Mg^{2+} or Ca^{2+} is to a certain extent exchangeable, as explained before. Consequently, *P. carnosum* could rather be considered as osmophilic than halophilic and much more independent of Na^+ than laboratory practice suggests. This is corroborated by the fact that meat contains e.g. high amounts of phosphorus (about 150–200 mg/100 g dry weight) and potassium (about 250 - >350 mg/100 g dry weight (Zarkadas et al., 1987; Pereira and Vicente, 2013); and the latter was found to be an appropriate substitute of Na^+ for osmophilic bacteria (Gibbons et al., 1969). Additionally, *P. carnosum* appears to be adapted to use organic osmolytes of meat for osmoregulation, since meat also contains high amounts of e.g. carnitine and glycine betaine (O'Byrne and Booth, 2002; Smith, 1996). This process has been evaluated by MacLeod and Onofrey who could show accustoming of a sodium-dependent (marine) *Pseudomonas* strain to successively dropping Na^+ availability in presence of a complex medium (MacLeod and Onofrey, 1963).

Altogether, *P. carnosum* is therefore suggested to be a piezo-sensitive but osmophilic terrestrial species and strains isolated from MAP salmon rather originate from cross-contamination. This is also supported by presence of metabolic traits associated with terrestrial habitats, e.g. acid production from glycogen, and lack of traits associated with marine habitats, e.g. motility and bioluminescence (Supplementary Table S7) (Fuertes-Perez et al., 2019; Hilgarth et al., 2018b). Additionally, *P. carnosum* strains from meat and MAP salmon have been found homogeneous regarding metabolic activity and growth and could not be physiologically divided in separate subpopulations before (Fuertes--Perez et al., 2019).

4.3. Tolerance of P. phosphoreum and P. carnosum to pressure-induced sublethal cell damages

We also investigated the effect of short HHP treatment on survival and halotolerance of the strains. Sublethal high pressure affects protein association and structures, membrane integrity and ribosomal functions, resulting in higher susceptibility for environmental stressors upon pressure release (for detailed review see (Vogel et al., 2003; Huang et al., 2014)). Here, treatment with 100 and 125 MPa and varying length did not reduce the viability of *P. carnosum* and *P. phosphoreum* cells distinctly. However, it affected the halotolerance especially of *P. phosphoreum* strains. Both, the *P. phosphoreum* strain from meat and the marine type strain showed reduced halotolerance upon treatment when compared to untreated cells, whereas *P. carnosum* strains did not. The potential different origin of *P. carnosum* and *P. phosphoreum* and the environmental differences of meat and marine habitats may explain why *P. phosphoreum* strains were significantly more vulnerable for pressure-induced sublethal cell damages. In this context, adaptation to constant hydrostatic pressure (<100 MPa) and resistance to pressure-induced sublethal damage must be distinguished. *P. phosphoreum* is supposed to have general adaptations to marine environment and its relatively steady living conditions. In accordance, sensitivity to sudden changes of the ambient pressure is suggested to be high, as already demonstrated for the sensitivity of marine bacteria to sudden changes of the salinity (ZoBell, 1941). Farmer 2006 additionally reports a weakening effect of Na^+ lack on the membrane of marine strains (Farmer and Hickman-Brenner, 2006) which may have resulted in reduced osmotolerance of the *P. phosphoreum* strains here. Also, structural differences of salt transport proteins may lead to different vulnerability of the osmotolerance systems of *P. phosphoreum* and *P. carnosum* to HHP. In this sense, 100 MPa pressure triggers protein misfolding and modification especially of multimeric proteins (Hörmann et al., 2006; Gross and Jaenicke, 1994). BADGE analysis revealed several different sodium transporters in *P. phosphoreum* and *P. carnosum* genomes. However, involvement of those transporters in

pressure-induced osmo-sensitivity remains speculative since the observed sensitivity could also be a result from damages in any of the 1318 genes that were unique in *P. phosphoreum* or from a transient loss of its membrane integrity.

4.4. Adaptation of P. phosphoreum and P. carnosum strains to different habitats

Besides their general sensitivity to HHP and high salinity, *P. carnosum* strains differed clearly regarding the extent of that sensitivity. Growth of strains from MAP salmon was significantly less reduced by HHP than growth of strains from meat. Additionally, halotolerance of the strains from MAP salmon was more heterogeneous, with one strain even showing halophilism comparable to a *P. phosphoreum* strain from meat. Since these observations are limited to a few representative strains, additional isolates from meat, fish and marine environment should be investigated in future studies. However, our observations already suggest remaining traits of a shared origin of the whole species *Photobacterium* from the sea. *P. carnosum* strains from MAP salmon were still less halotolerant and less piezotolerant than *P. phosphoreum* strains and their dynamics were relatively diverse. Consequently, hypothetical remaining traits of adaptation to marine environment in *P. carnosum* strains appear to have reduced functionality.

P. phosphoreum strains from meat also differed from the marine type strain. They were clearly less halotolerant and preferred lower NaCl concentrations for growth (lower halophilism). Fuertes-Perez et al., 2019 have also shown that these strains differ regarding carbon utilization, growth properties and metabolic activity (Fuertes-Perez et al., 2019). This may point at presence of different subpopulations of *P. phosphoreum* strains from meats and marine niches. In this context, *P. phosphoreum* can even become more dominant than *P. carnosum* on packaged and chilled meats despite its supposed marine background. Strains of the species were already found to grow faster than strains of *P. carnosum* in meat simulation medium (Fuertes-Perez et al., 2019) but may also take advantage of emerging adaptation towards the meat and meat processing environment. Its adaptation process is suggested to follow the development of *P. carnosum* strains, finally resulting in osmophilic strains with reduced halo- and piezotolerance.

Urbanczyk et al., 2010 have reported relatively large genomes and presence of high copy numbers of rRNA operons in photobacteria to facilitate fast translation by high numbers of ribosomes. They have concluded that photobacteria are thus able to adapt their metabolism in general quickly to periods of high nutrient availability and periods of nutrient lack (Urbanczyk et al., 2010; Klappenbach et al., 2000). This may additionally simplify the adaptation of *P. phosphoreum* and *P. carnosum* strains to their current habitat and may also explain the ability of these species to survive cross-contamination events.

4.5. Differences of relevant genetic aspects with respect to the isolation sources

Genomic analysis revealed several differences of *P. phosphoreum* and *P. carnosum* strains, supporting our hypothesis of separate development of the species. *P. phosphoreum* strains showed more characteristic genomic traits of adaptation to marine environments, e.g. more genes associated with sodium or cation transport, an intact *lux*-system resulting in bioluminescence of all analyzed strains and a full gene cluster for flagellar. Interestingly, size and organization of the detected flagellar cluster proved to be similar to the polar flagellar cluster detected in deep sea and shallow water isolates of *P. profundum* (Campanaro et al., 2005). However, the additional lateral flagellar cluster of *P. profundum* deep-sea isolates being relevant for motility at HHP (Campanaro et al., 2005) was missing in all *P. phosphoreum* strains. Hence, they may not be motile at HHP conditions at all. In *P. carnosum* genomes, several genes of the *lux*- and flagellar cluster were absent and especially strain TMW2.2148 from meat lacked around 80% of the flagellar genes, suggesting ongoing loss of the cluster in this species. In accordance, *P. carnosum* has been described as non-motile and non-luminescent species, whereas *P. phosphoreum* can be considered as motile and luminescent (Hilgarth et al., 2018b). However, presence of parts of the *lux*- and flagellar gene clusters, e.g. *luxU*, and *motAB,* in *P. carnosum* confirms our suggestion that this species shares the initially marine origin of the genus *Photobacterium*. Both species also shared most of the analyzed genes with relevance in pressure- and salt adaptation, except of some additional sodium/cation transporters found in *P. phosphoreum* strains. This suggests presence of modifications rather on transcription or translation level resulting in the observed differences in HHP- and halotolerance.

Strains of both species from meat had less unique ORFs than strains from MAP salmon/marine environment based on pair-wise BADGE comparison. Additionally, analysis revealed differences regarding secretion system IV (*P. phosphoreum*) and iron metabolism (*P. carnosum*). This differentiation corresponds to our conclusions from the growth experiments at HHP and high salinity, also revealing significant differences of strains from meat and MAP salmon/marine environment. It confirms development of the strains towards their respective habitat with ongoing separation in subpopulations. However, this hypothesis should be validated by a more detailed genetic analysis based on a broader selection of *P. carnosum* and *P. phosphoreum* isolates. Interestingly, elements of secretion system IV were present in both, *P. phosphoreum* strains from meat and *P. carnosum* strain TMW2.2148 from meat and elements of iron metabolism were present in *P. carnosum* strains from MAP salmon and all *P. phosphoreum* strains. Secretion systems type IV are related to the conjugation system and mediate one-step transport of proteins and DNA (Schröder and Lanka, 2005). Detected ORFs allow full functionality since they covered all necessary elements for energy supply (*virB4, virB11*), inner and outer translocation complex (*virB8* and *virB10, virB9*) and extracellular pilus (*virB5 (*55*)*). Presence of this system may allow conjugation and consequently competitive advantages in the microbial community on meat. The *sufBCDSE*-operon present in *P. carnosum* strains from MAP salmon and all *P. phosphoreum* strains participates in sulfur transfer mechanisms and is induced by oxidative stress and iron limitation (Outten et al., 2003). In accordance, the strains also had several additional genes for iron uptake. Iron availability in marine environments is continuously very low (0,76 nmol/kg) (Tortell et al., 1999; Johnson et al., 1997) explaining the presence of multiple iron uptake systems for improved iron exploitation in *P. phosphoreum* strains. Salmon also contains much less iron than meat (0.8 mg/100 g vs. 0.37–2.44 mg/100 g) (Bohrer, 2017) suggesting iron deprivation conditions for *P. carnosum* strains on salmon and selective pressure for the presence of the *sufBCDSE*-operon in those. Comparing the iron uptake system of *P. carnosum* and *P. phosphoreum* revealed additional species-specific genes in the former. This may indicate in general a higher iron requirement of *P. carnosum* strains that may even result from adaptation towards the meat habitat.

5. Conclusions

This study compared *P. phosphoreum* and *P. carnosum* strains from different isolation sources by characterizing their halo- and piezotolerance as well as their genomic settings for these traits. *P. phosphoreum* strains were comparably piezo- and halotolerant and are thus suggested to be adapted to deep sea environment. Strains from meat appear to represent a less halophilic subpopulation that may exploit meat processing facilities as persistent niche, following their distribution into packaged meats as transient niche. In contrast, *P. carnosum* strains were comparably piezo- and halo-sensitive, thus showing loss of adaptation to deep sea environments and adaptation to (terrestrial) meat environments. Strains on MAP salmon should be considered as cross-contaminants from processing facilities rather than from marine origin. However, osmophilism of *P. carnosum* strains and presence of (non-functional) genomic traits associated with marine lifestyle (flagellar cluster, *lux*-operon) points at a shared evolutionary origin of

Photobacterium from marine environment. Future work may show if the observed differences of the two species also influence their relevance in meat spoilage.

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Author contributions

PH designed the study, performed the experiments and the data evaluation, and wrote the original draft of the manuscript. MH helped to draft the manuscript, helped with the data interpretation and supervised the work of PH. RV initiated the project and supervised the work of PH. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2020.103679.

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5.3 Influence of the packaging atmosphere and presence of co-contaminants on the growth of photobacteria on chicken meat

Since bacterial growth on spoiling meat is subject to interaction with co-contaminating species, the effect of co-contamination on *P. phosphoreum* strain TMW2.2103 and *P. carnosum* strain TMW2.2149 was evaluated. Persistence and recovery of strains of both species proved to be more successful with chicken meat than with beef or pork, and experiments were performed with MAP chicken breast in accordance. To enable separate evaluation of the effect of co-contamination and MAP, growth of the strains on meat was monitored in different gas mixtures first (air, 70% O_2/30% CO_2, 70% N_2/30%CO_2, 21% O_2/30% CO_2/49% N_2, 100% N_2). Thereby, combination of high O_2/high CO_2 content (70%/30%) turned out to reduce the growth of both strains significantly, whereas the impact of CO_2 alone was distinctly lower. This allows for speculations on an inhibitory impact of the cumulated effects of O_2 and CO_2 on *P. phosphoreum* and *P. carnosum* strains on meat, but a certain tolerance of these strains to CO_2. The latter can be assumed to account for growth advantages over less tolerant species, such as other Gram-negative meat spoilers. Since the observed impact of high O_2/high CO_2 atmosphere does not correspond to the actual cell counts reported for photobacteria on meat from retail, the overall impact of MAP on its growth is consequently suggested to be low. Presence of *Ps. fragi* or *B. thermosphacta* as co-contaminant influenced the growth *P. phosphoreum* and *P. carnosum* strains on meat distinctly. Observed effects were thereby independent of the type of MAP but highly dependent of the respective *Photobacterium* species. Growth of *P. phosphoreum* TMW2.2103 was reduced or unchanged by presence of co-contaminating species under MAP, suggesting competitive interaction in this case. In contrast, growth of *P. carnosum* TMW2.2149 was enhanced, pointing at commensalistic interaction instead. It provides support for the assumed stronger adaptation of *P. carnosum* strains towards the meat environment. Observed effects were reversed under air atmosphere, demonstrating fundamentally changed growth conditions and the importance of MAP for the competitive success of single species within the spoilage community. Strains of both species proved additionally able to grow and prevail over seven days on chicken meat in 70% O_2/30% CO_2 atmosphere, when a mixed spoilage community of seven common meat spoilers was present. It points at successful integration of *P. phosphoreum* and *P. carnosum* strains in the meat spoilage community.

Author contributions: Philippa Frederieke Hauschild performed all experiments and evaluated and visualized the resulting data. Additionally, she wrote the original draft of the manuscript and realized corrections that arose during the reviewing process.

Maik Hilgarth supervised the work of Philippa Frederieke Hauschild and helped to realize corrections that arose during the reviewing process. Rudi F. Vogel initiated the corresponding project, supervised the work of Philippa Frederieke Hauschild and reviewed this manuscript.

Supplementary files of this publication are provided in appendix section 12.3.

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Influence of the packaging atmosphere and presence of co-contaminants on the growth of photobacteria on chicken meat

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ABSTRACT

Fresh meat is commonly packaged in modified atmosphere to decelerate spoilage processes. The applied gas mixture affects the growth of spoilage organisms and selectively shapes the spoilage community. In this study, we investigated the impact of O_2 and CO_2 on the growth of *Photobacterium* (P.) *phosphoreum* and *P. carnosum* strains *in situ* on chicken meat by packaging under different modified atmospheres (air, 70% O_2/30% CO_2, 70% N_2/30% CO_2, 100% N_2). Combination of 70% O_2 and 30% CO_2 resulted in significant growth reduction of the analyzed strains, suggesting inhibitory effects of both gases in combination. In contrast, 30% CO_2 alone had only a minor effect and photobacteria are supposed to have a growth advantage over other meat spoilers in this atmosphere. Additionally, single growth of the strains in the different atmospheres was compared when challenged with the presence of *Pseudomonas* (*Ps.*) *fragi* or *Brochothrix* (*B.*) *thermosphacta* as prominent co-contaminants in different ratios (10:1, 1:1, 1:10). Presence of co-contaminants resulted in increased cell numbers of *P. carnosum* TMW2.2149 but reduced or unchanged cell numbers of *P. phosphoreum* TMW2.2103 in most packaging atmospheres. The initial ratio of photobacteria and co-contaminants defined the relative abundance during storage but did not change the type of the interaction. Our results suggest either a commensalistic (*P. carnosum*) or competitive interaction (*P. phosphoreum*) of photobacteria and co-contaminants on modified atmosphere packaged chicken, respectively. Furthermore, in a mix comprising seven prominent spoilers, strains of both *Photobacterium* species prevailed as a constant part of the spoilage microbiome during 7 days of refrigerated storage on chicken meat packaged under O_2/CO_2 atmosphere.

1. Introduction

Fresh meat is a highly perishable product that provides optimal growth conditions for many microorganisms. Among other parameters, its high water activity and nutrient availability allows rapid growth of a core community (Jay et al., 2005; Reid et al., 2017) that consists of bacteria and fungi from the animal and from environmental sources (Chaillou et al., 2015). However, storage conditions and application of modified atmosphere packaging (MAP) select for particular species of that core community, thus shaping its development during spoilage (Chaillou et al., 2015; Kerry and Tyuftin, 2017). Most reports hypothesize that CO_2 is the main inhibitive gas, which has bacteriostatic function especially on aerobic *Pseudomonas* spp. (Chaix et al., 2015; Erichsen and Molin, 1981). Generally, CO_2 decreases growth rates and extents lag phases of bacteria (Stier et al., 1981; Sutherland et al., 1977), presumably by its influence on the cellular pH, on decarboxylating enzymes and transport processes (Garcia-Gonzalez et al., 2007). While high levels of O_2 reduce bacterial growth by formation of reactive oxygen species (ROS (Amanatidou, 2001; Baez and Shiloach, 2014; Chaix et al., 2015; Day, 2002)), producers rather use O_2 to primarily maintain an appealing red meat color (Young et al., 1988) or inhibit strict anaerobes (Farber, 1991). Depending on the applied atmosphere, major spoilage bacteria on chilled meat are *Pseudomonas* spp. (especially *Ps. fragi*), *B. thermosphacta*, *Enterobacterales*, *Carnobacterium* spp. and other lactic acid bacteria (LAB) (Kerry and Tyuftin, 2017; Molin and Ternström, 1982; Nychas et al., 2008), while the latter show the highest CO_2 tolerance and *B. thermosphacta/Pseudomonas* the lowest on beef and pork (Blickstad et al., 1981; Erichsen and Molin, 1981). Lots of studies have been investigating the effect of different gases on mentioned species and their role in meat spoilage, however, little is known on how the genus *Photobacterium* reacts to packaging. Species of the genus have exclusively been known as marine isolates and fish spoilers (reviewed e. g. by (Dalgaard et al., 1997; Labella et al., 2017)) until culture-independent methods and finally a suitable cultivation protocol

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proved their frequent occurrence also on meat in high cell numbers of over 10^7 CFU/g (e.g. (Fuertes-Perez et al., 2019; Hilgarth et al., 2018a; Höll et al., 2019; Nieminen et al., 2016)). Recent research reports metabolic adaptation of the two most common *Photobacterium* species on meat, *P. carnosum* and *P. phosphoreum*, to the meat system (Hauschild et al., 2020). Additionally, a meta-transcriptomic study on naturally contaminated chicken has predicted only minor effects of the packaging atmosphere due to similar gene expression patterns (Höll et al., 2019). This is in agreement with the work of Dalgaard reporting comparably high tolerance of *P. phosphoreum* to CO_2 (Dalgaard, 1995). In high CO_2 and also in high CO_2/high O_2 atmosphere, major metabolic pathways have been found to be lipolysis including beta-oxidation of fatty acids and glycerol, as well as amino acid degradation resulting in biogenic amines production (Höll et al., 2019). The present study investigates for the first time in situ growth of *P. carnosum* and *P. phosphoreum* strains on chicken. Growth upon different atmospheric packaging compositions and in the presence or absence of prominent Gram-negative (*Pseudomonas*) and Gram-positive (*B. thermosphacta*) spoilers was studied.

2. Materials and methods

2.1. Microorganisms and cultivation

Experiments were done with up to three strains of *P. phosphoreum* (TMW2.2134 isolated from chicken; TMW2.2103 isolated from beef, TMW2.2140 isolated from pork (Fuertes-Perez et al., 2019)) and *P. carnosum* (TMW2.2169 isolated from turkey, TMW2.2021 isolated from chicken, TMW2.2148 isolated from beef, TMW2.2149 isolated from pork (Fuertes-Perez et al., 2019)). Additionally, *Ps. fragi* TMW2.2082, *Ps. lundensis* TMW2.2076, *Ps. weihenstephanensis* TMW2.1728, *B. thermosphacta* TMW2.1567, *L. gelidum* subsp. *gelidum* TMW2.1618, *L. gelidum* subsp. *gasicomitatum* TMW2.1619, *C. divergens* TMW2.1577 and *C. maltaromaticum* TMW2.1581 were used to build up a representative meat spoilage community.

Photobacterium strains were cultivated in meat-simulation medium (MSM, 2% (*w/v*) NaCl, 2% (w/v) meat extract) and other species in brain heart infusion medium (BHI; Roth, Karlsruhe, Germany). Plates of both media were prepared with 1.6% (w/v) agar and MSM plates were additionally supplemented with 7 mg/l (w/v) vancomycin for selectivity towards photobacteria. All cultures were pre-inoculated from frozen glycerol-stocks and incubated in an overnight culture aerobically at 15 °C. Inoculated meat slices were incubated at 4 °C after packaging.

2.2. Preparation of meat samples

Fresh meat from the supermarket was cut into slices with defined surfaces of 16 cm^2 and slices were then inoculated from both sides with bacterial suspensions. Experiments with chicken meat were conducted with breast filets, and experiments with pork and beef with pre-cut steaks. All meat slices were about 1 cm in height. Inoculation with multiple species or strains was done with cells from separate cultures that were mixed before inoculation. Mixtures were done based on the respective OD_{600} of the separate cultures to gain the desired cell counts per cm^2. If necessary, cultures with low OD_{600} were concentrated by centrifugation for 10 min at 4000 ×*g* and resuspension of the pellet in tenfold lower volume of MSM. All separate cultures were additionally plated to monitor the actual colony forming units (CFU) per ml. Inoculated meat was cooled down to 4 °C and packaged with a semi-automatic tray sealer (Rotarius VG, Variovac PS SystemPack GmbH, Zarrentin am Schaalsee, Germany) that was connected to a gas mixer for fluctuating gas flow (Witt-Gasetechnik, Witten, Germany). Applied gas mixtures were 70%_O_2/30%_CO_2, 70%_N_2/30%_CO_2, 100%_N_2, 21%_O_2/79%_N_2 (air) and 21%_O_2/30%_CO_2/49%_N_2. Gas composition of the packages was checked using a compact gas analyser PA 7.0 (Witt-Gasetechnik, Witten, Germany). All replicates shared one package, respectively. Utilized polypropylene trays had an ethylene-vinylalcohol polymer coating and a volumetric permeation rate of 0.25 cc.20 μ m^{-2}.day.atm (ES-Plastic, Hutthurn, Germany). Packaged meat was stored for up to 7 days at constant 4 °C, corresponding to the assigned shelf life.

2.3. Sampling of meat

Composition of the modified gas atmosphere was checked before opening packaged meat for taking a sample. Meat pieces were then transferred to sterile filter bags containing 5 ml of MSM and homogenized for 120 s with a bag mixer (Interscience, Saint Nom la Breteche, France). Serial dilutions of the resulting suspensions were plated on BHI-agar and MSM-agar and counted after 2–3 days of incubation at 15 °C.

2.4. Species- and strain identification

Species were identified with MALDI-TOF MS and a Microflex LT spectrometer (Bruker Corporation, Billerica, MA, USA) using direct transfer of cells followed by on-target extraction (Usbeck et al., 2013). Spectrometry profiles were then compared to an established in-house database for species identification. Strains were further distinguished with Randomly Amplified Polymorphic DNA (RAPD)-PCR followed by agarose gel electrophoresis (1.4% *w/v* agarose, 150 V, 2.5 h) with primer M13V (Ehrmann et al., 2003) and lambda DNA/*Eco*RI plus *Hin*dIII as marker (Thermo Scientific, Hampshire, England).

2.5. Growth with H_2O_2

Strains were cultivated in MSM, washed and diluted to $OD_{600} = 0.1$. The cultures were then mixed with 0.01–0.1% H_2O_2 (Merck, Darmstadt, Germany) as stated by (Kolbeck et al., 2019) and cultivated at 15 °C in 96-well plates (Sarstedt, Nümbrecht, Germany). Growth over 93 h was measured as OD_{600} with a FLUOstar microplate reader (BMG Labtech, Ortenberg, Germany). The minimum inhibitory concentration (MIC) of H_2O_2 was calculated as mean value of triplicates.

2.6. Statistical analysis

Data were analyzed using the software IBM SPSS Statistics v23.0.0.0. One-way analysis of variances (ANOVA) and post-hoc Tukey test were used to evaluate significance of the observed differences if the variances were considered to be homogeneous. In case of heterogeneous variances, data were analyzed using Welch-ANOVA and Games-Howell *post-hoc* tests. Significance was accepted with $p < 0.05$.

3. Results

3.1. Recovery from different meat types

P. phosphoreum and *P. carnosum* strains isolated from beef, chicken and pork were cultivated on all three meats to assess the influence of the isolation origin and respective meat type on their development. In general, significantly higher cell numbers of both species were recovered from chicken than from pork and beef (Fig. 1). Only *P. carnosum* TMW2.2149 cell counts showed no significance but high variability of the replicates instead (Fig. 1 statistical analysis marked with X-Z). Observed differences in cell counts were not correlated with the isolation source of a strain (Fig. 1 statistical analysis marked with a–c) and strains isolated from one meat type did not show preference for this meat type (Fig. 1 statistical analysis marked with X-Z). Most strains had their lowest cell counts on pork, showing a significant reduction of the initial cell numbers of up to 3.5 $\log_{10}$ CFU/cm^2 (*P. phosphoreum*) or 7 $\log_{10}$ CFU/cm^2 (*P. carnosum*).

Additionally, three strains of each species were mixed in equal proportion and both species were inoculated on chicken meat together with a mixed meat spoilage community of *Ps. fragi*, *Ps. lundensis*, *Ps. weihenstephanensis*, *B. thermosphacta*, *L. gelidum* subsp. *gelidum*, *L. gelidum* subs.

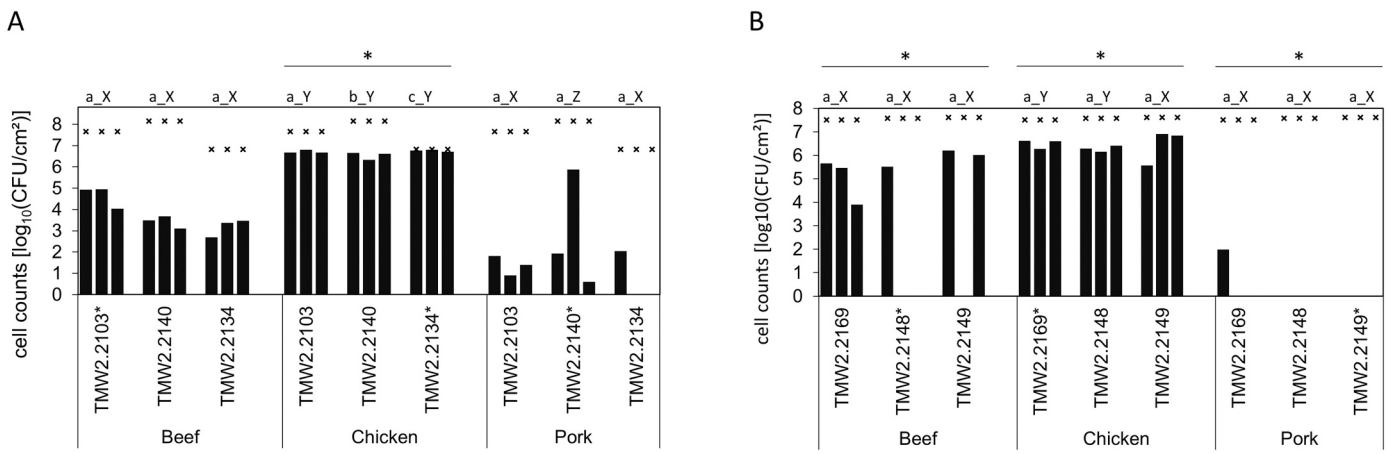

Fig. 1. Development of strains isolated from beef, chicken and pork over 6 days on beef, chicken and pork. Inoculated meat was packaged in 70%_O_2/30%_CO_2 atmosphere and stored at 4 °C. Crossmarks show the initial cell counts, bars show the cell counts after 6 days of $n = 3$ replicates. Strains that were originally isolated from the respective meat type are marked with an asterisk (*). Statistical analysis was done by grouping the strains of a species within one meat type (marked with a–c) and grouping the meat types for each strain (marked with X–Z). Detection limit was at 0.19 $\log_{10}$(CFU/cm^2). Significance was accepted with $p < 0.05$. **A** *P. phosphoreum* strains, **B** *P. carnosum* strains.

gasicomitatum, *C. maltaromaticum* and *C. divergens*. Species were isolated from the meat after 3 and 7 days and identified using MALDI-TOF MS. Further differentiation of the strains was done with RAPD-PCR. Strain differentiation showed shifted proportions compared to the initial composition and high cell numbers especially of *P. carnosum* TMW2.2149 and *P. phosphoreum* TMW2.2103, respectively (Supplementary Fig. S1). Consequently, these strains were selected for following experiments to study the influence of the gas atmosphere in detail.

3.2. Growth in different gas atmospheres

Four different gas atmospheres were selected to monitor their influence on the growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 on chicken. Packaging under air condition (21% O_2, 79% N_2) was compared to high-oxygen modified atmosphere packaging containing O_2/CO_2 (70%, 30%), oxygen-free MAP containing N_2/CO_2 (70%, 30%) and solely N_2 (100%). Initial cell counts were at 5.66–5.84 $\log_{10}$ CFU/cm^2 for *P. phosphoreum* TMW2.2103 and 4.53–4.81 $\log_{10}$CFU/cm^2 for *P. carnosum* TMW2.2149 in all atmospheres (Supplementary Table S2).

Both species reached significantly higher cell counts in air than in modified atmosphere over 7 days of incubation (Fig. 2). The counts of *P. phosphoreum* TMW2.2103 were at least 0.5 $\log_{10}$ CFU/cm^2 higher and the counts of *P. carnosum* TMW2.2149 at least 1 $\log_{10}$ CFU/cm^2 higher in air (compare Supplementary Table S2; *P. phosphoreum* TMW2.2103: 7.48 ± 0.056 $\log_{10}$ CFU/cm^2, *P. carnosum* TMW2.2149: 6.76 ± 0.175 $\log_{10}$ CFU/cm^2). Lowest cell counts were measured for both species in modified atmosphere containing 70% O_2 and 30% CO_2 (compare Supplementary Table S2; *P. phosphoreum* TMW2.2103: 6.03 ± 0.127 $\log_{10}$ CFU/cm^2, *P. carnosum* TMW2.2149: 3.74 ± 0.098 $\log_{10}$ CFU/cm^2). In this atmosphere, *P. phosphoreum* TMW2.2103 showed slightly increasing cell numbers that doubled within the given incubation time (Fig. 2A), whereas *P. carnosum* TMW2.2149 cell numbers declined (Fig. 2B). In most cases, maximum cell counts were reached at day 3 and dropped by about 1 $\log_{10}$ CFU/cm^2 from day 3 to 7.

Additionally, both species were cultivated in MAP that combined the oxygen concentration of air with high CO_2 concentration (21%_O_2, 30%_CO_2, 49%_N_2). Growth in this atmosphere was similar or only slightly reduced compared to the growth in air (Supplementary Fig. S2). The minimum inhibitory concentration of H_2O_2 was at 0.02% for *P. phosphoreum* TMW2.2103 and at 0.01% for *P. carnosum* TMW2.2149 (Supplementary Table S1).

3.3. Growth in a mixed model spoilage community on chicken

P. phosphoreum and *P. carnosum* were cultivated together with a mixed meat spoilage community in O_2/CO_2 atmosphere on chicken, mimicking the spoilage microbiome typically found on meat. Both *Photobacterium* species were continuously recovered as part of the spoilage community over 7 days of incubation (Fig. 3). *P. phosphoreum* made up to 20% and *P. carnosum* up to 8% of the total cell counts and both species reached its highest relative abundance after 3 days of incubation. Total cell numbers of the spoilage community increased significantly compared to the initial CFU, with a smaller increase from day 3 to 7. Maximum cell numbers were mostly detected after 7 days of incubation. The spoilage community changed in general from day 3 to 7 with only few species becoming more abundant (especially *B. thermosphacta*, *Pseudomonas* spp.) over other spoilers. Photobacteria prevailed as part of the microbiome throughout the storage time of 7 days within the inoculated meat samples. No photobacteria were detected in the spoilage community of the negative controls (data not shown).

3.4. Interaction of Photobacterium and P. fragi/B. thermosphacta in different gas atmospheres

Influence of two prominent co-contaminants, *Ps. fragi* and *B. thermosphacta,* on the growth of *Photobacterium* species was further investigated in a separate set of experiments without the whole consortium. *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 were challenged with presence of both co-contaminants separately at equal ratio (1:1) on chicken meat. Inoculated meat was incubated in the four atmospheres described in Section 3.2 (air, O_2/CO_2, N_2/CO_2, N_2).

Presence of *Ps. fragi* or *B. thermosphacta* did not result in significant changes of the maximum cell counts of *P. phosphoreum* TMW2.2103 in most cases (Fig. 4). Only in air atmosphere, *P. phosphoreum* cell counts were significantly increased by presence of *Ps. fragi* after 7 days (Fig. 4D). The overall development of the cell counts also did not change in N_2/CO_2 and N_2 atmosphere if a co-contaminant was present, except of a slightly but significantly stronger reduction at day 7 (Fig. 4B and C). However, presence of *Ps. fragi* or *B. thermosphacta* resulted in decreasing cell numbers of *P. phosphoreum* TMW2.2103 in O_2/CO_2 atmosphere over 7 days, whereas cell numbers increased in absence of a co-contaminant (Fig. 4A). In comparison of the overall trends shown by the cell counts during the first 3 days of incubation, presence of *Ps. fragi* or *B. thermosphacta* either led to growth impairment or no significant

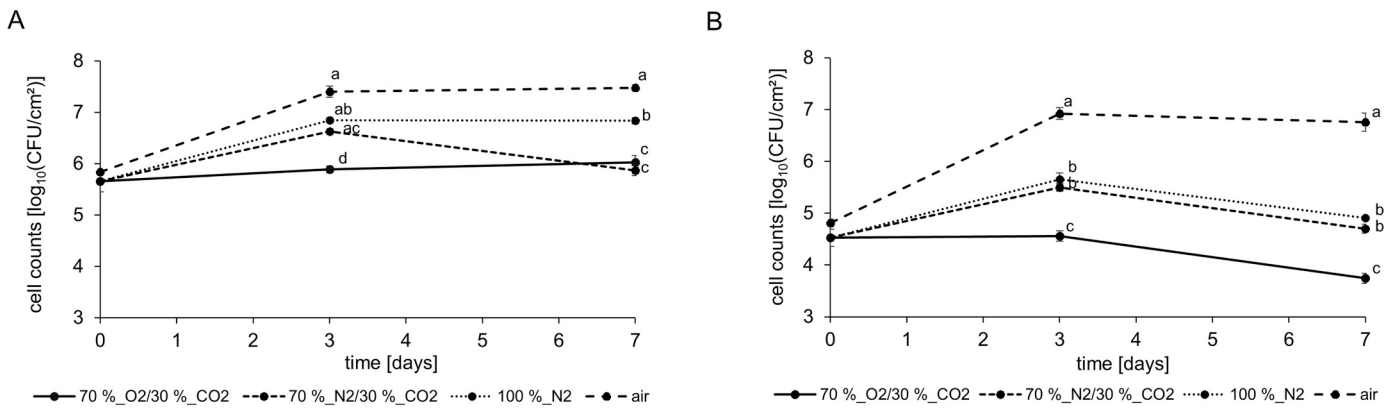

Fig. 2. Growth of *P. phosphoreum* and *P. carnosum* on chicken meat packaged under different gas atmospheres. Meat was incubated at 4 °C and the gas composition of the atmospheres was checked before taking a sample. Values were grouped by statistical significance (a–d) and significance was accepted with $p < 0.05$. N = 3. **A** *P. phosphoreum* TMW2.2103 **B** *P. carnosum* TMW2.2149.

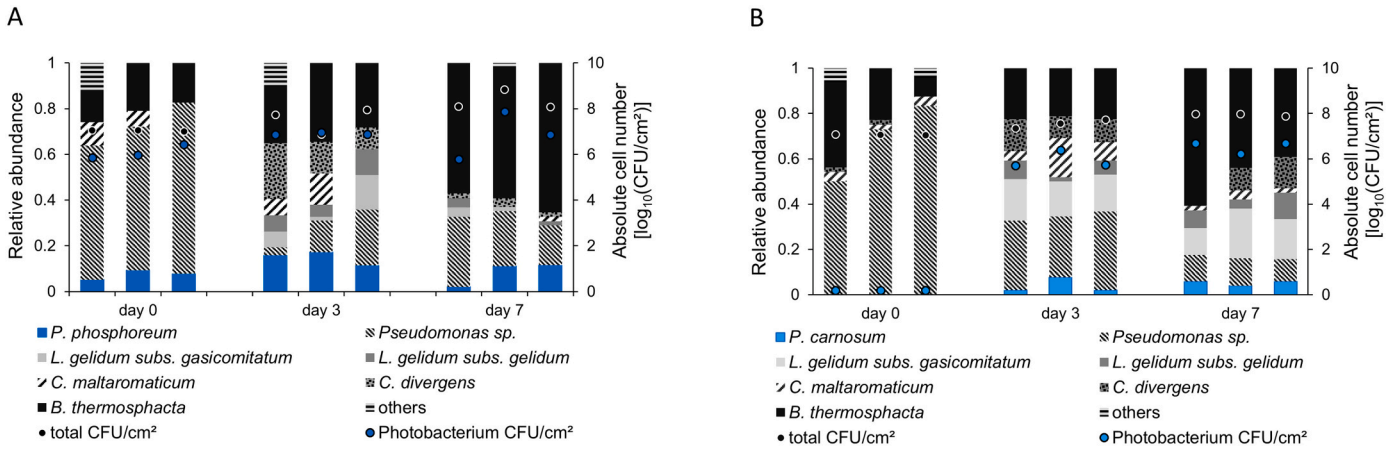

Fig. 3. Development of photobacteria in a mixed in situ spoilage community on chicken, packaged with 70%_O_2/30%_CO_2 atmosphere. Species were cultivated in separate culture, mixed and used for inoculation with 6 $\log_{10}$(CFU/cm^2), respectively. Species were identified with MALDI-TOF MS. N = 3 per day. Detection limit was at 0.19 $\log_{10}$(CFU/cm^2). **A** *P. phosphoreum*, **B** *P. carnosum.*

change for *P. phosphoreum* TMW2.2103 in MAP (Table 1). In contrast, it resulted mostly in growth improvement in air.

In all atmospheres except of air, cell numbers of *P. carnosum* TMW2.2149 were significantly higher in presence of either *Ps. fragi* or *B. thermosphacta* (Fig. 5). This was observed in all cases after 7 days of incubation and in few cases already after 3 days of incubation, e.g. with *Ps. fragi* in N_2 atmosphere (Fig. 5C). Additionally, presence of a co-contaminant also resulted in many cases in higher maximum cell counts of *P. carnosum* TMW2.2149 in all atmospheres except of air. To some extent the increase occurred in combination with growth delay, resulting in higher cell counts at day 7 than at day 3. This was found e.g. in O_2/CO_2 atmosphere (Fig. 5A). In contrast, presence of a co-contaminant did not influence the cell counts of *P. carnosum* TMW2.2149 significantly if the meat was packaged in air (Fig. 5D). Evaluation of the overall trends of the cell counts over 3 days showed that air was the only packaging resulting in growth impairment of *P. carnosum* TMW2.2149 by the presence of a co-contaminant (Table 1). In MAP atmosphere presence of a co-contaminant improved growth of the strain in most cases.

P. phosphoreum and *P. carnosum* strains were also challenged with both co-contaminant in over-represented ratio (1:10) and under-represented ratio (10:1) (Supplementary Table S2). However, in general, mixing *Photobacterium* and co-contaminant in different ratio did not result in clearly different effects on the *Photobacterium* cell counts in comparison to a ratio of 1:1 (Supplementary Fig. S3, Table 1).

4. Discussion

Photobacterium strains have been frequently isolated from different meat types and recovered from different modified atmosphere compositions (Fuertes-Perez et al., 2019; Hilgarth et al., 2018a; Höll et al., 2019). The presented study investigates in situ growth of two common but insufficiently studied potent meat spoilers, *P. phosphoreum* and *P. carnosum*, by investigating the impact of different packaging gases and presence of co-contaminants on chicken.

4.1. Effect of the packaging gases on the growth of P. phosphoreum and P. carnosum on meat

While fresh poultry meat was solely packaged under oxygen-free atmosphere in the past, high O_2/CO_2 atmosphere packaging is also employed nowadays (Belcher, 2006; Eilert, 2005; Rossaint et al., 2015). Therefore, influence of the atmosphere on the growth of photobacteria is of great importance. While Devlieghere *et al.* state high susceptibility of *P. phosphoreum* (Devlieghere and Debevere, 2000), Dalgaard has reported high CO_2 tolerance on fish products compared to other Gram-negatives (Dalgaard, 1995). The CO_2 tolerance of Gram-negative bacteria is in many cases much lower than that of Gram-positives (Molin, 1983), however, tolerance of *P. phosphoreum* has been shown to be comparably high (Dalgaard, 1995). In accordance, growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 on chicken

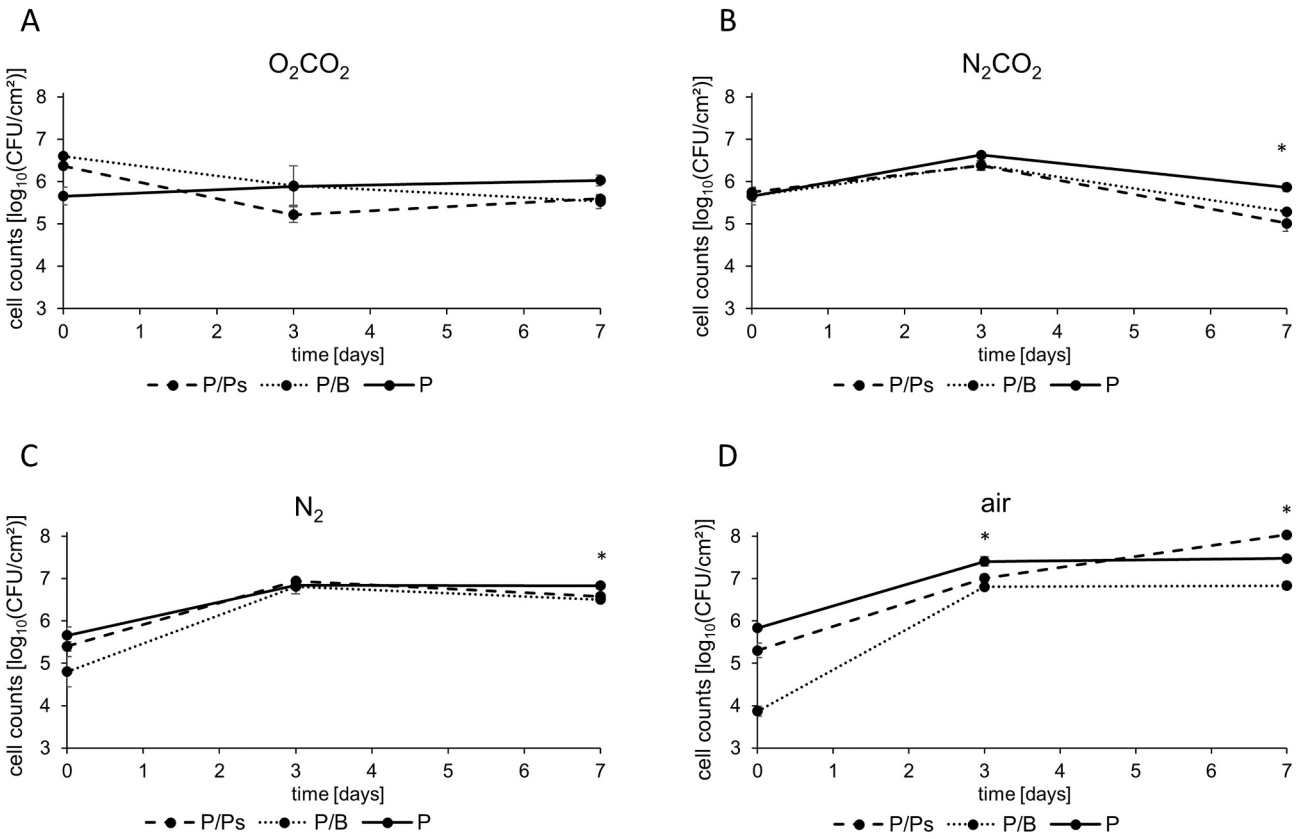

Fig. 4. Development of the *P. phosphoreum* TMW2.2103 cell counts when growing alone and in combination with *Ps. fragi* or *B. thermosphacta* under different atmospheres. Cultivation was started with *Photobacterium* and co-contaminant in 1:1 ratio. Significance was accepted with $p < 0.05$, $N = 3$. P/Ps – *P. phosphoreum* vs. *Ps. fragi*, P/B – *P. phosphoreum* vs. *B. thermosphacta*, P – *P. phosphoreum* alone. **A** 70%_O_2/30%_CO_2 atmosphere, **B** 70%_N_2/30%_CO_2, **C** 100%_N_2 atmosphere, **D** 21%_O_2/79%_N_2 atmosphere (air).

Table 1
Influence of the presence of *Ps. fragi* and *B. thermosphacta* on the cell numbers of *Photobacterium* at day 3. Species were mixed, inoculated on chicken meat and incubated in four atmospheres for 7 days. The effect of *Ps. fragi* and *B. thermosphacta* on *Photobacterium* was evaluated as log_{10}(CFU/cm^2) at day 3 in comparison to the CFUs of *P. phosphoreum* and *P. carnosum* alone. + = significantly improved growth with co-contaminant, − = significantly reduced growth with co-contaminant; +− = no significant change by co-contaminant; g = delayed growth (shifted maximum cell count), r = delayed reduction; $N = 3$, $p < 0.05$.

Species	Co-contaminant	Ratio (species:co-contaminant)	Effect on the cell number on day 3			
			O_2/CO_2	N_2/CO_2	N_2	air
P. phosphoreum	*Ps. fragi*	1:10	+−	−	+−^{r}	−^{g}
	Ps. fragi	1:1	−	+−	+−^{r}	+−^{g}
	Ps. fragi	10:1	−	−	−	+
	B. thermosphacta	1:10	+−	−	−	−^{g}
	B. thermosphacta	1:1	+−	+−	+−	+
	B. thermosphacta	10:1	−	−	−	+−
P. carnosum	*Ps. fragi*	1:10	+−^{g}	+	+	−
	Ps. fragi	1:1	+−^{g}	+-	+	−
	Ps. fragi	10:1	−^{g}	+−^{g}	+−	−
	B. thermosphacta	1:10	+	+	+−^{g}	−
	B. thermosphacta	1:1	−^{g}	+	−	−
	B. thermosphacta	10:1	+	+−^{g}	+−	−

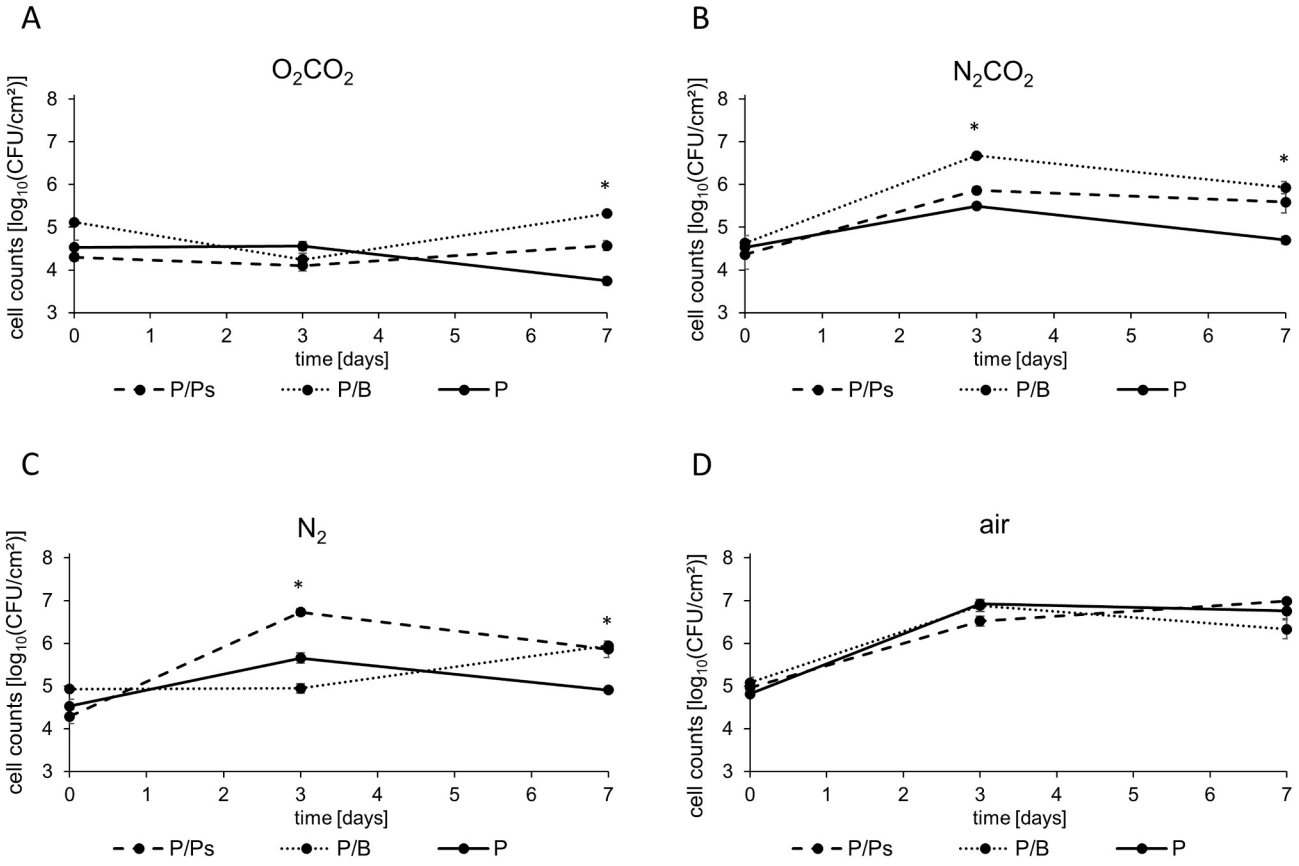

Fig. 5. Development of the *P. carnosum* TMW2.2149 cell counts when growing alone and in combination with *Ps. fragi* or *B. thermosphacta* under different atmospheres. Cultivation was started with *Photobacterium* and co-contaminant in 1:1 ratio. Significance was accepted with $p < 0.05$, $N = 3$. P/Ps – *P. carnosum* vs. *Ps. fragi*, P/B – *P. carnosum* vs. *B. thermosphacta*, P – *P. carnosum* alone. **A** 70%_O_2/30%_CO_2 atmosphere, **B** 70%_N_2/30%_CO_2, **C** 100%_N_2 atmosphere, **D** 21%_O_2/79%_N_2 atmosphere (air).

was only slightly reduced by 30% CO_2 in our study, suggesting to result in a growth advantage over other Gram-negative spoilers.

Both *Photobacterium* strains reached significantly higher cell counts in air than in anoxic atmosphere, which can be explained by higher energy conversion rates of aerobic respiration. However, high levels of O_2 resulted in significantly lower cell counts in an atmosphere with 70% O_2, which has been reported also in other studies (Guldager et al., 1998; López-Caballero et al., 2002). While the inhibitory effect of O_2 has been suggested to originate from its high reactivity and the formation of ROS and subsequently H_2O_2, the mode of action in high O_2 MAP is not yet confirmed (Chaix et al., 2015). Tolerance of photobacteria towards H_2O_2 was comparable or slightly lower to several LAB and much lower compared to *B. thermosphacta* (Supplementary Table S1). However, this can only partially explain the observed O_2 sensitivity, since LAB with H_2O_2 tolerance similar to *Photobacterium* were not sensitive to high O_2 concentrations (Kolbeck et al., 2019).

It is known that CO_2 can reduce the aerobic respiration rate and that the bacteriostatic effect of CO_2 sometimes depends on the presence of O_2 (Gill and Tan, 1980), suggesting a synergistic effect. Therefore, we tested growth in an atmosphere comprising 30% CO_2 + 21% O_2 + 49% N_2. Cell counts of both *Photobacterium* species in this atmosphere were reduced compared to the ones in air, but the effect was low compared to the significant effect of high O_2 concentration (Supplementary Fig. S2). This suggests a subordinate synergistic effect of CO_2 and O_2, which is only present under high-oxygen concentrations.

Still, *Photobacterium* frequently reach cell numbers high enough to be relevant for spoilage ($>10^7$ CFU/g) on naturally contaminated meat packaged under high O_2/CO_2 atmosphere (Hilgarth et al., 2018a). However, deliberate inoculation of meat appears to not fully represent the spoilage system on naturally-contaminated meat from retail. Indeed, chemical changes occurring in naturally contaminated meat and fish can differ significantly from the ones in deliberately contaminated products. In this context, especially differences in formation and depletion of organic acids (lactic acid, formic acid, acetic acid) and volatile compounds have been reported (Koutsoumanis and Nychas, 1999; Tsigarida and Nychas, 2001).

In this study, *P. phosphoreum* and *P. carnosum* strains grew well in absence of O_2 and presence of CO_2, demonstrating their importance also for vacuum and anoxically packaged meats. It is enabled by their versatile metabolic capabilities comprising aerobic and anaerobic respiration with alternative electron donors, as well as a fermentative lifestyle (Höll et al., 2019; Zhang et al., 2016).

4.2. Growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 in an in situ meat spoilage consortium

The overall relative abundance of photobacteria within the spoilage consortium remained constant over 7 days, even though the proportion of other species varied (e.g. *Pseudomonas* spp. and *B. thermosphacta*). This is in accordance with the previously proven importance of *Photobacterium* to meat spoilage. As mentioned in Section 4.1, the spoilage system in our experiments appears to be slightly different from the natural consortium. This could explain why we did not observe dominance of *Photobacterium* in our experiments, while these organisms are found to often dominate the spoilage microbiome in retail packages of different meat types.

4.3. Effect of the presence of Ps. fragi/B. thermosphacta as co-contaminant on the growth of P. phosphoreum and P. carnosum

Pseudomonas fragi and *B. thermosphacta* constituted the largest part of the whole inoculated microbiome during storage and were consequently selected for detailed investigation of the interaction with *Photobacterium*.

Presence of either *Ps. fragi* or *B. thermosphacta* resulted in enhanced growth of *P. carnosum* TMW2.2149 but impaired growth of *P. phosphoreum* TMW2.2103 in MAP in almost all cases (Table 1). This suggests a general growth advantage for *P. carnosum* in presence of other meat spoilers and a disadvantage for *P. phosphoreum*. Indeed, this supports the hypothesis of a different origin and relation to the meat spoilage microbiota of *P. phosphoreum* and *P. carnosum*. While the interaction of *P. phosphoreum* to *Ps. fragi* or *B. thermosphacta* appears competitive on meat, the interaction of *P. carnosum* appears rather commensal as suggested for a natural member of the spoilage microbiota. The spoilage community of packaged meat from retail is often dominated by either *P. phosphoreum* or *P. carnosum*. (Fuertes-Perez et al., 2019; Hilgarth et al., 2018a). Our data suggest that this might result from presence and effect of co-contaminants as for example *Ps. fragi* and/or *B. thermosphacta*.

Enhanced adaptation of *P. carnosum* to the growth in meat spoilage systems and less adaptation of *P. phosphoreum* has been previously suggested (Fuertes-Perez et al., 2019; Hauschild et al., 2020). Improved growth of meat spoilers in presence of additional species has already been shown e.g. for *B. thermosphacta* and *Ps. fragi* (Papadopoulou et al., 2020; Russo et al., 2006) and a synergistic spoilage effect has been proposed (Höll et al., 2019). However, the effect can be highly species specific (Russo et al., 2006). Based on the present data, the species-specific interaction is independent of the presence of high levels of O_2 and also of the presence of CO_2. The reduced growth of *P. phosphoreum* TMW2.2103 in presence of a co-contaminant is suggested to result from its less versatile substrate utilization compared to *P. carnosum* (Fuertes-Perez et al., 2019) rather than from strong inhibitory influence of e.g. bacteriocins (cf. (Schillinger et al., 1991; Hilgarth et al., 2018b)).

Interestingly, the observations discussed above were reversed in air atmosphere. Growth of *P. carnosum* TMW2.2149 was not influenced or impaired by the presence of a co-contaminant but growth of *P. phosphoreum* TMW2.2103 was. This atmosphere provides optimal growth conditions for all four species, however, *P. carnosum* grows much slower than *Ps. fragi* and *B. thermosphacta*. The resulting overgrowth by the co-contaminant is supposed to limit *P. carnosum* in this atmosphere. *P. phosphoreum* grows faster than *P. carnosum*, thus being able to grow along with the co-contaminants. Improved growth of *P. phosphoreum* TMW2.2103 in this case could result from extracellular enzymes secreted by the co-contaminant that facilitate substrate degradation (e.g. lipases) or from pH-elevation (especially by *Ps. fragi*), which improves growth conditions for *P. phosphoreum*. Inoculation of meat with photobacteria and co-contaminants in over- and under-represented ratio defined the relative abundance of the organisms throughout storage, which is in accordance with literature reporting an influence of the initial cell number on the dominance during spoilage (Gram et al., 2002; Russo et al., 2006). However, the different ratios did not change the general type of the interaction.

In conclusion, growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 on fresh meat is significantly and strongly influenced by the applied packaging atmosphere. While combination of high O_2 and high CO_2 content results in the strongest growth reduction of both species, CO_2 alone has a minor effect.

However, no atmosphere caused a full inhibition of photobacterial growth, emphasizing their metabolic versatility and the challenge to reduce their contamination level. In addition, co-contaminating *Ps. fragi/B. thermosphacta* can result in a mild but significant impact on the growth of *Photobacterium*. Its impact is suggested to be positive (commensalistic) for *P. carnosum* TMW2.2149 and neutral or negative (competitive) for *P. phosphoreum* TMW2.2103.

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CRediT authorship contribution statement

Hauschild, Philippa: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization.

Vogel, Rudi: writing (review and editing), project administration, funding acquisition.

Hilgarth, Maik: conceptualization, writing (review and editing), supervision, project administration, funding acquisition.

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Declaration of competing interest

The authors declare no conflict of interest.

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5.4 Transcriptomic analysis of the response of *Photobacterium phosphoreum* and *Photobacterium carnosum* to co-contaminants on chicken meat (submitted manuscript)

The impact of presence of *Ps. fragi*/*B. thermosphacta* as co-contaminants on the metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 was studied on the level of transcription. For this purpose, photobacteria were cultivated in presence and absence of the co-contaminant on chicken meat packaged under 70% O_2/30% CO_2 modified atmosphere or air atmosphere. Cultivation was followed by RNA isolation and sequencing and resulting sequences were evaluated regarding significant regulation. In modified atmosphere, presence of either co-contaminant was predicted to trigger enhanced substrate competition for *P. phosphoreum* TMW2.2103 resulting from the focus on fermentative metabolism. This was demonstrated by the downregulation of respiratory enzymes and the upregulation of fermentative enzymes. It is in accordance with the growth reduction demonstrated by our previous study for this strain under these conditions. *P. phosphoreum* TMW2.2103 is therefore suggested to recognize co-contaminants as additional stressor under MAP. In contrast, presence of either co-contaminant is predicted to enhance the substrate exploitation by *P. phosphoreum* TMW2.2103 under air atmosphere. Enhanced lipolytic and proteolytic activity of the co-contaminants is assumed to improve the availability of extracellular fatty acids and amino acids in this context. Observations are in accordance with the improved growth of this strain under these conditions as demonstrated in our previous work. Despite of the assumed marine provenance of *P. phosphoreum*, observations depict the ability for successful cooperation within the meat spoilage community. Growth improvement of *P. carnosum* TMW2.2149 in presence of co-contaminants and MAP, as shown in our previous study, could be linked to the upregulation of several pathways by this strain. It suggests that *P. carnosum* TMW2.2149 recognizes presence of co-contaminants as beneficial under MAP and that it enhances its metabolic activity in accordance. Under air atmosphere, growth reduction of *P. carnosum* TMW2.2149 in presence of co-contaminants has been demonstrated by our previous study. Absence of relevant regulation by the strain suggests inability to cope with this situation and predicts that *P. carnosum* TMW2.2149 is outcompeted by the co-contaminants due to their higher growth rate. This study therefore suggests different strategies of *P. phosphoreum* and *P. carnosum* to succesfully integrate into the meat spoilage consortium. The competitiveness of either species thereby depends of the type of packaging atmosphere and presence of co-contaminants in combination. Both factors are assumed to be relevant contributors to the prevalence of either *P. phosphoreum* or *P. carnosum* on spoiling meat. This fits the previous observation that either *P. carnosum* or *P. phosphoreum* is found on spoiled meats.

Author contributions: Philippa Frederieke Hauschild performed all experiments and evaluated and visualized the resulting data. Additionally, she wrote the original draft of the manuscript and realized corrections that arose during the reviewing process.

Maik Hilgarth supervised the work of Philippa Frederieke Hauschild and helped to realize corrections that arose during the reviewing process. Rudi F. Vogel initiated the corresponding project, supervised the work of Philippa Frederieke Hauschild and reviewed this manuscript.

Supplementary files and figures of this publication are provided in appendix section 12.4.

Transcriptomic analysis of the response of *Photobacterium phosphoreum* and *Photobacterium carnosum* to co-contaminants on chicken meat

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Abstract

Background: *Photobacterium* (*P.*) *phosphoreum* and *P. carnosum* are persistent members of the spoilage community on meats and their growth is influenced by the presence or absence of co-contaminants. This study investigated the impact of the presence of *Brochothrix (B.) thermosphacta* and *Pseudomonas (Ps.) fragi* on the transcriptomes of *P. phosphoreum* and *P. carnosum* on chicken meat stored under modified atmosphere (70% O_2/30% CO_2) as well as under air atmosphere (21% O_2/79% N_2).

Results: *P. phosphoreum* TMW2.2103 responded to modified atmosphere with a reduced number of transcripts related to cell division and an enhanced number of transcripts related to oxidative stress response. Concomitantly, analysis revealed upregulation of fermentative routes and downregulation of respiratory routes. Therefore, enhanced substrate competition in presence of co-contaminants and modified atmosphere is predicted for this strain. In contrast, the strain upregulated respiratory routes in air atmosphere and is predicted to benefit from improved accessibility of free fatty acids/amino acids due to the metabolism of the co-contaminants. Observations are in agreement with the growth reduction of *P. phosphoreum* TMW2.2103 in presence of modified atmosphere/co-contaminant and the growth improvement in presence of air/co-contaminant demonstrated in our previous study. For *P. carnosum* TMW2.2149 analysis revealed downregulation of the respiration and upregulation of the pyruvate metabolism under modified atmosphere. Presence of modified atmosphere/co-contaminant resulted in an increased number of transcripts of multiple metabolic routes, e.g. pentose-, or amino acid metabolism. This is in accordance with the enhanced growth demonstrated in our previous work. *P. carnosum* TMW2.2149 regulated the expression of only few minor genes in presence of air/co-contaminant, which is congruent with the inability of this strain to cope with the fast growth of *B. thermosphacta* and *Ps. fragi* in this situation.

Conclusions: Observed differential regulation of the gene expression revealed different strategies of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 to react to co-contaminants under modified atmosphere. This suggests different adaptation of both species

to the meat environment as demonstrated in our previous work on adaptation to hydrostatic pressure and NaCl.

Keywords: *Photobacterium, Pseudomonas fragi, Brochothrix thermosphacta*, interaction, modified atmosphere packaging, meat spoilage, transcriptomics

Background

Growth of photobacteria has been associated with spoilage on several types of meat, fish and seafood (e.g. (1-4)). The ability of *P. phosphoreum* and *P. carnosum* to thereby grow also on modified atmosphere packaged (MAP) products (4, 5) increases their relevance in the field of food loss and waste. MAP has been developed in the meat industry to prevent fast growth especially of aerobic Gram-negative bacteria on meat, such as pseudomonads (6, 7) that would otherwise cause rapid spoilage. Common modified atmospheres combine O_2, CO_2 and N_2 in different mixtures, with combinations of high levels of O_2 (70-80%) and high levels of CO_2 (20-30%) representing the most common ones (8-10). Both gases, O_2 and CO_2, hold the potential to affect the bacterial metabolism. While increased levels of O_2 may generate oxidative stress, it may also improve the activity of metabolic pathways that require this gas for functionality (11, 12). Increased levels of CO_2 have been reported to inhibit bacterial growth, supposedly by reducing membrane functionality, altering the cellular pH and influencing (decarboxylating) enzymes (13, 14). The resulting stress situation in presence of both gases on packaged meat comes together with the necessity for spoilage bacteria to assert within the spoilage community. Interaction can thereby either enhance the stress situation, for example by nutrient competition, or it can improve the growth conditions, for example by cooperative substrate exploitation. In this context, *Ps. fragi* and *B. thermosphacta* are both known to interact with other bacterial spoilers and their metabolism in modified atmosphere has been characterized in transcriptomic- and proteomic studies (15, 16). *Ps. fragi* has been described as dominant competitor in iron exploitation on fish, due to its ability to produce highly efficient siderophores (17, 18). Additionally, the comparable fast glucose utilization of *Pseudomonas*

results in decisive competitive advantages (17, 19). However, its metabolic activity can also result in enhanced growth of co-contaminants by facilitated substrate disposability, especially related to protein exploitation (20, 21). *B. thermosphacta* can impair the growth conditions for competitive species e.g. by production of acetic acid (22).

Very little is known about the interaction of photobacteria with other species and available reports mostly have a descriptive character. Presence of different lactic acid bacteria for example has been observed to reduce growth of photobacteria on salmon (e.g. (23, 24)). Information on the underlying mechanisms of interaction is limited to a metatranscriptomic study, which has provided first insight in different metabolic lifestyles of meat spoiling microbiota members (25). This study predicted a very limited response of photobacteria to different atmospheres. However, it could not differentiate between different species of photobacteria, namely as *P. carnosum* had not been described by then (26). Also, while *B. thermosphacta* and other Gram-positive spoilers were present, *Pseudomonas* spp. were merely absent in those samples.

The present study therefore investigated for the first time details of the response of different *Photobacterium* species to common meat spoiling species, based on transcriptomic data. Combined application of MAP with 70% O_2/30% CO_2 and presence of an additional Gram-positive (*B. thermosphacta*) or Gram-negative (*Ps. fragi*) meat spoiling bacterium as co-contaminant has been observed to result in growth reduction of *P. phosphoreum* but growth improvement of *P. carnosum* strains before. The opposite effect has been detected for air atmosphere with 21% O_2/79% N_2 (5). These observations were based only on cell counts, while mechanistic insight into adaptation strategies and gene regulation could not be obtained. In the present study, the effect of combined modified atmosphere and presence of *Ps. fragi*/*B. thermosphacta* as co-contaminant on the transcription of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 was investigated on chicken meat. Thereby, experimental setup was comparable to the one described by Hauschild *et al*. (5), to enable direct comparison of observed growth effects and effects on the transcription.

Materials and Methods

Microorganisms and cultivation

Analysis was performed with *Photobacterium* strains *P. phosphoreum* TMW2.2103 (Accession number WMCZ01) and *P. carnosum* TMW2.2149 (Accession number WMDL01) (27). *Ps. fragi* TMW2.2082 (Accession number JAAEBR000000000) and *B. thermosphacta* TMW2.2101 (Accession number RSDU000000001) from the TMW strain collection were employed as co-contaminants. *Photobacterium* strains were cultivated in liquid medium containing 20 g/l meat extract and 20 g/l NaCl and other species in brain heart infusion medium (BHI; Roth, Karlsruhe, Germany). Cultures were inoculated from frozen glycerol-stocks and incubated aerobically at 15 °C. For preparation of agar plates, 16 g/l agar-agar was added to the medium. Selectivity towards photobacteria was achieved by the addition of 7 mg/l (w/v) vancomycin.

Inoculation of meat

The general procedure followed the protocol of Hauschild *et al*. (5). In brief, fresh skinless chicken breast was obtained from a local supermarket and inoculated with bacterial suspension. Thereby, *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 were inoculated together with *Ps. fragi* TMW2.2082 or *B. thermosphacta* TMW2.2101, respectively, and incubated in two defined atmospheres (70% O_2/30% CO_2 referred to as 'modified atmosphere' and 21% O_2/79% N_2 referred to as 'air atmosphere'). Photobacteria were additionally inoculated alone and incubated in both atmospheres as a control. With this, the experimental setup comprised 4 experimental groups and 2 control groups per *Photobacterium* species. *Photobacterium* cultures were incubated for 72 h before inoculation and *Ps. fragi* and *B. thermosphacta* cultures for 24 h. Inoculation of the chicken meat was done from both sides based on the OD at 600 nm of respective bacterial cultures and in accordance to the meat surface area as described in Hauschild *et al.* (5). OD_{600} and surface area were utilized to adjust the initial colony forming units (CFU) per cm^2 to 6 log_{10} to enable interaction. Biological triplicates were performed with chicken breast fillets from one package, respectively. Each

inoculated chicken breast was placed in a polypropylene tray with ethylene vinylalcohol polymer coating and a volumetric permeation rate of 0.25 cc.20µ m^{-2}.day.atm (ES-Plastic, Hutthurn, Germany). Trays were then packaged with a semi-automatic tray sealer (Rotarius VG, Variovac PS SystemPack GmbH, Zarrentin am Schaalsee, Germany). After packaging, meat was stored for 3 days at 4 °C until sampling.

RNA isolation

Bacterial RNA was isolated from the incubated meat based on the provided protocol of the Qiagen RNeasy Mini Kit (Qiagen N.V. Hilden, Germany) and the work of Höll *et al* (25). Chicken breast was washed twice with in total 5 ml Invitrogen RNAlater solution (ThermoFisher Scientific, Waltham, MA, USA) per sample. Liquid was collected and centrifuged at 9000x g for 10 minutes. Afterwards, supernatant was removed, cell pellets were mixed with 50 mg/ml lysozyme (24,000 kU/mL, SERVA Electrophoresis GmbH, Heidelberg, Germany) and 20 µl proteinase K solution (20 mg/ml, ThermoFisher Scientific, Waltham, MA, USA) and incubated at 37 °C for 1 h. The following steps were performed as stated in the Qiagen RNeasy Mini Kit protocol with some modifications. Lysis buffer was mixed with 1 % β-mercaptoethanol and added. Cell disruption was improved by an additional homogenization step: for this, samples were mixed with small-size glass beads and shaken 5 times for 1 min at 4 m/s in a high-speed benchtop homogenizer (MP FastPrep-24, Thermo Fisher Scientific). After each homogenization cycle, the samples were kept on ice for 1 min and finally centrifuged for 3 min at maximum speed. The supernatant was then transferred to fresh tubes and handled as specified by the protocol. DNase digestion was done following the instructions for on-column DNAse digestion and the RNAse free DNase set of Qiagen (Qiagen N.V. Hilden, Germany). The final elution step was repeated by adding the former eluate a second time to the column. Eluted products were kept carefully on ice. Quality of the obtained RNA was measured with a NanoDrop spectrophotometer (NanoDrop 1000 3.6.0 PeQLab Biotechnologie GmbH, Erlangen, Germany) and RNAse free water as blank measurement. The RNA concentration of

147 the samples was up to 3593 ng/µl and was evaluated as sufficient for sequencing by Eurofins
148 Genomics GmbH (Konstanz, Germany). The RNA content of selected samples was
149 additionally confirmed by agarose gelelectrophoresis compared to a positive control.
150 The 260nm/280nm values of the samples were 2.0-2.27 and the 260nm/230nm values
151 were 1.36-2.43.

152

153 **Sequencing and bioinformatic analyses**

154 Sequencing was performed by Eurofins Genomics GmbH (Konstanz, Germany) with Illumina
155 HiSeq2500 method. Raw read counts for the analysis were obtained with featureCounts (28)
156 counting only reads that were overlapping 'CDS' features that had unique mapping positions
157 and that showed a mapping quality score of ≥10. Paired-end reads were included only once
158 and in case of reads with multiple mapping results, only the feature with the highest number of
159 matching bases was accepted. Normalization of the read counts followed the Trimmed Mean
160 of M-values method. The sequenced reads were mapped to the reference genomes of the
161 strains (accession number WMCZ01 and WMDL01). Mapping was done with BWA-MEM (29).
162 The sequencing output is provided in additional table 1.

163 The presented analysis of the transcription was done based on the provided values for
164 $\log_2$(counts per million) (logCPM, expression level) and $\log_2$(fold change) (logFC, regulation).
165 Regulation with logFC ≥ 2 or ≤ -2 and $p < 0.05$ was regarded as significant and included in the
166 analysis (additional figure 1, 2). Presence or absence of genes in the genomes of the strains
167 was analyzed using Basic Local Alignment Search Tool (BLAST, (30)).

168

169 **Data interpretation**

170 Regulation in response to MAP and presence of a co-contaminant was evaluated as
171 comparison of the level of transcription of the experimental group compared to a control group.
172 In absence of a co-contaminant, comparison of MAP samples *vs*. air samples revealed the

effect of the atmosphere. In presence of a co-contaminant, comparison of the samples with co-contaminant *vs.* the samples without co-contaminant revealed the effect of the co-contamination in the respective atmosphere.

Results and discussion

Transcription of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 was investigated in presence or absence of *B. thermosphacta*/*Ps. fragi* on chicken breast filet packaged under air (21% O_2/79% N_2) or MAP (70% O_2/30% CO_2). Additionally, general expression of central metabolic pathways and the respective genomic settings of the strains was evaluated independently of differential regulation.

Data interpretation followed the correlation of the physiological and transcriptional data as presented in table 1, to enable correlation of observations on the transcriptional level with the effects on the cell counts published within the scope of our previous study (5). Detection of differential transcript numbers in combination with reduced growth were interpreted to imply that the strain cannot cope with the given stress situation. In contrast, differential transcript numbers combined with unchanged growth performance were interpreted to imply that the strain copes successfully with the given stress situation. Combination of differential transcript numbers and enhanced growth was interpreted as a benefit of a strain from the given situation (table 1). Absence of differential transcript numbers in combination with reduced growth was interpreted as inability of the organism to cope with or adapt to the respective stress. If absence of differential transcript numbers came together with unchanged growth performance, it was assumed that the strain was not affected (table 1).

At the time point of RNA isolation, cell counts of *P. phosphoreum* TMW2.2103 were at 7.14±0.32 $\log_{10}$(CFU/cm^2) in air atmosphere and 6.41±0.14 $\log_{10}$(CFU/cm^2) in modified atmosphere. Cell counts of *P. carnosum* TMW2.2149 were at 6.1±0.11 $\log_{10}$(CFU/cm^2) in air atmosphere and 6.17±0.12 $\log_{10}$(CFU/cm^2) in modified atmosphere. *Ps. fragi* TMW2.2082 reached 7.23±0.22 $\log_{10}$(CFU/cm^2) in air atmosphere and 6.3±0.21 $\log_{10}$(CFU/cm^2) in modified

atmosphere. *B. thermosphacta* TMW2.2101 cell counts reached 7.66±0.17 $log_{10}(CFU/cm^2)$ in air atmosphere and 7.26±0.2 $log_{10}(CFU/cm^2)$ in modified atmosphere.

Genomic setting of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 for metabolizing substrates on chicken meat

Analysis of the genomes of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 revealed presence of all genes of the Embden-Meyerhoff-Parnas- and pentose phosphate pathway for carbohydrate utilization, as well as all necessary genes for gluconeogenesis (additional figure 3_1). Predicted pathways of the strains are thereby in agreement with the metabolic routes that have been predicted by Höll *et al.* as general for photobacteria from naturally contaminated chicken samples (25) and also by Fuertes *et al.* (31). Of the Entner-Doudoroff pathway, the key enzyme 2-dehydro-3-deoxyphosphogluconate aldolase was present. However, both genomes lacked phosphogluconate dehydratase, which performs the initial conversion of 6-phosphogluconate to 2-dehydro-3-deoxyphosphogluconate, thus cutting the pathway off from the oxidative part of the pentose phosphate route (additional figure 3_1). This is in accordance with the observations of Hilgarth (32). The pathway is therefore assumed to be a remnant with reduced functionality here, while it is present in some other strains of meat-spoiling photobacteria (31).

Several phosphotransferase systems for generation of phosphorylated substrates could be detected, including those for glucose, fructose, mannose and maltose. Except of the latter, these sugars have been described as available substrates on meat before (33-35). Both genomes harbor genes to convert pyruvate into lactate, acetate, ethanol, CO_2, formate and malate, thus enabling fermentation of the mixed acid type (36). Additionally, all enzymes for production of acetoin, which can be oxidized to diacetyl, were detected, whereas enzymes for production of butane-2,3-diol were not. Glycerol utilization was facilitated by glycerol dehydrogenase. Genes encoding pyruvate oxidase and glycerol oxidase were absent in the genomes. Both of these produce hydrogen peroxide and thereby oxidative stress (37, 38), and

their absence indicates that these bacteria rather would avoid oxidative stress then exploit these pathways for additional energy gain from respective substrates.

Both genomes harbored all enzymes of the tricarboxylic acid cycle and glyoxylate cycle (additional figure 3_2). Lipid exploitation ability was detected in form of genes for extracellular lipases, for complete β-oxidation mechanism and for glycerol metabolism. The genes for glycerol degradation included aerobic and anaerobic enzymes (additional figure 3_3), whereas the anaerobic fatty acid degradation pathway was incomplete and lacked known homologs to the long chain fatty acid CoA ligase FadK (compare (39)). Analysis revealed presence also of the arginine deiminase system and several genes for production of biogenic amines (additional figure 3_4). Of those, only *P. phosphoreum* TMW2.2103 contained the genes encoding lysine decarboxylase and tyrosine decarboxylase. None of the genomes contained known homologs to genes for histidine decarboxylase (additional figure 3_5), which is in accordance with the literature (25, 40). Presence of a new histamine producing enzyme with similar activity to pyridoxal-5-phosphate dependent histidine decarboxylase in *P. phosphoreum* strains has been reported recently (41). However, *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 genomes lacked also this new gene sequence, whereas other strains of both species have been demonstrated to harbor it (31). This explains reports on the formation of histamine by photobacteria, despite the absence of known homologs to histamine decarboxylase genes (41, 42).

The genomic set for enzymes of the respiratory chain was complete in both strains with minor differences regarding presence and completeness of certain enzymatic complexes. The NADH dehydrogenase operon of respiratory complex I was found to be NuoA-N in *P. phosphoreum* TMW2.2103 and NqrBDEFM in *P. carnosum* TMW2.2149 (additional figure 3_6, 3_7). Thereby, both strains also contained an incomplete version of the other operon, respectively. Both operons have been shown to enable electron transfer, with several genes of the Nqr operon being not necessary for functionality (43, 44). If both operons are present, they can compensate each other as reported for *Shewanella*, however, NuoA-N has been suggested to work more efficiently than NqrA-M (45). The Nqr-NADH dehydrogenase complex couples

electron transport to Na^+ translocation (46) and can be thereby highly dependent of the availability of Na^+ (47, 48). Literature states its presence in many marine organisms (43), including e.g. *Vibrio cholerae* (44), which is in accordance with the marine background of *P. phosphoreum* and presence of remnants of a marine lifestyle that has been suggested for *P. phosphoreum* and *P. carnosum* (49).

Both strains were found to have a complete set of cytochrome C oxidase CydABX, CyoABCDE and an incomplete set of CoxABCD. *P. phosphoreum* additionally had genes of a cbb3-type cytochrome-c oxidase (CcoOPQN) (additional figure 3_6). A heme biosynthesis pathway was present in both strains. Besides of this, *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 also had genes for anaerobic respiration. Genes for utilization of fumarate, trimethylamine-N-oxide (TMAO), nitrate and sulfate as electron acceptor were detected in this context, which is in accordance with the literature (31). *P. phosphoreum* TMW2.2103 also had genes for dimethylsulfoxide (DMSO) reduction, but the operon lacked DMSO reductase dmsA. However, even if pathways for utilization of TMAO, nitrate, sulfate and DMSO were present, consequences for the growth on meat will be limited, since meat contains low amounts of these substrates as compared to fish (50-54). Presence of the pathways nevertheless supports the hypothesis of a marine background for *P. phosphoreum* and presence of remnants of a (former) marine lifestyle for *P. carnosum* (27, 49), since marine habitats are characterized by high availability of TMAO (55, 56), nitrate (57), sulfate (58), DMSO (59).

General gene expression of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149

Regarding gene expression, transcription of the genes for key enzymes of all described metabolic pathways was observed in all analyzed samples, which is in agreement with the observations of Höll *et al*. (25). With this, *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 are characterized by a broad set of metabolic pathways that are predicted to be active during varying situations. Furthermore, transcription also of genes for the production of biogenic amines, e.g. putrescine or γ-aminobutyric acid, confirms the ability of both species to

contribute to health issues during growth on MAP meat. From the total list of detected transcripts, the 100 records with highest expression levels (logCPM) were extracted for each strain. Thereby, average values were calculated for all samples of each strain, respectively. The 100 highest expression levels ranged between 9.994 - 9.192 logCPM for *P. phosphoreum* TMW2.2013 and 9.996 - 9.053 logCPM for *P. carnosum* TMW2.2149 and included key genes of pathways of the central metabolism (additional table 2). Extracted transcripts included the key enzyme of glycolysis, 6-phosphofructokinase PfkA, as well as the key enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase PckA, and the key enzyme for entering the pentose phosphate pathway, glucose-6-phosphate dehydrogenase. Differences between the strains comprised certain enzymes of the TCA cycle, the respiratory chain and the amino acid metabolism (additional table 2).

General regulation in response to presence of co-contaminants and packaging conditions

In general, only few enzymes and even less entire metabolic pathways were found to be regulated on the level of transcription for both *Photobacterium* strains and in all tested situations. The number of differentially regulated genes with logFC≥2/≤-2 (logFoldChange) and $p<0.05$ ranged from 26-90 for *P. phosphoreum* TMW2.2103 and from 18-71 for *P. carnosum* TMW2.2149 (table 2, additional table 3 and 4). This is in accordance with Höll *et al*. (25), who have also reported limited regulation on the level of the transcriptome for photobacteria from a natural spoilage consortium on MAP chicken meat. It can be assumed that photobacteria from meat have a limited set of genes that are regulated on the transcriptomic level. Instead, they are supposed to have a broad set of metabolic enzymes available on stand-by and ready to be employed if the substrate is available. Multidimensional plotting of the samples by their logFC revealed separate clustering especially of samples from air atmosphere and modified atmosphere for both *Photobacterium* species (additional figure 4). Triplicates clustered in most cases together.

Examination predicted differences in the transcription of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 in response to packaging atmosphere and presence of *B. thermosphacta*/*Ps. fragi* as co-contaminant. In accordance, growth of photobacteria on chicken meat has been shown to be influenced by both factors (5). Observed regulation differed in dependence of the tested co-contaminating species, whereas the effects on the cell counts, as described by Hauschild *et al*. (5), have been demonstrated to be independent of the species of the co-contaminant. Presence of *B. thermosphacta* e.g. resulted in most cases in higher transcript numbers of aerobic and anaerobic glycerol-3-phosphate utilization (glycerol-3-phosphate dehydrogenase, glycerophosphodiester phosphodiesterase, sn-glycerol-3-phosphate transporter, figure 1, table 3). This points either at an elevated availability of glycerol as substrate by the activity of *B. thermosphacta* or at an elevated competition. In this context, *B. thermosphacta* has been reported to upregulate glycerol exploitation in presence of O_2 (60), as well as to release lipases with increased activity on glycerol esters (15, 61, 62). Beside of this, presence of *B. thermosphacta* resulted in both atmospheres in downregulation of the key enzyme for gluconeogenesis, glyceraldehyde-3-phosphate dehydrogenase, by *P. phosphoreum* TMW2.2103 (additional table 3). Additionally, transfer of pyruvate and acetyl-CoA into leucine biosynthesis by 2-isopropylmalate synthase LeuA was predicted to be downregulated (compare pathways in additional figure 3_4). This regulation suggests saving of resources for energy generation via pyruvate metabolism in *P. phosphoreum* TMW2.2103, thus indicating substrate competition as a bottle neck.

General regulation of both *Photobacterium* strains affected the anaerobic respiration based on nitrate, TMAO, sulfate or DMSO under several conditions. This was detected e.g. for nitrate reductase enzymes NapA/NapF of *P. phosphoreum* TMW2.2103 with *Ps. fragi*/*B. thermosphacta* as competitor in air atmosphere, sulfite reductase enzyme CysI of *P. phosphoreum* TMW2.2103 with *Ps. fragi* in air atmosphere and TMAO reductase enzyme TorC of *P. phosphoreum* TMW2.2103 in absence of co-contamination in modified atmosphere (figure 2). However, this is predicted to have limited impact in the meat system due to the aforementioned low availability of these substrates on meat. Upregulation of enzymes

participating in nitrate reduction, as observed e.g. for *P. phosphoreum* TMW2.2103 in presence of co-contamination/air atmosphere (figure 2) is nevertheless in accordance with the demonstrated activity of nitrate reductase Nap independently of the availability of nitrate (63). Nap has been demonstrated to be active especially with low levels of nitrate (64) and to be present also during oxic conditions (65). The predicted activity of this enzyme is consequently in accordance with reports from the literature. It can be speculated that upregulation of Nap constitutes a preparation of the cells to shift from aerobic respiration to anaerobic respiration.

Downregulation of cytochrome C and cytochrome C oxidase subunit CoxB was detected for *P. carnosum* TMW2.2149 in presence of *B. thermosphacta*/MAP and of cytochrome C oxidase subunit CyoB in presence of *Ps. fragi*/MAP. For *P. phosphoreum* TMW2.2103, cytochrome C oxidase subunit CyoC was downregulated in presence of *Ps. fragi*/air atmosphere (figure 2). Downregulation of enzymes of the aerobic respiration will have distinct consequences on growth and metabolic activity. Reduced transcript numbers of single genes in respiratory clusters are thereby assumed to reduce the overall efficiency of aerobic respiration. In contrast to this, upregulation of the respiratory chain was observed for both photobacteria in presence of *Ps. fragi*/MAP (cytochrome c oxidase enzymes CoxC or CyoE, figure 2). Both *Photobacterium* strains harbor several clusters of cytochrome c oxidase and NADH dehydrogenase that are characterized by different functionality under specific environmental conditions, as explained before. The observed regulation predicts an adapted reaction in response to environmental parameters regarding activity of the respiratory chain, as it has been reported for other meat spoiling bacteria before (e.g. for *B. thermosphacta* (66)). However, predictions cannot elucidate the actual separate physiological impact of each of the packaging gases on respiration. Effects can result either from the impact of O_2 that is correlated with oxidative stress, or from the reaction to elevated CO_2, which is assumed to be correlated with several implications for the metabolism. Further studies have to be conducted to complete the data obtained in this study and to finally explain the respective impact of the gases in detail.

Regulation of the methionine metabolism was detected for *P. phosphoreum* TMW2.2103 in the presence of *B. thermosphacta*, pinpointing to an increased methionine pool (from putative

proteolysis) or else a methionine requirement. Methionine represents an essential amino acid that is required for protein biosynthesis and plays a role in protection of other amino acids from oxidation (67). The transcript numbers of enzymes that use methionine as substrate were reduced (Yjjw, Cfa), while they were increased for enzymes that provide methionine as substrate (e.g. import protein MetN, peptide-methionine oxide reductase MsrB, additional table 3). Of those, activation of *mrs*B has been described to signal acute stress before (67), suggesting presence rather of a specific methionine requirement here. Reduced availability of methionine in the environment is supposed to trigger strong response in *Photobacterium*, since strains especially of *P. phosphoreum* are known for their requirement for external methionine for growth (36, 68, 69). Therefore, competition for available methionine in the presence of *B. thermosphacta* is more likely than its enhanced availability. However, the reason for that requirement remains hitherto unknown. It has been reported that methionine synthase can be dependent of tetrahydofolate in its polyglutamate form (70, 71). The two strains investigated here possess all necessary steps for tetrahydrofolate generation in their genomes, however, they lack the gene for folylpolyglutamate synthetase that is necessary for formation of the poly-glutamate form. Consequently, this is suggested to result in requirement for external methionine.

Regulation of *P. phosphoreum* TMW2.2103 in presence of *Ps. fragi*/*B. thermosphacta* as co-contaminant under modified atmosphere

Analysis of the transcripts of *P. phosphoreum* TMW2.2103 during cultivation under MAP conditions predicts reduced activity of the respiratory chain and, accordingly, of the TCA cycle (figure 3). The comparison of the transcriptome of the strain under MAP conditions and air atmosphere showed a reduced number of transcripts of cytochrome C oxidase CoxC and a cytochrome C oxidase assembly protein in MAP (figure 2). This was concomitant with the reduced number of transcripts of a key enzyme of aerobic β-oxidation (long-chain fatty acid-CoA ligase FadD), which provides acetyl-CoA as substrate for the TCA. Observations are

interpreted to result from the increased concentration of ROS and therefore oxidative stress under 70% O_2/30% CO_2 atmosphere. Metabolic activity is predicted to be shifted to fermentation instead (figure 3), as shown by the upregulation of alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase subunit A under these conditions (additional table 3, figure 1). Fermentation-based metabolism as predicted for MAP is correlated with higher substrate competition (6, 72) due to redox balancing and lower energy output compared to respiration. Therefore, MAP conditions are assumed to result in enhanced substrate competition leading to growth reduction in presence of co-contaminants, as observed for *P. phosphoreum* TMW2.2103 in our previous study on that interaction (5). Limited substrate availability is assumed to lead to reduced intracellular oxidative stress in reverse. This can explain the absence of regulation targeting oxidative stress during the cultivation of *P. phosphoreum* TMW2.2103 in presence of a co-contaminant/MAP. Additionally, reduced intracellular oxidative stress and resulting absence of pressure to cope with it is assumed to account for the observed increased number of transcripts of aerobic β-oxidation in this strain in presence of *B. thermosphacta*/*Ps. fragi* and of the cytochrome C oxidase subunit 3 gene in presence of *Ps. fragi* (table 4, figure 2). The regulation is also in accordance with the predicted enhanced substrate competition. Hence, relevance especially of fatty acid exploitation for energy conservation and growth of *P. phosphoreum* TMW2.2103 can be speculated.

Additionally, presence of *B. thermosphacta*/*Ps. fragi* resulted in a reduced transcript number of the phage shock cluster PspABC in *P. phosphoreum* TW2.2103 under MAP conditions (additional table 3). This cluster has been described in the context of membrane stability and maintenance of the proton motive force during surface stress (73). The predicted reduced activity in the presence of high levels of CO_2 can therefore be assumed to cause increased vulnerability to the multiple effects of this gas (figure 3), since bacterial adaptation to CO_2 commonly includes an adapted membrane barrier function (74, 75). While the downregulation of this cluster explains the reported growth reduction of *P. phosphoreum* TMW2.2103, the reason for the downregulation remains unknown, since an upregulation would be a method to counteract the detrimental effects of the modified atmosphere.

In presence of *B. thermosphacta*, analysis also revealed regulation of the arginine metabolism. The number of transcripts was reduced for arginine deiminase ArcA, while it was enhanced for arginase ArgA (figure 4). The regulation is predicted to aim at a general decrease of reactions resulting in production of CO_2 and/or NH_3. *B. thermosphacta* has been suggested to produce enhanced levels of NH_3 in response to high levels of CO_2 (60). *P. phosphoreum* TW2.2103 can therefore be assumed to react to changes of the pH caused by the NH_3 production of its co-contaminant and to downregulate respective pathways itself. With this, the negative impact on the growth of *P. phosphoreum* TMW2.2103 by *B. thermosphacta*/*Ps. fragi* as co-contaminant combined with MAP (as described in (5)) is suggested to result from substrate competition and enhanced vulnerability to the effects of CO_2.

Regulation of *P. phosphoreum* TMW2.2103 in presence of *Ps. fragi*/*B. thermosphacta* as co-contaminant under air atmosphere.

We have observed different effects on the growth of *P. phosphoreum* TMW2.2103 dependent on the atmosphere in our previous study when a co-contaminant was present. Co-contamination has resulted in a negative or neutral impact under MAP, whereas under air atmosphere a positive impact was observed (5). This correlation is also reflected in the regulation observed in this study on the level of transcription.

The moderate O_2 concentration and absence of CO_2 in air atmosphere are supposed to trigger less oxidative stress for *P. phosphoreum* TMW2.2103 than MAP, thus facilitating substrate exploitation by respiration (compare table 4, growth under MAP alone). The increased number of transcripts of anaerobic respiration in this situation, e.g. observed for sulfite reductase CysI or nitrate reductase maturation protein NapF (table 4, figure 2) corresponds to the suggested metabolic activity based on respiration. Air atmosphere provides optimal growth conditions for various meat spoilers (76) and *B. thermosphacta*/*Ps. fragi* will consequently grow fast, utilizing their preferential substrates. *B. thermosphacta* exploits glucose first, followed by exploitation of a limited spectrum of amino acids and finally glycerol. *Ps. fragi* also utilizes glucose first,

followed by multiple glycolytic and metabolic intermediates and finally multiple amino acids (15, 19, 77, 78). *P. phosphoreum* TMW2.2103 supposedly benefits from an enhanced availability of free amino acids, fatty acids and glycerol, which result from the release and activity of lipases and proteases by *Ps. fragi* (61, 79, 80), and to a lesser extent by *B. thermosphacta* (15, 62). The latter has been demonstrated to possess high lipolytic activity in case of carbohydrate exhaustion (60). *P. phosphoreum* TMW2.2103 can be speculated to compete successfully for the available free fatty acids and glycerol moieties in accordance. It is assumed to cause the positive impact of co-contaminants on the growth of *P. phosphoreum* TMW2.2103 in air atmosphere (figure 3). Similar observations have been reported e.g. for *Listeria monocytogenes* in presence of *Ps. fluorescens* in literature (21). Lack of regulation associated with optimization of the substrate exploitation, e.g. enhanced substrate import, shows that *P. phosphoreum* TMW2.2103 already expresses an efficient set of genes in that respect. It is in accordance with the stand-by activity of multiple metabolic routes predicted for this strain as explained in the section 'General gene expression of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149'

P. phosphoreum TMW2.2103 downregulated multiple enzymes with minor impact on the metabolism during growth on meat in presence of *Ps. fragi*. Downregulation affected e.g. nutrient stress response (carbon starvation protein CstA), fructose and galactoside transport, conjugation (VirB10, TraY), and potassium export (KefB, KefG, additional table 3). The regulation is predicted to save energy for more relevant pathways. Concomitantly, activity of the arginine-deiminase-pathway (ADI pathway) for energy generation was predicted to be enhanced, as shown e.g. by a reduced number of transcripts of argininosuccinate synthase gene *arg*G or argininosuccinate lyase gene *arg*H (figure 4). Since *Ps. fragi* has been demonstrated to also employ an efficient ADI pathway (especially under anaerobic conditions (16)), this observation can also be interpreted as response to competitive arginine fermentation. Even though cytochrome c oxidase enzyme CyoC was downregulated by *P. phosphoreum* TMW2.2103 in presence of *Ps. fragi* (figure 2), it is assumed to represent also an energy saving regulation. Cyo has been described as predominant especially at high O_2

availability in *Escherichia coli* (81) and since *P. phosphoreum* TMW2.2103 possesses genes for multiple types of cytochrome C oxidase, the observed regulation is assumed to represent metabolic adjustment to the given O_2 content. The demonstrated enhanced growth of *P. phosphoreum* TMW2.2103 in presence of *Ps. fragi* and its dominance in case of dominant initial cell counts (5) show that the predicted regulation regarding energy investment are a successful strategy to compete *Ps. fragi*.

Regulation of *P. carnosum* TMW2.2149 in presence of *Ps. fragi*/*B. thermosphacta* as co-contaminant under modified atmosphere.

Although *P. carnosum* TMW2.2149 and *P. phosphoreum* TMW2.2103 are similar regarding their genomic setting, they differ distinctly on the level of transcription regarding their response to presence of a competitor. This is in agreement also with reported growth differences of strains of both species (compare (5)). Especially under MAP, *P. carnosum* TMW2.2149 differed from *P. phosphoreum* TMW2.2103. It regulated fewer genes and upregulated several ones already in action, whereas *P. phosphoreum* TMW2.2103 downregulated multiple genes (table 2, additional table 3). Differences between the strains observed on the level of transcription are in accordance with differences assumed for provenance and adaptation of both species as described by Hauschild *et al*. (49) and Fuertes-Perez *at al.* (27). These authors have reported i.a. higher tolerance to hydrostatic pressure and elevated NaCl concentration for *P. phosphoreum* than for *P. carnosum* strains, as well as a broader spectrum of fermentable carbohydrates for the latter species. Our observations are supposed to demonstrate presence of a general stress inducing setting for *P. phosphoreum* TMW2.2103 in presence of a co-contaminant/MAP (figure 3). Strains of this species have been described as more adapted to marine conditions and less adapted to meat conditions before (27, 49). In contrast, observations for *P. carnosum* TMW2.2149 are assumed to demonstrate presence of a situation representing suitable growth conditions in presence of a co-contaminant/MAP (figure

3). Strains of this species have been characterized as more adapted to meat conditions than strains of *P. phosphoreum* before (27, 49).

P. carnosum TMW2.2149 upregulated multiple central metabolic pathways in presence of *B. thermosphacta*. These included genes for e.g. ribokinase RbsK, which feeds the pentose phosphate pathway, as well as genes for general amino acid exploitation (e.g. *lys*O, *opp*C, C69 family dipeptidase). Furthermore, genes of several amino acid biosynthetic pathways and of diacetyl/acetoin production were upregulated (acetolactate synthase IlvM; additional table 4). As acetolactate at the same time is a precursor for leucine, isoleucine and valine biosynthesis, regulation of *ilv*M could also be related to the amino acid metabolism. Also, biotin biosynthesis was predicted to be enhanced, due to the increased transcript numbers of *bio*F, and *bio*C. The biotin biosynthesis pathway requires high amounts of energy and substrates (82, 83) and upregulation can thus be evaluated as costly. However, only few enzymes require biotin as cofactor (82, 84) and of those, only the gene for acetyl-Coa carboxylase, which contributes to fatty acid biosynthesis, was detected in the genome of *P. carnosum* TMW2.2149. It points at a particular relevance of fatty acid biosynthesis for this strain in presence of *B. thermosphacta* and MAP. Together with the increased number of transcripts observed for multiple genes of several metabolic routes, it demonstrates that the combination of MAP and co-contaminant allows for high anabolic and catabolic activity of *P. carnosum* TMW2.2149. In accordance, enhanced (energy) metabolism was also predicted for this strain in presence of *Ps. fragi*/MAP. Detected upregulation in that issue included e.g. 2-dehydro-3-deoxy-phosphogluconate aldolase Eda (Entner-Doudoroff pathway), protoheme IX farnesyltransferase CyoE (heme biosynthesis) and CoA pyrophosphatase (additional table 4). Consequently, the main factor that causes the previously demonstrated differences of *P. carnosum* TMW2.2149 and *P. phosphoreum* TMW2.2103 in growth can be speculated to be the perception of the co-contaminants as advantage or as additional stressor under MAP. *P. carnosum* TMW2.2149 is assumed to percept co-contamination rather as beneficial than as stressor, however, details on these benefits remain to be explored in future work. An

involvement of substrate or nutrient availability, pH value or reduced oxidative stress can be supposed.

Regulation of *P. carnosum* TMW2.2149 in presence of *Ps. fragi*/*B. thermosphacta* as co-contaminant under air atmosphere.

In air atmosphere, general reduction of the growth of *P. carnosum* TMW2.2149 has been reported by presence of *B. thermosphacta*/*Ps. fragi* as co-contaminant (5). However, analysis revealed only few differentially regulated genes that were additionally predicted to be of only minor importance (additional table 4). In that respect, presence of growth reduction but absence of regulation can be interpreted as inability to cope with the given situation (table 1, figure 3). Since *Ps. fragi* and *B. thermosphacta* grow fast in air atmosphere and since the growth rate of *P. carnosum* strains is rather low (compare e.g. (27)), the latter species is assumed to be outcompeted in this case. The lower cell counts that were observed for *P. carnosum* TMW2.2149 at the time point of RNA isolation compared to its co-contaminant support this assumption (approx. 1-1.5 log difference, compare first paragraph of results and discussion section).

Regulation of photobacteria under MAP compared to air atmosphere in absence of co-contaminants.

Based on the available data, possibilities are limited to correlate observed effects with the separate impact of O_2 or CO_2. However, it is possible to evaluate the combined impact of 70% O_2/30% CO_2 MAP on the transcription of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149. High O_2/high CO_2 gas mixtures represent a very common modified atmosphere in the meat industry (8, 9) and analysis of its effects on photobacteria can improve the evaluation of *Photobacterium* species in the context of meat spoilage.

Previous work has shown distinct growth reduction of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 on chicken meat by application of 70% O_2/30% CO_2 MAP (compared to air atmosphere (5)). In agreement with this, reduced transcript numbers were observed for enzymes of cell division (*P. phosphoreum* TMW2.2103, e.g. NrdEFG, FtsZ, WecA), transcription and other basic processes of cell viability (*P. carnosum* TMW2.2149, e.g. TadA, SelA, RnfH family protein, additional table 3, 4). Additionally, upregulation of enzymes associated with conjugation and adhesion (*P. phosphoreum* TMW2.2103: MshJ, VirB2; *P. carnosum* TMW2.2149: VirB9, VirB3, VirB2, MshP), together with downregulation of enzymes involved in chemotaxis and motility (FlhH, FliP, FlgH, FlgF, FliL) was detected. It can be interpreted as presence of impaired growth conditions for both strains under MAP and activity to partially cope with it (figure 3).

Data also predict presence of oxidative stress especially under high-oxygen MAP conditions for both strains. *P. phosphoreum* TMW2.2103 is predicted to shift its metabolic activity from respiration to fermentation, as discussed earlier, thus reducing the activity of enzymes that can contribute to oxidative stress by formation of ROS (85). Concomitantly with this, S-(hydroxymethyl) glutathione synthase Gfa, which participates in glutathione metabolism, was upregulated (additional table 3). In this context, glutathione metabolism has been described as suitable defense against ROS (86-88). Likewise, *P. carnosum* TMW2.2149 downregulated respiration, such as heme transport/storage (HutX, HutZ, TonB dependent receptor), and NADPH-dependent FMN reductase (additional table 4), but upregulated enzymes that feed the pyruvate metabolism. This included e.g. enzymes for glycerol degradation (GlpAB, GlpQ, GlpT, figure 1, table 4) and sugar exploitation (EbgA, MglA, EIIC, additional table 4). It is in agreement with the aforementioned observation that some genes coding for ROS-generating enzymes are absent in the genomes of both strains (compare section Genomic setting of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 for metabolizing substrates on chicken meat**).** In this context, other common meat spoilers, such as *B. thermosphacta* and *Pseudomonas*, are also known for activity to efficiently minimize oxidative stress in presence of O_2 (60, 89, 90). However, evaluation of the efficiency of the stress response of photobacteria

compared to the one of these species would require more detailed information on the separate effect of CO_2 and O_2. The lower tolerance of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 to H_2O_2 in comparison to *B. thermosphacta* (5) nevertheless suggests also higher vulnerability of both strains to oxidative stress.

Key enzymes TrpC/TrpF of the tryptophane biosynthesis were downregulated by MAP conditions in *P. phosphoreum* TMW2.2103 (figure 5). This is supposed to represent an example for the effect of the elevated CO_2 concentration. Thereby, presence of CO_2 is assumed to inhibit the conversion of pephrenate to phenylpyruvate or 4-hydroxy-phenylpyruvate and CO_2, respectively (figure 5), following the law of mass action (91). This is predicted to result in an increased chorismate pool and consequently in enhanced biosynthesis of tryptophane. Feedback inhibition of the tryptophane operon is then assumed to result in the observed downregulation. Feedback inhibition of the first enzyme of tryptophane biosynthesis, indole-3-glycerol-phosphate synthase TrpC, has already been confirmed for *Vibrio harveyi* in this context (92). In contrast, upregulation of the tryptophane biosynthesis in *P. phosphoreum* TMW2.2103 was detected in presence of *Ps. fragi* as co-contaminant (additional table 3). It points at low availability of tryptophane in this situation. The tryptophane content of meat has been reported to be low in comparison to other amino acids (1.2-1.4% of the amino acid composition of meat protein, (93)). Consequently, competition for available tryptophane can be assumed for *P. phosphoreum* TMW2.2103 and *Ps. fragi* and necessity (for the former) to synthesize this amino acid by itself.

Conclusions

This transcriptomic study reveals that *P. carnosum* and *P. phosphoreum* employ different but successful strategies to integrate into the meat spoiling consortium. It suggests that the combination of co-contaminants and type of MAP in a specific batch of meat strongly affects the prevalence of *P. carnosum* or *P. phosphoreum*. This fits the observation that either *P. carnosum* or *P. phosphoreum* is found on spoiled meats (27).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets generated during the current study are available in European Nucleotide Archive of the EMBL-EBI. The study accession number is PRJEB48739, the sample accession numbers are ERS8385781-8385816 All other data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflict of interest.

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Author contributions

PH: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing - original draft, writing - review & editing, visualization.
RFV: writing - review & editing, project administration, funding acquisition.
MH: writing - review & editing, conceptualization, supervision, project administration, funding acquisition.
All authors read and approved the final manuscript.

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Table 1 Visualization of the data interpretation. Data on the growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 obtained from (5) were combined with the data regarding transcription obtained within the scope of this study

growth	regulation	interpretation
unchanged	no	organism does not (need to) react
enhanced	no	organism is equipped (on standby) to change and benefits from new situation
reduced	no	organism cannot react
unchanged	yes	organism copes succesfully with stress factor
enhanced	yes	organism benefits from new situation
reduced	yes	organism cannot cope with stress factor

Table 2. Number of genes with relevant regulation in modified atmosphere or air atmosphere in presence or absence of co-contaminants. Data on the regulation under MAP was obtained by the comparison to the regulation under air atmosphere. Data on the regulation in presence of a co-contaminant was obtained by the comparison to the regulation in absence of the co-contaminant. Relevant regulation was accepted with logFC ≥2/≤-2 und $p<0.05$

	atmosphere	MAP	MAP	air	MAP	air
	co-contaminant	-	*Ps. fragi*		*B. thermosphacta*	
P. phosphoreum TMW2.2103	upregulated	22	16	12	41	22
	downregulated	41	10	33	49	44
P. carnosum TMW2.2149	upregulated	42	11	4	12	17
	downregulated	29	7	23	25	19

Table 3 Presence and direction of regulation for genes of the glycerol metabolism, aerobic and anaerobic respiration and arginine metabolism. Data on the regulation under MAP was obtained by the comparison to the regulation under air atmosphere. Data on the regulation in presence of a co-contaminant was obtained by the comparison to the regulation in absence of the co-contaminant. The respective reference pathways are depicted in figure

1,2 and 4. Significant regulation was accepted with LogFC ≥2/≤-2 and $p < 0.05$. n= 3. + = significant upregulation, - = significant downregulation.

reference figure	strain	*P. phosphoreum* TMW2.2103					*P. carnosum* TMW2.2149				
	co-contaminant	-	*Ps. fragi*		*B. thermosphacta*		-	*Ps. fragi*		*B. thermosphacta*	
	atmosphere	MAP	MAP	air	MAP	air	MAP	MAP	air	MAP	air
1	GlpK										
	GlpA					+	+				+
	GlpB						+				+
	GlpC					+				+	
	GldA					+					
	GlpT						+				+
	GlpQ						+				+
	FadD	-	+								
2	CoxB									-	
	CoxC	-	+								
	CyoB							-			
	CyoC			-							
	CyoE							+			
	CysI			+							
	Cytochrome C									-	
	Cytochrome C oxidase assembly protein	-									
	HmpA						-				
	NADH:ubiquionen oxidoreductase (unspecified)	+									
	NapA	-	+								
	NapB	-									
	NapD	-									
	NapF			+		+				-	
	NorV					-					
	NqrM			-							
	SdhC						+				+
	TorC	-									
4	ArcA				-						
	ArgA				+						
	ArgB			-							
	ArgC			-							
	ArgG			-							
	ArgH			-							
	ggt			+							
	GlmS	-		-			+				
	SpeG				-						

Table 4 Summary of the most relevant regulation

The regulation was regarded as relevant, if several genes of one cluster or pathway were affected, or if rate limiting- or key enzymes were affected. + = relevant upregulation, - = relevant downregulation.

strain	*P. phosphoreum* TMW2.2103					*P. carnosum* TMW2.2149				
atmosphere	MAP	air		MAP		MAP	air		MAP	
co-contaminant	-	*Ps. fragi*	*B. thermo-sphacta*	*Ps. fragi*	*B. thermo-sphacta*	-	*Ps. fragi*	*B. thermo-sphacta*	*Ps. fragi*	*B. thermo-sphacta*
cell division	-		±		+					
transcription						-				
purine/pyrimidine metabolism	+		+			+	-			
Entner Doudoroff pathway									+	
gluconeogenesis			-		-					
pentose phosphate pathway										+ (substrate)
sugar exploitation (other mechanisms)		-				+				
TCA										
respiratory chain	(-)	-						(+)		-
heme availability						-		-	+	
anaerobic respiration	- (nitrate, TMAO)	+ (nitrate, sulfate)	+ (nitrate)							- (nitrate)
formate degradation			-		-	-				
production of acetoin/diacetyl							-			+
β-oxidation	-			+	+					
glycerol utilization			+			+		+		+
biotin biosynthesis										+
tryptophane biosynthesis	-			+						
arginine deiminase cycle		+			-					
leucine/isoleucine/valine biosynthesis			-		-					
availability of methionine as substrate			+		+					
aminosugar/nucleotide sugar metabolism	-	-				+				
stress response		- (nutrient)	+ (nutrient)	- (membrane)	- (membrane)	+ (nutrient)	+ (oxidative)			
motility	-									
conjugation	+	-				+		+		
biofilm						+	-			
potassium export		-				+		+		
defense against ROS	+	+			+					

Figure captions

Figure 1 Detected regulation in the glycerol metabolism.

Relevant enzymes that were fully coded in the genomes are depicted in black, relevant enzymes that were not coded in the genomes are depicted in grey. Enzymes with significant regulation in at least one of the experimental groups are marked in red.Table 3 specifies the up-/downregulation of respective genes for all analyzed samples. Significant regulation was accepted with LogFC ≥2/≤-2 and $p < 0.05$. n= 3.

Figure 2 Detected regulation in the aerobic and anaerobic respiration.

Relevant enzymes that were fully coded in the genomes are depicted in black, relevant enzymes that were not coded in the genomes are depicted in grey. Enzymes with significant regulation in at least one of the experimental groups are marked in red. If genes were detected in only one of the strains, respective enzymes are shown in brackets. Table 3 specifies the up-/downregulation of respective genes for all analyzed samples. Significant regulation was accepted with LogFC ≥2/≤-2 and $p < 0.05$. n= 3.

Figure 3 Summary of the most important effects of MAP and co-contamination.

Effects that were observed as transcriptomic regulation are highlighted in grey and effects that were assumed based on the observed regulation is not highlighted. Increase (of number of transcripts or in general) is depicted as green arrow and reduction as red arrow. Additionally, the general interpretation of the data based on table 1 is shown as light green box (absence of a stress situation for the respective strain) or light red box (presence of a stress situation for the respective strain).

Figure 4 Detected regulation in the arginine metabolism.

926 Relevant enzymes that were fully coded in the genomes are depicted in black, relevant
927 enzymes that were not coded in the genomes are depicted in grey. Enzymes with significant
928 regulation in at least one of the experimental groups are marked in red. Enzyme activity that is
929 not finally confirmed in literature is parenthesized. Table 3 specifies the up-/downregulation of
930 respective genes for all analyzed samples. Significant regulation was accepted with LogFC
931 ≥2/≤-2 and $p < 0.05$. n= 3.

932

933 **Figure 5 Detected regulation of the tryptophane metabolism by *P. phosphoreum***
934 **TMW2.2103 under MAP.**

935 Relevant enzymes that were fully coded in the genomes are depicted in black, relevant
936 enzymes that were not coded in the genomes are depicted in grey. Enzymes with significant
937 regulation in at least one of the experimental groups are marked in red. Significant regulation
938 was accepted with LogFC ≥2/≤-2 and $p < 0.05$. n= 3.

939

940 **Additional files**

941 Additional figure 1 (.pptx) Scatter plot of LogFC versus average logCPM values of the *P.*
942 *phosphoreum* TMW2.2103 samples. A air atmosphere vs. modified atmosphere, B air
943 atmosphere vs. air atmosphere + *B. thermosphacta*, C air atmosphere vs. air atmosphere +
944 *Ps. fragi,* D modified atmosphere vs. modified atmosphere + *B. thermosphacta,* E modified
945 atmosphere vs. modified atmosphere + *Ps. fragi.*

946 Additional figure 2 (.pptx) Scatter plot of LogFC versus average logCPM values of the *P.*
947 *carnosum* TMW2.2149 samples. A air atmosphere vs. modified atmosphere, B air atmosphere
948 vs. air atmosphere + *B. thermosphacta*, C air atmosphere vs. air atmosphere + *Ps. fragi,* D
949 modified atmosphere vs. modified atmosphere + *B. thermosphacta,* E modified atmosphere
950 vs. modified atmosphere + *Ps. fragi.*

Additional figure 3_1 (.pptx) Predicted metabolic pathways of the carbohydrate metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 3_2 (.pptx) Predicted metabolic pathways of the tricarboxylic acid cycle and pyruvate metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 3_3 (.pptx) Predicted metabolic pathways of the fatty acid metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 3_4 (.pptx) Predicted metabolic pathways of the amino acid metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 except of tryptophane- and histidine metabolism. The analysis is based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey. Enzymatic activity that is not finally confirmed by the literature is parenthesized.

Additional figure 3_5 (.pptx) Predicted metabolic pathways of the tryptophane- and histidine metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149. The analysis is based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 3_6 (.pptx) Predicted metabolic pathways of the respiration of *P. phosphoreum* TMW2.2103 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 3_7 (.pptx) Predicted metabolic pathways of the respiration of *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.

Additional figure 4 (.pptx) Multidimensional scaling plot of the samples based on the logFC. (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *B. thermosphacta* in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *B. thermosphacta* in modified atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 in modified atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *Ps. fragi* in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *Ps. fragi* in modified atmosphere. **A** *P. phosphoreum* TMW2.2103. **B** *P. carnosum* TMW2 .2149. The figure is based on a scaling plot created by Eurofins Genomics GmbH (Konstanz, Germany) with the R script package edgeR.

Additional table 1 (.xlsx) Summary of the sequencing results. Data were obtained from Eurofins Genomics GmbH (Konstanz, Germany) and are based on sequencing with Illumina HiSeq2500.

Additional table 2 (.xlsx) Detected transcripts with highest average logCPM value. Transcripts that were observed in samples of both strains are marked in red. The average logCPM values were provided by Eurofins Genomics GmbH (Konstanz, Germany).

Additional table 3 (.xlsx) Detected significant regulation in *P. phosphoreum* TMW2.2103 samples. Significance was accepted with LogFC ≥ 2/≤-2 and $p < 0.05$.

Additional table 4 (.xlsx) Detected significant regulation in *P. carnosum* TMW2.2149 samples. Significance was accepted with LogFC ≥ 2/≤-2 and $p < 0.05$.

6 Discussion

This thesis explores the classification of *Photobacterium* species within the current knowledge on meat spoilage communities and deals with the coherence of their occurrence on meat and in marine environments. To outline these issues, distribution and diversity of *Photobacterium* species in the food industry was evaluated, with specific focus on *P. phosphoreum* and *P. iliopiscarium* due to their previously demonstrated marine background. Results suggested the meat processing environment as most likely source of contamination. Environmental adaptation was then investigated for the two most frequently detected species, *P. phosphoreum* and *P. carnosum*, in the context of possible niches on meat and in marine environment. This enables to correlate long-established knowledge on the role of photobacteria in marine environment and the recent discovery of this genus in association with meat. Adaptation towards marine environments proved to be stronger for *P. phosphoreum* strains than for *P. carnosum* strains, demonstrating different selective pressure in the past. The role of both species in meat spoilage was then further characterized by studies on its growth on meat, its response to MAP during growth on meat and its response to co-contamination with selected members of the meat spoilage community. Results allow for a prediction of the successful interaction of *P. phosphoreum* and *P. carnosum* strains with other meat spoilers and reveal application of MAP as possible competitive advantage for photobacteria in this context.

The following theses were derived from this work, which are discussed in the following chapters:

(1) *P. phosphoreum* contaminants on meat originate from the meat processing environment, where they can colonize cold, humid habitats.

(2) *P. phosphoreum* and *P. carnosum* share a marine provenance but differ regarding the extent of adaptation towards meat environment.

(3) The packaging atmosphere has limited impact on the growth of photobacteria on meat.

(4) Photobacteria integrate in the meat spoilage consortium and interact differently with co-contaminants.

(5) Photobacteria are relevant contributors to meat spoilage on MAP meats

6.1 *P. phosphoreum* contaminants on meat originate from the meat processing environment, where they can colonize cold, humid habitats

Despite of its well-documented marine background, association of *P. phosphoreum* with the meat environment, and to a certain extent also of *P. iliopiscarium*, is approved by multiple scientific studies to date, including this work. Presence of *P. phosphoreum* strains was demonstrated within the scope of this work for multiple types of meat, as well as for meat products and the meat processing environment ((Fuertes-Perez *et al.*, 2019), unpublished data concerning a beef/pork processing facility). It confirms ubiquitous occurrence of *P. phosphoreum* contaminations in the meat industry, which is in agreement with reported detection of contaminated samples in multiple independent shops in literature (Delhalle *et al.*, 2016). To investigate the source of *Photobacterium* contaminations in the meat industry, occurrence of *P. phosphoreum* and *P. iliopiscarium* on meat and other food products was recorded and isolates were characterized with respect to their biodiversity (Fuertes-Perez *et al.*, 2019). Obtained results constitute for the first time a coherent recording of distribution and diversity of *P. phosphoreum* and *P. iliopiscarium* isolates from meat environment.

The initial working hypothesis assumed the source of contamination to be located in the livestock farming or on the animal itself. However, given the observations recorded within the scope of this work, these niches appear rather unfavorable for colonization and were not further followed during this study. *P. phosphoreum* isolates from meat prefer low growth temperatures <20°C (Fuertes-Perez *et al.*, 2019; Hilgarth *et al.*, 2018a), which is in accordance with the environmental conditions that characterize habitats of marine strains of this species (Jebbar *et al.*, 2015). Additionally, *P. phosphoreum* strains from meat are sensitive to elevated temperature ≥30°C, as well as to reduced water activity. Recovery of viable cells from desiccated surfaces of different material was unsuccessful in this context (metal, polystyrene, latex, cotton cloth, desiccated solid medium, unpublished data). Similar observations were obtained for strains of the species *P. iliopiscarium*. Like *P. phosphoreum*, this species has already been isolated from marine environments prior to its detection on meat. However, it can be evaluated as less relevant to meat spoilage than *P. phosphoreum,* due to its only sporadic occurrence on meat and meat products (Fuertes-Perez *et al.*, 2019). With these preferences for temperature and water activity in mind, arising contamination from the livestock itself appears very unlikely. Association of *P. phosphoreum* and *P. iliopiscarium* with soil, subsequent relocation to the animal and concomitant contamination of the meat is evaluated as improbable route likewise. Reports on the detection of photobacteria in soil exist, but they are rare and limited to habitats with strong contamination by heavy metals or herbicides/saline (e.g. (Li *et al.*, 2019a; Mathew *et al.*, 2015); compare chapter 3.5). Yet, occurrence of *P. phosphoreum* on alive (marine) animals has been documented for cases providing appropriate ambient temperature and water activity, e.g. the

intestines of farmed salmon (Hovda *et al.*, 2012; Møretrø and Langsrud, 2017). Association of photobacteria with invertebrates in the soil or in/on livestock and concomitant transfer to meat has also been considered in the past. In this context, free-living nematodes have been designated as putative vectors for bacteria (Anderson *et al.*, 2006), even for foodborne species (Caldwell *et al.*, 2003). However, even though *P. angustum* has been demonstrated to colonize the mucus track produced by aquatic nematodes (Moens *et al.*, 2005), this topic is barely examined in literature and correlations remain speculative.

Results suggest the source of contamination of meat to be located in the meat processing facilities instead, as it has already been proposed for psychrotrophic LAB from MAP broiler products (Vihavainen *et al.*, 2007). These facilities provide low temperatures and appropriate humidity (Nollet, 2012; Pearce *et al.*, 2006; Rouger *et al.*, 2017), as well as unnoticed niches that can allow proliferation. Presence of *P. phosphoreum/P. iliopiscarium* populations in small and local niches within the processing workflow is assumed to cause fluctuating contamination that can accordingly explain reported variances in the initial abundancy of photobacteria on meat (reported e.g. by (Cauchie *et al.*, 2020; Duthoo *et al.*, 2021; Stoops *et al.*, 2015)). This assumption is supported by the demonstration of significantly different total cell counts on (beef) carcasses already during chilling (Reid *et al.*, 2017). Detection of photobacteria also on variable places within butcheries, sometimes on the meat itself and sometimes only on tools and surfaces (Stellato *et al.*, 2016), provides support for the importance of locally restricted microbial populations in contamination. In such cases, populations are suggested to be part of the established in-house microbiota of the respective facility or butchery.

In this context, occurrence of *Photobacterium* contaminants on meat appears to be influenced to a certain extent by the season. Its occurrence on MAP chicken, MAP pork and MAP beef samples from a selected retailer and brand was monitored over three years. Selective cultivation gave positive results especially in July and August (three of three times positive, table 4) and mostly negative results from November to April. It is in accordance with a study from Juszczuk-Kubiak *et al*. who have reported presence of photobacteria on VP ostrich meat in high abundance during August and October, but not during March (Juszczuk-Kubiak *et al.*, 2021). Oral communication with people in charge of the workflow in a meat processing facility revealed seasonal changes in the activity of local cooling systems. This concordance suggests the cooling system as possible niche for colonization and confirms the meat processing environment as major source of contamination. Furthermore, *Photobacterium* contaminants have been reported to occur also on cooked meat products (e.g. (Duthoo *et al.*, 2021)), despite of their reported sensitivity to elevated temperature (Dalgaard *et al.*, 1997; Fuertes-Perez *et al.*, 2019; Kanki *et al.*, 2004). It implies that established bacterial populations within the meat processing environment, such as *Photobacterium* populations associated with the cooling system, harbor a considerable risk for re-contamination.

Table 4 Occurrence of contaminated samples within a sampling row of selected meats dependent of the season. Meats were obtained every month from the same producer and brand and tested for photobacteria by selective cultivation, followed by species identification with MALDI TOF MS. X =positive sample; - =negative sample; (X) =positive sample of different brand.

	January	February	March	April	May	June	July	August	September	October	November	December
2017					X	X	X	X	X	X		
2018							X	X	X	X	(-)	(-)
2019	(-)	X	-	-	X	(X)	(X)	X	-			

Assuming the meat processing environment as major source of contamination, bacterial growth will be restricted to only few niches in this habitat, due to the frequent application of hygienic measures. Survival of photobacteria during established cleaning and disinfection measures has already been confirmed (Bagge-Ravn *et al.*, 2003), demonstrating the ability for successful colonization of such niches. Colonization of niches in the meat processing environment is assumed to be accompanied by phases of unfavorable growth conditions and nutrient limitation. However, *P. phosphoreum* strains are characterized by genomic adaptations enabling to cope with changing nutrient availability that could allow for persistence in this context. Adaptations include e.g. a high copy number of rRNA operons that enables a quick reaction to sudden nutrient availability (Klappenbach *et al.*, 2000; Urbanczyk *et al.*, 2010), or multiple metabolic alternatives for nutrient exploitation. Presence of the Embden-Meyerhof-Parnas pathway and pentose phosphate pathway, of TCA cycle and glyoxylate shortcut, of β-oxidation and glycerol degradation, and of multiple versions of respiratory complexes and a full heme biosynthesis pathway has been confirmed for photobacteria from meat (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). This statement includes also the species *P. carnosum*. Strains of *P. carnosum* occur in relevant quantity and in a relevant number of cases on spoiling meat, similar to *P. phosphoreum* (Fuertes-Perez *et al.*, 2019), thus bringing both species into focus for studies on growth and interaction during meat spoilage (Hauschild *et al.*, 2021a; Hauschild *et al.*, 2021b; Hauschild *et al.*, 2021c). The metabolic flexibility of *P. phosphoreum* and *P. carnosum* strains from meat can be considered as remnant of a marine lifestyle with phases of low nutrient availability in open water and phases of high nutrient availability in association with marine animals (Urbanczyk *et al.*, 2010). It reflects the accepted principle that metabolic (and phenotypic) versatility is advantageous at unstable environmental conditions, whereas stable environmental conditions favor specialization (Yamamichi *et al.*, 2011). In this context, other common meat spoilers are frequently limited to less functional metabolic pathways instead, e.g. *B. thermosphacta*, *L. gelidum*, *C. maltaromaticum* or *C. divergens* (Kolbeck, 2021). Beside of metabolic flexibility, adaptation to substrate limitation can also take place by production of storage compounds. *P. phosphoreum* has been described to accumulate up to 37% polyhydroxybutyrate of the dry weight during nutrient stress. Even if the described strain could not

use the compound as sole carbon and energy source in that case (Reichelt and Baumann, 1973; Thyssen and Ollevier, 2007), accumulated polyhydroxybutyrate may still be re-used and contribute to persistence in the meat processing environment in case of nutrient limitation.

Concomitant with flexibility of its metabolic routes, photobacteria exploit a rather narrow range of substrates as principle energy source (Thyssen and Ollevier, 2007), especially during fermentative metabolism (Bamann and Baumann, 1984; Baumann and Baumann, 1981). The substrate versatility has thereby been demonstrated to be highly species-specific (Baumann and Baumann, 1981). Especially *P. phosphoreum* strains from marine environments have been shown to exhibit low nutrient versatility in accordance, when compared to other marine photobacteria, e.g. *P. fischeri or P. mandapamensis* (Reichelt and Baumann, 1973). *P. phosphoreum* strains from meat were detected to ferment less substrates than *P. carnosum* strains (Fuertes-Perez *et al.*, 2019). The combination of narrow substrate spectrum and optimal capabilities to exploit it proves adaptation to habitats not only with limited substrate availability, but also limited choice of substrates. Such specialization can be required for the colonization of specific marine habitats, but could also function as strategy to avoid nutrient competition within the meat spoilage community or during colonization of niches in the meat processing facility. Successful competition can then depend on the availability of particular substrates that may also arise as side-products of the metabolic activity of other spoilers. Even low amounts of these substrates would allow for growth in this case (Bachmann *et al.*, 2016), implying successful competitiveness in accordance.

Dependence of a specific and rather narrow substrate spectrum can also explain the expansion of *P. phosphoreum*/*P. carnosum* from their supposedly original habitats in association with fish/seafood (Hauschild *et al.*, 2021a) to habitats on meat. Habitats on meat and fish/seafood share multiple nutritional elements, such as the protein content (approximately 16-24 g/100 g (Bohrer, 2017)) and amino acid composition (Milton, 1999), as well as the sodium (approximately 40-80 mg/100g), phosphorus (approximately 150-270 mg/100g), vitamin B12 and cholesterol content of the animal tissue (Bohrer, 2017). Especially strains of *P. phosphoreum* have been reported to be subject to a certain methionine requirement (Baumann and Baumann, 1977), and animal proteins contain in general higher amounts of methionine than plant-based proteins (Elango, 2020). A correlation of the occurrence of photobacteria on meat/fish/seafood and especially the methionine content of these products can be speculated in accordance (Hauschild *et al.*, 2021c). Additionally, meat and marine fish/seafood differ from other animal related foods by their high content of taurine (Schuller-Levis and Park, 2003). Bacteria can exploit taurine as carbon source by means of the enzymes taurine dehydrogenase or taurine-pyruvate transaminase, resulting in acetyl-CoA formation (Cook and Denger, 2006). These pathways are still poorly studied and presence of respective genes in photobacteria or closely related species has not been addressed in research so far. However, genomes

of *P. phosphoreum* and *P. carnosum* strains from meat harbor sequences that could be relevant in this context (unpublished data). Consequently, occurrence of photobacteria on other food products with variable substrate spectra appears as rather unlikely. Nevertheless, it is possible that photobacteria have been overlooked also in other fields of food spoilage so far, due to the unusual cultivation conditions that they require (compare e.g. (Hilgarth *et al.*, 2018a)). Suitable habitats beside of meat were suspected on other animal-related foods (e.g. milk), on protein-rich vegetables (e.g. sprouts), on (vegetable) MAP products (e.g. ready-to-eat salad), or on vegetable products with marine origin (e.g. algae). The respective samples analyzed during this study are summarized in Figure 4.

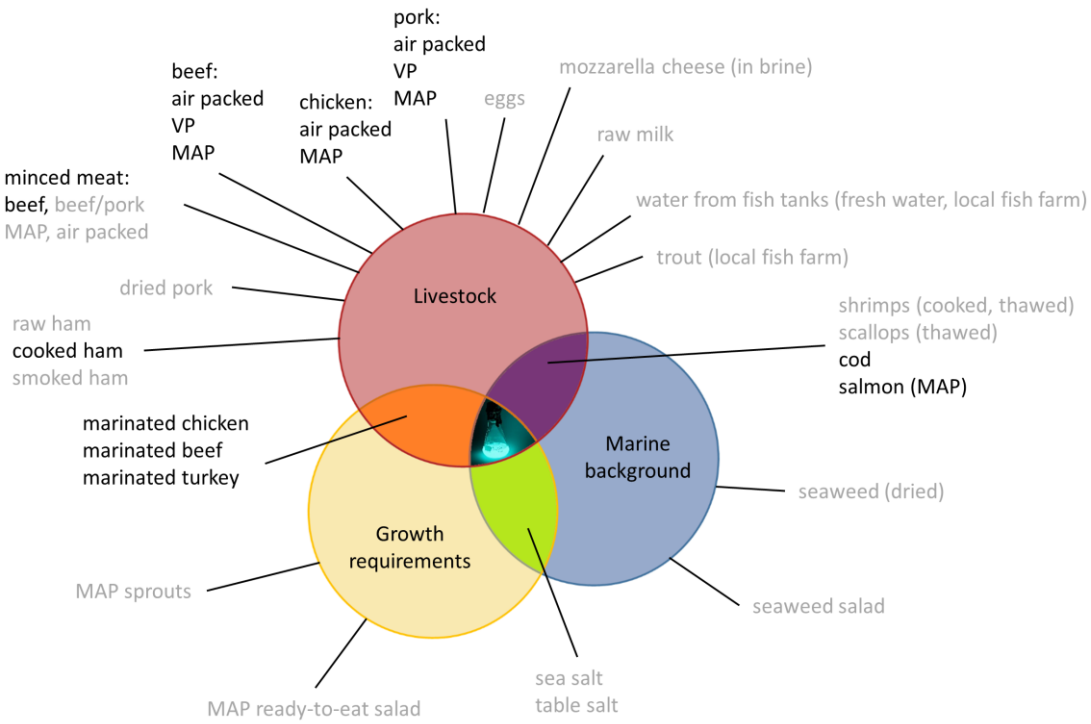

Figure 4 Food products and related samples that were analyzed for presence of photobacteria. Samples were obtained from local retailers, stored until they reached the expiry date and then plated on selective medium. Species were identified with MALDI-TOF MS. Samples that were negative for presence of photobacteria are depicted in grey. Samples that were positive for presence of photobacteria within the scope of this study or that have been stated to be positive in the literature (summarized in table 1), are depicted in black.

However, all products besides of meat that were tested during this study were negative for *P. phosphoreum* (and also for *P. carnosum* and *P. iliopiscarium*) (Fuertes-Perez *et al.*, 2019). It shows that the species is either specific to meat and seafood or was unable to access the processing chains of these products so far. Beside of their substrate specificity, confirmed interaction of *P. phosphoreum* and *P. carnosum* with meat spoiling species and its growth and persistence within meat spoilage communities (Hauschild *et al.*, 2021b) provides support rather for an association of photobacteria specifically with meat/fish/seafood in this context.

6.2 *P. phosphoreum* and *P. carnosum* share a marine provenance but differ regarding the extent of adaptation towards meat environment

Considering the frequently reported presence of *P. phosphoreum* in marine habitats (e.g. (Gornik *et al.*, 2013)) and the so far reported general absence of *P. carnosum*, their provenance as contaminants on meats and on fish/seafood arouses interest. Adaptation of isolates from both habitats to high hydrostatic pressure and elevated NaCl concentration was investigated to reveal differences of the two most relevant *Photobacterium* species on meats, *P. phosphoreum* and *P. carnosum*, in the context of their isolation source (Hauschild *et al.*, 2021a). Investigations generated for the first time insights into the correlation of the occurrence of *P. phosphoreum* and *P. carnosum* strains on meat and on fish/in marine environment.

Both species are assumed to share a certain marine background (Hauschild *et al.*, 2021a), since they are characterized by very similar genomic features, including several ones associated with marine lifestyles ((Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). Strains of both species harbor e.g. genes for motility and anaerobic respiration with nitrate, sulfate, TMAO and DMSO as characteristic substrates in marine environments (Barrett and Kwan, 1985; Karl and Letelier, 2009; Lee and De Mora, 1999; Pilson, 2012). However, isolates from meat and from marine environments indeed differed regarding their tolerance and requirements of high hydrostatic pressure and NaCl, as had been assumed in the working hypothesis. Furthermore, differences could be observed also on species level, with *P. phosphoreum* strains being in general more tolerant to conditions related to marine habitats (Hauschild *et al.*, 2021a). This is in accordance with differences detected in the response of both species to meat-specific co-contaminants. *P. carnosum* TMW2.214 was shown to benefit from co-contaminants on MAP meat, while *P. phosphoreum* TMW2.2103 did not (Hauschild *et al.*, 2021b). It demonstrates that *P. phosphoreum* and *P. carnosum* strains vary on a very fundamental level of their environmental adaptation and were subject to different selective pressure in the past. The major difference of both species in the context of their occurrence on meat is therefore suggested to be the point in time at which they accessed meat as habitat. *P. carnosum* is assumed to have reached it earlier than *P. phosphoreum*, resulting in stronger specialization to meat systems (Fuertes-Perez *et al.*, 2019; Hauschild *et al.*, 2021b; Hauschild *et al.*, 2021c) and ongoing loss of specializations to marine systems (Hauschild *et al.*, 2021a). *P. carnosum* could be consequently termed a rather terrestrial species that possesses remnants of a marine lifestyle.

The assumption of a shared initial marine provenance of both species raises the question of the continuance of *P. carnosum* in marine environment. Though, detection of this species on spoiled MAP salmon has been reported (Fuertes-Perez *et al.*, 2019; Le Doujet *et al.*, 2019; Sørensen *et al.*, 2020), rarity of this event and comparable low tolerance of obtained strains to elevated pressure and NaCl

concentration (Hauschild *et al.*, 2021a) suggest cross-contamination in at least one of the reported cases (compare (Fuertes-Perez *et al.*, 2019)). Another case is unable to provide clear separation of *P. carnosum* and *P. iliopiscarium* during identification (compare (Le Doujet *et al.*, 2019)), thus being subject to questionable reliability. Strains of *P. carnosum* are characterized in general by slower growth and higher vulnerability to multiple environmental factors than strains of *P. phosphoreum* (Fuertes-Perez *et al.*, 2019; Hilgarth *et al.*, 2018b; Sørensen *et al.*, 2020). It allows speculation on poor competitiveness of this species in marine habitats that could explain the documented absence of striking cell numbers of *P. carnosum*. In the context of specialization of *P. carnosum* to the meat environment and concomitant loss of adaptation towards marine environments, a study of Machado is of interest. These scientists have reported genomic adaptation of marine photobacteria in response to presence or absence of (alien) microbial competitors and concomitant lifestyles (Machado, 2017). Described adaptation thereby refers to genomic clusters for secondary metabolites. It implies marine photobacteria to be able to deal with both, presence and absence of an interacting community of other species. With regard to the positive impact of co-contaminants on *P. carnosum* TMW2.2149 on meat (Hauschild *et al.*, 2021b), a certain requirement of this strain for a co-contaminating community can be assumed and thus reduced capability for a free-living marine lifestyle. Despite all this, *P. carnosum* and *P. phosphoreum* both represent widespread meat spoilers (Fuertes-Perez *et al.*, 2019) with similar requirements and similar contributions to the spoilage process (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). With this in mind, differences regarding their initial exploration of meat as habitat and the arising extend of adaptation towards it can be considered as subordinate.

Competitive growth of *P. carnosum* on meat is assumed to be enabled by efficient adaptation to meat and resulting growth advantages, despite of its comparably low growth rate, long lag phase and greater vulnerability. In accordance, the major *Photobacterium* species on multiple spoiled meat samples from retail has turned out to be *P. carnosum* before (Fuertes-Perez *et al.*, 2019; Hilgarth *et al.*, 2018a). Dominant occurrence of only one *Photobacterium* species thereby raises the issue of the interaction within the genus *Photobacterium* on meat. Dominance of either *P. phosphoreum* or *P. carnosum* may be related either to initially leading cells numbers, as it has been detected to be relevant for other meat spoilers e.g. on beef carcasses (Reid *et al.*, 2017), or to higher competitiveness of one of the species under certain conditions. Since strains of both species share many genomic features (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c), presence at least of substrate competition can be expected in that respect. *P. phosphoreum* strains grow faster and fast growth has already been described as efficient strategy for dominance (Gill, 1976). However, a low growth rate can be compensated by higher efficiency in substrate utilization, e.g. due to higher affinity for the substrate (Veldkamp and Jannasch, 1972) or due to a broader substrate spectrum. The latter has been detected for *P. carnosum*

strains from meat when compared to *P. phosphoreum* strains (Fuertes-Perez *et al.*, 2019; Fuertes-Perez *et al.*, 2021). Consequently, strains of both species possess the potential for competitive growth and a decisive impact of the initial cell counts can be assumed in accordance. In the context of the comparably weak growth of *P. carnosum* strains during cultivation in laboratory conditions on meat (Hauschild *et al.*, 2021a; Hauschild *et al.*, 2021b) and the positive impact of co-contaminants (Hauschild *et al.*, 2021b), an additional impact of the spoilage community on competitive growth of *P. carnosum* can be speculated.

Dominance of *P. carnosum* especially on beef and chicken (Fuertes-Perez *et al.*, 2019; Hilgarth *et al.*, 2018a) and high amounts of *P. phosphoreum* especially on pork and fish (Fuertes-Perez *et al.*, 2019) questions the role of the meat type during successful competition. The persistence of several strains on chicken meat, beef and pork has been shown to be comparable for both species (Hauschild *et al.*, 2021b). However, laboratory conditions have already been proven incapable to imitate the spoilage system on meat from retail in studies with other meat spoiling bacteria (Koutsoumanis and Nychas, 1999; Tsigarida and Nychas, 2001). Poultry meat, beef and pork exhibit multiple differences with relevance for bacterial growth and it is impossible to trace single traits up to growth advantages for either *P. phosphoreum* or *P. carnosum*. Nevertheless, available data allow for general assumptions in this context. All meat types are colonized by specific microorganisms that originate from the livestock, thus providing a characteristic community for interaction, respectively (Bolton *et al.*, 2002; Pearce *et al.*, 2006; Stanbridge and Davies, 1998). Given the documented differences regarding the impact of co-contaminants on the growth of *P. phosphoreum* and *P. carnosum* strains (Hauschild *et al.*, 2021b; Hauschild *et al.*, 2021c), preferences regarding interacting species and with this the meat type may exist. Additionally, poultry meat, pork, beef and fish exhibit some variations with respect to the nutrient composition, even considering its general similarity. This concerns especially the availability of fat and the respective fatty acid composition (Belitz *et al.*, 1999; Bohrer, 2017; Cerny, 2012; Pereira and Vicente, 2013). Chicken meat contains for example approximately ten times higher concentrations of linoleic acid than beef (Belitz *et al.*, 1999). Consequently, dominance on a specific type of meat could result from the availability of specific lipids, even if no evident difference was detected regarding the genetic basis of fatty acid- and glycerol metabolism in *P. phosphoreum* and *P. carnosum* (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). In contrast to the suggested existence of preferences on species level, adaptation of strains towards a specific meat type was confuted (Fuertes-Perez *et al.*, 2019; Hauschild *et al.*, 2021b). Indeed, having in mind the assumed contamination of meat by established in-house populations in industry, such specialization would not be expected to occur unless colonies from spoiled meat and populations from the processing environment are in exchange. However, strain-specific differences could certainly result from different in-house microbiota.

The occurrence of *P. phosphoreum* and *P. carnosum* on meat and the occurrence in marine habitats are thus interpreted to be linked on a very fundamental level. Photobacteria are not the only meat contaminating bacteria that occur also on fish/seafood and contribute to spoilage. Several LAB have been detected in association with meat and also marine fish/fish products, e.g. *C. maltaromaticum* and *C. divergens* (Leroi *et al.*, 1998). Isolates of *C. maltaromaticum* and *C. divergens* from meat and fish have been reported to form no habitat-specific phenotypic cluster (Groth Laursen *et al.*, 2005), which is in accordance with the absence of habitat related sub-populations of *Photobacterium* strains from meat and fish (Fuertes-Perez *et al.*, 2019). However, information on the correlation of both habitats is limited for carnobacteria likewise and their primary habitat, as well as the impact of cross-contamination, remains unidentified (Groth Laursen *et al.*, 2005; Pilet *et al.*, 1995). Speculations on the involvement of cross-contamination events in the distribution of photobacteria are confined to only few hints in literature. Re-contamination of smoked salmon during the washing/smoking process has been observed (Chaillou *et al.*, 2015) and also survival during cleaning and disinfection (Bagge-Ravn *et al.*, 2003). Additionally, studies have demonstrated a general major contribution of cross-contamination to the final spoilage community of meat during processing (e.g. (Hinton Jr *et al.*, 2002; Hinton Jr *et al.*, 2004)). Even if some scientists have stated elimination of photobacteria by hygienic measures (Møretrø *et al.*, 2016), existence of cross-contamination appears consequently possible. It has been speculated before that seafood-associated microorganisms can contaminate diced bacon by the addition of sea salt (Chaillou *et al.*, 2015), however, photobacteria occur mainly on non-salted meats and do not survive on pure salt (sea salt or NaCl). Beside of the possibility for its existence, details of the process of cross-contamination during the contamination of meat and (marine) fish by photobacteria are therefore elusive.

The idea to correlate the addition of (sea) salt and the occurrence of photobacteria on meat implies the well-documented requirement for NaCl for successful cultivation of this genus. Strains of *P. phosphoreum* and *P. carnosum* (and also of *P. iliopiscarium*) from meat grow only in presence of NaCl in laboratory conditions (Fuertes-Perez *et al.*, 2019), with *P. phosphoreum* strains preferring higher amounts of NaCl than *P. carnosum* strains (Hauschild *et al.*, 2021a). The respiratory chain of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 exhibits some traits that could give an explanation in this regard. Presence of the NADH dehydrogenase Nqr was detected in both strains (Hauschild *et al.*, 2021c) and this enzyme requires sodium ions for optimal activity (Bogachev *et al.*, 2001; Neehaul *et al.*, 2012; Unemoto and Hayashi, 1993). Additionally, Nqr is of relevance for optimal activity of the entire respiratory chain especially of marine bacteria (Unemoto and Hayashi, 1993) and can therefore be hypothesized to trigger a certain dependency of sodium. It has been documented that some alternative ions, e.g. magnesium, can replace sodium to some degree regarding activation of Nqr (Unemoto *et al.*, 1977). This is in accordance also with statements on the ion dependency of

marine bacteria for growth (MacLeod, 1968; Reichelt and Baumann, 1974), thus supporting the assumption of a general relevance of Nqr regarding the sodium requirement of *P. phosphoreum* and *P. carnosum*.

The sodium content of meat is considerably lower than the one of sea water and the one provided in laboratory media for cultivation (<100 mg sodium per 100 g meat; (Ruusunen, 2005)). Since photobacteria nevertheless reach high cell numbers on meat, presence of alternative substances with similar function to sodium can be speculated. Meat contains multiple ions in higher concentration than sodium, such as potassium (>230-400 mg/100 g) or phosphorus (>130-290 mg/100 g), as well as low concentrations of ions that have been reported as suitable alternatives for sodium regarding growth of marine Gram-negatives, such as magnesium (15-30 mg/100 g) or calcium (3-7 mg/100 g) (Williams, 2007; Zarkadas *et al.*, 1987). Even though sodium ions appear to be preferred by photobacteria, the broad spectrum of alternative ions is assumed to allow for functionality of ion-dependent metabolic processes on meat (e.g. transport). Requirement of high concentrations of NaCl could also result from a dependency for osmotic regulation. In this context, halophilism should be carefully separated from osmophilism. Strains of *P. phosphoreum* preferred higher concentrations of NaCl for growth, whereas strains of *P. carnosum* preferred (and tolerated) lower concentrations in a complex medium. Accordingly, the former could be termed rather halophilic and the latter rather osmophilic, concomitant with their suggested difference in adaptation towards the meat (Hauschild *et al.*, 2021a). The osmoregulation of strains growing on meat can be speculated to involve organic osmolytes instead of NaCl. Major organic osmolytes available on meat are taurine (Huxtable, 1992), carnitine (Demarquoy *et al.*, 2004) or glycine betaine (O'Byrne and Booth, 2002). Since the availability of organic osmolytes on meat has been evaluated as sufficient for the osmoregulation of bacteria (O'Byrne and Booth, 2002), it can be assumed to form the basis for the growth of photobacteria in absence of high concentrations of NaCl.

6.3 The packaging atmosphere has limited impact on the growth of photobacteria on meat

Since packaging constitutes an essential method for dealing with spoilage bacteria today (reviewed e.g. by (Cenci-Goga *et al.*, 2020)), it necessitates evaluation of growth and metabolic activity of photobacteria also in the context of different packaging atmospheres. *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 were investigated regarding their growth on MAP chicken meat in presence and absence of co-contaminants (Hauschild *et al.*, 2021b). Additionally, metabolic activity in response to the atmosphere with greatest impact (70% O_2/30% CO_2) was analyzed on the level of

transcription (Hauschild *et al.*, 2021c). Presented studies enable a detailed examination of the influence of MAP on the growth of *P. phosphoreum* and *P. carnosum* strains on meat. Observations are in accordance with the predictions of (Höll *et al.*, 2019), who have studied the transcription of photobacteria in general in response to different modified atmospheres during growth in a natural spoilage consortium on chicken meat. Here, *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 turned out to be comparably tolerant to CO_2, even independently of present/absent co-contaminants (Hauschild *et al.*, 2021b). This is in agreement with the low impact of MAP predicted by Höll *et al*. (2019). Application of MAP in the meat industry aims mainly at a restriction of the growth of Gram-negative spoilers (Cenci-Goga *et al.*, 2020; Church, 1993). The comparably high tolerance of photobacteria especially to CO_2 therefore constitutes a major aspect of its relevance to meat spoilage. While vulnerability to combined high concentrations of CO_2 and O_2 was detected for strains of both species under laboratory conditions (Hauschild *et al.*, 2021b), proven occurrence of high cell numbers in this atmosphere on meat from retail (e.g. (Chen *et al.*, 2020; Fuertes-Perez *et al.*, 2019; Stoops *et al.*, 2015)) confirms its limited effectiveness towards growth of *Photobacterium*. Support has been obtained from the work of Bassey *et al*. who have reported increasing cell numbers of photobacteria in 70% O_2/30% CO_2 atmosphere during growth within the natural spoilage community of pork loins (Bassey *et al.*, 2021).

Even though effects are supposed to differ in the laboratory and on meat from retail, the general relation of *P. phosphoreum* and *P. carnosum* to O_2 and CO_2 is of interest. The major influence on the meat spoilage community has been attributed already to both gases before (Erichsen and Molin, 1981; Newton and Rigg, 1979). In any case, packaging gases exert a stronger selective pressure on spoilage bacteria than intrinsic factors of the meat itself (Pothakos *et al.*, 2014). Some photobacteria have been mentioned to react with reduced growth to (high levels of) O_2 (Dalgaard, 1995c; Guldager *et al.*, 1998; Nealson, 1978), which is in accordance with the effect observed for 70% O_2/30% CO_2 within the scope of this work (Hauschild *et al.*, 2021b). Possible explanations can be obtained from the performed analysis of the transcription of photobacteria under MAP. It revealed signs for enhanced oxidative stress in strains of *P. phosphoreum* and *P. carnosum* under these conditions (Hauschild *et al.*, 2021c). Undesirable oxidative reactions can concern lipids and fatty acids, DNA and RNA, as well as multiple enzymes of central metabolic pathways e.g. aconitase, alcohol dehydrogenase, ATPase or enolase in this context (Cabiscol *et al.*, 2000; Gardner *et al.*, 1994; Matallana-Surget *et al.*, 2013; Tamarit *et al.*, 1998). However, although presence of oxidative stress was detected in 70% O_2/30% CO_2 atmosphere, strains of both species possess also extensive features for optimal utilization of O_2. They have a functional heme biosynthesis pathway and also multiple versions of some complexes of the respiratory chain, e.g. NADH dehydrogenase (Nuo, Nqr; partial in some cases), cytochrome c oxidase (Cyo, Cyd, Cox, Cco; partial in some cases) or multiple types of cytochromes (type b, c, c1) (Fuertes-Perez *et al.*,

2021; Hauschild *et al.*, 2021c). A variable set of respiratory enzymes combined with presence of heme biosynthesis has been suggested to allow for high O_2 consumption per cell in this context before (as shown for *B. thermosphacta* by (Kolbeck *et al.*, 2019)). This is consistent with the growth improvement observed for *P. phosphoreum* and *P. carnosum* strains by presence of 21% O_2 in the packaging atmosphere of meat (Hauschild *et al.*, 2021b). It suggests that both species perform preferentially aerobic respiration for energy generation, even if transcriptomic data showed expression also of all necessary genes for anaerobic respiration and fermentation during growth on meat (Hauschild *et al.*, 2021c). Interestingly, other species have been demonstrated in this context to switch expression of their anaerobic respiration off, if >2% O_2 are available (Proctor and Gunsalus, 2000; Tseng *et al.*, 1996). It shows that *Photobacterium* strains exhibit a general strategy of stand-by activity regarding central metabolic pathways, which is in agreement with the suggested adaptations towards sudden substrate availability (compare chapter 6.2).

Utilization of O_2 can be further improved by the activity of the luciferase reaction expressed by many *P. phosphoreum* strains (Fuertes-Perez *et al.*, 2019), especially in case of low availability of this gas. If the environmental conditions do not allow cytochrome activity, either due to low O_2 availability or due to lack of iron, the luminescence reaction can function as terminal oxidase instead, thus allowing for oxidation of coenzymes and consequently respiration (Makemson and Hastings, 1986; Nealson and Hastings, 1979). The maximum capacity of the luciferase reaction can account for up to 20% of the absorbed O_2 in oxic conditions (reviewed by (Dunlap and Kita-Tsukamoto, 2006)). Therefore, its contribution is considered to provide a relevant growth advantage in case of low O_2 availability (Nealson and Hastings, 1979). It supports again a certain independence of photobacteria of the applied packaging gases in the context of MAP.

The effect of CO_2 on photobacteria from meat turned out to be comparably low (Fuertes-Perez *et al.*, 2019; Hauschild *et al.*, 2021b), despite of the well-documented negative impact of this gas on spoilage bacteria (Garcia-Gonzalez *et al.*, 2007; Gill and Tan, 1980; Sivertsvik, 2007). Support has been obtained from studies reporting relevant growth of photobacteria in high CO_2 modified atmosphere e.g. on roasted duck (Chen *et al.*, 2020) or minced pork (Cauchie *et al.*, 2020). Since other meat spoilers, such as *B. thermosphacta*, experience disadvantages in high CO_2 atmosphere, photobacteria as less sensitive species can consequently become dominant (Devlieghere and Debevere, 2000; Emborg *et al.*, 2002; Gornik *et al.*, 2013). Still, a certain growth reduction by high levels of CO_2 exists also for *P. phosphoreum* and *P. carnosum* strains from meat (Hauschild *et al.*, 2021b). The effect of CO_2 is not yet fully understood but may consist of multiple factors, including changes of membrane physiology and intracellular pH (Kolbeck *et al.*, 2021b; Sears and Eisenberg, 1961), as well as increased carboxylation/decarboxylation reactions and feedback inhibition of those (Leeson, 1987). Evidence for presence of at least the latter was demonstrated for *P. phosphoreum* TMW2.2103 on chicken meat

(Hauschild *et al.*, 2021c). Thus, CO_2 as well as O_2 as packaging gases trigger an effect on *P. phosphoreum* and *P. carnosum* strains from meat. Observations thereby deduce a direct impact of the gases itself, even given the assumed limited effectiveness on photobacteria on meat from retail. Additionally, an indirect impact via effects on co-contaminants may contribute, since sensitivity of co-contaminants to either one of the gases or both is well-documented (e.g. (Chaix *et al.*, 2015; Wang *et al.*, 2017)). The comparably high tolerance of photobacteria to MAP can be consequently assumed to result from presence of efficient defense strategies.

The modified atmosphere with greatest impact on the growth of *P. phosphoreum* and *P. carnosum* strains on meat was shown to combine high concentrations of O_2 (70%) and CO_2 (30%) (Hauschild *et al.*, 2021b). Since observed effects were thereby unproportionally strong, a synergistic mechanism of both gases appears likely. This might include e.g. triggered aerobic respiration by presence of O_2 and simultaneous inhibition of essential enzymes by CO_2. Reduced respiratory activity in presence of CO_2 has already been reported for other meat spoilers before, e.g. for *Pseudomonas* (Gill and Tan, 1980). However, the much weaker impact of moderate O_2 (21%)/high CO_2 (30%) atmosphere on the growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 (Hauschild *et al.*, 2021b) and the absence of a distinct effect of 70% O_2/ 30% CO_2 atmosphere on the transcript numbers of respiratory enzymes (Hauschild *et al.*, 2021c) refute a direct interrelation of both gases. Instead, it is the combined presence of two stressors that is assumed to demonstrate enhanced effectiveness. This assumption is in accordance with the commonly applied hurdle technique in industry, based on the effectiveness of the sum of multiple hurdles against bacterial growth (Erkmen and Bozoglu, 2016a). In this context, presence of high O_2 content has already been proposed to enhance the sensitivity of food-associated bacteria to additional hurdles, such as elevated CO_2 content (Amanatidou, 2001). The combined effect of high O_2/high CO_2 observed here could result from enhanced energy consumption for protection of oxidative stress and of CO_2 stress at a time. Additionally, the assumed stand-by activity of multiple metabolic routes in *P. phosphoreum* and *P. carnosum* (Hauschild *et al.*, 2021c) is expected to account for a considerable energetic burden, thus increasing the vulnerability to cumulated energetic expenses. *P. phosphoreum* strains exhibited sensitivity also to cumulated pressure- and osmotic stress in accordance (Hauschild *et al.*, 2021a). However, the strategy of stand-by activity may nevertheless come along with growth advantages in some cases, since it allows fast exploitation of O_2 and nutrients as soon as they are available, as described in chapter 6.2. Many niches in marine environment can be characterized by low levels of both in that respect (e.g.(Boyd and Hurd, 2009; Emerson and Bushinsky, 2014)), thus connecting remnants of a possible marine provenance of *P. phosphoreum* and *P. carnosum* strains with its consequences for the growth in meat systems.

Literature reports on a possible impact of modified atmosphere on the biofilm formation of *Vibrio parahaemolyticus* (Qian *et al.*, 2020), a species with relevance in seafood spoilage and close

phylogenetic relationship to the genus *Photobacterium* (Su and Liu, 2007). Since biofilm formation can play a role in resistance to hygiene interventions (Møretrø and Langsrud, 2017) and adhesion to the meat surface (Piette and Idziak, 1991), vulnerability of this process to MAP can affect the spoilage contribution of respective bacteria. However, even though modified atmosphere with 70% O_2/30% CO_2 influenced the growth of *P. phosphoreum* and *P. carnosum* strains on meat (Hauschild *et al.*, 2021b), its impact on the expression of genes with relevance in biofilm formation was confined to only few cases and irrelevant regulations (Hauschild *et al.*, 2021c). Consequently, the impact of MAP on biofilm formation of photobacteria on meat can be considered as low, which is in agreement with the suggested overall low impact of MAP on the growth of *P. phosphoreum* and *P. carnosum.*

6.4 Photobacteria integrate in the meat spoilage consortium and interact differently with co-contaminants

Investigation of *P. phosphoreum* and *P. carnosum* in single cultures is suitable to evaluate general capabilities. However, evaluation of their relevance to meat spoilage requires consideration of their integration within the spoilage community. Variability during the development of their cell counts on spoiling meat (compare introduction) implies presence of a factor with considerable impact on growth and assertiveness. A contribution of the interaction of *Photobacterium* strains with co-contaminants appears likely in this context. To explore the response of *P. phosphoreum* and *P. carnosum* strains to other meat spoilers, cells were cultivated alone and in presence of co-contaminants on meat in different modified atmospheres. Strains of both species grew in presence of the co-contaminant, adapted their gene expression in response and were dominant in case of high initial cell counts ((Hauschild *et al.*, 2021b; Hauschild *et al.*, 2021c). The studies prove for the first time interaction of both species with characteristic competitors of the meat system and successful competition at advantageous conditions.

Growth in presence of co-contaminants and accompanying regulation of genes was demonstrated to differ considerably between strains of *P. phosphoreum* and *P. carnosum* (Hauschild *et al.*, 2021b; Hauschild *et al.*, 2021c). It points out that the strains experience co-contamination in a very different manner. *P. phosphoreum* TMW2.2103 exhibited signs of elevated stress in presence of *Ps. fragi*/*B. thermosphacta* and MAP (Hauschild *et al.*, 2021c) and a tendency for reduced growth in accordance (Hauschild *et al.*, 2021b), whereas growth of *P. carnosum* TMW2.2149 was improved in this situation. The response of *P. phosphoreum* TMW2.2103 supports the assumption of a strong impact of cumulated stressors on photobacteria (compare chapter 6.3). In that sense, the distinguishing trait of *P. carnosum* TMW2.2149 is suggested to be absence of the recognition of *Ps. fragi* and *B.*

thermosphacta as additional stressor or competitor under MAP. It provides support for advanced adaptation of this species to persist within the meat spoilage consortium and is consequently in accordance with observations on its low tolerance to high hydrostatic pressure and elevated NaCl concentrations (Hauschild *et al.*, 2021a), as well as the loss of bioluminescence and an expanded substrate versatility (Fuertes-Perez *et al.*, 2019). Specific adaptation to the meat spoilage community has been reported also for other meat spoiling species beside of *P. carnosum*. *B. thermosphacta* for example shares its specific association with meat and meat processing with *P. carnosum* (Labadie, 1999) and has been demonstrated likewise to benefit from presence of other meat spoilers (e.g. *Pseudomonas,* enterobacteria; (Russo *et al.*, 2006)).

However, although the general responses of *P. phosphoreum* and *P. carnosum* strains to the presence of co-contaminants differed substantially, studies demonstrated for both species presence of situations in which they benefited from interaction. *P. carnosum* TMW2.2149 showed improved growth in presence of both co-contaminants and MAP, whereas *P. phosphoreum* TMW2.2103 benefited from co-contaminants under air atmosphere (Hauschild *et al.*, 2021b). This proves the ability for successful integration into the spoilage consortium for both strains and suggests a certain extent of adaptation towards the growth conditions of the meat system. Indeed, it is consequently questionable, if laboratory conditions and recreated meat spoilage systems are able to provide a stress-free growth environment for these bacteria at all. Growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 on meat in laboratory conditions (Hauschild *et al.*, 2021b) showed relevant discrepancy to actual *Photobacterium* cell counts on meat from retail (Fuertes-Perez *et al.*, 2019; Höll *et al.*, 2019), implying elevated stress in the former situation. Weak growth of photobacteria in laboratory conditions has also been reported for manually inoculated cold-smoked salmon before (Leroi *et al.*, 2015). Thus, observations suggest laboratory conditions to be unfavorable to a certain extent. Photobacteria can be considered rather as highly adapted regarding their environmental preferences and the provision of optimal growth conditions can be evaluated as challenging in consequence. Much information is available concerning factors with relevance in successful cultivation of photobacteria, such as presence of NaCl (Moi *et al.*, 2017), low temperature and narrow limits to exceed it (Farmer III and Hickman-Brenner, 2006; Reichelt and Baumann, 1973), presence of methionine (Baumann and Baumann, 1977), and in some cases even elevated ambient pressure (Nogi *et al.*, 1998). In accordance, some so far unknown factors may exist that photobacteria require for optimal growth and that are unintentionally eliminated during reduction of the meat spoilage system to a manageable dimension. Since one of the main differences of laboratory conditions compared to meat from retail is its simplified spoilage community (compare e.g. (Gribble and Brightwell, 2013; Russo *et al.*, 2006), and given the growth improvement observed for strains of *P. phosphoreum* and *P. carnosum* by co-contamination under air/modified atmosphere, interaction is believed to be essential.

Additional support is obtained from the observation that modified atmosphere with 70% O_2/30% CO_2 reduced the growth of *P. phosphoreum*/*P. carnosum* strains on meat only, if they grew without a natural spoilage community (compare (Hauschild *et al.*, 2021b)). In presence of such a community, *Photobacterium* cell counts have been shown to increase considerably from 0.96 log_{10}(CFU/g) to 4.06 log_{10}(CFU/g) within 28 days on super-chilled pork loin (Bassey *et al.*, 2021).

Regarding the type of interaction, observations suggest rather an indirect interaction of *P. phosphoreum*/*P. carnosum* strains and *B. thermosphacta*/*Ps. fragi* strains than a direct one. In case of comparable initial cell counts of *Photobacterium* and co-contaminant, none of them became dominant within 7 days (Hauschild *et al.*, 2021b), thus pointing at absence of quick direct inhibition. However, dominant initial cell counts of one species results in dominance of this species also at the end of spoilage (Hauschild *et al.*, 2021b). Correlation of dominance and high initial cell number has already been suggested in literature before (Gram and Huss, 1996), even referring to the growth of photobacteria on meats (Stoops *et al.*, 2015). In this context, also the assertiveness of *Pseudomonas* and *B. thermosphacta* as common co-contaminants of photobacteria has been documented to depend on their initial cell density in co-culture (Gill and Newton, 1977). These observations highlight the essential role of the initial contamination for the subsequent spoilage process.

High cell numbers of photobacteria have been reported to occur especially during the late stage of spoilage in multiple cases (e.g. (Jääskeläinen *et al.*, 2016; Reynisson *et al.*, 2009)). Mostly, these cases have been characterized by low CO_2 content in the packaging atmosphere and photobacteria could therefore not benefit from the inhibitory effect of this gas on other species. However, beside of observations on the effect of CO_2, these cases also allow for speculations on the competition strategies of *P. phosphoreum* and *P. carnosum* strains on meat. Since competitive advantages at low temperature are mainly subject to high growth rates (Gill and Newton, 1977), and since the growth rates of photobacteria from meat are comparably low (Fuertes-Perez *et al.*, 2019), other species will be more competitive during early spoilage, as long as the CO_2 content is low. Nevertheless, photobacteria have been shown to become dominant at some point. This observation suggests on the one hand persistence of *P. phosphoreum* and *P. carnosum* strains within the spoilage community even if competitors prevail. On the other hand, it implies onset of competitive advantages for these species during late spoilage. The late stage of meat spoilage is characterized by progressive exhaustion of glucose and its derivates (Gill, 1976). Bacterial growth that depends on these substrates will be limited by the diffusion rate of glucose from deeper tissue in this case (Garcia-Lopez *et al.*, 1998; Gill, 1976). Even if the genus *Photobacterium* has been mentioned in context of high levels of carbohydrate metabolism before (Li *et al.*, 2019b), strains of the species possess multiple metabolic alternatives to the utilization of glucose. Presence e.g. of a functional ADI pathway, of fatty acid- and glycerol degradation pathways and of gluconeogenesis (Hauschild *et al.*, 2021c) could therefore enable

photobacteria to avoid the growth-limiting competition for glucose during late spoilage. This can allow photobacteria to still reach high cell numbers even when growth of species with greater glucose dependency is limited.

With that in mind, little competition of photobacteria and *B. thermosphacta* is assumed if the glucose availability is sufficient. This, however, is only the case in the initial phase of meat spoilage. Comparably high dependency of the latter of glucose and few amino acids (Nychas and Skandamis, 2005) and its high dependency of O_2 (Kolbeck *et al.*, 2019) distinguishes its growth requirements clearly from photobacteria and their suggested growth strategy and independency of O_2. Even more, the lipolytic activity of *B. thermosphacta* in oxic conditions (Höll *et al.*, 2020; Kolbeck, 2021) can even provide free fatty acids and glycerol as possible substrates for the growth of photobacteria. However, since *B. thermosphacta* and photobacteria both possess the ability for efficient glycerol- and fatty acid exploitation (Hauschild *et al.*, 2021c; Höll *et al.*, 2020; Kolbeck *et al.*, 2020), substrate competition is expected to occur in case of limited carbohydrate availability, i.e. upon early depletion of glucose and glycogen in the early phase of meat spoilage. It predicts an antagonistic response of photobacteria to *B. thermosphacta* in this case that differs to the substrate spectrum-based co-existence that has been proposed for the interaction of several LAB and *B. thermosphacta* (Kolbeck *et al.*, 2020).

Pseudomonads and photobacteria share large parts of their substrate spectra (Fuertes-Perez *et al.*, 2021; Gill and Newton, 1977; Hauschild *et al.*, 2021c; Nychas and Skandamis, 2005), and substrate exhaustion is therefore likely to occur. Competition for available substrates was suggested in this context during growth of *P. phosphoreum* TMW2.2103 in co-contamination with *Ps. fragi* on MAP chicken meat (Hauschild *et al.*, 2021c). In general, literature provides multiple hints on a specific competitive interaction of photobacteria and pseudomonads on meat and dominance of either genus in accordance. High abundance of photobacteria has been demonstrated to come along with low abundance of pseudomonads and the other way round on chicken carcasses (Yu *et al.*, 2019), minced pork (Cauchie *et al.*, 2020), pork (Li *et al.*, 2019b), ostrich meat (Juszczuk-Kubiak *et al.*, 2021), donkey meat (Wei *et al.*, 2021) and also multiple cooked chicken and cooked ham samples (Duthoo *et al.*, 2021). Additionally, within a row of spoiling beef samples, an increased number of samples contaminated with relevant cell counts of photobacteria has been detected to come along with a reduced number of samples contaminated with relevant cell counts of *Pseudomonas* (Pennacchia *et al.*, 2011). It supports presence of (substrate) competition of both species, as well as involvement of another factor that constitutes the basis for assertion of either genus. The expression of the respiratory chain of *Pseudomonas* has been suggested to be vulnerable to the effect of CO_2 (compare chapter 3.4), whereas the effect on the expression of the respiratory chain of photobacteria (and therefore on their growth) can be predicted to be low (compare chapter 6.3). Consequently, the ability for respiration-based metabolism and concomitant high energy yield also in presence of CO_2 can be speculated to

result in competitive advantages for the latter genus under MAP. However, given the occurrence of both, photobacteria and pseudomonads, as dominant genus on meat, a certain contribution also of the initial cell number to assertion can be assumed.

P. phosphoreum TMW2.2103 and *P. carnosum* TMW2.2149 from meat were shown to possess multiple decarboxylases for production of amines and also metabolic pathways for production of ammonia (e.g. ADI pathway, (Hauschild *et al.*, 2021c)). Both, amines and ammonia, can be involved in the regulation of the (intracellular) pH in this context and contribute to withstand acid stress (Bao *et al.*, 2020; Höll *et al.*, 2020; Ryan *et al.*, 2009). The ability of photobacteria to alkalize could contribute to its comparably high tolerance to CO_2 by counteracting the pH reduction caused by this gas. Dissolved CO_2 turns partially into carbonic acid that can dissociate into carbonate and protons and consequently lower the pH (Daniels *et al.*, 1985; Farber, 1991). However, even if photobacteria are able to balance the pH itself, it will require energy investment. Consequently, presence of *Pseudomonas* and its ability to grow very fast and concomitantly elevate the external pH on meat (e.g. (Papadopoulou *et al.*, 2020)) can provide suitable pH conditions without the need for photobacteria to invest energy in this issue. In such cases, presence of *Pseudomonas* as co-contaminant could imply a certain growth benefit aside from the assumed substrate competition. This is in accordance with observed growth improvement e.g. of *P. phosphoreum* TMW2.2103 in presence of *Ps. fragi* under air atmosphere (Hauschild *et al.*, 2021b).

The pH value could also be involved in the response of photobacteria to acid-producing LAB. Literature documents a certain growth reduction of photobacteria in some cases of co-contamination e.g. with *C. divergens* or *Latilactobacillus sakei* (formerly *Lactobacillus sakei*; (Zheng *et al*., 2020)) (Joffraud *et al.*, 2006; Jorgensen *et al.*, 2000). LAB are well-known for strong pH reduction on meat (Pothakos *et al.*, 2015) that has been associated with competitive advantages during spoilage by an inhibitory effect on competitors before (Gram and Dalgaard, 2002; Jorgensen *et al.*, 2000). Interaction of photobacteria and LAB has already been evaluated, based on the application of the latter as protective culture in this context. Reduced production of off-odors by *P. phosphoreum* has been detected on cold-smoked salmon e.g. in presence of *Leuconostoc gelidum,* whereas presence of *Lactococcus piscium* had only little effect (Leroi *et al.*, 2015). Interaction of *P. phosphoreum* and *P. carnosum* especially with the latter is of interest to meat spoilage due to the proposed suitability of *Lactococcus piscium* for prevention of bacterial spoilage on meats (Hilgarth *et al.*, 2018c). An inhibitory impact of *Lactococcus piscium* on photobacteria has been demonstrated, but has been proven to be highly strain-specific (e.g. (Matamoros *et al.*, 2009)). Beside of the so far unexplored direct inhibitory effect of *Lactococcus piscium* on photobacteria, both share a broad substrate spectrum including utilization of glycerol (Andreevskaya *et al.*, 2015; Fuertes-Perez *et al.*, 2021). Additionally, both possess the ability for dominance also on high CO_2-atmosphere packaged meats (Chaillou *et al.*, 2015; Saraoui *et al.*, 2016).

These similarities raise the possibility of substrate competition and also of competitive growth in high CO_2 atmosphere. Aiming at an inhibition of *Photobacterium* contaminants on meat, utilization of LAB and especially *Lactococcus piscium* could therefore prove suitable especially for MAP products. The extent of the speculated substrate competition nevertheless remains to be elucidated, since the rather plant-related carbohydrate substrate spectrum of *Lactococcus piscium* (Andreevskaya *et al.*, 2015) and the more pronounced amino acid utilization of photobacteria and its additional ability to exploit ribose (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c) do not exclude the possibility of co-existence. However, given the suggested importance of interaction for the growth of photobacteria on meat, utilization of competitive species can be evaluated as promising strategy to limit the contribution of photobacteria to meat spoilage.

6.5 Photobacteria are relevant contributors to meat spoilage on MAP meats

Even if *Photobacterium* strains are known for efficient production of biogenic amines (Bjornsdottir-Butler *et al.*, 2018; Bjornsdottir-Butler *et al.*, 2020), their main importance in the context of meat spoilage does not only result from exceptional spoilage activity. It is rather their ability to persist and grow on meats, even if MAP/VP and careful chilling as common methods for spoilage retardation are applied. Since both methods represent approved standards in the meat industry to date, occurrence of photobacteria can be regarded as subsequent issue of an otherwise successful spoilage management.

Bacterial spoilage comprises perceptible and non-perceptible changes of the meat, of which several can result from growth and activity of *P. phosphoreum* and *P. carnosum*. Both species reach cell counts >8 $\log_{10}$(CFU/g) on meat (Fuertes-Perez *et al.*, 2019) and can therefore trigger visible changes of the meat appearance already by growth itself (Figure 5). Cell densities of ≥6 $\log_{10}$(cells/g) or (cells/ml) have been reported to result in biofilm formation and consequently apparent slime formation on the meat surface (Gram *et al.*, 1999; McKee, 2012). Additionally, visible colonies can develop in this context, contributing to an overall impression of spoilage (reviewed e.g. by (Ayres, 1960; Jay *et al.*, 2005)). Apart from this, *P. phosphoreum* and *P. carnosum* strains from meat possess extracellular lipases and multiple metabolic routes for (intracellular) protein exploitation (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). Lipid- and protein degradation will change the visual impression of the meat surface likewise (e.g. (Nowak and Piotrowska, 2012)). Based on the suggested preference of *P. phosphoreum* and *P. carnosum* strains for fatty acid- and protein metabolism during interaction in the meat spoilage community (compare chapter 6.4, (Hauschild *et al.*, 2021c)), a relevant contribution of these species to lipid- and protein degradation seems likely in this context. Their spoilage contribution can therefore be speculated to depend rather on the interaction with the spoilage community than directly on the

applied modified atmosphere. This assumption is in accordance with the literature attributing a particular relevance to the interaction within spoilage communities during the development of spoilage (Pothakos *et al.*, 2015).

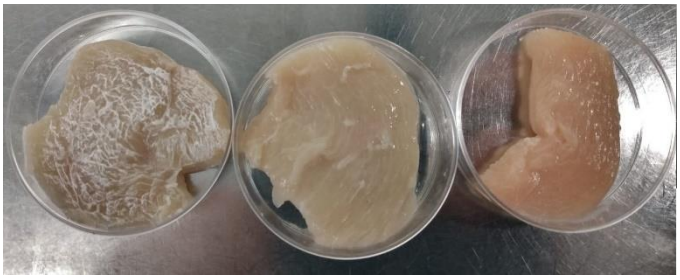

Figure 5 Visual changes of the surface of chicken meat by growth of P. phosphoreum *and* P. carnosum *strains. Meat was incubated for 7 days at 4°C in modified atmosphere with 21% O2/30% CO2/49% N2. Left:* P. phosphoreum *TMW2.2103. Middle*: P. carnosum *TMW2.2149. Right: negative control.*

Products of lipid- and protein degradation can cause strong off-odors and off-taste (Flores, 2017; Nowak and Piotrowska, 2012). Since *P. phosphoreum* and *P. carnosum* strains from meat are able to metabolize substrates to multiple volatiles, including e.g. acetoin, diacetyl, H_2S and putrescine (Hauschild *et al.*, 2021c), participation in off-odor formation on spoiling meat can be assumed. Support has been obtained by reports on the production of multiple volatiles by *P. phosphoreum* on spoiled salmon samples (Macé *et al.*, 2014). Regarding formation of TMA as central volatile during fish spoilage, strains of *P. phosphoreum* and *P. carnosum* were shown to express the necessary genes on MAP chicken (Hauschild *et al.*, 2021c). Even if meat contains less of the substrate TMAO than marine fish (Bekhit *et al.*, 2021; Cho *et al.*, 2017), the spoilage contribution of photobacteria on meat can consequently be assumed to include TMA production. However, given the differences in substrate availability on meat and fish, off-odors and also other signs of spoilage caused by photobacteria are speculated to differ on both products.

The available data allow also for speculation on the role of *P. phosphoreum* and *P. carnosum* in issues regarding food safety. Strains of both species from meat express genes for the production of multiple biogenic amines during growth on MAP chicken meat (Hauschild *et al.*, 2021c) and are consequently assumed to contribute to the health concerning effects of these substances (compare chapter 3.7). Participation of *P. phosphoreum* in histamine formation is still under consideration in this context. Reports on absence of the common histidine decarboxylase gene (Fuertes-Perez *et al.*, 2021; Höll *et al.*, 2019) and histamine-producing strains that were wrongly assigned to this species on the one hand (Ast *et al.*, 2007; Bjornsdottir-Butler *et al.*, 2016), face reports on significant histamine production by this species on the other hand (Bjornsdottir-Butler *et al.*, 2018; Emborg and Dalgaard, 2006). Recent investigations have demonstrated that *P. phosphoreum* expresses an enzyme with histidine

decarboxylating activity and only 12% sequence identity to previously known histidine decarboxylase genes (Bjornsdottir-Butler *et al.*, 2020). It is consequently likely that this specific gene has been overlooked in the past (e.g. by (Höll *et al.*, 2019)) and that strains of the species are indeed able to produce histamine during spoilage. Support has been obtained from the work of Fuertes-Perez who have confirmed presence of the new histidine decarboxylase gene in *P. phosphoreum* strains isolated from meat (Fuertes-Perez *et al.*, 2021). Histamine formation depends on the availability of histidine as substrate (Taylor and Woychik, 1982) and the NaCl concentration and pH value of the environment (Emborg *et al.*, 2002; Takahashi *et al.*, 2015). It implies differences in histamine formation by *P. phosphoreum* strains in dependence of the meat product, as it has been suggested for fish products before (Emborg *et al.*, 2002). Strain *P. phosphoreum* TMW2.2103 from meat expresses also genes for putrescine- and cadaverine production on MAP chicken (Hauschild *et al.*, 2021c), and these products can enhance the toxicity of histamine as explained before. Strains of *P. carnosum* have been shown to lack the well-known gene for histidine decarboxylase (Fuertes-Perez *et al.*, 2021; Hauschild *et al.*, 2021c). However, with respect to the novel enzyme sequence discovered by (Bjornsdottir-Butler *et al.*, 2020), and also given the lack of actual measurements of histamine formation, it is impossible to exclude the possibility of histidine decarboxylation by *P. carnosum*. Fuertes *et al.* have identified one out of 15 investigated *P. carnosum* strain isolated from MAP salmon that possessed the novel histidine decarboxylase gene, thus supporting this statement. In comparison 11 of 26 investigated strains of *P. phosphoreum*, harbored that gene. Consequently, the risk for histamine formation can be predicted to be lower in presence of *P. carnosum* than in presence of *P. phosphoreum*. Still, the discovery of other yet undiscovered, non-homologous genes in *P. carnosum* may change that view in the future.

The greatest spoilage potential on chilled meat in aerobic conditions has been attributed to Gram-negative bacteria, e.g. pseudomonads, *Acinetobacter* and *Moraxella*, and inhibition of those has been mentioned as most promising strategy to retard spoilage (Garcia-Lopez *et al.*, 1998). In this context and given their assumed tolerance to MAP, tolerance also to alternative methods for inhibition affects the relevance of *P. phosphoreum* and *P. carnosum* in meat spoilage. The most efficient method to eliminate *Photobacterium* contaminations on meat products is assumed to be freezing so far. Freezing has been demonstrated to remove *P. phosphoreum* entirely from MAP cod (Guldager *et al.*, 1998), MAP salmon (Emborg *et al.*, 2002) and Japanese dried sardine (Kanki *et al.*, 2004) and to result in enhanced shelf life due to lower TMA- and biogenic amine production thereof (Emborg *et al.*, 2002; Guldager *et al.*, 1998). It is in accordance with absence of photobacteria also on previously frozen seafood (Fuertes-Perez *et al.*, 2019) and represents consequently a promising strategy for elimination. However, freezing is not applicable in all cases and can come along with negative sensory changes of the meat, e.g. surface dehydration and oxidative rancidity (Nollet, 2012). Other methods beside of freezing have been proven to be active to a certain extent against *Photobacterium* contaminations.

Application of essential oils (Mejlholm and Dalgaard, 2002), citrus fruit peel (Yavuzer and Kuley, 2020), grape seed-/chestnut-/olive-extract (Pini *et al.*, 2020) or tea polyphenol (Li *et al.*, 2022) have reduced growth of photobacteria in other studies before. These compounds additionally harbor the potential for utilization as natural ingredients during marination in the meat industry. Chemical inhibition, e.g. by ethylendiamintetraacetate (EDTA), has also been demonstrated to inhibit *P. phosphoreum* and to extent shelf life of MAP cod (Dalgaard *et al.*, 1998). However, EDTA is in focus of criticism due to its possible role in colon inflammation and –cancer, even if it is still accepted as food additive to date (Evstatiev *et al.*, 2021). NaCl as very common additive in the meat industry due to its low cost and multiple beneficial properties (Odeyemi *et al.*, 2020) can be assumed to be inefficient against photobacteria. Given their NaCl requirement and, in some cases, even preference of a high NaCl availability (Hauschild *et al.*, 2021a), this intervention could even be advantageous for the growth of *P. phosphoreum* and *P. carnosum* on meats.

Efficient reduction of the spoilage contribution of *P. phosphoreum* and *P. carnosum* on meats is therefore assumed to be mainly subject to a reduction of the contamination during processing. New strategies could nevertheless turn the impact of co-contamination on the growth and metabolic activity of both species to account, as it is already in focus of examinations on protective cultures.

7 Conclusions

The presented data allow for a prediction of the provenance of *Photobacterium* species isolated from meat, of likely contamination routes and concomitantly of possibilities to control the contamination in industrial production. Furthermore, it is possible to estimate the response of photobacteria to presence of *Ps. fragi* and *B. thermosphacta* as competitors on spoiling meat based on these data. This represents a decisive step for understanding the integration of the genus *Photobacterium* into the spoilage consortium of meat. The relevance of *P. phosphoreum* and *P. carnosum* to meat spoilage is also subject to its divergence to established knowledge about other Gram-negative meat spoiling bacteria. Their particular growth requirements complicate detection, and the control of their growth by application of MAP is comparably inefficient.

8 Abbreviations

A – surface
ADI – arginine deiminase
ANOVA – analysis of variances
B. – *Brochothrix*
BHI – brain heart infusion medium
BLAST – basic local alignment search tool
C. – *Carnobacterium*
°C – degree Celsius
CFU – colony forming units
cm – centimeter
CO_2 – carbon dioxide
DSMZ – Deutsche Stammsammlung von Mikroorganismen und Zellkulturen
EDTA – ethylenediaminetetraacetic acid
g – gram
h – hour
HHP – high hydrostatic pressure
KEGG – Kyoto encyclopedia of genes and genomes
l – liter
L. – *Leuconostoc*
LAB – lactic acid bacteria
logCPM – $\log_2$(counts per million)
logFC – $\log_2$(fold change)
MALDI-TOF MS – Matrix assisted laser desorption/ionization –time of flight mass spectrometry
MAP – modified atmosphere packaging
µ – micro (10^{-6})
min – minutes
ml – milliliter
mM - millimolar
MPa – Megapascal
MSM – meat simulation medium
Mt – million metric tonnes
N_2 – nitrogen
NCBI – National center for biotechnology information
nm – nanometers
O_2 – oxygen
OD_{600} – optical density at 600 nm
P. – *Photobacterium*
PCR – polymerase chain reaction
pH – negative decimal logarithm of hydrogen ion activity
Ps. – *Pseudomonas*
RAPD – randomly amplified polymorphic DNA
ROS – reactive oxygen species
rpm – revolutions per minute
s – seconds
SSO – specific spoilage organisms
ssp. – subspecies
TBE – Tris-HCL boric acid EDTA buffer
TCA – tricarboxylic acid
TMA – trimethylamine
TMAO – trimethylamine oxide
TMW – Technische Mikrobiologie Weihenstephan
Tris - Tris(hydroxymethyl)-aminomethane
UPGMW – unweighted pair group method with arithmetic mean
VP – vacuum packaging

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Sandra, thank you for everything that we shared in the last years. It is said that challenges weld together and I would dare to say that photobacteria are enough to create Siamese twins. In any case, it sometimes felt to me as working with another pair of my own hands when we were entertaining our fussy little lord rulers, pardon, lab rulers together. So, I guess, this proverb is true. Let’s keep in mind that there are very creative ways of blowing things up, that it is not the white mice secretly ruling the world but rather the photobacteria, and that one sometimes need a friend shouting ‘Fly, you fools’ to escape in difficult moments.

Sandra, danke für die vielen Ideen, die wir zusammen haben wahr werden lassen. Mit dir kann man Pferde stehlen gehen, dann lachend über eine Bergwiese davon wandern und schließlich am Lagerfeuer hitzig darüber diskutieren, ob man Gas A weglassen kann, um den Effekt von Gas B auf Mechanismus C sichtbar zu machen und damit Hypothese XY zu bestätigen. Es war eigentlich unwichtig, ob wir Luftballons und Luftschlangen verteilt, Wagen mit Goldfolie verziert, Überraschungskuchen gebacken oder im Büro Stangenbohnen gezogen haben, ob wir einen Tischkicker oder ein Abenteuer organisieren wollten: wir haben uns immer prima ergänzt!

Und meiner Familie. Was wäre ich nur ohne euch?

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11 List of publications and presentations

Publications in peer-reviewed journals

Hauschild, P., Röttig, A., Madkour M.H., Al-Ansari, A., Almakishah, N.H., Steinbüchel, A., 2017. Lipid accumulation in prokaryotic microorganisms from arid habitats. *Appl Microbiol Biotechn* 101, 2203-2216. https://doi.org/10.1007/s00253-017-8149-0

Röttig, A., **Hauschild, P.,** Madkour M.H., Al-Ansari, A., Almakishah, N.H., Steinbüchel, A., 2016. Analysis and optimization of triacylglycerol synthesis in novel oleaginous *Rhodococcus* and *Streptomyces* strains isolated from desert soil. J Biotechn 225, 48-56. https://doi.org/10.1016/j.jbiotec.2016.03.040

Fuertes-Perez, S.*, **Hauschild, P.*,** Hilgarth, M., Vogel, R.F., 2019. Biodiversity of *Photobacterium* spp. isolated from meats. *Front Microbiol* 10, 2399. https://doi.org/10.3389/fmicb.2019.02399
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Hauschild, P., Hilgarth, M., Vogel, R.F., 2021. Hydrostatic pressure- and halotolerance of *Photobacterium phosphoreum* and *P. carnosum* isolated from spoiled meat and salmon. *Food Microbiol* 99, 103679. https://doi.org/10.1016/j.fm.2020.103679.

Hauschild, P., Vogel, R.F., Hilgarth, M., 2021. Influence of the packaging atmosphere and presence of co-contaminants on the growth of photobacteria on chicken meat. *Int J Food Microbiol* 351, 109264. https://doi.org/10.1016/j.ijfoodmicro.2021.109264

Hauschild, P., Vogel, R.F., Hilgarth, M., 2021. Transcriptomic analysis of the response of *Photobacterium phosphoreum* and *Photobacterium carnosum* to co-contaminants on chicken meat (submitted).

Conference contributions

Hauschild, P., 2019. Identifizierung von *Photobacterium* spp. als unterschätzte Verderber auf Fleisch. Oral presentation at the 18. Fachsymposium für Lebensmittelmikrobiologe und –hygiene. Presented on 08.10.2019 in Kiel, Germany.

Hauschild, P., Hilgarth, M., 2019. Biodiversität von *Lactococcus piscium* und Nutzbarmachung als Schutzkultur. Oral presentation at the 18. Fachsymposium für Lebensmittelmikrobiologe und –hygiene. Presented on 08.10.2019 in Kiel, Germany.

Hauschild, P., 2019. Biodiversity of *Photobacterium phosphoreum* and *Photobacterium iliopiscarium* on meats. Poster presentation at the international conference Microbial Diversity 2019. Presented on 26.09.2019 in Catania, Italy.

Oral presentations at meetings of the steering committee (AiF 20113N)

Hauschild, P., Hilgarth, M., Vogel, R.F., 2018. Kontrolle psychrophiler Photobakterien beim Fleischverderb. Oral presentation at the annual project meeting of the AiF steering committee. Presented on 29.11.2018 in Freising, Germany.

Hauschild, P., Hilgarth, M., Vogel, R.F., 2019. Vorkommen, Nachweis und Biodiversität von *Photobacterium* spp. im Fleisch-System. Oral presentation at the annual project meeting of the AiF steering committee. Presented on 28.11.2019 in Freising, Germany.

Hauschild, P., Hilgarth, M., Vogel, R.F., 2020. Anpassung und Interaktion von *Photobacterium* spp. Oral presentation at the annual project meeting of the AiF steering committee. Presented on 26.11.2020 online in Freising, Germany.

Hauschild, P., Hilgarth, M., Vogel, R.F., 2021. Wachstum und Interaktion psychrophiler Photobakterien im Fleisch-System. Oral presentation at the annual project meeting of the AiF steering committee. Presented on 20.07.2021 online in Freising, Germany.

12 Appendix

12.1 Supplementary files to publication 1

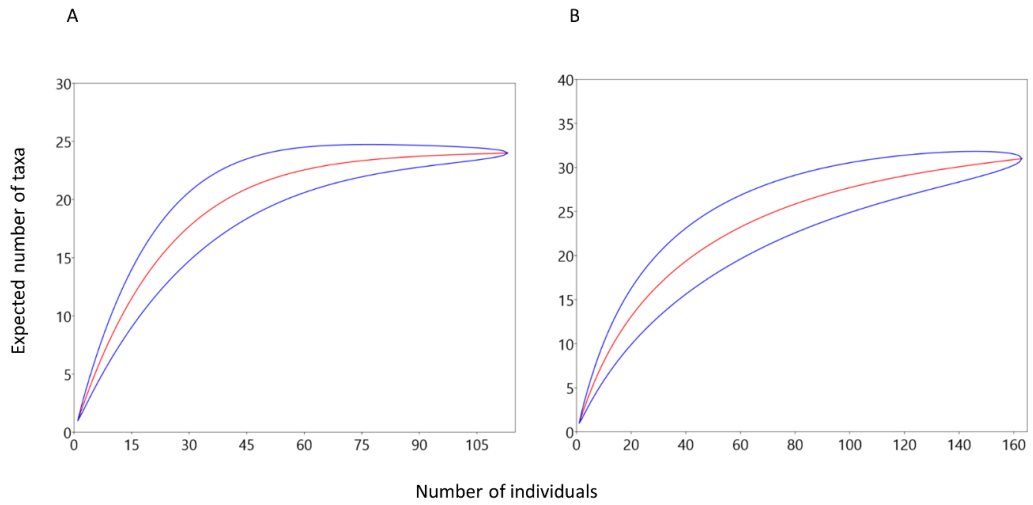


Figure S1 Rarefaction analysis visualization of A. *P. phosphoreum* and B. *P. carnosum*.
The blue line shows 95% confidence interval.

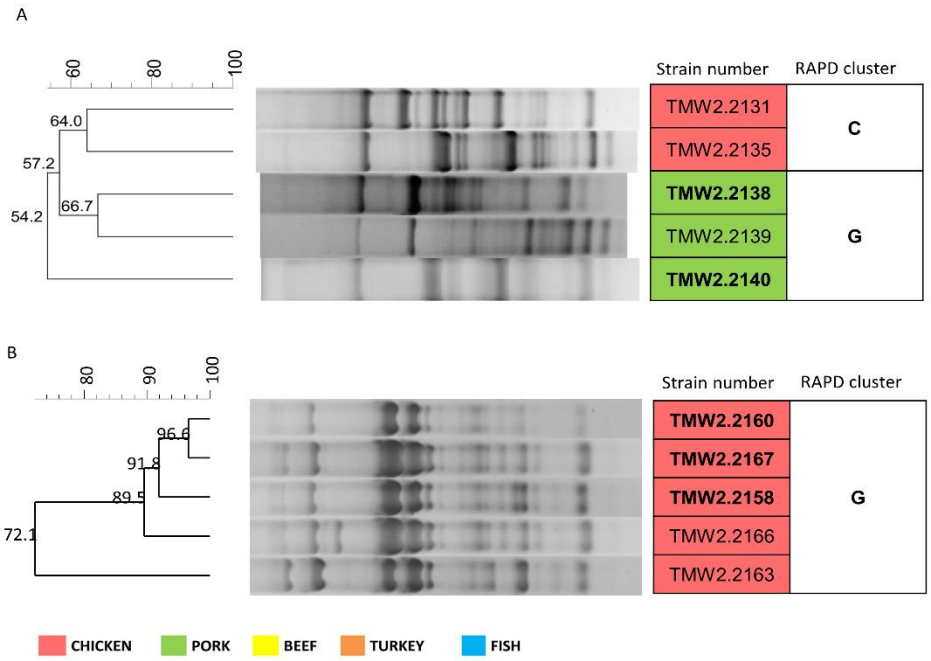


Figure S2 RAPD-clustering of the most similar strains of *P. phosphoreum* and *P. carnosum* used for the preliminary strain selection.
Hierarchical clustering was calculated with the unweighted pair group method with arithmetic mean (UPGMA), Dice similarity coefficient and 1 % tolerance. The RAPD-clustering of all selected isolates in the manuscript shows high similarity of strains TMW2.2138, TMW2.2140 from *P. phosphoreum* and strains TMW2.2160, TMW2.2167, TMW2.2158 from *P. carnosum,* respectively. However, initial comparison of all recovered isolates showed clear differences of the mentioned strains from A *P. phosphoreum* and B *P. carnosum*. Therefore, isolates were kept for the further study.

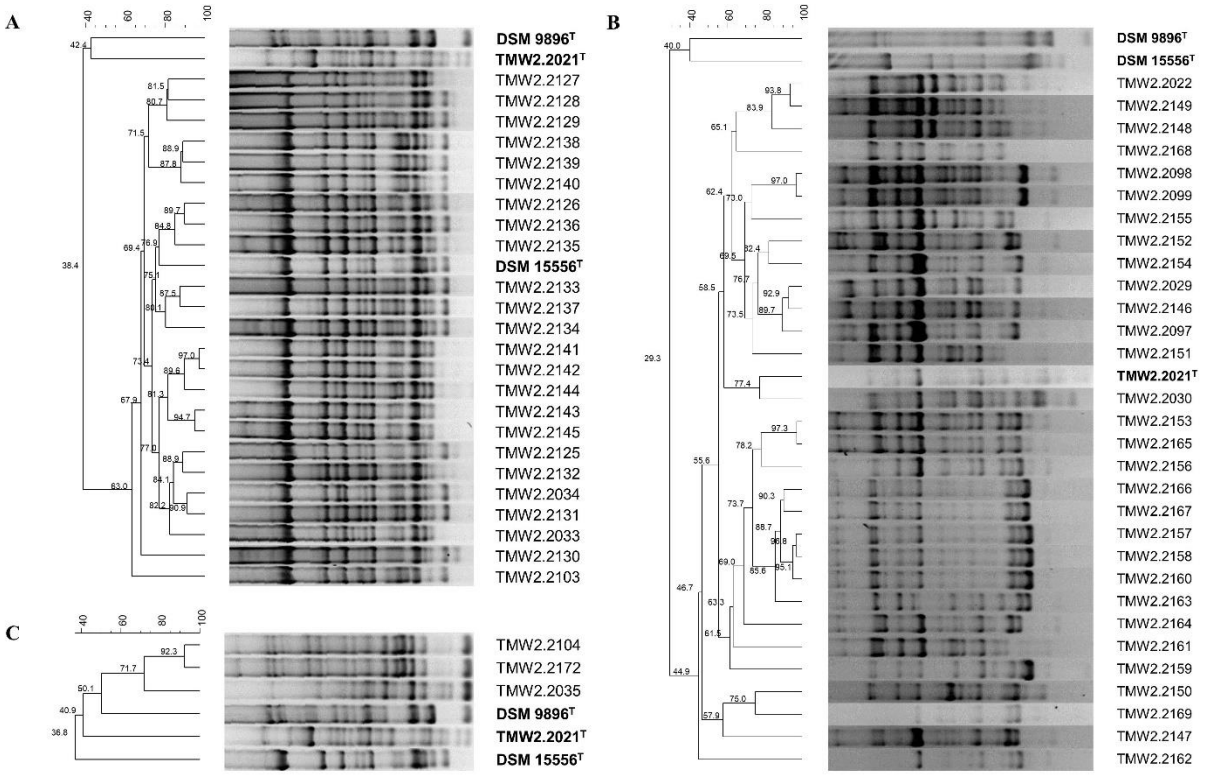


Figure S3 RAPD-clustering of the selected strains with additional primer M14V.
Strain differentiation based on primer M13V was confirmed with additional primer M14V. Hierarchical clustering was calculated with the unweighted pair group method with arithmetic mean (UPGMA), Dice similarity coefficient and 1 % tolerance. The similarity values are shown at the nodes of the tree. **A** *P. phosphoreum*, type strain DSM 15556^T, **B** *P. carnosum*, type strain TMW2.2021^T, **C** *P. iliopiscarium*, type strain DSM 9896^T.

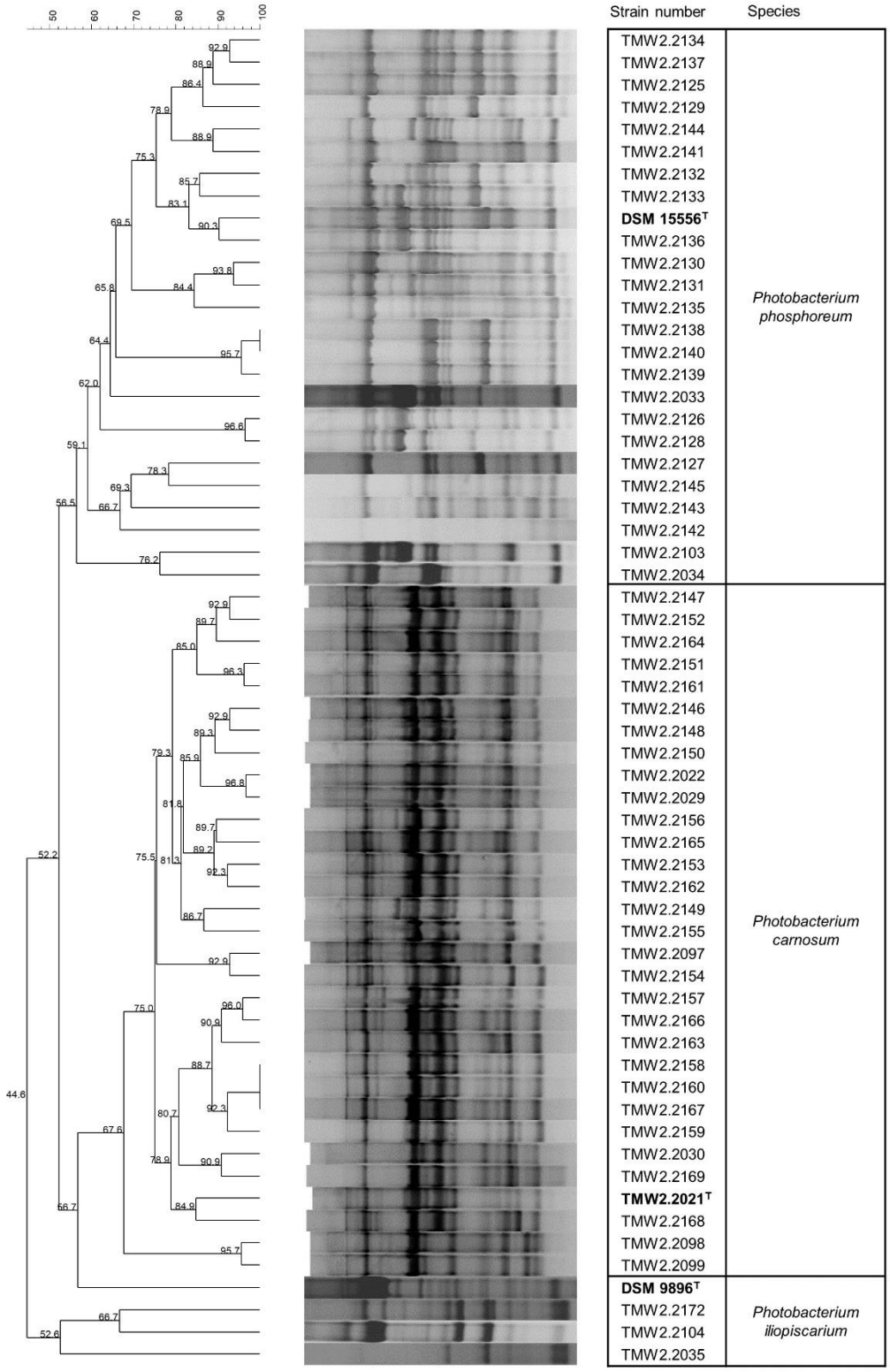


Figure S4 RAPD-clustering of all the selected strains of the three species of photobacteria together. Hierarchical clustering was calculated with the unweighted pair group method with arithmetic mean (UPGMA), Dice similarity coefficient and 1% tolerance. Similarity values are shown at the nodes of the tree. All strains of one species cluster together, and apart from the strains belonging to another species.

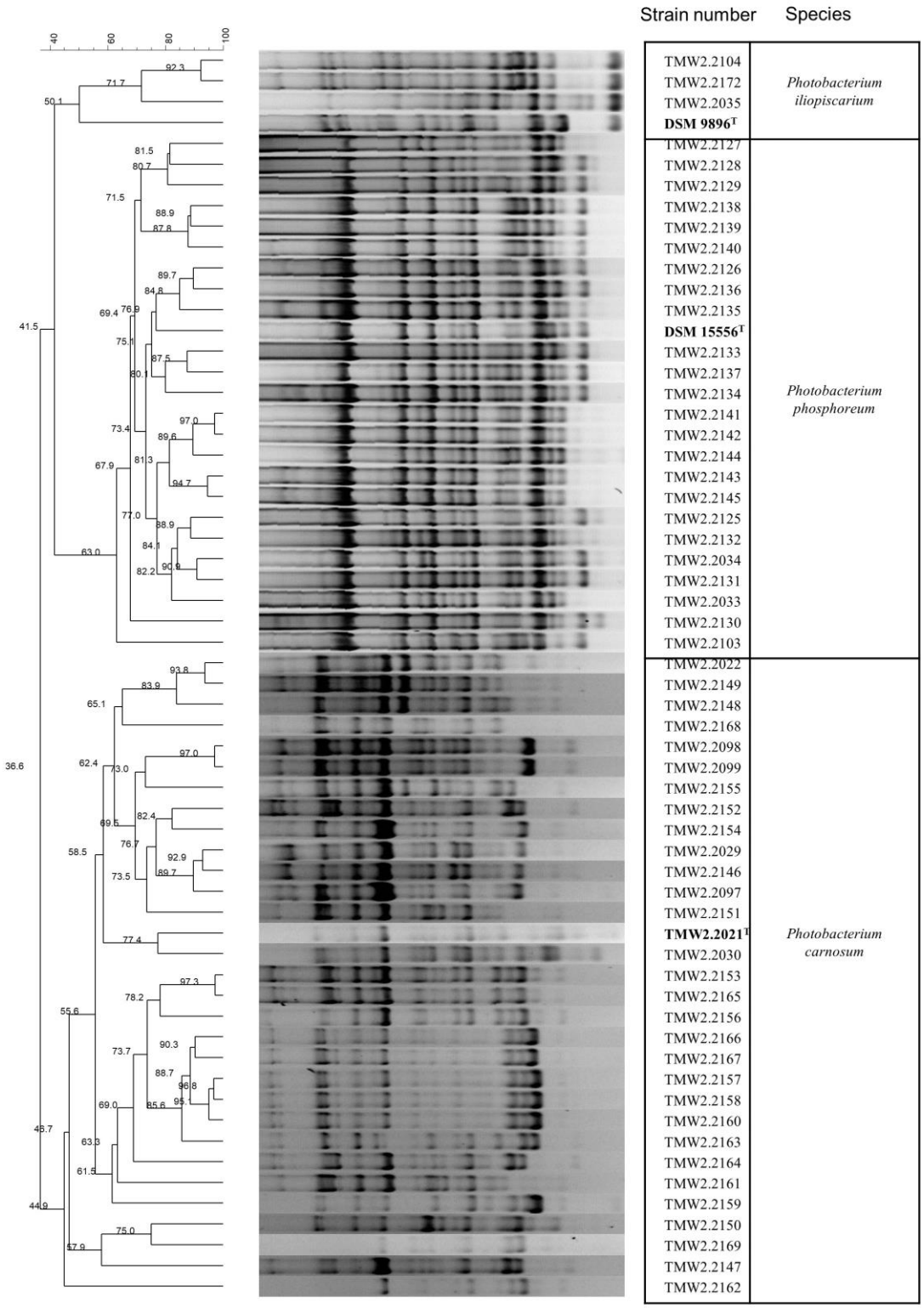


Figure S5 RAPD-clustering of the selected strains of all three species with additional primer M14V. Species differentiation based on primer M13V was confirmed with additional primer M14V. Hierarchical clustering was calculated with the unweighted pair group method with arithmetic mean (UPGMA), Dice similarity coefficient and 1 % tolerance. The similarity values are shown at the nodes of the tree. Selected strains of all three species were included. *P. phosphoreum*, type strain DSM 15556^T, *P. carnosum*, type strain TMW2.2021^T, *P. iliopiscarium*, type strain DSM 9896^T.

Table S1 Strains origin

Origin by type of packaging, type of meat, and sample, of the strains included in the study. The number of isolates screened refers to the number of isolates from that species that were recovered from the sample, and compared by RAPD PCR approach. The number of strains refers to the amount of strains obtained from the recovered isolates.

Package	Meat type	Contaminated/ Sampled	Sample	*P. carnosum* isolates screened	*P. carnosum* strains	TMW	*P. phosphoreum* isolates screened	*P. phosphoreum* strains	TMW	*P. iliopiscarium* isolates screened	*P. iliopiscarium* strains	TMW
MAP	Chicken*	5/15	1	-	-	-	4	2	TMW2.2033 TMW2.2034	1	1	TMW2.2035
			2	21	2	TMW2.2021** TMW2.2022** TMW2.2030** TMW2.2146 TMW2.2147	-	-	-	-	-	-
			3			TMW2.2029**	-	-	-	-	-	-
MAP	Beef*	2/2	1	-	-	-	2	1	TMW2.2103	-	-	-
MAP	Pork*	2/9	1	7	2	TMW2.2097 TMW2.2149	-	-	-	1	1	TMW2.2104
			2	-	-	-	-	-	-	1	1	TMW2.2172
MAP	Marinated chicken	2/3	1	99	15	TMW2.2151 TMW2.2152 TMW2.2153 TMW2.2155 TMW2.2156 TMW2.2157 TMW2.2158 TMW2.2159 TMW2.2160 TMW2.2161 TMW2.2162 TMW2.2163 TMW2.2164 TMW2.2165 TMW2.2166	26	12	TMW2.2134 TMW2.2137 TMW2.2129 TMW2.2132 TMW2.2133 TMW2.2136 TMW2.2130 TMW2.2131 TMW2.2135 TMW2.2127 TMW2.2126 TMW2.2128	-	-	-
			2	27	3	TMW2.2167 TMW2.2168 TMW2.2154	-	-	-	-	-	-
MAP	Marinated beef	1/3	1	-	-	-	35	5	TMW2.2144 TMW2.2141 TMW2.2145 TMW2.2142 TMW2.2143	-	-	-
MAP	Salmon	6/6	1	3	1	TMW2.2099	27	-	-	-	-	-
			2	1	1	TMW2.2098	10	-	-	-	-	-
Air	Chicken	1/4	1	3	1	TMW2.2150	-	-	-	-	-	-
Air	Beef	1/3	1	1	1	TMW2.2148	-	-	-	-	-	-
Air	Pork	1/3	1	-	-	-	7	3	TMW2.2138 TMW2.2139 TMW2.2140	-	-	-
Air	Marinated turkey	1/3	1	1	1	TMW2.2169	2	1	TMW2.2125	-	-	-

*Samples marked belong to the previous study by Hilgarth *et al.* (Hilgarth *et al.*, 2018a).

**Marked strains were obtained fromt he previous work by Hilgarth *et al.* (Hilgarth *et al.*, 2018b), from the *P. carnosum* sp. nov. species description.

Table S2. Antibiotic inhibition zone of *P. phosphoreum*.

Diameter values in mm of the inhibition zone observed for every antibiotic and each of the selected isolates of *P. phosphoreum*. The diameter of the antibiotic discs was measured as 6 mm, and therefore values of 6 in the table represent no inhibition zone observed.

Strain	Clindamycin DA 2 µg	Norfloxacin NOR 10 µg	Nalidixic acid NA 30 µg	Ampicillin AMP 10 µg	Sulphonamides S3 300 µg	Trimetoprim W 5 µg	Penicillin G P 5 µg	Streptomycin S 25 µg	Apramycin APR 15 µg	Rifampicin RD 5 µg	Gentamycin CN 10 µg	Kanamycin K 30 µg	Chloramphenicol C 30 µg	Erythromycin E 15 µg	Tetracyclin TE 30 µg
TMW 2.2103	6	22	19	6	6	6	6	10	6	13	15	14	35	13	6
TMW 2.2033	6	25	20	6	6	6	6	11	8	12	15	14	31	10	7
TMW 2.2034	6	25	20	6	6	6	6	10	6	11	14	13	27	8	6
TMW 2.2126	6	32	25	8	6	6	6	7	6	14	7	6	36	11	21
TMW 2.2127	6	34	25	6	6	6	6	10	6	13	8	6	38	11	6
TMW 2.2128	6	25	22	6	6	25	6	8	6	12	10	7	36	17	6
TMW 2.2138	6	25	20	11	6	6	6	13	11	18	16	14	20	24	6
TMW 2.2139	6	32	9	12	6	6	12	12	9	15	14	16	25	21	6
TMW 2.2140	6	33	25	11	6	6	10	11	10	17	10	12	20	25	6
TMW 2.2125	6	30	10	6	6	6	6	16	11	15	17	20	16	14	6
DSM 15556^T	6	29	9	6	6	6	6	12	10	13	13	20	13	15	6
TMW 2.2141	6	26	20	6	6	6	6	12	6	16	18	16	30	15	6
TMW 2.2142	6	25	10	6	6	6	6	12	10	12	23	20	15	17	6
TMW 2.2143	6	24	8	6	6	6	6	13	6	11	11	9	12	8	6
TMW 2.2144	6	21	9	6	6	6	6	10	10	14	14	22	18	11	6
TMW 2.2145	6	28	8	6	6	6	6	9	11	12	10	6	6	9	6
TMW 2.2129	6	21	20	6	6	6	6	14	8	15	18	15	23	12	6
TMW 2.2130	6	22	20	10	6	6	6	12	6	13	15	15	17	8	6
TMW 2.2131	6	31	8	6	22	23	6	18	11	10	19	20	16	15	6
TMW 2.2132	6	25	9	6	6	6	6	9	10	16	16	14	17	17	6
TMW 2.2133	6	26	8	6	6	6	6	8	6	13	15	11	15	10	6
TMW 2.2134	6	14	11	8	6	6	6	12	9	9	12	6	16	8	6
TMW 2.2135	6	26	8	9	6	6	6	6	10	11	17	13	13	7	6
TMW 2.2136	6	28	18	6	9	26	6	14	10	22	14	18	37	22	6
TMW 2.2137	6	21	18	6	6	6	6	6	9	12	12	11	6	8	6

Table S3. Antibiotic inhibition zone of *P. carnosum*.

Diameter values in mm of the inhibition zone observed for every antibiotic and each of the selected isolates of *P. carnosum*. The diameter of the antibiotic discs was measured as 6 mm, and therefore values of 6 in the table represent no inhibition zone observed.

Strain	Clindamycin DA 2 µg	Norfloxacin NOR 10 µg	Nalidixic acid NA 30 µg	Ampicillin AMP 10 µg	Sulphonamides S3 300 µg	Trimetoprim W 5 µg	Penicillin G P 5 µg	Streptomycin S 25 µg	Apramycin APR 15 µg	Rifampicin RD 5 µg	Gentamycin CN 10 µg	Kanamycin K 30 µg	Chloramphenicol C 30 µg	Erythromycin E 15 µg	Tetracyclin TE 30 µg
TMW 2.2021^T	6	26	40	20	6	28	6	20	16	26	20	30	46	12	12
TMW 2.2022	6	32	18	18	6	28	6	14	6	16	20	18	38	14	18
TMW 2.2029	6	36	28	32	6	16	10	20	6	22	22	14	46	8	26
TMW 2.2030	6	32	24	20	6	32	6	22	12	20	20	20	44	10	18
TMW 2.2098	6	36	26	24	6	36	6	22	6	24	24	16	40	16	22
TMW 2.2099	6	32	28	18	6	30	6	16	10	22	26	20	42	14	20
TMW 2.2097	6	38	28	24	6	36	6	28	6	20	20	18	50	12	24
TMW 2.2146	6	36	26	28	6	26	10	14	6	20	12	18	40	6	20
TMW 2.2147	6	28	22	10	6	24	6	14	10	20	16	28	42	6	14
TMW 2.2148	6	44	24	20	6	38	6	14	6	20	12	12	44	20	20
TMW 2.2149	6	32	22	26	6	36	6	12	6	18	14	16	44	18	20
TMW 2.2150	6	40	30	18	6	34	6	18	6	16	14	18	48	16	22
TMW 2.2151	6	44	26	6	6	20	6	12	6	24	12	24	44	18	16
TMW 2.2152	6	36	26	30	6	24	6	16	6	22	16	10	40	20	16
TMW 2.2153	6	40	22	24	6	22	6	22	16	26	18	22	44	16	18
TMW 2.2154	6	44	28	22	6	34	6	18	16	30	30	20	50	26	22
TMW 2.2155	6	46	30	24	32	38	6	24	6	18	18	14	48	24	20
TMW 2.2156	6	42	24	26	6	30	6	20	6	24	18	16	42	16	6
TMW 2.2157	6	24	20	6	6	28	6	14	16	22	18	20	36	16	18
TMW 2.2158	6	26	24	22	30	24	6	16	14	24	18	24	40	14	20
TMW 2.2159	6	40	38	26	6	38	6	22	18	18	22	24	50	14	6
TMW 2.2160	6	24	26	20	6	32	6	12	10	22	20	16	42	16	20
TMW 2.2161	6	38	24	6	6	32	6	12	6	20	12	16	42	22	18
TMW 2.2162	6	44	24	22	6	32	6	20	12	22	18	20	40	16	12
TMW 2.2163	6	26	26	24	26	32	6	14	6	16	18	16	38	14	6
TMW 2.2164	6	36	26	16	6	24	6	6	6	32	16	16	42	22	18
TMW 2.2165	6	32	22	20	6	36	6	12	12	18	26	20	42	16	18
TMW 2.2166	6	32	26	24	6	34	6	24	12	20	16	18	38	18	18
TMW 2.2167	6	30	26	22	6	34	6	16	6	22	18	14	44	20	20
TMW 2.2168	6	28	28	28	6	36	6	14	12	20	18	16	48	16	6
TMW 2.2169	6	32	22	6	6	26	6	14	6	18	18	16	40	14	18

Table S4. Antibiotic inhibition zone of *P. iliopiscarium*.

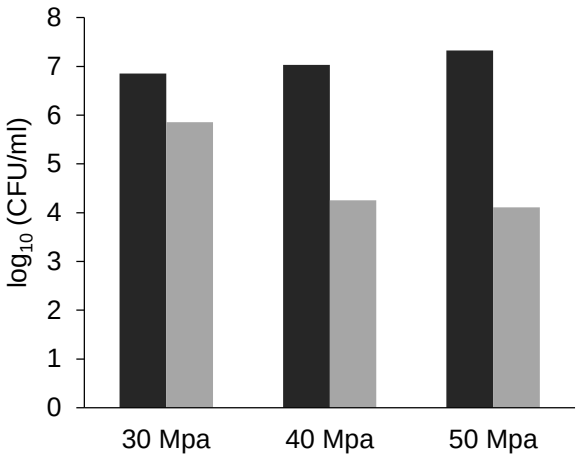
Diameter values in mm of the inhibition zone observed for every antibiotic and each of the selected isolates of *P. iliopiscarium*. The diameter of the antibiotic discs was measured as 6 mm, and therefore values of 6 in the table represent no inhibition zone observed.

Strain	Clindamycin DA 2 µg	Norfloxacin NOR 10 µg	Nalidixic acid NA 30 µg	Ampicillin AMP 10 µg	Sulphonamides S3 300 µg	Trimetoprim W 5 µg	Penicillin G P 5 µg	Streptomycin S 25 µg	Apramycin APR 15 µg	Rifampicin RD 5 µg	Gentamycin CN 10 µg	Kanamycin K 30 µg	Chloramphenicol C 30 µg	Erythromycin E 15 µg	Tetracyclin TE 30 µg
DSM 9896^T	6	20	18	9	6	20	6	10	6	15	12	13	34	6	10
TMW 2.2035	6	20	18	6	6	6	6	9	6	11	12	10	33	10	6
TMW 2.2104	6	24	19	14	6	6	6	9	10	14	11	11	35	6	8
TMW 2.2172	6	20	19	15	6	6	15	13	9	18	16	16	33	10	6

Table S5. Comparison of positive metabolic reactions in API50ch and APIzym between type strain and the rest of isolates of the species.
Summary of the positive reactions found in the selected *Photobacterium* strains. For each species, the table shows the results recorded for the type strain, and the results observed in at least one of the other strains of the species. Marked in light red are the differences observed between each of the type strains and the rest of the strains. In the case of *P. phosphoreum* and *P. iliopiscarium*, it additionally represents the differences between the sea-related type strain and the meat-related strains. Positive reactions are marked with a „+“ sign, negative reactions are marked with a „-“ sign, while weakly positive reactions are marked with a „w“.

Reaction	*P. phosphoreum*		*P. carnosum*		*P. iliopiscarium*	
	DSM 15556^T	Species	TMW 2.2021^T	Species	DSM 9896^T	Species
Alkaline phosphatase	+	+	+	+	+	+
Esterase (C 4)	-	+/w	-	w/-	-	w/-
Esterase Lipase (C 8)	-	+/w	-	w/-	-	w
Leucine arylamidase	+	+	+	+	+	+
Valine arylamidase	+	+/w/-	-	w/-	w	-
Cystine arylamidase	-	+/w/-	-	-	-	-
Trypsin	+	+/w/-	-	+/w/-	w	w/-
Acid phosphatase	+	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	-	+	+	+/w	+	+
β-galactosidase	+	+/w/-	-	+/w/-	-	-
β-glucuronidase	-	+/-	-	-	-	-
α-glucosidase	-	-	+	+/w/-	-	-
N-acetyl-β-glucosaminidase	+	+	+	+/-	+	+/-
Glycerol	+	-	w	+/w/-	+	w/-
D-ribose	+	+/w	+	+	+	+
D-galactose	+	+/w/-	+	+	+	+
D-glucose	+	+	+	+	+	+
D-fructose	+	+/w	+	+	+	+
D-mannose	+	+	+	+	+	+
Methyl-αD-glucopyranoside	-	-	-	+/w/-	-	-
N-acetylglucosamine	+	+	+	+	+	+
Esculin	+	+/-	+	+/-	+	+/-
D-cellobiose	-	-	-	+/-	-	-
D-maltose	+	+/w/-	+	+	+	+
D-lactose	w	-	-	+/-	-	-
D-melibiose	w	-	-	-	-	-
D-saccharose	-	-	-	+/-	-	-
Starch	-	-	+	+/w	-	+/w
Glycogen	-	-	-	+/w/-	-	-
Gentiobiose	-	-	-	+/-	-	-
D-turanose	-	-	-	+/-	-	-
L-fucose	-	-	-	+/-	-	-
Potassium 2-ketogluconate	w	w/-	w	w/-	-	w/-
Potassium 5-ketogluconate	w	w/-	-	-	-	w/-

12.2 Supplementary files to publication 2



Supplementary figure S1 Growth of *P. carnosum* strain TMW2.2021^T at 30 MPa, 40 MPa and 50 MPa. Cultures were plated after 72 h of cultivation at atmospheric pressure (negative control, (■)) and cultivation at high pressure (■).

Supplementary table S2 Statistical comparison of the cell counts of *P. carnosum* and *P. phosphoreum* strains after treatment with 40 MPa pressure.

Cell counts are shown as log10(CFU/ml). Significant differences were accepted with $p < 0.05$ and are grouped by their significance. *P. phosphoreum*: TMW2.2134, TMW2.2125 (strains from meat, red), DSM 15556T (marine type strain, blue); *P. carnosum*: TMW2.2021T, TMW2.2148 (strains from meat, red), TMW2.2098, TMW2.2186 (strains from MAP salmon, blue).

***P. phosphoreum* strains**

strain	TMW2.2134	TMW2.2125	DSM 15556^T
cell counts	7.34 ± 0.34^{ab}	7.69 ± 0.08^{a}	6.93 ± 0.36^{b}

***P. carnosum* strains**

strain	TMW2.2021^T	TMW2.2148	TMW2.2098	TMW2.2186
cell counts	4.55 ± 1.15^{a}	4.83 ± 0.23^{b}	6.07 ± 0.41^{ac}	6.48 ± 0.28^{c}

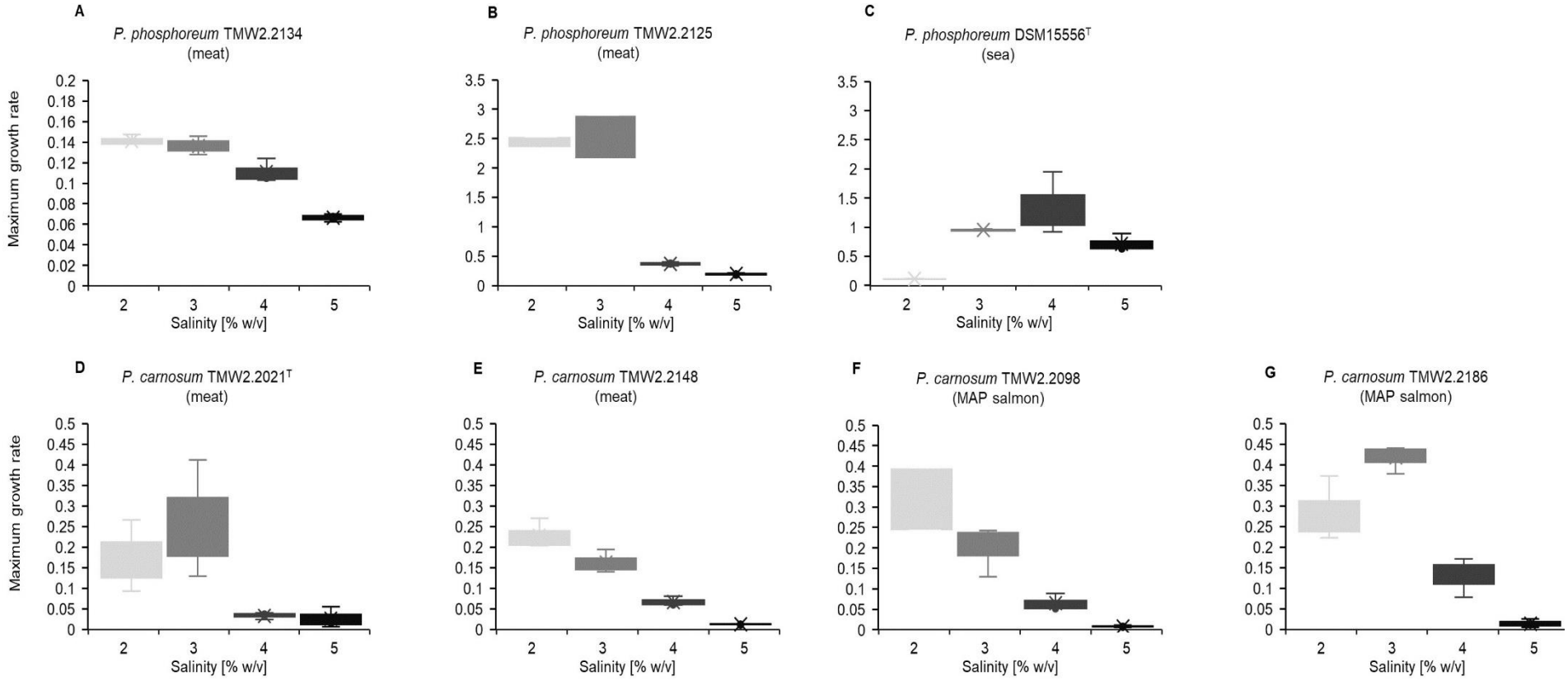
***P. carnosum* strains vs. *P. phosphoreum* strains**

strain	TMW2.2134	TMW2.2125	DSM 15556^T	TMW2.2021^T	TMW2.2148	TMW2.2098	TMW2.2186
cell counts	7.34 ± 0.34^{ab}	7.69 ± 0.08^{a}	6.93 ± 0.36$^{be(ce)}$	4.55 ± 1.15^{c}	4.83 ± 0.23^{d}	6.07 ± 0.41$^{(ce)}$	6.48 ± 0.28^{e}

Supplementary table S3 Statistical comparison of the salt tolerance of P. carnosum and *P. phosphoreum* strains.

Tolerance was calculated as difference of the maximum OD600 relative to the total maximum OD600 that was reached by the strain. Significant difference of the relative values was accepted with $p < 0.05$. Values were grouped row-wise by their significance. The assay included tolerance to 2 %, 3 %, 4 % and 5 % (w/v) NaCl.

Species	*P. carnosum*				*P. phosphoreum*		
Isolation source	MAP salmon		meat		sea	meat	
Strain	**TMW2. 2098**	**TMW2. 2186**	**TMW2. 2021^T**	**TMW2. 2148**	**DSM 15556^T**	**TMW2. 2134**	**TMW2. 2125**
2 % NaCl	1 ± 0^a	0.799 ± 0.041^b	1 ± 0^a	1 ± 0^a	0.85 ± 0.024^b	1 ± 0^a	0.936 ± 0.019^c
3 % NaCl	0.853 ± 0.002^a	1 ± 0^b	0.962 ± 0.034^{bc}	0.983 ± 0.0237^c	0.93 ± 0.04^c	0.989 ± 0.006^c	1 ± 0^b
4 % NaCl	0.543 ± 0.016^a	0.706 ± 0.067^{bc}	0.34 ± 0.041^d	0.593 ± 0.024^c	1 ± 0^e	0.886 ± 0.012^f	0.732 ± 0.013^b
5 % NaCl	0.07 ± 0.014^a	0.139 ± 0.005^b	0.2 ± 0.038^{cb}	0.194 ± 0.02^c	0.859 ± 0.042^d	0.727 ± 0.014^e	0.523 ± 0.017^f



Supplementary figure S4 Maximum growth rate of *P. phosphoreum* and *P. carnosum* strains in liquid medium with different salt content.
Strains were cultivated in HS-MSM (2 % NaCl (■)), MSM with 3 % NaCl (■), MSM with 4 % NaCl (■) and MSM with 5 % NaCl (■). **A - C** *P. phosphoreum*: TMW2.2134 (meat), TMW2.2125 (meat), DSM15556^T (sea); **D – G** *P. carnosum*: TMW2.2021^T (meat), TMW2.2148 (meat), TMW2.2098 (MAP salmon), TMW2.2186 (MAP salmon). Error bars show the standard deviation of N = 3, no error bars are shown in case of N = 2. Growth rate was calculated following the grofit package (logistic modeling, gompertz modeling or model free spline method (TMW2.2134 only)).

Supplementary table S5 Cell size of representative strains after cultivation at different NaCl concentration.

Cells were cultivated in MSM with 2 - 5 % (w/v) NaCl until they reached their maximum OD_{600}. Cell size is shown as mean value of the surface area in µm of N = 10 cells, respectively. No significant difference was detected regarding the cell size of each strain.

NaCl concentration	TMW2.2134	TMW2.2021^T
2%	3.577±0.22	4.271±0.12
3%	3.954±0.19	4.698±0.25
4%	4.08±0.22	4.558±0.11
5%	3.573±0.21	4.526±0.24

Supplementary table S6 Statistical comparison of the relative growth reduction of P. carnosum and P. phosphoreum strains on salt rich agar after pressure treatment.

Cells were treated with 125 MPa hydrostatic pressure and plated on MSM with 2 % NaCl (HS-MSM), 3 % NaCl and 3.5 % NaCl. Values show the relative development of the cell counts per ml compared to non-treated cultures, with positive values representing a reduction of the cell counts. Significant differences of the reduction were accepted with $p < 0.05$ and values were grouped row-wise by their significance. P. phosphoreum: TMW2.2134, TMW2.2125 (strains from meat), DSM 15556T (marine type strain); P. carnosum: TMW2.2021T, TMW2.2148 (strains from meat), TMW2.2098, TMW2.2186 (strains from MAP salmon)

A Media in comparison

	NaCl [% w/v]	2%	3%	3.50%
P. phosphoreum	TMW2.2134	-0.077 ± 0.29^{a}	0.94 ± 0.028^{b}	93.59 ± 4.34^{b}
	DSM15556^T	0.24 ± 0.18^{a}	0.78 ± 0.072^{b}	0.85 ± 0.036^{b}
P. carnosum	TMW2.2021^T	0.17 ± 0.19^{c}	0.50 ± 0.087^{c}	0.77 ± 0.019^{c}
	TMW2.2098	0.041 ± 0.049^{c}	0.33 ± 0.07^{c}	0.73 ± 0.063^{c}

B Strains in comparison

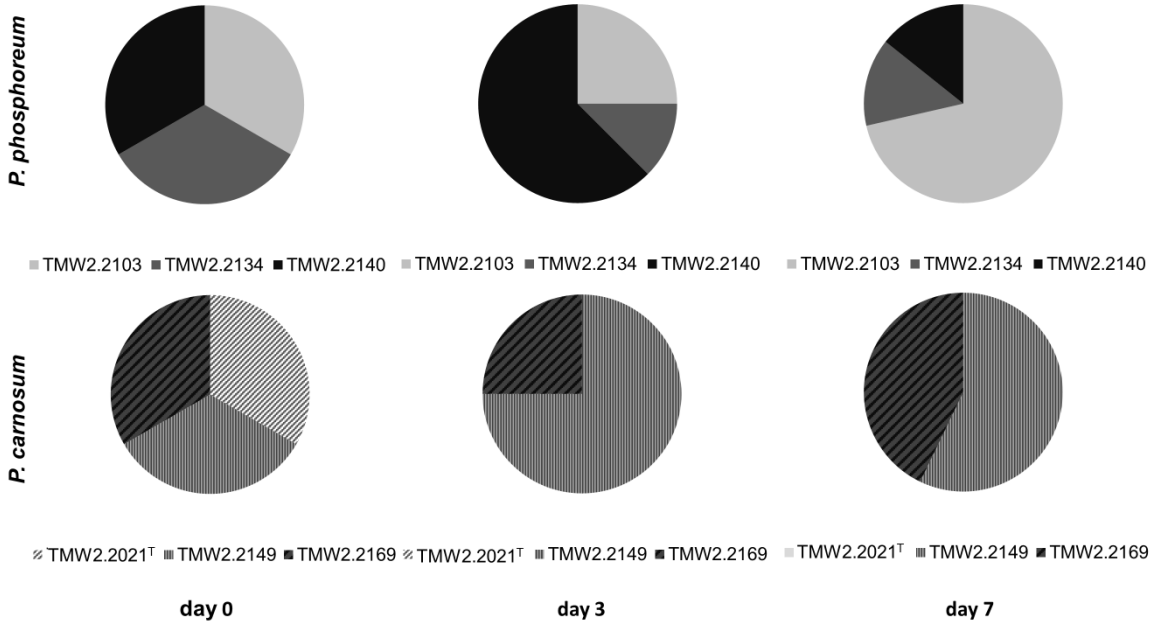
		TMW2.2134	DSM15556^T	TMW2.2021^T	TMW2.2098
NaCl [% w/v]	2%	-0.077 ± 0.29^{a}	0.24 ± 0.18^{a}	0.17 ± 0.19^{a}	0.041 ± 0.049^{a}
	3%	0.94 ± 0.028^{ab}	0.78 ± 0.072^{ab}	0.50 ± 0.087^{a}	0.33 ± 0.07^{b}
	3.50%	93.59 ± 4.34^{ab}	0.85 ± 0.036^{ab}	0.77 ± 0.019^{a}	0.73 ± 0.063^{b}

Supplementary table S7 Presence of ORFs with putative relevance in adaptation to marine environment in *P. phosphoreum* and *P. carnosum* strains.
Analysis was done with blastp (NCBI) using respective sequences of *P. profundum* strain SS9 unless otherwise stated. ORFs with striking differences regarding metabolism or presence only in selected strains are additionally included. Present, was accepted with >70 % identity and query cover in blastp analysis; putative presence was accepted with 50 - 70 % identity and query cover in blastp analysis and marked with brackets; absence was accepted with <50 % identity and query cover in blastp analysis. *P. phosphoreum*: TMW2.2134 from meat, TMW2.2125 from meat, DSM15556T from the sea; *P. carnosum*: TMW2.2021T from meat, TMW2.2148 from meat, TMW2.2098 from MAP salmon, TMW2.2186 from MAP salmon.

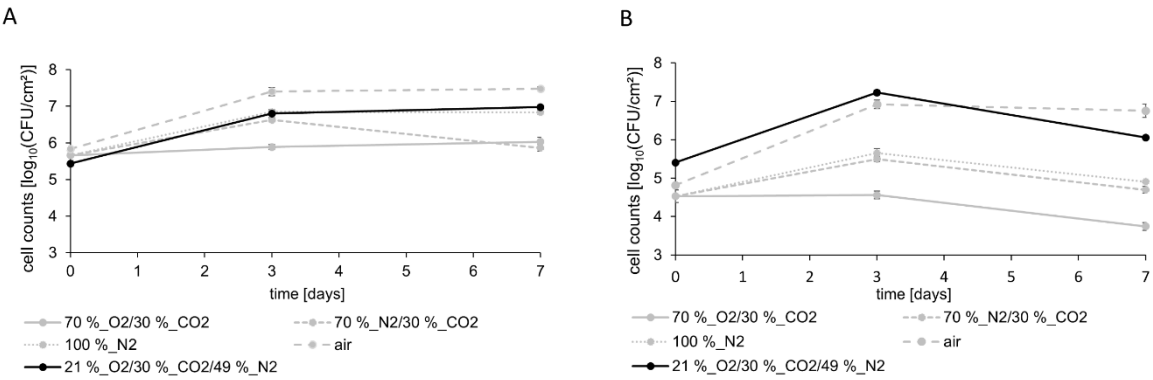
Category	Protein annotation	KEGG orthology number	Reference and notes; Accession number	*P. carnosum*				*P. phosphoreum*		
				TMW2.2021^T	TMW2.2148	TMW2.2098	TMW2.2186	TMW2.2134	TMW2.2125	DSM 15556^T
				from meat		from MAP salmon		from meat		from the sea
Pressure response	TMAO reductase system sensor histidine kinase/response regulator TorS	K07647	Sensor protein associated with pressure resistance (11); WP_011217973.1	(CIK00_11775)	(GLP11_10490)	(GLP27_10420)	(GLP20_00180)	(GLP25_05500)	(GLP32_10810)	(CTM77_08085)
	Molecular chaperone Dnak	K06204	(32, 60, 61); WP_107281016.1	CIK00_07030	GLP11_05470	GLP27_07250	GLP20_06725	GLP25_12215	GLP32_20605	CTM77_06435
	Molecular chaperone DnaJ	K03686	(32, 61); WP_011217465.1	CIK00_07025	GLP11_05465	GLP27_07255	GLP20_06730	GLP25_12210	GLP32_20600	CTM77_06440
	Molecular chaperone GroEL	K04077	(32, 60, 61); CAG21672.1	CIK00_17160	GLP11_14385	GLP27_16650	GLP20_14640	GLP25_17870	GLP32_17565	CTM77_10420
	Co-chaperone GroES	K04078	(62); PSV59559.1	CIK00_17155	GLP11_14380	GLP27_16645	GLP20_14645	GLP25_17865	GLP32_17560	CTM77_10415
	Outer membrane protein OmpH	K06142	Pressure adaptation, induced at HHP (63); Sequence of *P. phosphoreum* DSM 15556^T; WP_107305034.1	CIK00_15835	GLP11_12815	GLP27_14410	GLP20_16880	GLP25_16745	GLP32_19840	CTM77_14955
	Porin-like protein OmpL	K22110	Antagonist of OmpH, repressed at HHP (64); Sequence of *P. phosphoreum* DSM 15556^T; CEO40251.1	CIK00_07350	(GLP11_05890)	GLP27_06910	GLP20_06355	GLP25_12530	GLP32_10360	CTM77_06065
	Transcription activator system ToxR/ToxS	K10921, K10922	Pressure response of the membrane, regulate OmpL (10, 65); Sequence of *P. phosphoreum* DSM 15556^T; CEO39867.1, CEO39868.1	CIK00_01735, CIK00_01740	GLP11_01885, GLP11_01890	GLP27_03330, GLP27_03325	GLP20_02275, GLP20_02270	GLP25_04685, GLP25_04680	GLP32_01520, GLP32_01525	CTM77_04790, CTM77_04795
	RNA polymerase sigma factor RpoE	K03088	Pressure induced stress response (66, 67); AAA65226.1	CIK00_17800	GLP11_13950	GLP27_11315	GLP20_13600	GLP25_15840	GLP32_16265	CTM77_16510
	Anti-sigma E factor RseA	K03597	Regulation of the ompL/ompH system, regulation of RpoE (67, 68); WP_011219669.1, WP_011219668.1, WP_011219667.1	CIK00_17795	GLP11_13945	GLP27_11320	GLP20_13595	GLP25_15845	GLP32_16270	CTM77_16515
	Sigma-E factor regulatory protein RseB	K03598		CIK00_17790	GLP11_13940	GLP27_11325	GLP20_13590	GLP25_15850	GLP32_16275	CTM77_16520
	Transcriptional regulator RseC	K03803		(CIK00_17785)	(GLP11_13935)	(GLP27_11330)	(GLP20_13585)	(GLP25_15855)	(GLP32_16280)	(CTM77_16525)
	Exodeoxyribonuclease V subunit alpha, RecD	K03581	Required for growth at HHP in *P. profundum* (69); WP_006232632.1	(CIK00_15650)	(GLP11_12990)	(GLP27_14235)	(GLP20_17640)	(GLP25_16545)	(GLP32_19415)	(CTM77_14755)
Salt response	Outer membrane protein OmpW	K07275	Antagonistic salt tolerance system in *P. damselae* (70, 71); WP_006230905.1, ABB54459.1	-	-	-	-	-	-	-
	Major outer membrane protein OmpV	K07274		-	-	-	-	-	-	-
	RNA polymerase sigma factor RpoS	K03087	Induces resistance to several stresses (72, 73); WP_041394512.1	CIK00_17680	GLP11_13830	GLP27_11435	GLP20_13480	GLP25_ 15960, (GLP25_14910)	GLP32_16385 (GLP32_03245)	CTM77_16630 (CTM77_09900)
	Two-component system response regulator OmpR	K02483	Porin system induced by osmotic stress (74, 75); WP_011217001.1, WP_011217002.1	CIK00_06735	GLP11_06960	GLP27_07535	GLP20_07010	GLP25_11920	GLP32_09255	CTM77_18465
	Two-component system sensor histidine kinase EnvZ	K07638		CIK00_06730	GLP11_06955	GLP27_07540	GLP20_07015	GLP25_11915	GLP32_09265	CTM77_18470
	Porin OmpC/OmpF	K09475, K09476		-	-	-	-	-	-	-
	Sodium-dependent transporter	-	This study, sequence of *P. phosphoreum;* PSV65920.1	-	-	-	-	GLP25_00420	GLP32_04570	CTM77_20325
	Bile acid:sodium symporter family protein	-	This study, sequence of *P. phosphoreum;* PSV70248.1	-	-	-	-	GLP25_07625	GLP32_16005	CTM77_12860
	Sodium/proline symporter putP	K11928	This study, sequence of *P. phosphoreum;* PSV70349.1, PSV73104.1	-	-	-	-	GLP25_01970, GLP25_13025	GLP32_04685 , GLP32_04060	CTM77_12440. CTM77_02225
	Dicarboxylate/amino acid:cation symporter	-	This study, sequence of *P. phosphoreum;* PSV73451.1	-	-	-	-	GLP25_11145	GLP32_00915	CTM77_00625
	Dicarboxylate/amino acid:cation symporter	-	This study, sequence of *P. phosphoreum;* PSV72743.1	-	-	-	-	GLP25_08680	GLP32_11375	CTM77_03120
	Cation:proton antiporter	-	This study, sequence of *P. phosphoreum;* PSV70735.1	-	-	-	-	GLP25_02810	GLP32_05760	CTM77_11175
	Na(+)/H(+) antiporter NhaB	K03314	This study; WP_107295101.1	CIK00_09375	GLP11_08375	GLP27_09155	GLP20_00905	GLP25_06310	GLP32_14605	CTM77_04015

Motility	Chemotaxis protein CheY	K03413	Polar flagellar cluster *P. profundum* (12); PSV64574.1,	CIK00_02060	-	GLP27_02990	GLP20_01950	GLP25_04270	GLP32_058755	CTM77_05195
	Protein phosphatase CheZ	K03414	CAG19352.1,	(CIK00_02055)	-	(GLP27_02995)	(GLP20_01955)	GLP25_04275	GLP32_08760	CTM77_05190
	Chemotaxis protein CheA	K03407	WP_011217529.1,	(CIK00_02050)	-	(GLP27_03000)	(GLP20_01960)	(GLP25_04280)	(GLP32_08765)	(CTM77_05185)
	Chemotaxis response regulator protein-Glutamate methylesterase CheB	K03412	CAG19354.1,	CIK00_02045	GLP11_02180	GLP27_03005	GLP20_01965	GLP25_04285	GLP32_08770	CTM77_05180
	Chemotaxis protein CheW	K03408	PSV64568.1,	CIK00_02030	-	GLP27_03020	GLP20_01980	GLP25_04300	GLP32_08785	CTM77_05165
	Flagellar protein FlgN	K02399	WP_045026950.1 (Sequence of *P. phosphoreum*),	CIK00_02280	GLP11_02200	GLP27_02770	GLP20_01730	GLP25_04050	GLP32_08535	CTM77_05415
	Flagellar biosynthesis anti-sigma factor FlgM	K02398	WP_011217645.1,	(CIK00_02275)	(GLP11_02195)	(GLP27_02775)	(GLP20_01735)	(GLP25_04055)	(GLP32_08540)	(CTM77_05410)
	Flagellar basal body P-ring formation protein FlgA	K02386	WP_011217646.1,	(CIK00_02270)	-	(GLP27_02780)	(GLP20_01740)	(GLP25_04060)	(GLP32_08545)	(CTM77_05405)
	Putative polar flagellar protein FlaK	K07991	CAG19329.1,	CIK00_02170	GLP11_02185	-	GLP20_01840	GLP25_04160	GLP32_08645	CTM77_05305
	Flagellin flaA	K07324	ODA22432.1 (Sequence of *P. damselae*),	(CIK00_02195)	-	(GLP27_02855)	(GLP20_01815)	GLP25_04135	(GLP32_08620)	CTM77_05330
	RNA polymerase sigma factor FliA	K02405	PSV64575.1,	CIK00_02065	-	GLP27_02985	GLP20_01945	GLP25_04265	GLP32_08750	CTM77_05200
	Flagellar hook-length control protein FliK	K02414	WP_036803302.1	-	-	-	-	(GLP25_04208)	(GLP32_08690)	(CTM77_05260)
	Flagellar motor protein MotA	K02556	(12), Sequence of *P. phosphoreum*; PTB31111.1	CIK00_12265	GLP11_03605	GLP27_05545	GLP20_09510	GLP25_10080	GLP32_07910	CTM77_15815
	Flagellar motor protein PomA	-	(12); WP_011217559.1	CIK00_02580	GLP11_02540	GLP27_18515	GLP20_01425	GLP25_03670	GLP32_08160	CTM77_17615
	Flagellar motor protein MotB	K02557	(12), Sequence of *P. phosphoreum*; PTB34546.1	CIK00_02575	GLP11_02535	GLP27_18520	GLP20_01430	GLP25_03675	GLP32_08165	CTM77_17620
	Putative sodium-type flagellar protein MotX	K21217	(12), Sequence of *P. phosphoreum*; PTB31725.1	CIK00_10565	GLP11_16200	GLP27_15810	GLP20_15145	GLP25_18950	GLP32_18055	CTM77_10575
	Putative sodium-type flagellar protein MotY	K21218	(12), Sequence of *P. phosphoreum*; CEO38623.1	CIK00_09540	GLP11_08210	GLP27_09330	GLP20_00740	GLP25_06120	GLP32_14860	CTM77_04275
Bioluminscence	Phosphorelay protein LuxU	K10911	Sequence of *P. phosphoreum,* (76); CEO38635.1	CIK00_09595	GLP11_08155	GLP27_09385	GLP20_00685	GLP25_06055	GLP32_15360	CTM77_04340
	Activated long-chain acyl hydrolase LuxD	K15853	Sequence of *P. phosphoreum,* (3); WP_107238702.1	-	-	-	-	GLP25_13010	GLP32_04075	CTM77_02240
	quorum sensing regulator LuxR	K07782	Sequence of *P. phosphoreum,* (76); CEO38104.1	CIK00_09050	GLP11_08690	GLP27_08845	GLP20_08315	GLP25_14150	GLP32_12540	CTM77_06740
	Alpha subunit luciferase LuxA	K00494	Sequence of *P. phosphoreum*, (3); BAU80908.1	-	-	-	-	GLP25_13005	GLP32_04080	CTM77_02245
	Beta subunit luciferase LuxB	K15854	Sequence of *P. phosphoreum,* (3); BAU80909.1	-	-	-	-	GLP25_13000	GLP32_04085	CTM77_02250
Metabolism	Proline/glycine betaine ABC transporter permease	-	This study, Sequence of *P. phosphoreum*; PSV67650.1	-	-	-	-	GLP25_02955	GLP32_05905	CTM77_18295
	Glycine betaine/L-proline ABC transporter ATP-binding protein	-	This study, Sequence of *P. phosphoreum*; PSV67651.1	-	-	-	-	GLP25_02960	GLP32_05910	CTM77_18300
	Fe-S cluster assembly protein SufD	K09015	This study; Sequence of *P. phosphoreum*; WP_107295572.1	-	-	(GLP27_17530)	(GLP20_16140)	GLP25_07700	GLP32_16080	CTM77_12935
	SufS family cysteine desulfurase	K11717	This study; Sequence of *P. phosphoreum*; WP_065192912.1	-	-	(GLP27_17535)	(GLP20_16135)	GLP25_07705	GLP32_16085	CTM77_12940
	Fe-S cluster assembly ATPase SufC	K09013	This study; Sequence of *P. phosphoreum*; WP_107303381.1	-	-	GLP27_17525	GLP20_16145	GLP25_07695	GLP32_16075	CTM77_12930
	Fe-S cluster assembly protein SufB	K09014	This study; Sequence of *P. phosphoreum*; WP_107274087.1	-	-	GLP27_17520	GLP20_16150	GLP25_07690	GLP32_16070	CTM77_12925
	P-type DNA transfer ATPase VirB11	K03196	This study; Sequence of *P. phosphoreum*; WP_107197938.1	-	(GLP11_16605)	-	-	(GLP25_19585)	(GLP32_19220)	-
	Type IV secretion system protein VirB10	K03195	This study; Sequence of *P. phosphoreum*; WP_107197939.1	-	(GLP11_16600)	-	-	(GLP25_19580)	(GLP32_19225)	-
	P-type conjugative transfer protein VirB9	K03204	This study; Sequence of *P. phosphoreum*; PSW07910.1	-	(GLP11_16595)	-	-	(GLP25_19575)	(GLP32_19230)	-
	Type IV secretion system protein VirB8	K03203	This study; Sequence of *P. phosphoreum*; WP_107197941.1	-	(GLP11_16590)	-	-	(GLP25_19775)	(GLP32_19235)	-
	VirB4 family type IV secretion/conjugal transfer ATPase	K03199	This study; Sequence of *P. phosphoreum*; WP_107197942.1	-	(GLP11_16585)	-	-	(GLP25_19565)	(GLP32_19240)	-
	P-type DNA transfer protein VirB5	K03200	This study; Sequence of *P. phosphoreum*; WP_107197915.1	-	(GLP11_16670)	-	-	(GLP25_19650)	(GLP32_19155)	-
	Iron-sulfur cluster assembly accessory protein	-	This study; Sequence of *P. carnosum*; GLP27_17515	-	-	GLP27_17515	GLP20_16155	(GLP25_07685)	(GLP32_16065)	(CTM77_12920)
	Manganese/iron ABC transporter ATP-binding protein	-	This study; Sequence of *P. carnosum*; GLP27_17560	-	-	GLP27_17560	GLP20_16110	GLP25_01700	GLP32_02905	CTM77_17185
	Iron chelate uptake ABC transporter family permease subunit	-	This study; Sequence of *P. carnosum*; GLP27_17555	-	-	GLP27_17555	GLP20_16115	GLP25_01695	GLP32_02900	CTM77_17180
	Iron chelate uptake ABC transporter family permease subunit	-	This study; Sequence of *P. carnosum*; GLP27_17550	-	-	GLP27_17550	GLP20_16120	(GLP25_01690)	(GLP32_02895)	CTM77_17175

12.3 Supplementary files to publication 3

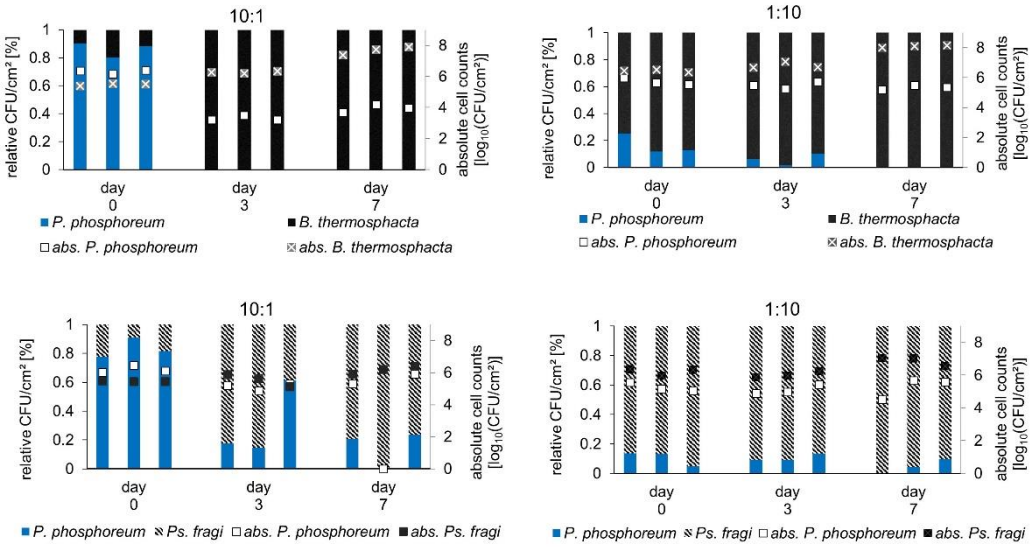


Supplementary Figure S1: Intraspecies assertiveness of *P. phosphoreum* and P*. carnosum strains*. Strains were cultivated on chicken in O_2/CO_2 atmosphere together with a spoilage community of *Ps. fragi* TMW2.2082, *Ps. lundensis* TMW2.2076, *Ps. weihenstephanensis* TMW2.1728, *B. thermosphacta* TMW2.2101, *L. gelidum* subsp. *gelidum* TMW2.1618, *L. gelidum* subsp. *gasicomitatum* TMW2.1619, *C. divergens* TMW2.1577 and *C. maltaromaticum* TMW2.1581. Species were identified with MALDI-TOF MS and strains were differentiated by RAPD-PCR fingerprinting.



Supplementary Figure S2 Growth of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 in presence of 21 % O_2 and 30 % CO_2 compared to its growth under MAP and air packaging. Meat was incubated at 4 °C. N = 3. **A** *P. phosphoreum* TMW2.2103 **B** *P. carnosum* TMW2.2149.

A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1:10
relative CFU/cm² [%]
absolute cell counts [log10(CFU/cm²)]
day 0
day 3
day 7
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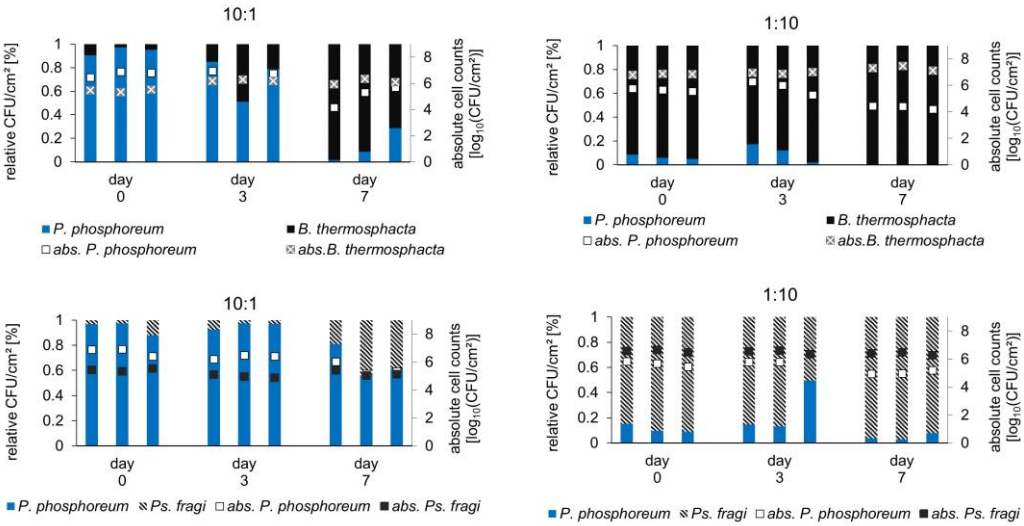
B. thermosphacta
abs. P. phosphoreum
abs. B. thermosphacta
Ps. fragi
abs. Ps. fragi

B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absolute cell counts [log10(CFU/cm²)]
day 0
day 3
day 7
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B. thermosphacta
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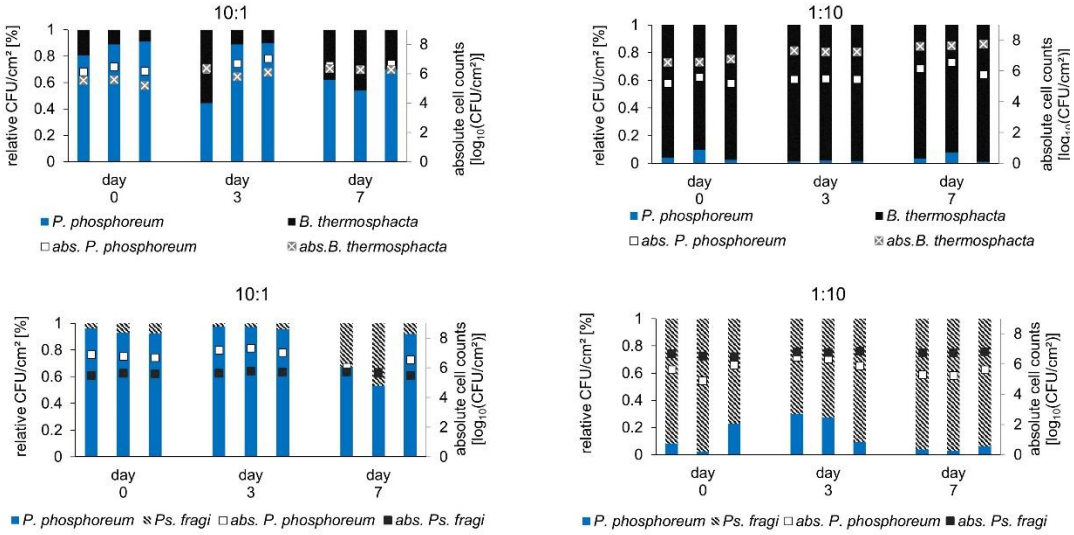
abs.B. thermosphacta
Ps. fragi
abs. Ps. fragi

C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B. thermosphacta
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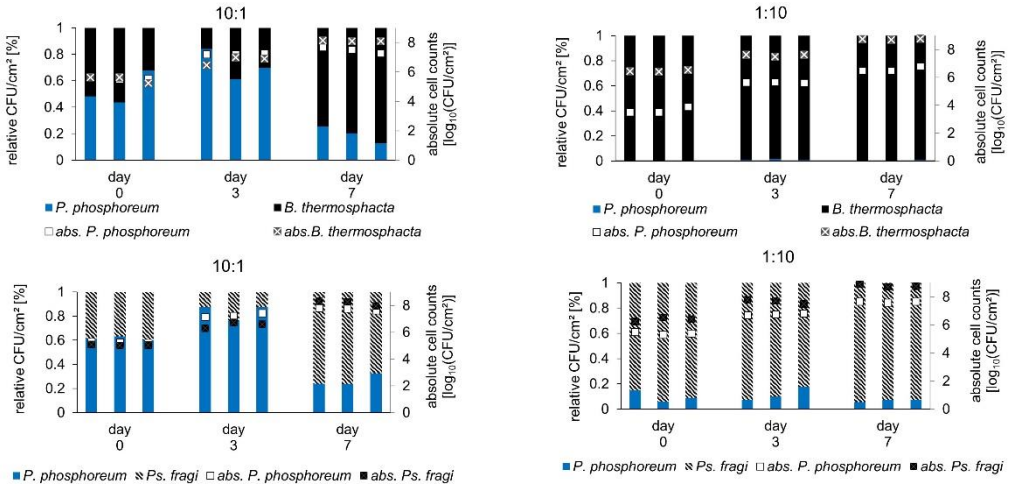
abs.B. thermosphacta
Ps. fragi
abs. Ps. fragi

D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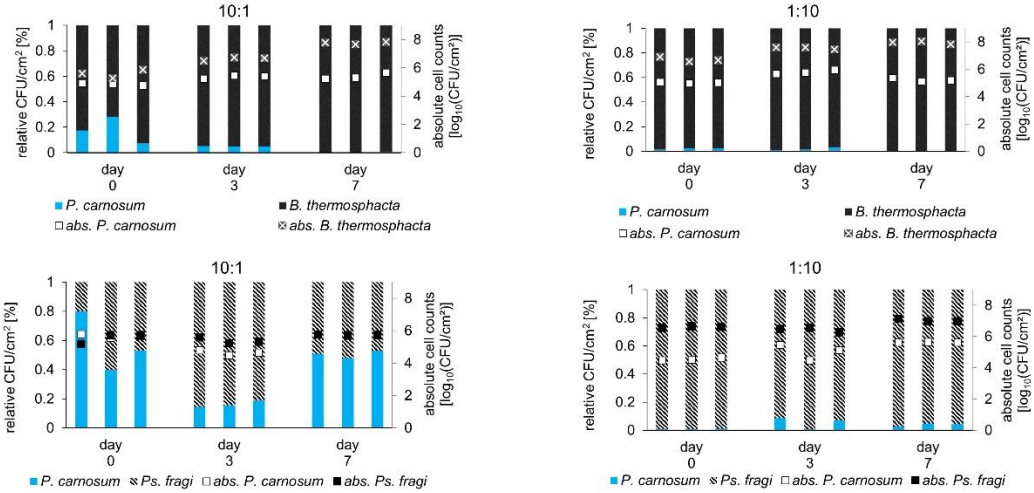
relative CFU/cm² [%]
absolute cell counts [log10(CFU/cm²)]
day 0
day 3
day 7
P. phosphoreum
B. thermosphacta
abs. P. phosphoreum
abs.B. thermosphacta
Ps. fragi
abs. Ps. fragi

E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1:10
relative CFU/cm² [%]
absolute cell counts [log10(CFU/cm²)]
day 0
day 3
day 7
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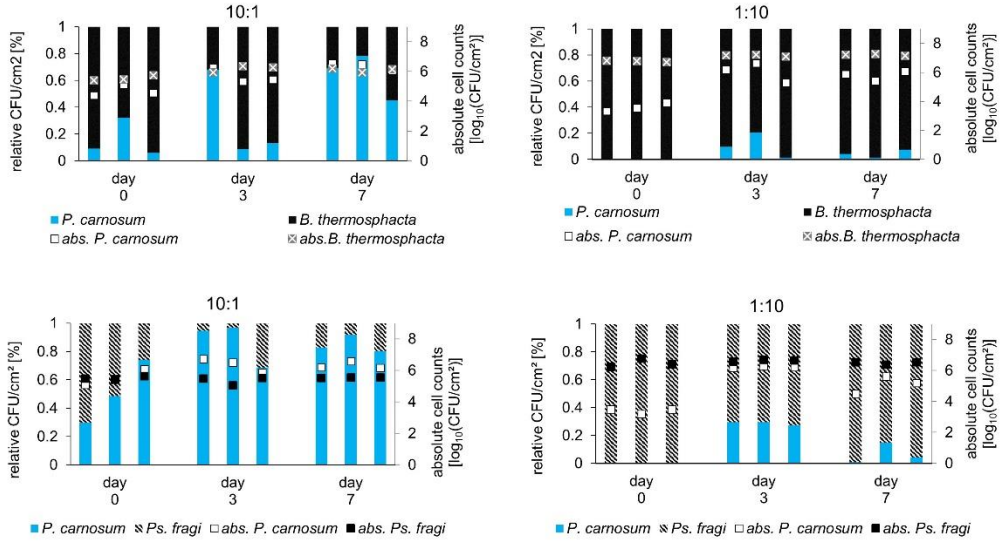
B. thermosphacta
abs. P. carnosum
abs. B. thermosphacta
Ps. fragi
abs. Ps. fragi

F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relative CFU/cm2 [%]
relative CFU/cm² [%]
absolute cell counts [log10(CFU/cm²)]
day 0
day 3
day 7
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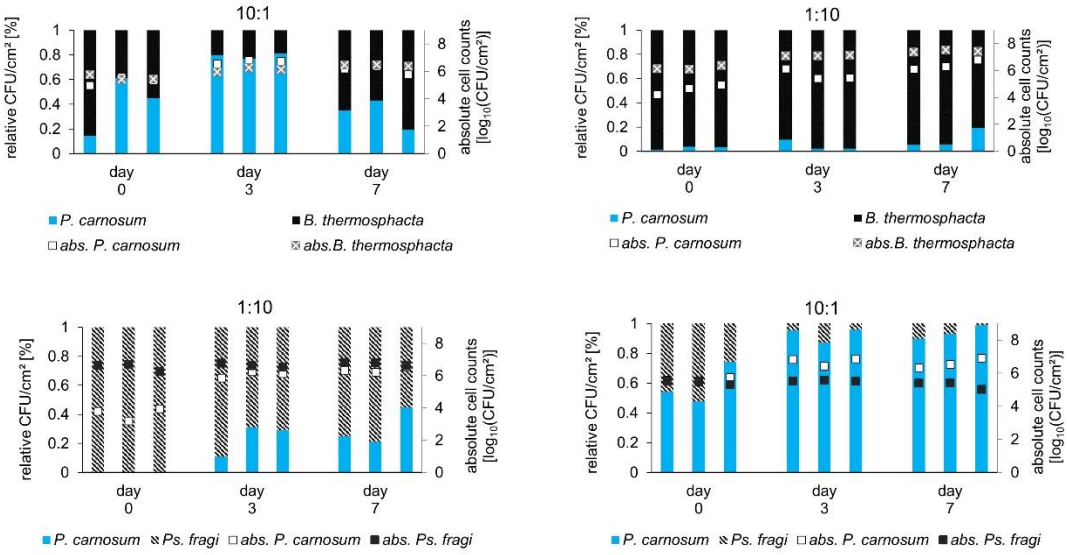
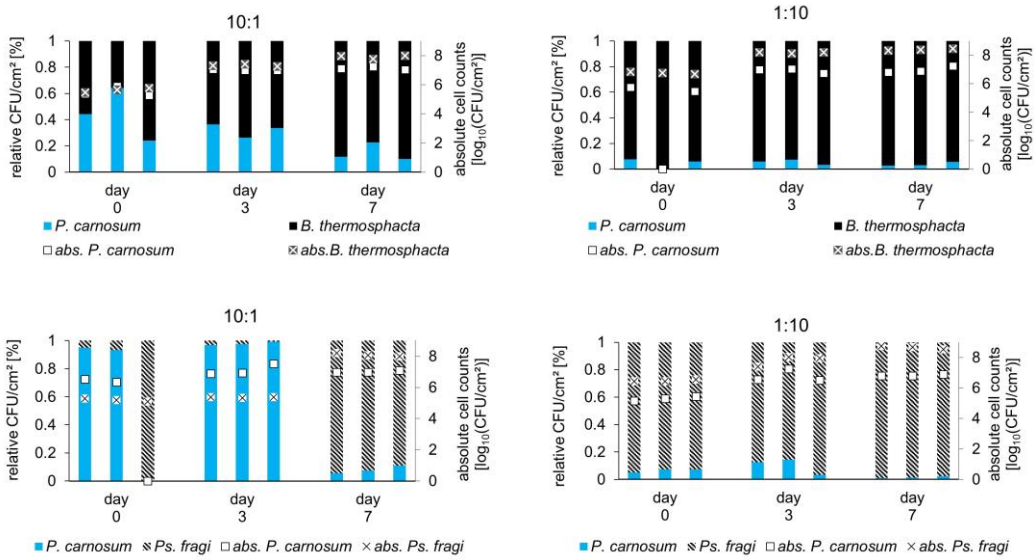
abs.B. thermosphacta
Ps. fragi
abs. Ps. fragi

G



H



Supplementary Figure S3 Growth of *P. phosphoreum* and *P. carnosum* in presence of *Ps. fragi* or *B. thermosphacta* in over- and under-represented ratio (*Photobacterium*:co-contaminant). Experiments were done in triplicates on chicken packaged in different atmospheres. **A** *P. phosphoreum* TMW2.2103 in O_2/CO_2 atmosphere **B** *P. phosphoreum* TMW2.2103 in N_2/CO_2 atmosphere **C** *P. phosphoreum* TMW2.2103 in N_2 atmosphere **D** *P. phosphoreum* TMW2.2103 under air **E** *P. carnosum* TMW2.2149 in O_2/CO_2 atmosphere **F** *P. carnosum* TMW2.2149 in N_2/CO_2 atmosphere **G** *P. carnosum* TMW2.2149 in N_2 atmosphere **H** *P. carnosum* TMW2.2149 under air.

Supplementary table S1 Susceptibility of strains of *P. phosphoreum* and *P. carnosum* to exogenous H_2O_2 in comparison to meat spoilers from the literature.

Species	MIC [%]	Reference
P. phosphoreum TMW2.2103	0.02	this work
P. carnosum TMW2.2149	0.01	this work
B. thermosphacta TMW2.2101	0.09	Kolbeck *et al.* 2019
Leuconostoc gelidum ssp. *gelidum* TMW2.1618	0.016	Kolbeck *et al.* 2019
Leuconostoc gelidum ssp. *gasicomitatum* TMW2.1619	0.02	Kolbeck *et al.* 2019
Carnobacterium maltaromaticum TMW2.1581	0.024	Kolbeck *et al.* 2019
Carnobacterium divergens TMW2.1577	0.012	Kolbeck *et al.* 2019

Supplementary Table S2: Absolute cell numbers of the growth and interaction experiments.

Three independent experiments were performed per *Photobacterium* species: growth alone in different gas atmospheres, growth concomitant with *Ps. fragi* in different gas atmospheres and growth concomitant with *B. thermosphacta* in different gas atmospheres. Cell counts are shown as mean values of triplicates.

***Photobacterium* species**	**Experiment**	**Gas atmosphere**	**Species**	**Ratio (*Photobacterium*/co-contaminant)**	**cell counts [log_{10}(CFU/cm^2)]**			**Standard error**		
					day 0	**day 3**	**day 7**	**day 0**	**day 3**	**day 7**
P. phosphoreum	Growth alone	70 %_O_2/30 % CO_2	*P. phosphoreum*	-	5.658753952	5.889102332	6.028235283	0.205292619	0.068255125	0.127190592
		70 %_N_2/30 % CO_2	*P. phosphoreum*	-	5.658753952	6.627569056	5.865265593	0.205292619	0.029606616	0.095026351
		100 %_N_2	*P. phosphoreum*	-	5.658753952	6.846004223	6.834292003	0.205292619	0.045488976	0.059396389
		21 %_O_2/79 %_N_2 (air)	*P. phosphoreum*	-	5.835332599	7.403214862	7.47724621	0.023456898	0.109312392	0.056236126
	interaction	70 %_O_2/30 % CO_2	*P. phosphoreum*		5.247509488	5.096343418	5.252517112	0.128360481	0.135169673	0.309878823
			Ps. fragi	1:10	6.223314488	6.025492279	6.873507022	0.102770198	0.087340614	0.12994193
			P. phosphoreum		5.728540435	5.505991274	5.342872703	0.11167053	0.112550501	0.070963496
			B. thermosphacta		6.45323041	6.81376487	8.089076226	0.039857851	0.099411796	0.035266101
			P. phosphoreum		6.372288957	5.220213775	5.595193354	0.04474099	0.190194465	0.081929987
			Ps. fragi	1:1	5.240563831	6.076281016	6.843571144	0.359580038	0.052568426	0.077571455
			P. phosphoreum		6.603338138	5.905424816	5.52830687	0.039929403	0.465220443	0.159469934
			B. thermosphacta		6.758043592	7.160398597	8.193152571	0.073196098	0.115041438	0.085343533
			P. phosphoreum		6.194973537	5.131457812	3.733517803	0.104640245	0.101069288	1.530426987
			Ps. fragi	10:1	5.464345028	5.543256595	6.161516688	0.012453612	0.184582936	0.119793113
			P. phosphoreum		6.30530461	3.294163358	3.945577528	0.064532859	0.081929987	0.123713844
			B. thermosphacta		5.483407345	6.26892312	7.672540908	0.035215348	0.034482924	0.130024744
		70 %_N_2/30 % CO_2	*P. phosphoreum*		5.649107431	5.969810567	5.038495395	0.089586187	0.151199194	0.063628025
			Ps. fragi	1:10	6.56760764	6.502470838	6.385501558	0.043364767	0.063048532	0.050897938
			P. phosphoreum		5.648882815	5.845554255	4.355727168	0.045975087	0.244380048	0.066356771
			B. thermosphacta		6.829498344	6.935655886	7.290883756	0.018351904	0.03176048	0.087732587
			P. phosphoreum		5.749675031	6.38098103	5.017808727	0.08910223	0.116981047	0.194837416
			Ps. fragi	1:1	5.504779845	5.552420811	5.528186182	0.035597347	0.029705362	0.138118924
			P. phosphoreum		5.667325636	6.388144002	5.29049823	0.133865391	0.109802605	0.045520003
			B. thermosphacta		5.736616336	6.451782247	6.598485231	0.071103723	0.012225909	0.036497198
			P. phosphoreum		6.736275312	6.362203352	5.504779845	0.138253535	0.07243786	0.217672081
			Ps. fragi	10:1	5.433486572	4.976873667	5.190065784	0.046419872	0.051391674	0.087733955

	P. phosphoreum		6.719028872	6.664022874	5.047002545	0.112442907	0.151774803	0.377618563
	B. thermosphacta		5.44285367	6.216475126	6.117932152	0.043242186	0.030030534	0.095069775
100 %_N_2	*P. phosphoreum*		5.486660625	6.224352233	5.394651382	0.253686646	0.141303172	0.102006016
	Ps. fragi	1:10	6.559919205	6.818050777	6.759170173	0.047744375	0.026646681	0.028453983
	P. phosphoreum		5.332144475	5.487062192	6.168992812	0.11294144	0.011829863	0.186199809
	B. thermosphacta		6.634394251	7.2800299	7.671646783	0.056998725	0.019504093	0.037903168
	P. phosphoreum		5.401982814	6.943854261	6.579578492	0.110285207	0.030742193	0.070374029
	Ps. fragi	1:1	5.539495986	6.112952036	6.205443025	0.080720727	0.09006396	0.055108299
	P. phosphoreum		4.80358795	6.812415222	6.507789617	0.354095514	0.171182271	0.04792597
	B. thermosphacta		5.828183355	6.588005793	6.798586986	0.026375565	0.033075966	0.047653011
	P. phosphoreum		6.779997833	7.186699009	6.097811977	0.049480989	0.068048292	0.203323921
	Ps. fragi	10:1	5.575862705	5.707467463	5.620375674	0.035048837	0.037694243	0.054964676
	P. phosphoreum		6.278910861	6.672349884	6.512157994	0.088814073	0.182304529	0.073131199
	B. thermosphacta		5.440607589	6.087567105	6.297370305	0.101596828	0.13535422	0.019897232
21 %_O_2/79 %_N_2 (air)	*P. phosphoreum*		5.40018447	6.762760079	7.650225312	0.044106264	0.030246457	0.016914518
	Ps. fragi	1:10	6.395020128	7.672009661	8.788537426	0.065822751	0.074213768	0.045523961
	P. phosphoreum		3.627496691	5.640237571	6.595193354	0.108305552	0.014651521	0.081929987
	B. thermosphacta		6.469868062	7.581739201	8.744562203	0.027721823	0.035472359	0.014405986
	P. phosphoreum		5.302939671	7.009952394	8.034448587	0.172033822	0.088613803	0.054338383
	Ps. fragi	1:1	5.259423782	6.484052179	8.428407492	0.053431467	0.101893296	0.022949678
	P. phosphoreum		3.871627945	6.806349505	6.835246455	0.116632837	0.008548301	0.088209367
	B. thermosphacta		5.53398465	7.504068469	8.280510489	0.016378283	0.074705568	0.036195122
	P. phosphoreum		5.243037224	7.267439981	7.732568156	0.019260674	0.069987914	0.039690736
	Ps. fragi	10:1	5.037414522	6.541607452	8.174429804	0.020749825	0.100521361	0.089427992
	P. phosphoreum		5.560126852	7.220213775	7.486660625	0.021942116	0.021550406	0.094559086
	B. thermosphacta		5.500458186	6.794132332	8.117856818	0.108349985	0.132985343	0.008346995

P. carnosum	Growth alone	70 %_O_2/30 % CO_2	*P. carnosum*	-	4.527153359	4.559224888	3.742379247	0.165275794	0.102871085	0.098224011
		70 %_N_2/30 % CO_2	*P. carnosum*	-	4.527153359	5.497624464	4.697548445	0.165275794	0.069612515	0.084551629
		100 %_N_2	*P. carnosum*	-	4.527153359	5.652435505	4.908035347	0.165275794	0.121550167	0.02258714
		21 %_O_2/79 %_N_2 (air)	*P. carnosum*	-	4.812930858	6.922163999	6.755387283	0.07129409	0.111085393	0.174607078
	interaction	70 %_O_2/30 % CO_2	*P. carnosum*		4.56734467	5.038495395	5.634972815	0.043403871	0.234752627	0.015183758
			Ps. fragi	1:10	6.612316559	6.441133052	7.033836585	0.024302696	0.070114634	0.044651523
			P. carnosum		5.011949182	5.801644769	5.228717332	0.020187753	0.072774007	0.060651066
			B. thermosphacta		6.701518639	7.54988198	7.940219807	0.08161179	0.041342522	0.053234597
			P. carnosum		4.299771522	4.101364667	4.56734467	0.090649029	0.125031238	0.114064581
			Ps. fragi	1:1	5.325668291	5.772438123	5.895424945	0.04081894	0.099208908	0.008547764
			P. carnosum		5.116116201	4.242529371	5.319345678	0.008624544	0.143226556	0.054964676
			B. thermosphacta		5.38562629	6.557415669	7.698416714	0.061654997	0.056440224	0.038027661
			P. carnosum		5.700516802	4.65389044	5.752486087	0.060158	0.07129409	0.022009057
			Ps. fragi	10:1	5.543559367	5.372335786	5.74593263	0.143074851	0.084098752	0.006568356
			P. carnosum		4.850074375	5.360748417	5.40370476	0.04386292	0.052648848	0.110069693
			B. thermosphacta		5.579457501	6.635110228	7.759458453	0.138070717	0.058036468	0.043919287
		70 %_N_2/30 % CO_2	*P. carnosum*		3.39450669	6.240236432	5.099154474	0.081929987	0.028205646	0.265388027
			Ps. fragi	1:10	6.465058157	6.633397015	6.4807989	0.127157449	0.02638059	0.045269679
			P. carnosum		3.596095318	6.035933118	5.774466385	0.131353326	0.328814817	0.1616562
			B. thermosphacta		6.767940827	7.163700558	7.21382083	0.023271459	0.030748556	0.027225456
			P. carnosum		4.358538224	5.863103411	5.591082109	0.336742655	0.087051594	0.253038342
			Ps. fragi	1:1	5.493120651	5.456919563	5.700516802	0.079448487	0.006390745	0.067682556
			P. carnosum		4.631071313	6.674233159	5.927170421	0.179659263	0.021022166	0.141381117
			B. thermosphacta		5.841836079	6.295316869	6.322248178	0.038745249	0.134908315	0.029733376
			P. carnosum		5.528765713	6.358183301	6.311214199	0.240422099	0.211993035	0.115039038
			Ps. fragi	10:1	5.512606877	5.342510338	5.529508245	0.053629812	0.124363729	0.005498665
			P. carnosum		4.700516802	5.68368216	6.372291236	0.186448054	0.237497078	0.122373323
			B. thermosphacta		5.540212054	6.192365091	6.09664399	0.087482505	0.107849166	0.067009106
		100 %_N_2	*P. carnosum*		3.65389044	6.088882094	6.370467827	0.1923542	0.098567197	0.07203912
			Ps. fragi	1:10	6.542104432	6.639546568	6.749618996	0.111140851	0.057337927	0.050860314
			P. carnosum		4.618192382	5.674876385	6.400379214	0.16700329	0.18346667	0.167557421
			B. thermosphacta		6.200261372	7.106972831	7.43581495	0.069356886	0.013801028	0.038357901

	P. carnosum	1:1	4.285973961	6.729895498	5.864100855	0.161290401	0.07785984	0.191542335
	Ps. fragi		5.70114485	6.229845246	5.961893029	0.055698512	0.040122077	0.067060284
	P. carnosum		4.931482088	4.944779124	5.943046269	0.047154398	0.106196655	0.04463482
	B. thermosphacta		5.321638792	6.574251013	7.324914867	0.14806129	0.103667285	0.032327351
	P. carnosum	10:1	5.617376533	6.715185919	6.609658909	0.065702951	0.119276289	0.141324953
	Ps. fragi		5.453348469	5.548014021	5.280701262	0.059625327	0.018483203	0.106654478
	P. carnosum		5.305043798	6.707273196	6.107870196	0.14402067	0.062921787	0.151362739
	B. thermosphacta		5.528076902	6.123097211	6.445778311	0.095708989	0.076980912	0.031414482
21 %_O_2/79 %_N_2 (air)	*P. carnosum*	1:10	5.301639482	6.769558633	6.828183355	0.070997482	0.200031127	0.026375565
	Ps. fragi		6.468028586	7.791321276	8.65248268	0.035729595	0.161609826	0.056664088
	P. carnosum		3.736820566	6.918922968	6.971702945	1.526831788	0.07471397	0.109564836
	B. thermosphacta		6.753417171	8.176526501	8.391416508	0.037018437	0.017747256	0.028705136
	P. carnosum	1:1	4.961055682	6.519788835	6.987223773	0.041540689	0.117472246	0.048512818
	Ps. fragi		5.098636851	6.146264191	8.281807859	0.040524597	0.164750446	0.048331794
	P. carnosum		5.077579364	6.880487497	6.33048693	0.128480664	0.134192776	0.219479551
	B. thermosphacta		5.749793254	7.443860868	7.809018057	0.009720542	0.060379582	0.10151174
	P. carnosum	10:1	4.29206359	7.105771457	7.013617522	1.752838352	0.167924543	0.034004017
	Ps. fragi		5.187910437	5.359923544	8.090433829	0.041575167	0.008154956	0.048515446
	P. carnosum		5.511900862	7.007117156	7.129863331	0.157166088	0.022835201	0.048959968
	B. thermosphacta		5.629051769	7.335011199	7.9129884	0.071120308	0.037349134	0.056927936

12.4 Supplementary files and figures to publication 4 (submitted)

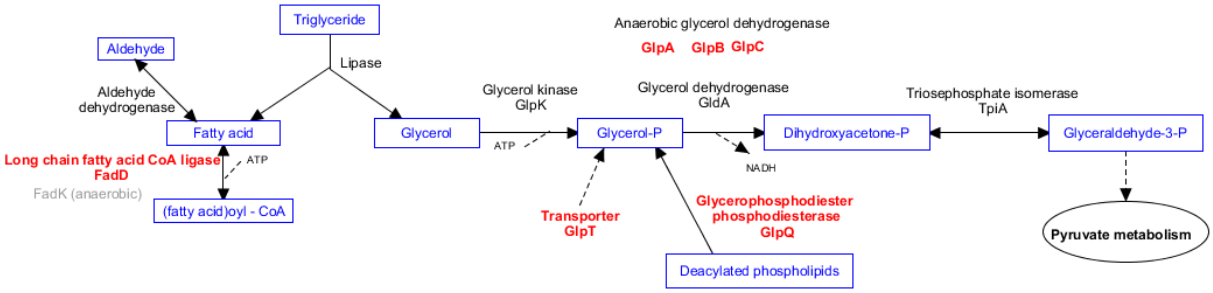


Figure 1

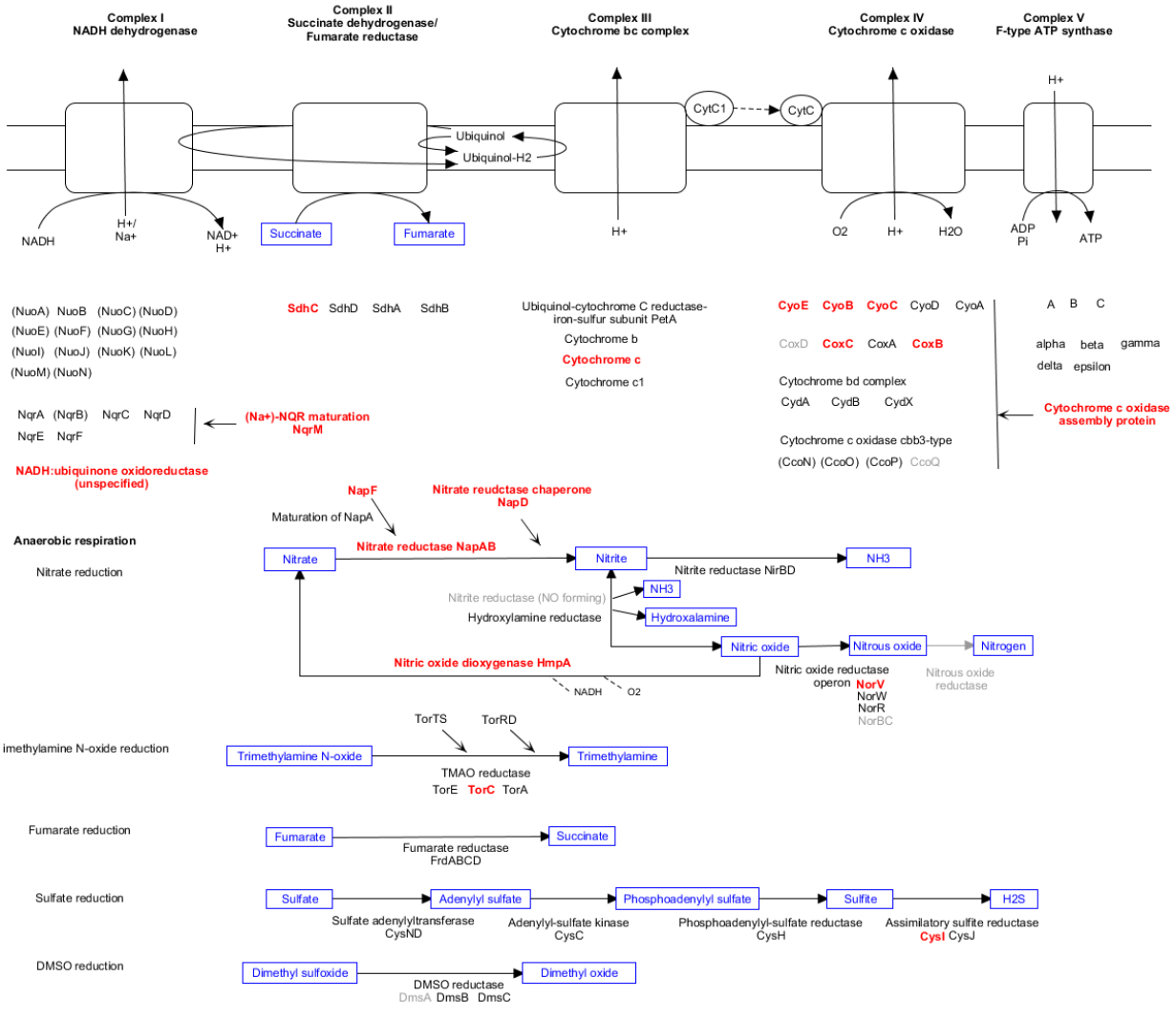


Figure 2

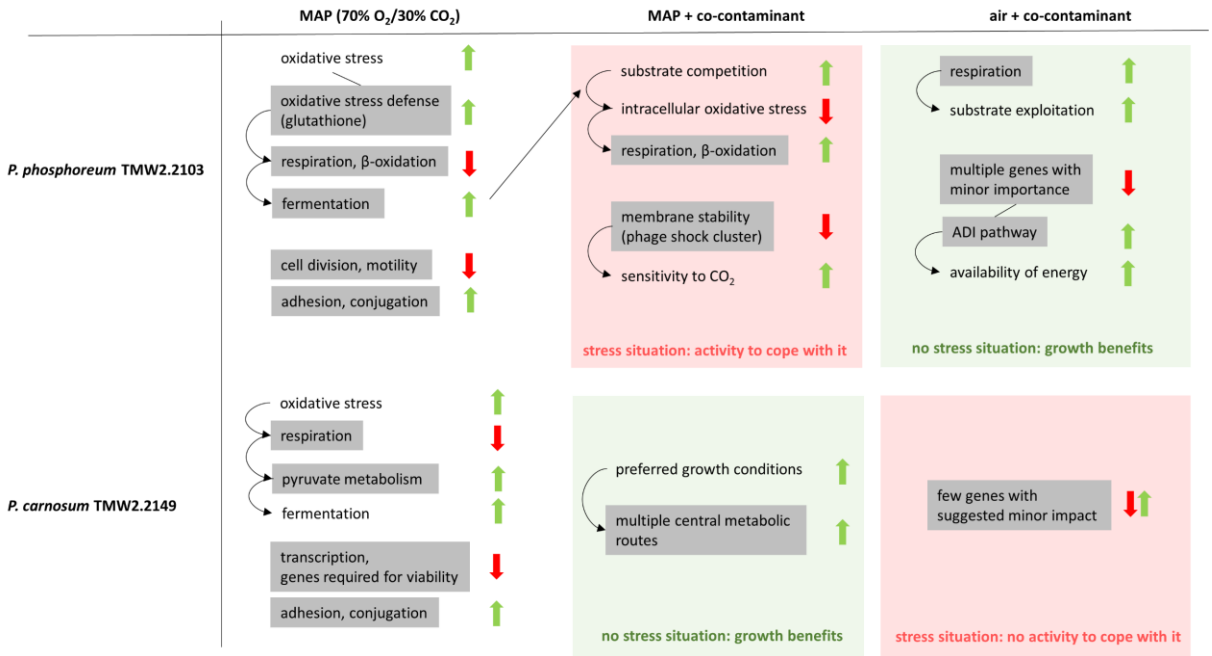


Figure 3

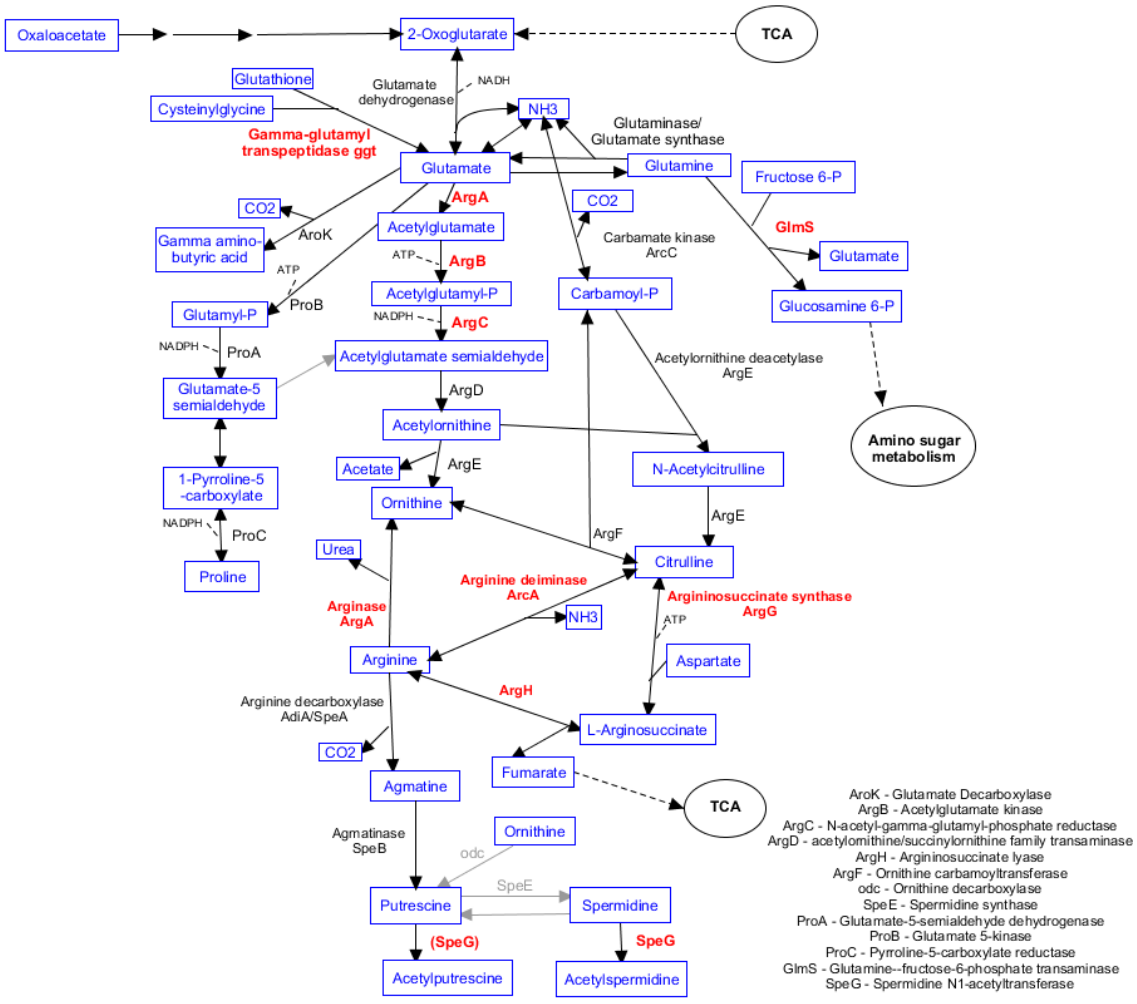


Figure 4

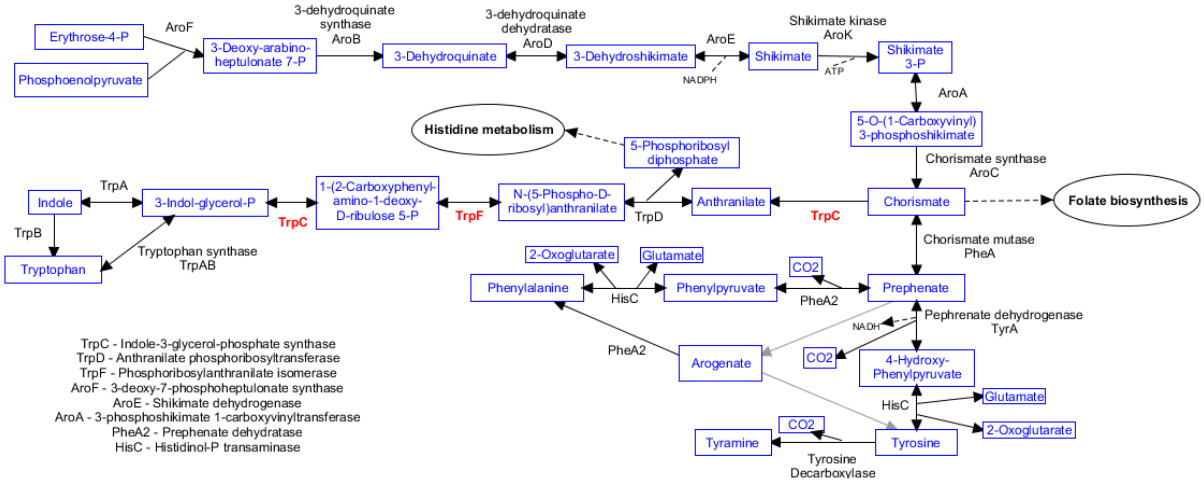
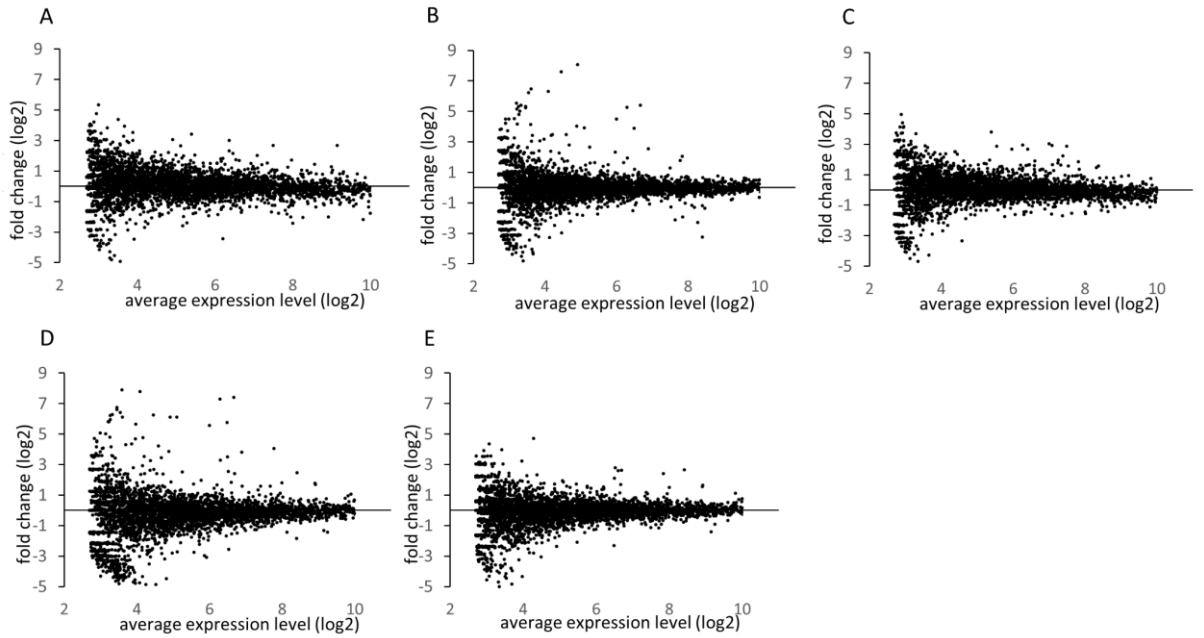
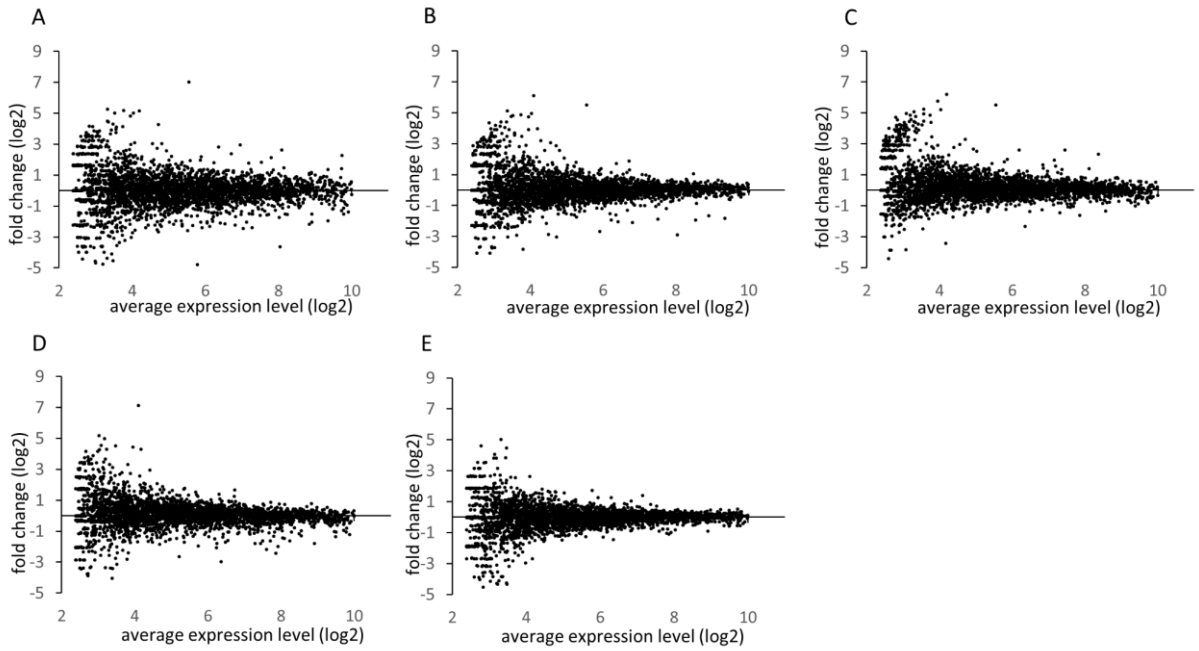


Figure 5

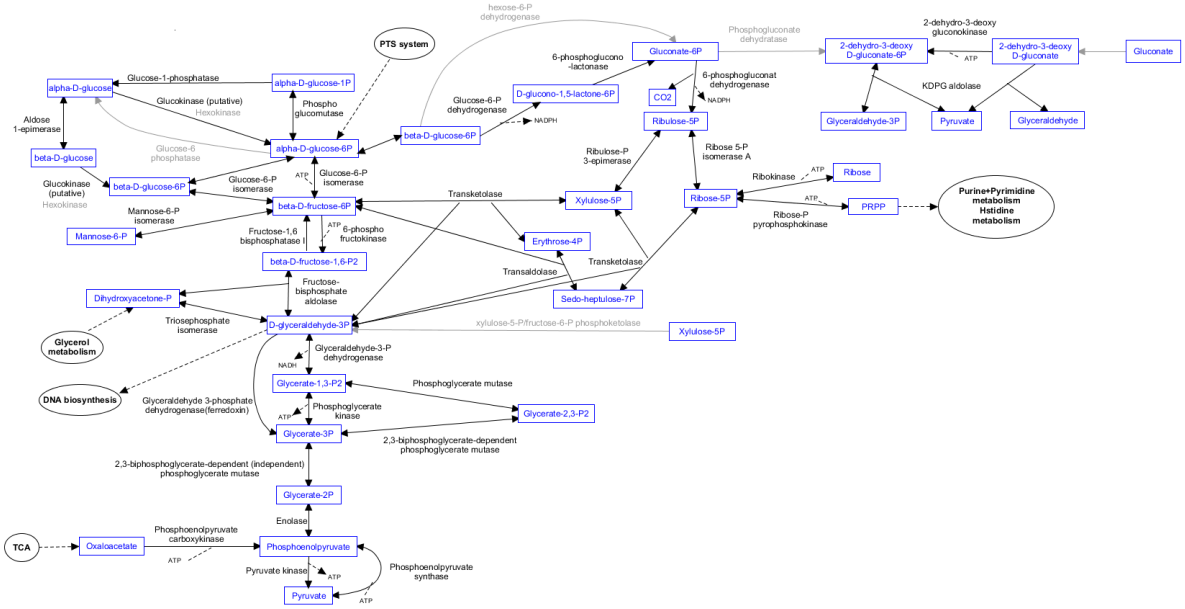


Additional figure 1 Scatter plot of LogFC versus average logCPM values of the *P. phosphoreum* TMW2.2103 samples.

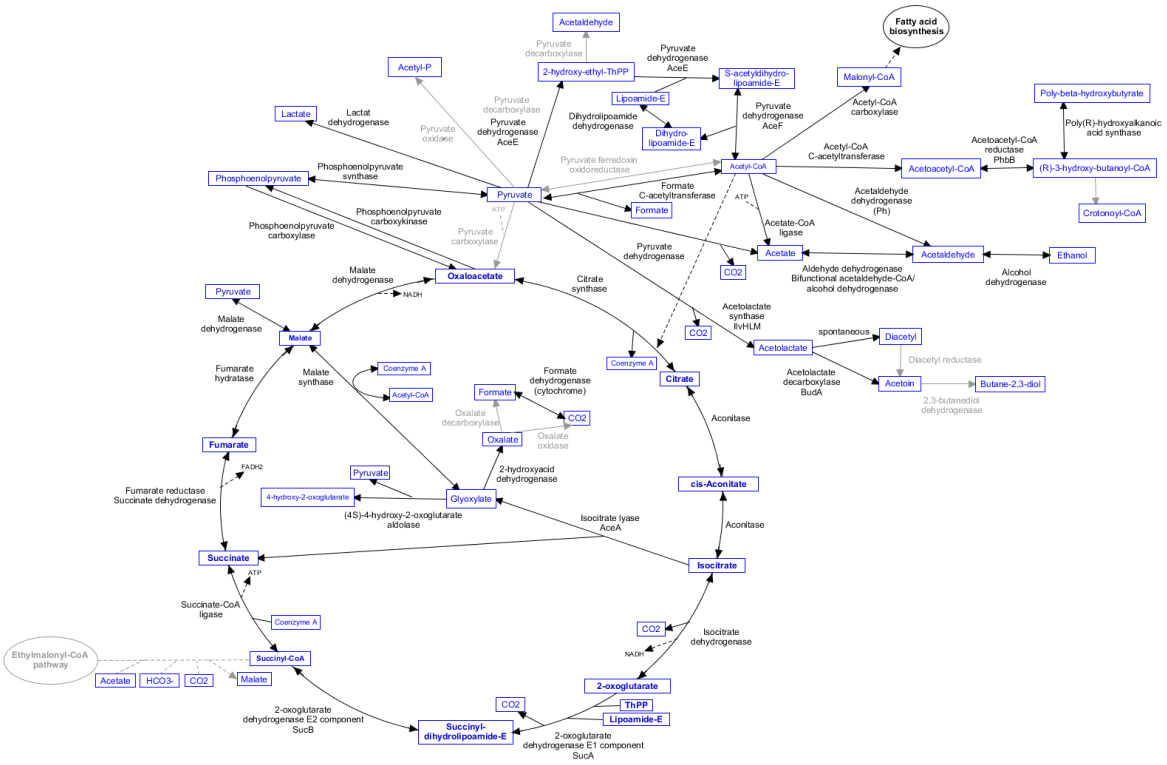
A air atmosphere vs. modified atmosphere, B air atmosphere vs. air atmosphere + *B. thermosphacta*, C air atmosphere vs. air atmosphere + *Ps. fragi,* D modified atmosphere vs. modified atmosphere + *B. thermosphacta,* E modified atmosphere vs. modified atmosphere + *Ps. fragi.*



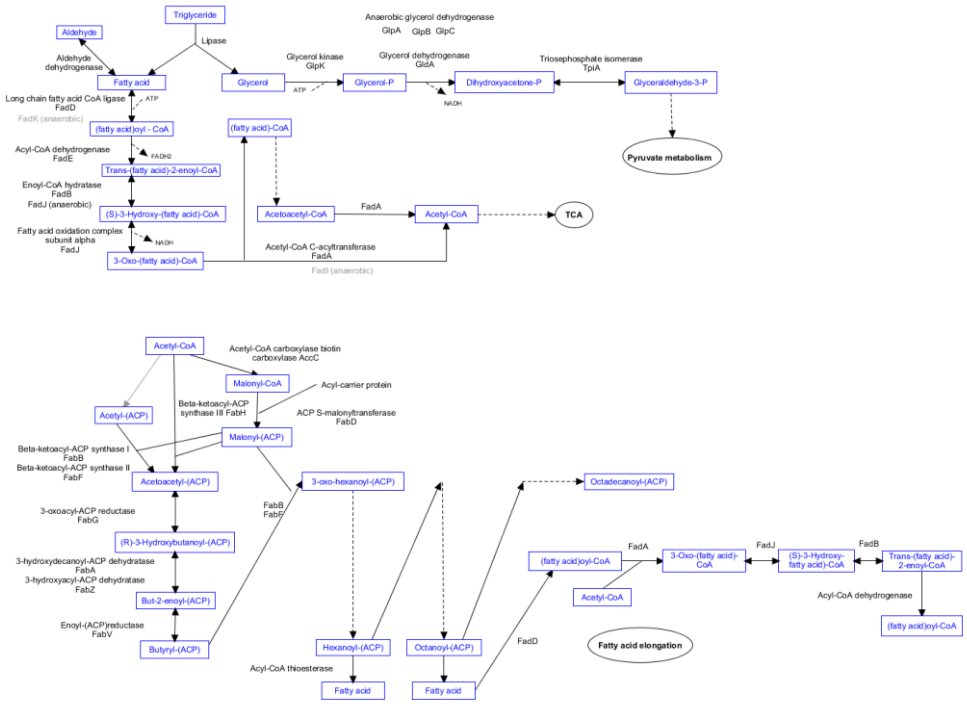
Additional figure 2 Scatter plot of LogFC versus average logCPM values of the *P. carnosum* TMW2.2149 samples.
A air atmosphere vs. modified atmosphere, B air atmosphere vs. air atmosphere + *B. thermosphacta*, C air atmosphere vs. air atmosphere + *Ps. fragi,* D modified atmosphere vs. modified atmosphere + *B. thermosphacta,* E modified atmosphere vs. modified atmosphere + *Ps. fragi.*



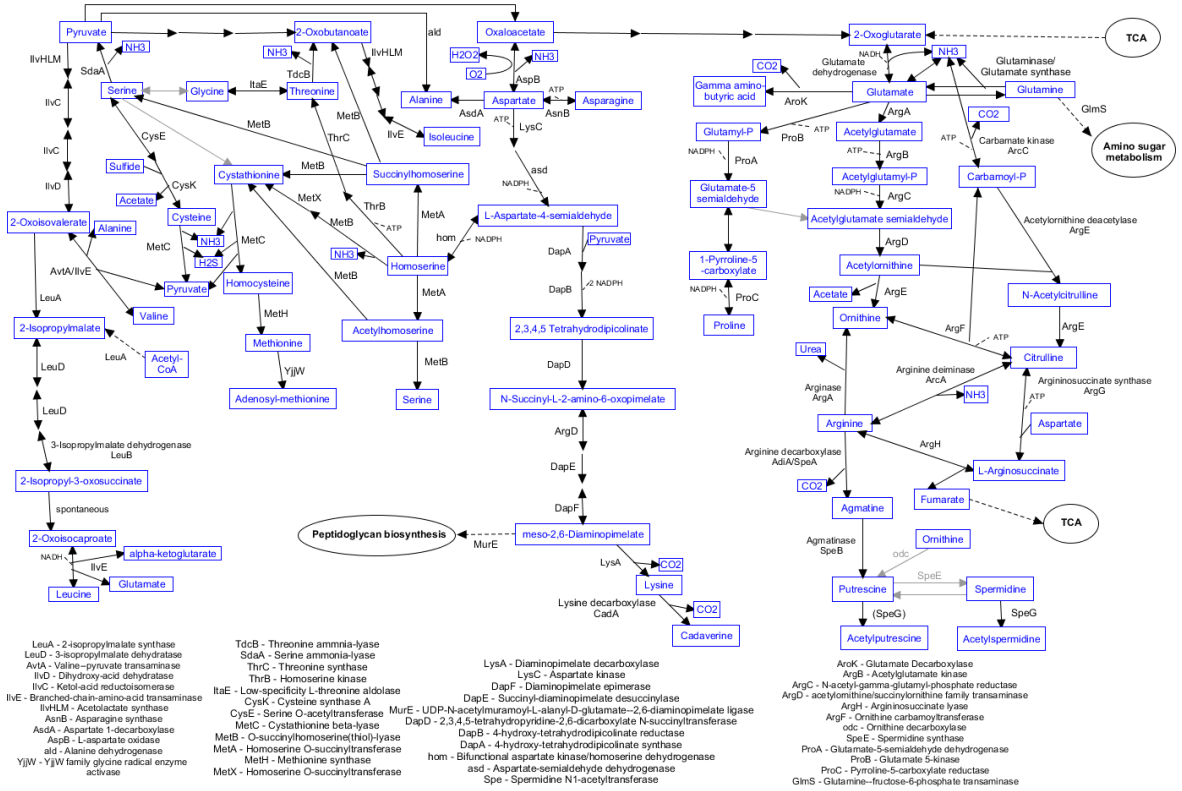
Additional figure 3_1 Predicted metabolic pathways of the carbohydrate metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes.
Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



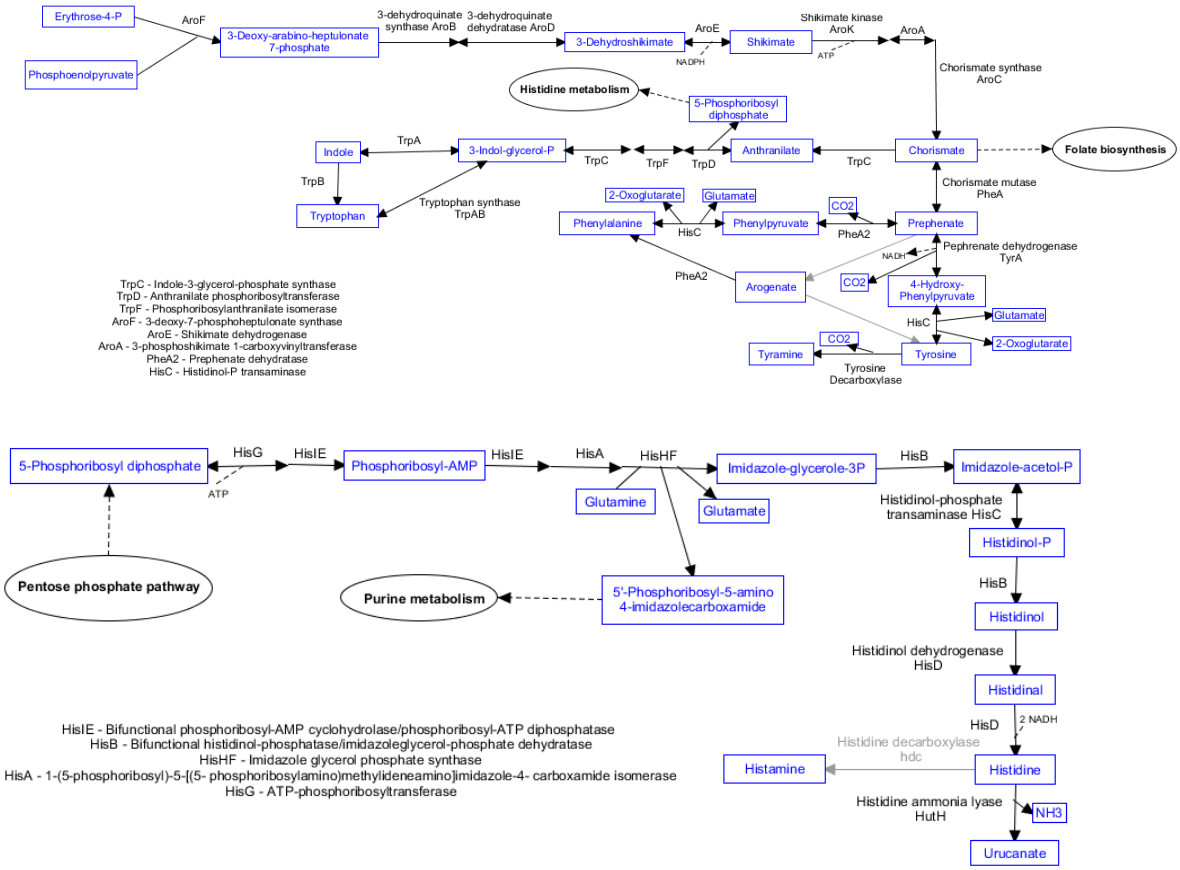
Additional figure 3_2 Predicted metabolic pathways of the tricarboxylic acid cycle and pyruvate metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



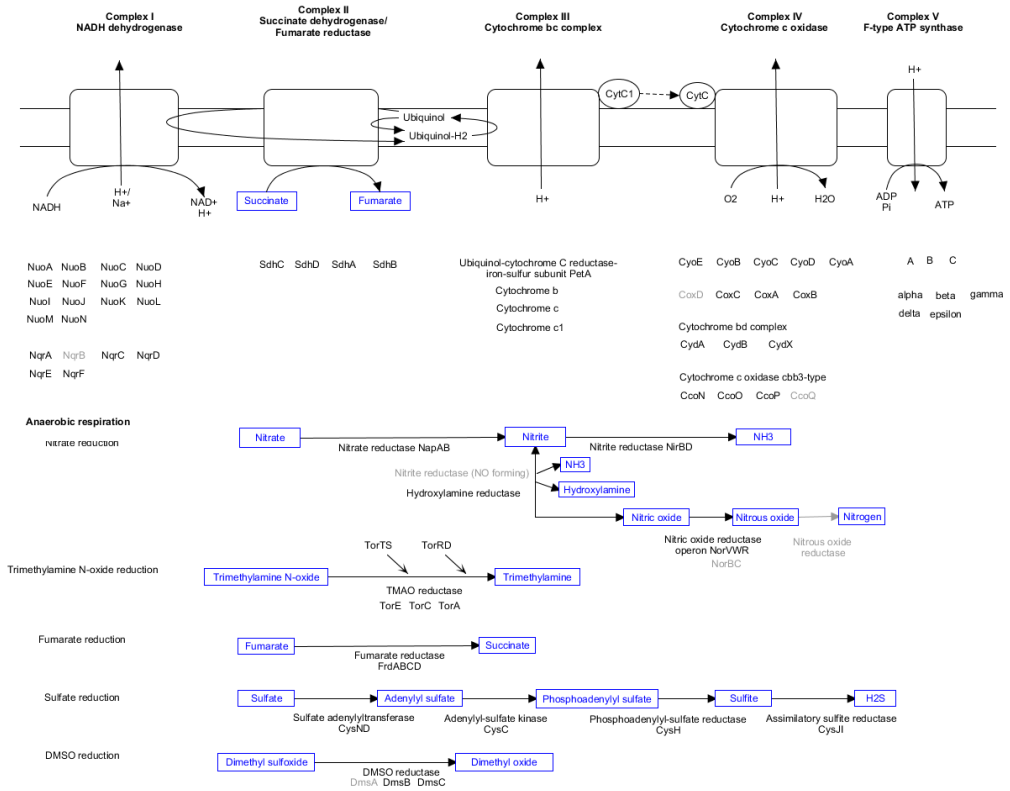
Additional figure 3_3 Predicted metabolic pathways of the fatty acid metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



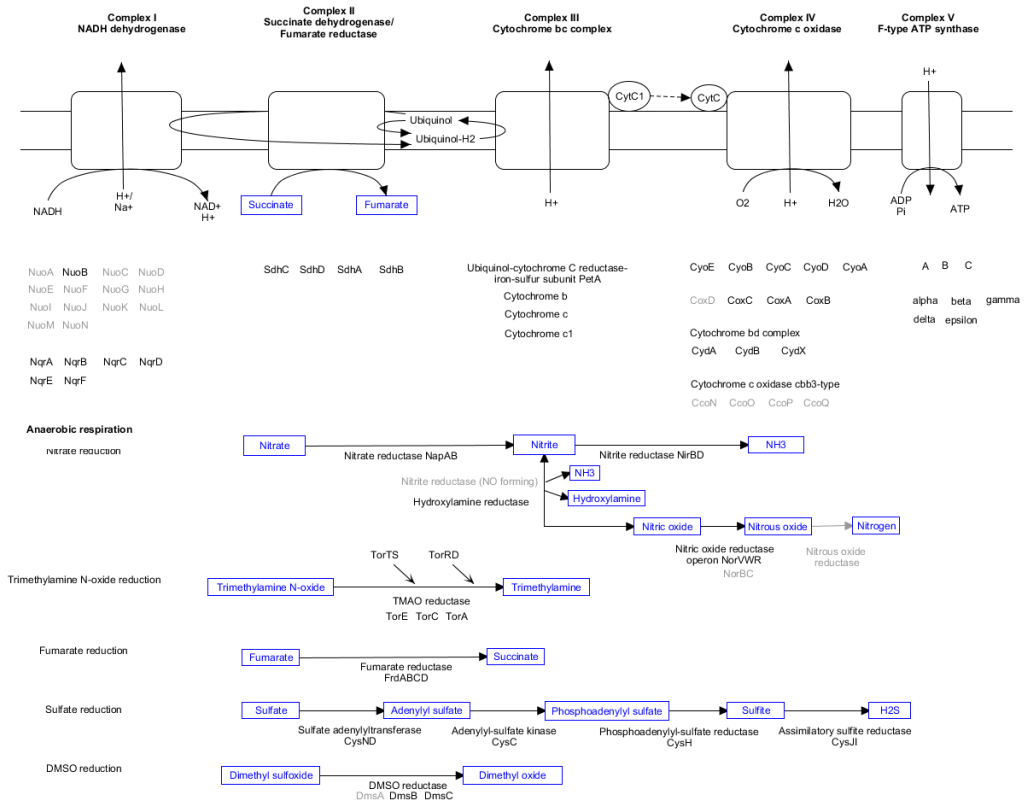
Additional figure 3_4 Predicted metabolic pathways of the amino acid metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 except of tryptophane- and histidine metabolism. The analysis is based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey. Enzymatic activity that is not finally confirmed by the literature is parenthesized.



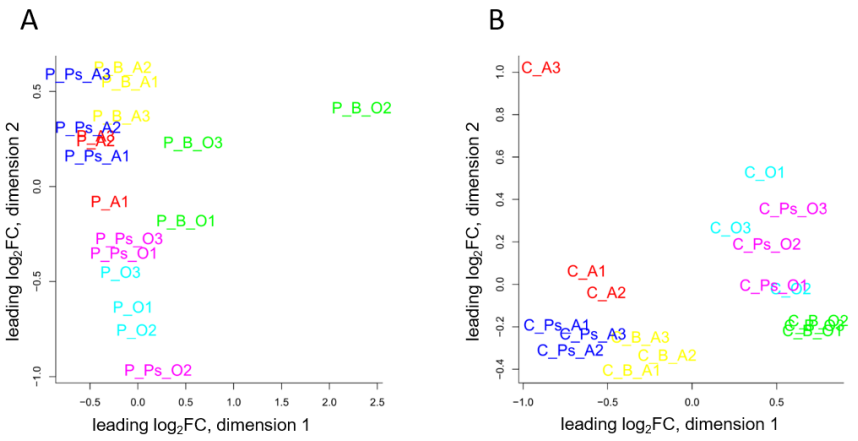
Additional figure 3_5 Predicted metabolic pathways of the tryptophane- and histidine metabolism of *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149. The analysis is based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



Additional figure 3_6 Predicted metabolic pathways of the respiration of *P. phosphoreum* TMW2.2103 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



Additional figure 3_7 Predicted metabolic pathways of the respiration of *P. carnosum* TMW2.2149 based on presence or absence of genes. Genomes were analyzed for presence of the respective gene sequences by means of BLAST. Enzymes of present genes are depicted in black, enzymes of absent genes with relevant function are depicted in grey.



Additional figure 4 Multidimensional scaling plot of the samples based on the logFC. (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *B. thermosphacta* in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *B. thermosphacta* in modified atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 in modified atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *Ps. fragi* in air atmosphere, (■) *P. phosphoreum* TMW2.2103/*P. carnosum* TMW2.2149 vs. *Ps. fragi* in modified atmosphere. **A** *P. phosphoreum* TMW2.2103. **B** *P. carnosum* TMW2 .2149. The figure is based on a scaling plot created by Eurofins Genomics GmbH (Konstanz, Germany) with the R script package edgeR.

Additional table 1 Summary of the sequencing results.
Data were obtained from Eurofins Genomics GmbH (Konstanz, Germany) and are based on sequencing with Illumina HiSeq2500.

strain	atmosphere	co-contaminant	Reads mapped [Mio]	Unique [Mio]	Reference covered [Mb]	Mean read coverage
P. phosphoreum						
TMW2.2103	air	-	17.71 (69.3%)	17.71 (69.3%)	3.34 (69.2%)	770.4
	air	-	31.94 (86.4%)	31.94 (86.4%)	3.46 (71.6%)	1,308.5
	air	-	32.80 (80.8%)	32.79 (80.8%)	3.39 (70.3%)	1,375.0
	MAP	-	16.98 (56.8%)	16.98 (56.8%)	3.22 (66.8%)	752.3
	MAP	-	19.49 (64.6%)	19.49 (64.6%)	3.33 (69.0%)	829.9
	MAP	-	9.86 (43.0%)	9.86 (43.0%)	2.73 (56.6%)	511.4
	air	*Ps. fragi*	19.69 (75.5%)	19.68 (75.5%)	3.14 (65.1%)	848.3
	air	*Ps. fragi*	15.66 (75.1%)	15.66 (75.1%)	2.72 (56.3%)	778.7
	air	*Ps. fragi*	19.55 (72.3%)	19.55 (72.3%)	2.90 (60.1%)	891
	MAP	*Ps. fragi*	14.95 (58.8%)	14.95 (58.8%)	2.68 (55.6%)	776.7
	MAP	*Ps. fragi*	10.01 (46.1%)	10.01 (46.1%)	2.63 (54.5%)	519.5
	MAP	*Ps. fragi*	17.10 (65.3%)	17.10 (65.3%)	2.66 (55.1%)	902.2
	air	*B. thermosphacta*	16.45 (75.4%)	16.45 (75.4%)	2.61 (54.1%)	862.5
	air	*B. thermosphacta*	17.90 (69.5%)	17.90 (69.4%)	2.80 (58.1%)	845.8
	air	*B. thermosphacta*	24.44 (74.1%)	24.44 (74.1%)	2.96 (61.3%)	1,145.2
	MAP	*B. thermosphacta*	12.54 (47.3%)	12.54 (47.2%)	2.45 (50.7%)	635.8
	MAP	*B. thermosphacta*	2.93 (44.5%)	2.93 (44.5%)	1.43 (29.7%)	249.3
	MAP	*B. thermosphacta*	12.46 (36.0%)	12.46 (36.0%)	2.22 (46.1%)	690.6
P. carnosum						
TMW2.2149	air	-	41.86 (85.2%)	41.86 (85.2%)	2.33 (58.6%)	2393.1
	air	-	27.38 (84.8%)	27.37 (84.7%)	2.48 (62.5%)	1,509.0
	air	-	27.65 (84.7%)	27.65 (84.7%)	2.08 (52.3%)	1,807.3
	MAP	-	30.05 (79.2%)	30.04 (79.2%)	2.48 (62.5%)	1,645.6
	MAP	-	23.82 (78.1%)	23.82 (78.0%)	2.92 (73.4%)	1,131.3
	MAP	-	28.04 (78.5%)	28.03 (78.5%)	2.71 (68.2%)	1,420.1
	air	*Ps. fragi*	48.97 (84.4%)	48.95 (84.4%)	3.27 (82.4%)	1,951.6
	air	*Ps. fragi*	42.51 (81.6%)	42.49 (81.6%)	3.33 (83.8%)	1,714.6
	air	*Ps. fragi*	26.30 (79.1%)	26.29 (79.1%)	2.97 (74.8%)	1,206.0
	MAP	*Ps. fragi*	22.35 (63.1%)	22.34 (63.0%)	2.78 (70.0%)	1,087.9
	MAP	*Ps. fragi*	26.29 (70.3%)	26.28 (70.2%)	2.71 (68.3%)	1,320.5
	MAP	*Ps. fragi*	28.59 (77.0%)	28.58 (77.0%)	2.66 (67.1%)	1,468.7
	air	*B. thermosphacta*	24.50 (77.5%)	24.49 (77.5%)	2.28 (57.5%)	1,438.5
	air	*B. thermosphacta*	40.03 (78.2%)	40.01 (78.1%)	2.82 (70.9%)	1,849.3
	air	*B. thermosphacta*	26.12 (75.7%)	26.10 (75.6%)	3.01 (75.6%)	1,173.0
	MAP	*B. thermosphacta*	18.25 (69.7%)	18.24 (69.6%)	2.80 (70.5%)	860.5
	MAP	*B. thermosphacta*	22.81 (73.2%)	22.79 (73.1%)	3.14 (79.0%)	973.2
	MAP	*B. thermosphacta*	23.70 (73.4%)	23.68 (73.4%)	2.92 (73.6%)	1,075.3

Additional table 2 Detected transcripts with highest average logCPM value.
Transcripts that were observed in samples of both strains are marked in red. The average logCPM values were provided by Eurofins Genomics GmbH (Konstanz, Germany).

P. phosphoreum TMW.2103	log_2CPM	*P. carnosum* TMW2.2149	log_2CPM
growth		**growth**	
septum site-determining protein MinD GLP34_00500	9.269	DNA primase DnaG GLP09_08840	9.269
membrane protein insertase YidC GLP34_16710	9.726	lipoprotein leucine-zipper GLP09_13575	9.485
transcription termination factor Rho GLP34_20630	9.746	membrane protein insertase YidC GLP09_13065	9.118
transcription termination/antitermination protein NusG (Rho-dependent) GLP34_19600	9.697	outer membrane protein assembly factor BamA GLP09_12850	9.536
recombinase RecA GLP34_17870	9.328	septum site-determining protein MinD GLP09_00995	9.507
DNA replication/repair protein RecF GLP34_16665	9.227	type I DNA topoisomerase TopA GLP09_01420	9.983
translation/RNA catabolism		UDP-3-O-acyl-N-acetylglucosamine deacetylase LpxC GLP09_06940	9.530
23S rRNA (uridine(2552)-2'-O)-methyltransferase RlmE GLP34_09085	9.946	exoribonuclease II GLP09_16585	9.331
23S rRNA pseudouridine(2605) synthase RluB GLP34_00905	9.395	transcription termination factor Rho GLP09_15970	9.839
30S ribosomal protein S20 RpsT GLP34_09160	9.982	transcription termination/antitermination protein NusG (Rho-dependent) GLP09_14610	9.979
50S ribosomal protein L27 RpmA GLP34_19370	9.763	recombinase RecA GLP09_10810	9.554
50S ribosomal protein L31 RpmE GLP34_10650	9.994	**translation, RNA catabolism**	
50S ribosomal protein L34 RpmH GLP34_16695	9.609	16S rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))- dimethyltransferase RsmA GLP09_08725	9.156
ribosome biogenesis GTPase Der GLP34_15890	9.549	23S rRNA pseudouridine(2605) synthase RluB GLP09_01400	9.129
alanine--tRNA ligase AlaS GLP34_17860	9.683	30S ribosomal protein S20 RpsT GLP09_05770	9.792
glycine--tRNA ligase subunit alpha GlyQ GLP34_16645	9.607	30S ribosomal protein S6--L-glutamate ligase RimK GLP09_06600	9.474
leucine--tRNA ligase LeuS GLP34_18635	9.597	50S ribosomal protein L31 RpmE GLP09_04285	9.533
tRNA (N6-isopentenyl adenosine(37)-C2)-methylthiotransferase MiaB GLP34_18670	9.273	50S ribosomal protein L33 RpmG GLP09_04060	9.710
tRNA dihydrouridine synthase DusB GLP34_19475	9.119	ATP-dependent RNA helicase RhlB GLP09_15980	9.679
tRNA uridine-5-carboxymethylaminomethyl(34) synthesis enzyme MnmG GLP34_16745	9.660	glutamate--tRNA ligase GltX GLP09_00370	9.996
ATP-dependent RNA helicase RhlB GLP34_20620	9.579	glutamine--tRNA ligase GlnS GLP09_00810	9.816
transport		glycine--tRNA ligase subunit alpha GlyQ GLP09_13000	9.545
OmpA family protein GLP34_16640	9.556	leucine--tRNA ligase LeuS GLP09_15855	9.477
outer membrane channel protein TolC GLP34_15370	9.477	methionine--tRNA ligase MetG GLP09_09610	9.345
secretion/conjugation		ribosome biogenesis GTPase Der GLP09_12575	9.475
type II secretion system protein M GLP34_14815	9.847	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis enzyme MnmG GLP09_13100	9.789

Fe-S cluster	
Fe-S cluster assembly scaffold IscU GLP34_15810	9.858
Fe-S cluster assembly transcriptional regulator IscR GLP34_15800	9.591
Fe-S protein assembly chaperone HscA GLP34_15825	9.283
carbohydrate transport	
HPr family phosphocarrier protein GLP34_10060	9.943
glycolysis, gluconeogenesis	
2,3-diphosphoglycerate-dependent phosphoglycerate mutase GpmA GLP34_02570	9.732
6-phosphofructokinase PfkA GLP34_10725	9.478
phosphoenolpyruvate carboxykinase (ATP) PckA GLP34_14880	9.210
pentose phosphate pathway	
glucose-6-phosphate dehydrogenase GLP34_17350	9.481
TCA	
phosphoenolpyruvate carboxylase GLP34_10625	9.608
succinate dehydrogenase/fumarate reductase iron-sulfur subunit GLP34_19710	9.529
respiratory chain	
ATP synthase subunit I GLP34_03600	9.212
cytochrome bd-I ubiquinol oxidase subunit CydA GLP34_09530	9.460
cytochrome d ubiquinol oxidase subunit II CydB GLP34_00685	9.795
F0F1 ATP synthase subunit A AtpB GLP34_03605	9.302
F0F1 ATP synthase subunit A AtpB GLP34_16770	9.561
F0F1 ATP synthase subunit alpha AtpA GLP34_16790	9.695
F0F1 ATP synthase subunit B AtpF GLP34_03615	9.290
F0F1 ATP synthase subunit beta AtpD GLP34_16800	9.612
F0F1 ATP synthase subunit gamma AtpG GLP34_03630	9.662
NADH:ubiquinone reductase (Na(+)-transporting) subunit F NqrF GLP34_10215	9.780
hemoglobin metabolism	
porphobilinogen synthase HemB GLP34_15100	9.787
energy supply	
GTPase HflX GLP34_05125	9.192
Obg family GTPase CgtA GLP34_19360	9.245
pyuvate metabolism	
formate dehydrogenase subunit alpha GLP34_07595	9.218
tyrosine--tRNA ligase TyrS GLP09_11935	9.300
transport	9.301
autotransporter domain-containing protein GLP09_12360	9.177
secretion, conjugation	
type I secretion C-terminal target domain-containing protein GLP09_17580	9.053
cofactors	
hydrogenase 2 large subunit GLP09_04920	9.743
Fe-S cluster	
iron-sulfur cluster assembly protein IscA GLP09_12500	9.195
general carbohydrate metabolism	
alpha-D-glucose phosphate-specific phosphoglucomutase GLP09_00835	9.181
carbohydrate transport	
PTS glucose transporter subunit IIA GLP09_00320	9.569
PTS sugar transporter subunit IIABC GLP09_09455	9.373
HPr family phosphocarrier protein GLP09_00330	9.402
sugar transporter GLP09_02915	9.132
glycolysis, gluconeogenesis	
phosphoenolpyruvate carboxykinase (ATP) PckA GLP09_11795	9.869
6-phosphofructokinase PfkA GLP09_04215	9.320
adenylate kinase GLP09_00765	9.262
pentose phosphate pathway	
glucose-6-phosphate dehydrogenase GLP09_14865	9.723
acetate/propionate family kinase GLP09_02735	9.990
acetate/propionate family kinase GLP09_08345	9.106
TCA	
succinate dehydrogenase/fumarate reductase iron-sulfur subunit FrdB GLP09_14380	9.786
fumarate hydratase GLP09_01730	9.784
oxaloacetate-decarboxylating malate dehydrogenase MaeA GLP09_10425	9.509
anaerobic C4-dicarboxylate transporter GLP09_14445	9.497
phosphoenolpyruvate carboxylase GLP09_04305	9.352
2-hydroxyacid dehydrogenase GLP09_09405	9.728
respiratory chain	
c-type cytochrome GLP09_14310	9.707

	iron-containing alcohol dehydrogenase GLP34_07740	9.810
	pyruvate dehydrogenase complex transcriptional repressor PdhR GLP34_11350	9.611
fatty acid metabolism		
	acetyl-CoA carboxylase biotin carboxylase subunit AccC GLP34_19610	9.668
	acyl carrier protein AcpP GLP34_06505	9.706
amino acid metabolism		
	aminopeptidase PepB GLP34_15840	9.228
	aspartate kinase GLP34_17855	9.294
	ATP-dependent Clp endopeptidase proteolytic subunit ClpP GLP34_19780	9.621
	ATP-dependent Clp protease ATP-binding subunit ClpA GLP34_06300	9.457
	beta-Ala-His dipeptidase PepD GLP34_10195	9.862
	C69 family dipeptidase GLP34_03420	9.989
	glutaminase A GlsA GLP34_15940	9.204
	glycine C-acetyltransferase GLP34_02365	9.213
	HslU--HslV peptidase ATPase subunit HslU GLP34_10675	9.756
	L-threonine 3-dehydrogenase YiaY GLP34_02370	9.276
	peptidylprolyl isomerase GLP34_10445	9.416
	peptidylprolyl isomerase PpiD GLP34_19760	9.409
	phosphatidylserine decarboxylase GLP34_12740	9.381
	preprotein translocase subunit SecE GLP34_19605	9.204
	signal recognition particle protein GLP34_20115	9.262
	twin-arginine translocase subunit TatA GLP34_15120	9.290
	twin-arginine translocase subunit TatC GLP34_15110	9.820
	type 1 glutamine amidotransferase GLP34_03805	9.317
	type I methionyl aminopeptidase Map GLP34_16135	9.530
aminotransferasen		
	aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme GLP34_10355	9.951
purine and pyrimidine metabolism		
	nucleoside-specific channel-forming protein Tsx GLP34_10270	9.566
Purines	purine-nucleoside phosphorylase DeoD GLP34_08925	9.742
	IMP dehydrogenase GuaB GLP34_15910	9.958
	guanylate kinase GLP34_10925	9.752
	glutamine-hydrolyzing GMP synthase GuaA GLP34_15915	9.628
	adenylosuccinate synthase GLP34_19165	9.257
	cytochrome d ubiquinol oxidase subunit II CydB GLP09_01175	9.482
	F0F1 ATP synthase subunit C AtpE GLP09_08400	9.718
	Na(+)-translocating NADH-quinone reductase subunit A GLP09_00155	9.611
	NADH:ubiquinone reductase (Na(+)-transporting) subunit B GLP09_00160	9.104
	NADH:ubiquinone reductase (Na(+)-transporting) subunit F NqrF GLP09_00180	9.416
	hydrogenase small subunit GLP09_04925	9.337
butanoate metabolism		
	acetolactate decarboxylase BudA GLP09_17550	9.262
energy supply		
	GTPase Era GLP09_10910	9.205
pyuvate metabolism		
	alpha-hydroxy-acid oxidizing protein GLP09_15205	9.825
	esterase FrsA GLP09_00215	9.139
	pyruvate dehydrogenase complex transcriptional repressor PdhR GLP09_06875	9.475
fatty acid metabolism		
	acetyl-CoA carboxylase biotin carboxylase subunit AccC GLP09_14485	9.172
	acetyl-CoA carboxylase carboxyl transferase subunit alpha AccA GLP09_12890	9.506
	acyl carrier protein AcpP GLP09_09515	9.335
	beta-ketoacyl-ACP synthase I FabB GLP09_03100	9.612
amino acid metabolism		
	asparagine synthase B GLP09_00780	9.347
	aspartate kinase GLP09_10795	9.362
	ATP-dependent Clp protease ATP-binding subunit ClpA GLP09_03655	9.138
	beta-Ala-His dipeptidase PepD GLP09_00200	9.603
	bifunctional glutathionylspermidine amidase/synthase GLP09_05035	9.714
	carboxy terminal-processing peptidase GLP09_15330	9.946
	ClpXP protease specificity-enhancing factor SspB GLP09_07045	9.398
	cysteine synthase A CysK GLP09_00335	9.270
	FKBP-type peptidyl-prolyl cis-trans isomerase FkpA GLP09_15600	9.763
	L-threonine 3-dehydrogenase YiaY GLP09_01310	9.727
	preprotein translocase subunit SecE GLP09_14615	9.135
	shikimate kinase AroK GLP09_04370	9.088
	type I methionyl aminopeptidase Map GLP09_12805	9.817
aminotransferasen		

Pyrimidines	UMP kinase PyrH GLP34_16150	9.208
stress response		
oxidative	catalase GLP34_07970	9.247
	Dyp-type peroxidase GLP34_04655	9.719
	glutathione synthase GshB GLP34_20440	9.341
	redoxin family protein GLP34_10600	9.686
nutrient	bifunctional GTP diphosphokinase/guanosine-3',5'-bis pyrophosphate 3'-pyrophosphohydrolase SpoT GLP34_10935	9.214
cold/heat shock	ribosome-associated translation inhibitor RaiA GLP34_12745	9.546
	co-chaperone GroES GLP34_19670	9.543
other	autonomous glycyl radical cofactor GrcA GLP34_09290	9.899
	RNA chaperone ProQ GLP34_19010	9.463
unknown function		
	acid phosphatase AphA GLP34_04980	9.225
	hypothetical protein GLP34_13460	9.238
	iron-sulfur cluster-binding protein GLP34_01555	9.993
	TIGR00153 family protein GLP34_15345	9.406
	YicC family protein GLP34_10920	9.824

	aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme GLP09_00035	9.194
purine and pyrimidine metabolism		
Purines	adenylosuccinate synthase GLP09_15475	9.326
	glutamine-hydrolyzing GMP synthase GuaA GLP09_12600	9.486
	guanylate kinase GLP09_04015	9.058
	IMP dehydrogenase GuaB GLP09_12595	9.911
	purine-nucleoside phosphorylase DeoD GLP09_05555	9.387
Pyrimidines	uracil phosphoribosyltransferase Upp GLP09_15735	9.311
stress response		
oxidative	alkyl hydroperoxide reductase subunit F AhpF GLP09_05500	9.782
	Dyp-type peroxidase GLP09_06330	9.532
	glutathione synthase GshB GLP09_17395	9.266
	S-(hydroxymethyl)glutathione synthase Gfa GLP09_17575	9.386
osmotic	small-conductance mechanosensitive channel MscS GLP09_11045	9.482
cold/heat shock	co-chaperone GroES GLP09_14425	9.875
	Fe-S protein assembly chaperone HscA GLP09_12510	9.652
	nucleotide exchange factor GrpE GLP09_16365	9.252
	ATP-dependent protease subunit HslV GLP09_04265	9.695
	DnaJ domain-containing protein GLP09_06700	9.234
other	RNA chaperone ProQ GLP09_15325	9.395
unknown function		
	DUF2076 family protein GLP09_16535	9.476
	hypothetical protein GLP09_11420	9.434
	TIGR00153 family protein GLP09_08905	9.467
	YicC family protein GLP09_04020	9.566
	iron-sulfur cluster-binding protein GLP09_02025	9.364

Additional table 3 Detected significant regulation in *P. phosphoreum* TMW2.2103 samples. Significance was accepted with LogFC ≥ 2/≤-2 and p < 0.05.

		atmosphere	MAP	air	air	MAP	MAP
		co-contaminant	-	*Ps. fragi*	*B. thermosphacta*	*Ps. fragi*	*B. thermosphacta*
growth							
	class 1b ribonucleoside-diphosphate reductase subunit alpha nrdE GLP34_12975		-5.360				4.315
	ribonucleoside-diphosphate reductase subunit beta nrdF GLP34_12970		-3.838			4.563	
	anaerobic ribonucleoside-triphosphate reductase-activating protein nrdG GLP34_04895		-4.388				
	cell division protein FtsZ GLP34_12645		-2.070				2.607
	cell division protein ftsL GLP34_11475				2.967		
	UDP-N-acetylglucosamine--undecaprenyl-phosphate N-acetylglucosaminephosphotransferase wecA GLP34_09945		-2.866				
	UDP-N-acetylglucosamine 2-epimerase wecB GLP34_02330				-5.027		
	oligosaccharide flippase family protein GLP34_20740				-3.437		-2.198
	polysaccharide export protein GLP34_20580				4.249		
	capsular polysaccharide biosynthesis protein GLP34_20600						5.509
	capsule polysaccharide biosynthesis protein GLP34_20550						
	membrane-bound lytic murein transglycosylase C mltC GLP34_07350		-2.163				
	glycosyltransferase GLP34_18860				-6.485		-2.418
	glycosyltransferase GLP34_17250						2.564
	sugar-transfer associated ATP-grasp protein GLP34_09970						-4.065
	RecT family protein GLP34_14295				-5.406		-3.451
	tyrosine-type recombinase/integrase GLP34_17745						-2.509
	DNA gyrase subunit B GLP34_03555						2.730
	DNA-binding protein HU GLP34_14325			-4.963			
	putative adenosine monophosphate-protein transferase Fic GLP34_02460				2.491		
translation/RNA catabolism							
	ribosomal protein S5-alanine N-acetyltransferase rimJ GLP34_01145						3.044
	prolyl-tRNA synthetase associated domain-containing protein GLP34_08150				-3.064		-4.292
	tRNA (N6-threonylcarbamoyladenosine(37)-N6)-methyltransferase tsaA GLP34_16250						2.608
	MBL fold metallo-hydrolase GLP34_21055						-2.014
	ribonuclease H rnhA GLP34_18470			-2.151			
	YigZ family protein GLP34_16600				3.265		
transport							
ion	glutathione-regulated potassium-efflux system ancillary protein KefG GLP34_10460		-2.279	-2.904			
	glutathione-regulated potassium-efflux system ancillary protein KefB GLP34_00280			-2.336			

	efflux RND transporter periplasmic adaptor subunit GLP34_01750	-2.272				
	efflux RND transporter periplasmic adaptor subunit GLP34_03905					3.182
	divalent cation tolerance protein CutA GLP34_13780	-2.097				
	copper homeostasis protein CutC GLP34_15735	2.592				
	efflux RND transporter periplasmic adaptor subunit GLP34_14070				-2.036	
	iron chelate uptake ABC transporter family permease GLP34_11935			-8.065		-3.816
	ABC transporter ATP-binding protein GLP34_08960		-3.001		-2.865	
	phosphate import ATP-binding protein pstB GLP34_15725					5.024
general	ABC transporter permease subunit GLP34_01095					2.619
	ABC transporter permease subunit GLP34_01735			4.333		
	ABC transporter permease subunit GLP34_02815	4.912				
	ABC transporter permease subunit GLP34_05410	6.516			-4.723	
	ABC transporter permease subunit GLP34_07820	-2.124				
	ABC transporter substrate-binding protein GLP34_07855		-2.894			
	ATP-binding cassette domain-containing protein GLP34_08020					
	ATP-binding cassette domain-containing protein GLP34_16735			-2.277		-2.807
	porin GLP34_00165			-3.187		
	MFS transporter GLP34_03650	-2.705				
	transmembrane transporter (MFS) tsgA GLP34_21075					2.562
	OmpA family protein GLP34_07515		4.043			
	OmpA family protein GLP34_18315	-2.185				
secretion/conjugation						
	MSHA pilus biogenesis protein MshJ GLP34_11780	4.703				
	TraY domain-containing protein GLP34_18235		-2.456			
	type IV conjugative transfer system coupling protein TraD virD4 GLP34_14680			-4.498		
	type IV secretion protein A TrbC/VIRB2 pilin GLP34_18255	2.439				-2.467
	type IV secretion system protein VirB10 GLP34_18280		-2.508			
cofactors/vitamins						
	riboflavin synthase subunit alpha GLP34_01810					2.668
	sodium/pantothenate symporter panF GLP34_19465					5.545
	sulfur carrier protein thiS GLP34_15165				4.329	
Fe-S cluster						
	Fe-S cluster assembly protein sufB GLP34_12995		3.345			
motility						
	flagellar basal body L-ring protein flgH GLP34_09865	-3.045				

	flagellar basal-body rod protein FlgF GLP34_09875	-3.484			4.313	
	flagellar biosynthesis protein FlhA GLP34_09720	-3.527				
	flagellar biosynthetic protein FliP GLP34_09740	-3.039				
	methyl-accepting chemotaxis protein (MCP) GLP34_01255			-5.275		-6.108
biofilm						
	diguanylate cyclase GLP34_01520	-2.412	-2.783			
	diguanylate cyclase GLP34_16055					4.854
	fimbrial protein GLP34_00240					-6.222
	Ig-like domain-containing protein GLP34_11860	-2.223				
signaling						
	EAL domain-containing protein GLP34_05415			2.399		
	cyclic nucleotide-binding domain-containing protein GLP34_21165			2.661		
aromatic compounds						
	carboxymuconolactone decarboxylase family protein GLP34_13395	-2.171				
general carbohydrate metabolism						
	chitinase GLP34_12590			-3.479		-3.566
	DeoR family transcriptional regulator GLP34_16815		-3.802			
	glycoside hydrolase family 9 protein GLP34_09445					4.576
	glycoside hydrolase family 92 protein GLP34_03185					4.443
	sulfur carrier protein fdhD GLP34_02850			-3.896		-7.405
carbohydrate transport						
	EAL domain-containing protein EIIC GLP34_01010			-5.414		-6.122
	melibiose:sodium transporter, symporter melB GLP34_12730		-2.373			
	sugar ABC transporter substrate-binding protein GLP34_02105					-5.807
glycolysis, gluconeogenesis						
	ArsJ-associated glyceraldehyde-3-phosphate dehydrogenase GLP34_13110			-3.932		-3.514
TCA						
	sodium:dicarboxylate symporter family GLP34_17295	4.537				
pyuvate metabolism						
	iron-containing alcohol dehydrogenase GLP34_07740	2.171				
respiratory chain						
	(Na+)-NQR maturation NqrM GLP34_18040		-2.385			
	cytochrome bo(3) ubiquinol oxidase subunit 3 cyoC GLP34_12340		-3.687			
	cytochrome c oxidase assembly protein GLP34_11040	-4.764				
	cytochrome c oxidase GLP34_07525					-4.351

	cytochrome c oxidase subunit 3 GLP34_11035	-2.271			4.993	
	molybdopterin-dependent oxidoreductase GLP34_00100			4.512		
	NADH:ubiquinone oxidoreductase GLP34_13875	2.439				
	heme ABC transporter ATP-binding protein GLP34_13410		2.905			
anaerobic respiration						
nitrate	periplasmic nitrate reductase electron transfer subunit napB GLP34_10110	-2.715				
	periplasmic nitrate reductase subunit alpha napA GLP34_10115	-2.060			2.090	
	nitrate reductase protein napD GLP34_10120	-2.807				
	ferredoxin-type protein napF GLP34_10125		4.262	4.237		
	anaerobic nitric oxide reductase flavorubredoxin norV GLP34_03265			-5.320		
Sulfate	assimilatory sulfite reductase (NADPH) hemoprotein subunit cysI GLP34_19280		2.670			
TMAO	cytochrome c-type protein torC GLP34_01415	-2.779				
fatty acid metabolism						
	long-chain fatty acid--CoA ligase FadD GLP34_07300	-3.950			3.778	3.023
	beta-ketoacyl synthase GLP34_07865	-2.025				
	3-ketoacyl-ACP reductase FabG GLP34_13770		-4.417			
	anaerobic glycerol-3-phosphate dehydrogenase subunit A glpA GLP34_17475	2.047		2.286		
	anaerobic glycerol-3-phosphate dehydrogenase subunit C glpC GLP34_17485			2.124		
	glycerol-3-phosphate dehydrogenase gldA GLP34_11080, GLP34_10755			3.248		
amino acid metabolism						
biosynthesis/metabolism	2-isopropylmalate synthase leuA GLP34_15235			-2.664		-7.283
	3-isopropylmalate dehydrogenase leuB GLP34_15230					5.682
	argininosuccinate lyase argH GLP34_10605		-2.228			
	argininosuccinate synthase argG GLP34_10610		-2.921			
	cysteine desulfurase-like protein GLP34_12670					5.226
	LysR family transcriptional regulator GLP34_12145					2.321
	LysR family transcriptional regulator GLP34_17665			-5.170		-2.629
	bifunctional indole-3-glycerol-phosphate synthase TrpC/phosphoribosylanthranilate isomerase TrpF GLP34_00875	-2.284			2.487	
	selenocysteine-specific translation elongation factor selB GLP34_15580			-2.789		-6.283
	YjjI family glycine radical enzyme GLP34_02625		-3.671	-2.343		
	glutamine--fructose-6-phosphate aminotransferase glmS GLP34_16820	-2.696	-2.862			
	YjjW family glycine radical enzyme activase yjjW GLP34_0262		-2.515	-2.279		
	cyclopropane fatty acyl phospholipid synthase cfa GLP34_17715					-2.554
decarboxylation/biogenic amines	pyridoxal-dependent decarboxylase GLP34_08315	-2.065				

	lysine decarboxylase cadA GLP34_15540	-2.826				
	spermidine N1-acetyltransferase speG GLP34_12140					-6.264
	N-acetyl-gamma-glutamyl-phosphate reductase argC GLP34_10620		-2.687			
	acetylglutamate kinase argB GLP34_10615		-2.945			
	arginase GLP34_02255					4.363
degradation	zincin-like metallopeptidase GLP34_18380		-3.187			
	peptidase M66 GLP34_21090			-3.040		
	leucyl/phenylalanyl-tRNA--protein transferase GLP34_06315					-4.554
	metalloendopeptidase GLP34_06925	3.469				
	peptidase C45 GLP34_02835	3.797	2.086			
ADI pathway	arginine deiminase arcA GLP34_15445					-7.895
transport	amino acid carrier protein GLP34_09270					5.006
	amino acid permease GLP34_13210		-2.375			
	amino acid permease GLP34_21570	-2.165				
	methionine import ATP-binding protein metN GLP34_16265			2.095		
	oligopeptide ABC transporter ATP-binding protein oppF GLP34_06250		-2.320			
	peptidase domain containing ABC transporter GLP34_05670	-2.909				
	biopolymer transporter ExbD GLP34_13425	3.165				
	GNAT family N-acetyltransferase GLP34_01300					4.348
	GNAT family N-acetyltransferase GLP34_02205			4.045		
	GNAT family N-acetyltransferase GLP34_02440					-5.758
modification	YdiU family protein GLP34_02575	-3.003				
aminotransferasen						
	aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme GLP34_05765					2.218
dioxygenases						
	2oxoglutarate-Fe dioxygenase GLP34_12675		2.108			
	2-oxoglutarate-dependent dioxygenase GLP34_13095			4.825		
oxidoreductases						
	LLM class flavin-dependent oxidoreductase GLP34_07295				2.511	
	oxidoreductase GLP34_07980					5.104
aminosugar metabolism, glycosaminoglycans						
	acylneuraminate cytidylyltransferase GLP34_20730	-2.516				
	N-acetylneuraminate synthase neuB GLP34_20725					2.474
	sulfatase-like hydrolase/transferase GLP34_06990	4.800				
purine and pyrimidine metabolism						

	purine-nucleoside phosphorylase deoD GLP34_08925	2.856		2.044		
	purine permease GLP34_03235				4.162	
	pyrimidine 5'-nucleotidase GLP34_04830				2.133	
stress response						
oxidative	MerR family transcriptional regulator GLP34_13975	-3.024				
	peptide-methionine (R)-S-oxide reductase msrB GLP34_05900					2.973
	redoxin domain-containing protein GLP34_12355			-2.653		
	gamma-glutamyltranspeptidase ggt GLP34_15000		2.325			
	S-(hydroxymethyl)glutathione synthase gfa GLP34_19860	2.415				
nutrient	carbon starvation protein A cstA GLP34_20140		-2.145			
	type II toxin-antitoxin system RelE/ParE family toxin GLP34_15665			2.386		-3.285
	BrnT family toxin GLP34_15675			3.221		
	Hok/Gef family protein GLP34_18410			-2.059	-2.398	
envelope	phage shock envelope stress response membrane protein pspB GLP34_01115				-2.620	-6.607
	phage shock envelope stress response membrane protein pspC GLP34_01120				-2.783	-6.411
	phage shock protein pspA GLP34_01110				-2.659	-6.741
osmotic	BCCT family transporter GLP34_03210		4.684			
	betaine/proline/choline family ABC transporter ATP-binding protein GLP34_04545			-4.042		-4.773
	extracellular solute-binding protein GLP34_04565					-4.691
	TAXI family TRAP transporter solute-binding subunit GLP34_19955			-2.710		
cold/heat shock	rhodanese-like domain-containing protein GLP34_19420	-2.128				
	cold-shock protein GLP34_19050	2.004				
	chaperone dnaK GLP34_05145					-7.788
other	transposase tnpB GLP34_21495		4.480			
	sel1 repeat family protein GLP34_07525			-7.608		
	iron-regulated protein A IrpA GLP34_01460					4.805
folate metabolism						
	dihydrofolate reductase folA GLP34_19345					2.115
	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase folK GLP34_11255					3.087
unknown function						
	AAA domain-containing protein GLP34_16575			-4.805		-2.939
	alpha/beta fold hydrolase GLP34_11895					-3.842
	alpha/beta hydrolase GLP34_21060			-4.176		
	ATPase GLP34_14770					4.344
	cellulose metabolism/membrane activity GLP34_12680	-2.947				

DUF1107 family protein GLP34_19435			-2.391		
DUF1481 domain-containing protein GLP34_19525					2.473
DUF1488 family protein GLP34_16960					2.511
DUF3283 family protein GLP34_07210	2.303				
DUF3305 domain-containing protein GLP34_02855		-2.244			
DUF411 domain-containing protein GLP34_02755			-3.257		-5.576
DUF445 domain-containing protein GLP34_17305		-2.041			
DUF454 family protein GLP34_00180	-3.296		-3.442		
DUF465 domain-containing protein GLP34_11900		-2.855			
DUF502 domain-containing protein GLP34_09165					2.213
DUF547 domain-containing protein GLP34_15215					5.673
FRG domain-containing protein GLP34_13265	2.562				
helix-turn-helix domain-containing protein GLP34_12490 (gene expression regulation)		-2.577			
hydrogenase 2 large subunit GLP34_13515 ferredoxin reduction/oxidation?					
hydrolase GLP34_10470		-2.199			
hypothetical protein GLP34_00005			-2.269		
hypothetical protein GLP34_01035			-4.501		-6.119
hypothetical protein GLP34_01510				4.824	
hypothetical protein GLP34_01605		5.076			
hypothetical protein GLP34_01980	-2.378				
hypothetical protein GLP34_03250			-2.398		
hypothetical protein GLP34_03435			-5.326		-5.015
hypothetical protein GLP34_03710			2.204		
hypothetical protein GLP34_04350	-2.005		-2.425		
hypothetical protein GLP34_05655					4.352
hypothetical protein GLP34_05990 promotor					2.823
hypothetical protein GLP34_06370			3.097		
hypothetical protein GLP34_07905					4.527
hypothetical protein GLP34_08140				2.516	2.616
hypothetical protein GLP34_08245					4.266
hypothetical protein GLP34_08555					-4.234
hypothetical protein GLP34_08715		-2.801			
hypothetical protein GLP34_10025				2.090	
hypothetical protein GLP34_10365	2.380				
hypothetical protein GLP34_10875					2.821

hypothetical protein GLP34_12365		4.221			
hypothetical protein GLP34_12700	2.333				
hypothetical protein GLP34_13040			-4.535		-3.532
hypothetical protein GLP34_13710				4.706	
hypothetical protein GLP34_14510			-4.505		
hypothetical protein GLP34_14575			-6.303		-3.432
hypothetical protein GLP34_14915			4.382		
hypothetical protein GLP34_16435			-4		-3.272
hypothetical protein GLP34_16500					-3.005
hypothetical protein GLP34_18315		-2.401			
hypothetical protein GLP34_18375				-2.600	
hypothetical protein GLP34_18385	2.066			-2.326	-2.421
hypothetical protein GLP34_18885			-5.233		-2.408
hypothetical protein GLP34_18915		-2.475			
hypothetical protein GLP34_19070	4.293				
hypothetical protein GLP34_20010			-6.237		-2.297
hypothetical protein GLP34_21110				4.461	
NlpC/P60 family protein GLP34_17375			2.686		
SanA protein GLP34_02655				2.318	
sulfurtransferase GLP34_02305			-5.549		-5.807
TIGR03899 family protein GLP34_19445					-2.364

Additional table 4 Detected significant regulation in *P. carnosum* TMW2.2149 samples. Significance was accepted with LogFC ≥ 2/≤-2 and p < 0.05.

		atmosphere	MAP	air	air	MAP	MAP
		co-contaminant	-	*Ps. fragi*	*B. thermosphacta*	*Ps. fragi*	*B. thermosphacta*
growth							
	cell division protein FtsZ GLP09_05265						-2.178
	deoxyribodipyrimidine photo-lyase phrB GLP09_09660			-4.946			
	DNA topoisomerase 3 topB GLP09_16205		2.659		3.045		
	glycosyltransferase GLP09_02200		-2.213				
	LgrA family protein GLP09_05360		-3.025				
	exonuclease GLP09_06720						-4.548
	nucleotidyltransferase GLP09_07870			-5.236			
	peptidoglycan DD-metalloendopeptidase family protein GLP09_05530		-2.202				
	transcription elongation factor GLP09_13865		-5.173				
	endonuclease/exonuclease/phosphatase family protein GLP09_06535						-7.133
	PTS N-acetylgalactosamine transporter subunit IIC GLP09_14185						2.747
	N-6 DNA methylase GLP09_05450			2.725			
translation, RNA catabolism							
	L-seryl-tRNA(Sec) selenium transferase selA GLP09_11365		-5.262				
	RnfH family protein GLP09_16385		-2.344				
	tRNA-specific adenosine deaminase tadA GLP09_00010		-2.585	-2.647	-2.059		
	uracil permease uraA GLP09_15740		2.316				
transport							
ion	TonB-dependent hemoglobin/transferrin/lactoferrin family receptor GLP09_05030		-2.186				
	DUF21 domain-containing protein GLP09_02110		-2.021				
	glutathione-regulated potassium-efflux system ancillary protein kefG GLP09_04470		2.223		2.366		
	DASS family sodium-coupled anion symporter GLP09_10435		4.786				
	phosphate-binding protein pstS GLP09_12355						-2.103
other	MFS transporter GLP09_00125		-5.158	-6.195			
	NCS2 family permease GLP09_00205		2.027				
	porin GLP09_11140			-2.592			
	YeeE/YedE family protein GLP09_13960			2.487			
	transporter GLP09_03215					4.108	
	ABC transporter permease subunit GLP09_10250						-3.104
secretion, conjugation							

	conjugal transfer protein traR GLP09_00720	-7.023				
	type IV secretion, P-type conjugative transfer protein virB9 GLP09_16185	3.561		5.996		
	type IV secretion protein virB3 GLP09_16170	4.662				
	type IV secretion system pilus biogenesis assembly protein pilO GLP09_04355	-4.450				
	large polyvalent protein-associated domain 7 containing protein GLP09_16230	2.180				
	type IV secretion system trbC/virB2 pilin GLP09_16165	3.230				
	type IV secretion/conjugal transfer ATPase virB4 family GLP09_16175			2.870		
	type IV secretion system protein virB10 GLP09_16190			6.052		
	DNA internalization-related competence protein comEC/rec2 GLP09_01850				2.286	
cofactors						
	adenosylcobinamide-phosphate synthase GLP09_05975			-3.972		
	bifunctional hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase thiD GLP09_07680			3.029		
	CoA pyrophosphatase GLP09_01720				4.151	
	malonyl-ACP O-methyltransferase bioC GLP09_02060	-2.263				2.018
	8-amino-7-oxononanoate synthase bioF GLP09_02055					3.131
motility						
	flagellar protein fliL GLP09_03870	-4.139		-4.870		
	methyl-accepting chemotaxis signaling protein GLP09_17255			2.108		
biofilm						
	Mannose-sensitive agglutinin biogenesis protein GLP09_07245	2.402				
	diguanylate cyclase GLP09_03425		-3.023		2.840	
	ExeM/NucH family extracellular endonuclease GLP09_11145		-2.593			-5.170
	diguanylate cyclase GLP09_05615					-2.007
	L-fucose:H+ symporter permease fucP GLP09_08485					2.423
	EAL domain-containing protein GLP09_16005				2.806	
general carbohydrate metabolism						
	beta-galactosidase ebgA GLP09_15090	2.010				
	sugar O-acetyltransferase (maltose) GLP09_07805		-4.867			
	family 20 glycosylhydrolase GLP09_06065			-3.200		-2.052
	arabinose-binding domain protein of araC transcription regulator GLP09_13995				-4.624	
	ribokinase rbsK GLP09_08480					2.170
carbohydrate transport						
	galactose/methyl galactoside ABC transporter ATP-binding protein mglA GLP09_13535	2.384				
	phosphotransferase system protein EIIC GLP09_06555	2.581				
	transcriptional regulator uhpA GLP09_01960		-2.711			

TCA						
	GPR1/FUN34/yaaH family protein GLP09_01700	2.187		2.304		
	malate-2H(+)/Na(+)-lactate antiporter nhaC GLP09_03470		-2.832			
	2-hydroxyacid dehydrogenase GLP09_09405	-2.276				
pyuvate metabolism						
	formate dehydrogenase accessory protein fdhE GLP09_12285	-3.033	-2.952	-3.068		
	formate hydrogenlyase maturation protein hycH GLP09_11325	-3.975				
Entner Doudoroff pathway						
	bifunctional 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxy-phosphogluconate aldolase eda GLP09_08535				4.334	
respiratory chain						
	succinate dehydrogenase cytochrome b556 subunit sdhC GLP09_00860	2.230		2.149		
	cytochrome C GLP09_00290					-2.939
	cytochrome bo(3) ubiquinol oxidase subunit 1 cyoB GLP09_01170				-4.071	
	cytochrome c oxidase subunit 2 coxB GLP09_03895					-3.110
	NADPH-dependent FMN reductase GLP09_16965	-4.810	-5.506	-5.516		
hemoglobin metabolism						
	heme utilization protein hutX GLP09_04795	-2.255				
	heme utilization protein hutZ GLP09_04800	-4.264	-3.291	-2.862		
	protoheme IX farnesyltransferase cyoE GLP09_03935				4.528	
anaerobic respiration						
nitrate	ferredoxin-type protein napF GLP09_00270					-3.297
butanoate metabolism						
	acetolactate synthase 2 small subunit ilvM GLP09_13200		-3.452			2.806
fatty acid metabolism						
	anaerobic glycerol-3-phosphate dehydrogenase subunit A glpA GLP09_14745	2.229		2.089		
	anaerobic glycerol-3-phosphate dehydrogenase subunit B glpB GLP09_14740	2.101		2.032		
	anaerobic glycerol-3-phosphate dehydrogenase subunit C glpC GLP09_14735					2.070
	glycerophosphodiester phosphodiesterase glpQ GLP09_01095	2.396		2.685		
	sn-glycerol-3-phosphate transporter glpT GLP09_03855	3.642		2.901		
amino acid metabolism						
biosynthesis	bifunctional phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP diphosphatase hisIE GLP09_01030	-2.734				
	L-asparaginase 2 ansB	2.458				
	LysR family transcriptional regulator GLP09_09150				-2.220	
	LysR family transcriptional regulator GLP09_09910					-4.983

	peptidylprolyl isomerase GLP09_13455				4.290	
	glutamine--fructose-6-phosphate aminotransferase glmS GLP09_13175	2.163				
degradation	transglutaminase-like cysteine peptidase GLP09_17705	3.357		2.330		
	peptidase T pepT GLP09_17040			-5.109		
	C69 family dipeptidase GLP09_05170					2.001
transport	amino acid carrier protein GLP09_05880	2.029				
	amino acid permease GLP09_02115	-2.973				
	amino acid permease GLP09_15080	4.753				
	ATP-binding cassette domain-containing protein GLP09_06045			-4.765		
	lysine exporter lysO GLP09_09430					2.403
	HAAAP family serine/threonine permease GLP09_02430		-4.201			
	MIP family channel protein GLP09_04245					2.647
	NAAT family transporter GLP09_00680					-2.189
	oligopeptide ABC transporter ATP-binding protein oppD GLP09_03610					-2.006
	oligopeptide ABC transporter permease oppC GLP09_03605					2.228
	tyrosine transporter GLP09_14830					-2.335
modification	GNAT family N-acetyltransferase GLP09_10000			-2.907		-2.720
	GNAT family N-acetyltransferase GLP09_10630					-2.095
purine and pyrimidine metabolism						
	nucleoside hydrolase GLP09_11970	2.158				
Purines	5-(carboxyamino)imidazole ribonucleotide synthase purK GLP09_13290	2.013				
	NACHT domain-containing protein GLP09_13980		-4.783	-4.403		
	phosphoribosylformylglycinamidine cyclo-ligase purM GLP09_15730	2.598				
	phosphoribosylglycinamide formyltransferase purN GLP09_15725	2.231				
Pyrimidines	dihydroorotate dehydrogenase (quinone) pyrD GLP09_15355	2.383				
	aspartate carbamoyltransferase pyrB GLP09_08990		-2.335			
Stress response						
oxidative	serine/threonine protein kinase GLP09_08270	2.045				
	glyoxalase protein GLP09_00640		3.877			
nutrient	acetoacetyl-CoA reductase phbB GLP09_06110	2.098				
cold/heat shock	cold-shock protein GLP09_15545				-5.024	
other	flavohemoprotein hmpA GLP09_15485	-2.085				
	Type I toxin/antitoxin system protein GLP09_16310		2.328			
	nitrate/nitrite two-component system sensor histidine kinase narQ GLP09_00265		-5.203	-4.947		
	metalloregulator arsR/smtB family transcription factor GLP09_14975				2.299	

Folate metabolism						
	methylenetetrahydrofolate reductase metF GLP09_13520	-2.812	-5.760			
Unknown						
	AAA domain-containing protein GLP09_14060	-4.503				
	AAA family ATPase GLP09_09915			2.935		
	alkene reductase GLP09_06160	-2.621				
	alpha/beta fold hydrolase GLP09_07875	2.652				
	DUF1439 domain-containing protein GLP09_09820	2.404				
	DUF2057 domain-containing protein GLP09_00360	2.178				
	DUF2238 domain-containing protein GLP09_10050					-4.263
	DUF3389 family protein GLP09_12070				-2.550	
	DUF692 family protein GLP09_11665					-2.498
	GAF domain-containing protein GLP09_15320		-2.001			
	helix-turn-helix domain-containing protein GLP09_09865 gene expression	4.531			-4.463	-4.509
	hypothetical protein GLP09_00630	-2.111				
	hypothetical protein GLP09_02155	-3.093				
	hypothetical protein GLP09_02365	-2.945				
	hypothetical protein GLP09_05405	2.310				
	hypothetical protein GLP09_06080	2.537				
	hypothetical protein GLP09_06445	2.727				
	hypothetical protein GLP09_06590			-2.116		
	hypothetical protein GLP09_07745		-5.079		-2.724	
	hypothetical protein GLP09_07830	2.276				
	hypothetical protein GLP09_08360			-2.579		-4.293
	hypothetical protein GLP09_09895					2.576
	hypothetical protein GLP09_11150	3.038				
	hypothetical protein GLP09_11260		-2.141			
	hypothetical protein GLP09_12045					
	hypothetical protein GLP09_13640	2.249				
	hypothetical protein GLP09_14105					-4.309
	hypothetical protein GLP09_14935				2.745	
	hypothetical protein GLP09_16505	2.371				
	hypothetical protein GLP09_17815			2.049		
	response regulator GLP09_11085			-2.804		
	sigma-54-dependent Fis family transcriptional regulator GLP09_06530			-4.732		-4.427

StbB GLP09_16240		3.051		-4.163
VOC family protein GLP09_16830			4.324	
YwbE family protein GLP09_09885	-2.759	-2.863		
ATP-binding cassette domain-containing protein GLP09_04725				-2.593
ATP-binding cassette domain-containing protein GLP09_13090				-2.006

12.5 Details on material and methods

12.5.1 Cultivation of bacteria

12.5.1.1 Used species and strains

All *Photobacterium* strains except of the type strains of *P. phosphoreum* and *P. iliopiscarium* were isolated on location prior to or during the performance of this study. Strains belonging to other species than *Photobacterium* spp. were obtained as agar plate from the local strain collection. All strains that were used in the context of this work are listed in Table 5.

Table 5 Utilized strains and their respective isolation source. Bolt letters highlight Photobacterium *strains that were examined in detail regarding growth and response to co-contamination on meat. TMW – Technische Mikrobiologie Weihenstephan, DSMZ – Deutsche Stammsammlung von Mikroorganismen und Zellkulturen (German strain collection).*

Species	Strain number	Source
P. phosphoreum	DSM15556^T	DSMZ; marine habitat
	TMW2.2103	MAP beef
	TMW2.2033	MAP chicken
	TMW2.2034	MAP chicken
	TMW2.2125	Air-packed turkey
	TMW2.2126	MAP chicken
	TMW2.2127	MAP chicken
	TMW2.2128	MAP chicken
	TMW2.2129	MAP chicken
	TMW2.2130	MAP chicken
	TMW2.2131	MAP chicken
	TMW2.2132	MAP chicken
	TMW2.2133	MAP chicken
	TMW2.2134	MAP chicken
	TMW2.2135	MAP chicken
	TMW2.2136	MAP chicken
	TMW2.2137	MAP chicken
	TMW2.2138	Air-packed pork
	TMW2.2139	Air-packed pork
	TMW2.2140	Air-packed pork
	TMW2.2141	Vacuum-packed beef
	TMW2.2142	Vacuum-packed beef
	TMW2.2143	Vacuum-packed beef
	TMW2.2144	Vacuum-packed beef
	TMW2.2145	Vacuum-packed beef
P. carnosum	TMW2.2021^T	MAP chicken
	TMW2.2098	MAP salmon
	TMW2.2148	Air-packed beef
	TMW2.2149	MAP pork
	TMW2.2169	Air-packed turkey
	TMW2.2186	MAP salmon
P. iliopiscarium	DSM9896T	DSMZ; pyloric ceca of herring
	TMW2.2035	MAP chicken
	TMW2.2104	MAP pork
	TMW2.2172	MAP pork
Ps. fragi	TMW2.2082	Minced beef
Ps. lundensis	TMW2.2076	Minced beef
Ps. weihenstephanensis	TMW2.1728	Beef steak
B. thermosphacta	TMW2.2101	Chicken breast
Leuconostoc (L.) gelidum ssp. gelidum	TMW2.1618	Chicken breast
L. gelidum ssp. gasicomitatum	TMW2.1619	Beef steak
Carnobacterium (C.) divergens	TMW2.1577	Chicken breast
C. maltaromaticum	TMW2.1581	Chicken breast

12.5.1.2 Cultivation media

All media were prepared with deionized water. Marine Broth 2216 (MB, DIFCO, VWR, Darmstadt, Germany) was prepared as standard isolation medium for photobacteria, utilizing 37.4 g/l w/v of the broth powder. The broth powder contained 5 g/l peptone, 1 g/l yeast extract, 19.45 g/l sodium chloride, 0.1 g/l ferric citrate, 5.9 g/l magnesium chloride, 3.24 g/l magnesium sulfate, 1.8 g/l calcium chloride, 0.55 g/l potassium chloride, 0.16 g/l sodium bicarbonate, 0.08 g/l potassium bromide, 34 mg/l strontium chloride, 22 mg/l boric acid, 4 mg/l sodium silicate, 2.4 mg/l sodium fluoride, 1.6 mg/l ammonium nitrate, and 8 mg/l disodium phosphate. The medium was supplemented with 3 g/l w/v meat extract (Merck, Darmstadt, Germany). According to the instructions from the manufacturer the pH of MB was adjusted to pH 7.6.

Adapted meat simulation medium (MSM) was used as standard cultivation medium for photobacteria. It contained 20 g/l w/v meat extract (Merck, Darmstadt, Germany) and 20 g/l w/v NaCl (Roth, Karlsruhe, Germany) with pH 7.6. This medium was also used for dilution, washing and all further handling of *Photobacterium* cells. If selectivity towards photobacteria was necessary, 7 mg/l w/v vancomycin hydrochloride (AppliChem GmbH, Darmstadt, Germany) was added after sterilization, as soon as the temperature of the medium fell below 50°C. The standard cultivation medium for all species except of photobacteria was brain-heart-infusion broth (BHI; Roth, Karlsruhe, Germany). According to the manufacturer, the medium was prepared with 37.5 g w/v of the broth powder.

All media were sterilized by autoclave sterilization at 121°C for 15-20 min (1 bar overpressure). Liquid media were allowed to cool down afterwards and kept at room temperature, whereas media containing agar-agar were poured into petri dishes with 7.8 cm diameter (Sarstedt, Nümbrecht, Germany). Solid medium was thereby prepared by addition of 16 g/l w/v agar-agar (Roth, Karlsruhe, Germany) before sterilization. Plates were then stored at 4°C until usage. For testing motility, cultivation was done with soft agar instead. Therefore, MSM was mixed with 3 g/l w/v agar-agar and poured after sterilization in 15 ml screw cap tubes (Sarstedt, Nümbrecht, Germany).

12.5.1.3 Isolation and identification

Selective isolation of photobacteria followed the isolation protocol of Hilgarth (Hilgarth *et al.*, 2018a) with some modifications. Fresh food products were bought in local shops of the area of Freising, Germany, and put on ice for transport. Study included shops belonging to supermarket chains as well as small local stores. Products were stored at 4°C until the desired sampling point was reached. Afterwards, products were cut in pieces under sterile working conditions and selected pieces were compiled to a representative sample. Samples were weighed, mixed with up to 100 ml of liquid MB in sterile filter bags (Interscience, Saint Nom la Breteche, France) and homogenized for 2 min in a bag

mixer (Interscience, Saint Nom la Breteche, France). Serial dilutions of the resulting liquid were then plated with glass beats (2.85-3.45 mm Roth, Karlsruhe, Germany) on selective MB. Plates were cultivated at 15°C. Cell numbers were evaluated based on the Total Viable Counts (TVC) on agar plates and referred to the utilized sample weight, following the equation

$$CFU\ per\ g = \frac{CFU * dilution\ factor * V(medium\ in\ ml) * 20}{m(meat\ in\ g)}$$

Thereby, plates with 25-250 CFUs were selected for evaluation as stated by Tomasiewicz (Tomasiewicz *et al.*, 1980). Species identity was reviewed using MALDI-TOF MS and a Microflex LT spectrometer (Bruker Corporation, Billerica, MA, USA). Thereby, cells were directly transferred to the target and prepared on-target for measurement with the method of Usbeck (Usbeck *et al.*, 2013). Measured spectra of the low-molecular sub-proteome were compared to the local database of reference mass spectrometry profiles for species identification. Species were considered as correctly identified, if a score value of ≥ 2.0 depicted reliable species identification. Further differentiation on strain level was done by RAPD-PCR fingerprinting. Strains to be kept were picked and transferred to fresh agar plates.

12.5.1.4 Cultivation procedure and storage

All *Photobacterium* strains were cultivated aerobically at 15°C with MB or MSM. Strains of other species were cultivated aerobically at 15°C with BHI. Thereby, all cultures were placed in Lovibond thermostatic cabinets (Tintometer GmbH, Dortmund, Germany) to ensure constant cultivation temperature. The temperature was monitored using laboratory thermometers. *Photobacterium* cultures were inoculated exclusively from plate, whereas cultures of other species were also inoculated from cryo stocks. Unless otherwise stated, cultures were inoculated until they reached the exponential phase, which was observed after up to approximately 72 h in case of photobacteria. For short-term storage up to one month plates were sealed with parafilm and kept at 4°C. For long-term storage, cells were cultivated in liquid culture until they reached the exponential phase and centrifuged for 10 min at 4000x g. Cell pellets were washed with fresh medium, mixed with 34% w/v glycerol and stored at -80°C.

12.5.2 Sampling in meat processing facilities

To explore the occurrence of photobacteria in the meat processing environment, different types of samples were taken within two different processing plants. The first performed slaughter and further processing of chicken, while the second obtained pre-processed cattle- and swine halves for final

processing. Meat, offal and product samples were collected with sterile forceps, transferred to screw cap tubes containing MSM and mixed vigorously. Air samples were taken with a Spin Air microbial air sampler (Neutec Group, Thermo Fisher Scientific, Ottawa, USA) and MSM agar plates for cell collection. Swab samples were taken with cotton swabs from different surfaces and tools during ongoing processing work. Thereby, swabs were taken from a surface area of 10x10 cm, if applicable. After taking the sample, cotton swabs were placed in screw cap tubes containing 5 ml MSM and mixed vigorously. Additionally, several already packaged meat products were obtained that had been produced during the day. All samples were carried on ice and plated the same day on selective and non-selective medium. Remaining sample volumes were incubated at 4°C for enrichment and plated after another 72 h again. Half of the obtained already packaged meat products were sampled the same day and another half after reaching the expiry date.

12.5.3 Phenotypic characterization of bacteria

Isolated strains of *P. phosphoreum* and *P. iliopiscarium* were evaluated regarding their general phenotype. Results of the analysis were clustered hierarchically with a heatmapper tool using average linkage and Euclidean distance (www2.heatmapper.ca/expression/). Additionally, phenotypic traits of selected strains of *P. phosphoreum* and *P. carnosum* isolated from meat were explored in the context of their relevance in meat spoilage.

12.5.3.1 Recording of general growth parameters in liquid medium

Growth curves were recorded for all isolated *P. phosphoreum* and *P. iliopiscarium* strains from meat, as well as for the respective type strains from the DSMZ. For this, cells from overnight cultures were centrifuged for 10 min at 4000x g, washed with 2% w/v NaCl solution and re-suspended in fresh medium. Afterwards, 25 ml MSM was inoculated with the prepared cells to an initial OD_{600} of 0.05. Cultures were agitated at 4°C and growth was monitored by periodic measurements of the OD_{600}. As soon as the cultures reached the end of the exponential phase, measurement of the pH was included. The final impact of the strain on the pH was thereby evaluated based on the pH at the maximum OD_{600}. Measurement was performed with a 766 Laboratory pH Meter (Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany). To ensure purity of the cultures, all strains were plated and re-checked afterwards regarding species identity. Relevant growth parameters, such as lag phase and maximum growth rate, were then determined by evaluating the obtained data with RStudio v1.1.463 and grofit package v.1.1.1-1 (Kahm *et al.*, 2010). Analysis was thereby based on three replicates of each culture. Statistical analysis was performed with IBM SPSS Statistics v23.0.0.0. Normality of the obtained data was checked with the Shapiro-Wilk test and homogeneity of variances with the Levene test.

Significance of observed differences was determined by means of one-way ANOVA and HSD *post-hoc* Tukey test, if homogeneity of variances was confirmed. In case of heterogeneity of variances, Welch-ANOVA and *post-hoc* Games Howell test were chosen instead. Observations were regarded as significant if $p < 0.05$.

12.5.3.2 Evaluation of motility and bioluminescence

Motility of all *P. phosphoreum* and *P. iliopiscarium* strains was analyzed by the soft-agar stab method. Therefore, sterile inoculation loops were dipped into fresh cultures of the strains and then stabbed into screw cap tubes filled with soft-agar. Tubes were incubated straight at 15°C and motility was assessed based on the visible turbidity after 72 h. Bioluminescence was evaluated by visual comparison of cells on agar plates. For this, overnight cultures of all strains were diluted to the same OD_{600} and plated on MSM. Light emission was assessed in darkness after 72 h of incubation, involving the extent of the observed growth.

12.5.3.3 Evaluation of antibiotic resistances

For evaluating the resistance to antibiotics, all strains of *P. phosphoreum* and *P. iliopiscarium* were inoculated homogenously on agar plates by means of cotton swabs. Antibiotics were applied in form of discs (Oxod, Thermo Scientific, Hampshire, United Kingdom) that were operating by diffusion. The discs contained, respectively, 30 µg chloramphenicol; 10 µg norfloxacin; 300 µg sulfonamides; 25 µg apramycin; 5 µg penicillin G; 2 µg clindamycin; 30 µg nalidixic acid; 5 µg trimethoprim; 10 µg ampicillin; 25 µg streptomycin; 5 µg rifampicin; 10 µg gentamycin; 30 µg kanamycin; 15 µg erythromycin or 30 µg tetracycline. The diameter of the resulting inhibition zones in the bacterial lawns was used to assess the extent of sensitivity of the respective strain.

12.5.3.4 Metabolic characterization by API tests

General metabolic abilities of *P. phosphoreum* and *P. iliopiscarium* from meat were exemplarily examined for selected strains of the species. Examinations focused on fermentation of carbohydrate substrates (API 50CH test; bioMérieux, Marcy-l'Étoile, France) and enzymatic activity (API ZYM test; bioMérieux, Marcy-l'Étoile, France). API 50CH test followed the instructions of the manufacturer with the adaptations described in Hilgarth *et al*. (Hilgarth *et al.*, 2018b). Thereby, washed cells from overnight cultures were re-suspended in MB containing 0.17 g/l bromocresol purple, 1 ml/l Tween 80 and 56 mg/l manganese sulphate and used to inoculate the test strips. For the API ZYM test, cells were

re-suspended in 0.85% w/v NaCl solution. Preparation followed the instructions of the manufacturer with adaptations by (Hilgarth *et al.*, 2018b). Strips were incubated for up to 24 h at 15°C.

12.5.3.5 H_2O_2 tolerance assay

Sensitivity to extracellular H_2O_2 was monitored with *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149 to explore their general sensitivity to high O_2 concentrations. Thereby, overnight cultures were washed, diluted to OD_{600} = 0.1 and transferred to 96-well plates (Sarstedt, Nümbrecht, Germany). Afterwards, 0.01–0.1% v/v H_2O_2 of a 30% v/v stock solution (Merck, Darmstadt, Germany) was added as stated by (Kolbeck *et al.*, 2019). During handling of H_2O_2 containing solutions the working place was carefully protected from light. Prepared plates were placed on ice immediately. Growth of the strains at 15°C was measured as OD_{600} with a FLUOstar microplate reader (BMG Labtech, Ortenberg, Germany). The minimum inhibitory concentration (MIC) of H_2O_2 was determined for each strain as mean value of triplicates and utilized for evaluation of the sensitivity.

12.5.4 Influence of different NaCl concentrations on the growth

Strains of *P. phosphoreum* (TMW2.2134, TMW2.2125, DSM15556^T) and *P. carnosum* (TMW2.2021^T, TMW2.2148, TMW2.2098, TMW2.2186) were cultivated aerobically in liquid MSM with different NaCl content. Thereby, the standard concentration of 2% w/v NaCl was compared to MSM with 3%, 4% or 5% w/v NaCl to evaluate salt tolerance and -requirement. Washed cells from overnight cultures were utilized to inoculate 20 ml of fresh medium to OD_{600} = 0.02. Growth at 15°C was then monitored by measuring the development of the OD_{600}, until the cultures reached the stationary phase. Salt tolerance was determined by the maximum OD_{600} as highest possible yield that was reached by each strain in dependency of the salt concentration. Additionally, maximum growth rate and lag phase were calculated with RStudio v1.1.463. All cultures were additionally plated after finishing the experiment to verify their purity.

Effects of the salt concentration on the cell shape were explored by light microscopy in order to verify the usage of the OD as valid growth parameter. Thereby, cell surfaces (π*length*width) at maximum OD_{600} were measured for two representative strains (*P. phosphoreum* TMW2.2134 and *P. carnosum* 2.2021^T) in each medium. Microscopy was performed with an Axiostar plus microscope (Zeiss, Jena, Germany) at 1000x magnification without staining. For reliable measurement, pictures of multiple cells from each culture condition were taken using the software ZEN (Zeiss, Jena, Germany) and depicted cells were sized hereafter. Effects of the salt concentration on the cell size were assessed as significant differences of cells from low-salt cultures and cells from elevated-salt cultures.

12.5.5 Influence of hydrostatic pressure on growth and survival

Growth and survival at HHP was examined for *P. phosphoreum* TMW2.2134 and TMW2.2125 isolated from meat and DSM15556^T isolated from marine environment, as well as *P. carnosum* TMW2.2021^T, TMW2.2148 isolated from meat and TMW2.2098 and TMW2.2186 isolated from fish. Experiments were performed in 1.8 ml cryo vials (Nunc, Thermo Fisher Scientific) and with 30-125 MPa hydrostatic pressure. Cryo vials were thereby filled to the brim with culture suspension, closed carefully and placed in an 8 ml pressure vessel filled with Bis(2-ethylhexyl) sebacate (Nr. 84822; Sigma-Aldrich, USA) for pressure transmission. The pressure vessel (type MV2-13, Unipress, Warsaw, Poland) possessed a double wall for temperature control. Temperature was kept by a circulating oil bath (Witeg Labortechnik GmbH, Wertheim, Germany) filled with silicon oil (Sil 180, Fisher Scientific, New Hampshire, USA). Accuracy of the present temperature was monitored by a type K thermocouple that led through the lid of the pressure vessel. After closing the vessel, hydrostatic pressure was built up with a manual pump that was linked to a pressure intensifier system (Unipress, Warsaw, Poland). To minimize adiabatic heating, pressure increase was thereby stretched to 20s and pressure release to 30s.

12.5.5.1 Examination of growth and survival at hydrostatic pressure

Washed overnight cultures of all strains were re-suspended in fresh medium and diluted to OD_{600} = 0.01 to survey the growth of the strains at HHP. Cultures were transferred to respective cryo vials and pressurized with 30 – 50 MPa for 72 h. Temperature was thereby hold at 15°C. Vials with concomitant reference cultures were incubated at 0.1 MPa (1 atmosphere) at 15°C. After pressure release, serial dilutions of all cultures were plated immediately on MSM to count the CFU as quantitative criterion for growth at HHP.

In order to test survivability at HHP, strains were additionally cultivated until they reached the exponential phase and plated on MSM to evaluate the initial cell numbers. Afterwards, cultures were treated for 5 - 60 min with 100 - 125 MPa. Temperature was thereby set to 10°C with a variance of ± 5°C during pressure building and release. After pressure release, vials were carefully put on ice to avoid additional temperature stress and plated in serial dilution on MSM. Survivability was assessed based on significant differences of the cell counts prior to- and after pressure exposure.

12.5.5.2 Evaluation of the effect of hydrostatic pressure on osmotolerance

The impact of HHP on the cellular osmotolerance was examined with *P. phosphoreum* strain TMW2.2134 and DSM15556^T and *P. carnosum* TMW2.2021^{T}and TMW2.2098. The experiment aimed at detecting sublethal cell injury resulting from HHP treatment that is overlooked by methods based on the evaluation of survivability. Strains were cultured until they reached exponential phase and transferred to cryo vials for pressure application. HHP of 125 MPa was applied for 5 min at 10°C with a temperature variance of ± 5°C during pressure building and release. Cells were carried on ice after pressure release. Serial dilutions of treated cultures as well as untreated control cultures were then plated on MSM agar with 2% (standard concentration), 3% and 3.5% w/v NaCl and incubated at 15°C. Colonies were counted after 72 h of incubation or, if necessary, after up to 2 weeks of incubation. The effect of HHP on the osmotolerance of the cells was evaluated based on significant differences of the cell counts of treated and untreated cells.

12.5.6 Growth experiments on fresh meats

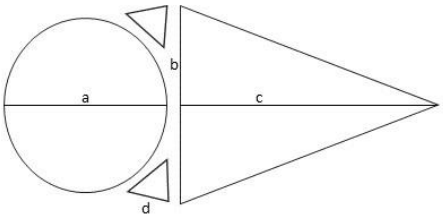
The present work includes studies on the growth of *P. phosphoreum* and *P. carnosum* strains on different types of fresh meat, in different modified atmospheres and in correlation with presence or absence of competitive species. All experiments were performed in triplicates.

12.5.6.1 Cultivation on meat in different packaging atmospheres

Fresh meat from local supermarkets from the area of Freising, Germany, was obtained for cultivation of photobacteria on meat. For cultivation on poultry meat, skinless MAP chicken breast was utilized and for cultivation on pork and beef MAP pre-cut steaks. The original packaging atmosphere of all obtained products was 70% O_2/30% CO_2 and remaining shelf life was at least 4 days. All packages originated from the same producer and all replicates (N=3) originated from the same package to ensure maximum comparability. Meat was stored at 4°C until usage. For inoculation, meat was cut aseptically with sterile scalpels (Braun, BA211, Thermo Fisher Scientific, Schwerte, Germany) in squares with defined surface of 16 cm^2 and approximately 1 cm height. Each square was placed in a petri dish and inoculated from both sides with bacterial suspension.

Suspensions were prepared from overnight cultures of the strains. Thereby, OD_{600} was measured and utilized to calculate the respective volume of the culture to reach the desired cell counts per cm^2 meat surface. If the inoculation volume per side exceeded 80 µl, the respective culture was concentrated by centrifugation for 10 min at 4000x g and resuspension in the 10x smaller volume of fresh medium. Additionally, all cultures were plated to evaluate the CFUs per ml prior to the inoculation. Growth

experiments on different meat types were performed with strains TMW2.2103, TMW2.2143 and TMW2.2140 of *P. phosphoreum* and TMW2.2148, TMW2.2169 and TMW2.2149 of *P. carnosum*, isolated from beef, chicken and pork, respectively. Growth experiments in different packaging atmospheres and also interaction studies were performed with *P. phosphoreum* TMW2.2103 and *P. carnosum* TMW2.2149. For recovery of RNA samples, entire chicken breast fillets without skin were utilized instead of cut meat squares. In this case, the required inoculation volume was calculated based on the meat surface on hand. The meat surface A_{total} was thereby calculated as presented in Figure 6. Cultures for inoculation of entire chicken breast fillets were only concentrated, if the necessary inoculation volume exceeded 300 µl per side.



$$A_{total} = [(0.5*a)^2*\pi] + [0.5*b*c] + [2*d^2]$$

Figure 6 Required measurements on skinless chicken breast fillets for calculating the respective surface area A_{total}.The obtained area was then utilized to calculate the inoculation volume for the desired initial cell counts per cm^2.

Inoculation was done with sterile inoculation loops and sterile forceps to handle the meat pieces. Petri dishes with meat slices were placed in polypropylene trays with ethylene-vinylalcohol polymer coating and a volumetric permeation rate of 0.25 cc.20 µ m^{-2}.day.atm (ES-Plastic, Hutthurn, Germany) and cooled down to 4°C. Afterwards, trays were sealed with a semi-automatic tray sealer (Rotarius VG, Variovac PS SystemPack GmbH, Zarrentin am Schaalsee, Germany) connected to a gas mixer for fluctuating gas flow (Witt-Gasetechnik, Witten, Germany). Sealing temperature was 175°C. Applied gas mixtures were 70%_O_2/30%_CO_2, 70%_N_2/30%_CO_2, 100%_N_2, 21%_O_2/79%_N_2 (designated 'air atmosphere') and 21%_O_2/30%_CO_2/49%_N_2. Inoculated and packaged meat was incubated at 4°C. Before opening a package for taking a sample, composition of the present gas atmosphere was measured with a compact gas analyzer PA 7.0 (Witt-Gasetechnik, Witten, Germany). Meat pieces were sampled following the procedure described in chapter 12.6.1.3. Divergently of the described procedure, only 5 ml of MSM was thereby utilized for homogenization of each piece of meat. Growth of the respective strains was assessed as the maximum cell numbers that were reached within the given incubation time of 7 days.

12.5.6.2 Studies on the response of photobacteria to other meat spoilers

If the experimental design required inoculation of meat with multiple strains to study interaction, all strains were cultivated separately and mixed right before inoculation according to their respective OD_{600}, as described in chapter 12.6.6.1. Growth of photobacteria within an interacting meat spoilage community was studied with the additional meat spoiling species listed in chapter 12.6.1, table 5. Thereby, three strains of *P. phosphoreum* (TMW2.2134, TMW2.2103, TMW2.2140) and *P. carnosum* (TMW2.2021^T, TMW2.2149, TMW2.2169) with appropriate growth performance and recovery were mixed and challenged with the presence of the compiled community on chicken meat. Species were identified with MALDI-TOF MS and further strain identification was done with RAPD-PCR as presented in chapter 12.6.1.3. Development of the CFUs over 6 days of incubation on MAP meat was utilized to assess growth and persistence.

For studies on the response of photobacteria to selected Gram-negative and Gram-positive meat spoilers, strains of *Ps. fragi* and *B. thermosphacta* were chosen. Both were inoculated together with *P. phosphoreum* and *P. carnosum* strains in equal (1:1) ratio of the initial cell counts, as well as in over-represented (1:10) and under-represented (10:1) ratio on chicken meat. Presence of interaction was evaluated as the development of the cell counts over 7 days of incubation in comparison to its development when incubated alone. Experiments were done in modified atmospheres containing 70%_O_2/30%_CO_2, 70%_N_2/30%_CO_2, 100%_N_2 or 21%_O_2/79%_N_2 (air). The response to co-contamination was further studied based on the transcriptome of *P. phosphoreum* and *P. carnosum* in presence and absence of *Ps. fragi* or *B. thermosphacta*. For this, strains were inoculated on entire chicken breast fillets and on pieces of chicken meat as described in chapter 12.6.6.1. Inoculated meat was cooled down to 4°C and packaged with 70%_O_2/30%_CO_2 and 21%_O_2/79%_N_2 (air) atmosphere. After 3 days of incubation, RNA was isolated from the inoculated breast fillets and CFUs were counted based on the inoculated meat pieces.

12.5.7 Genomic characterization of bacteria

Genomic characterization of bacteria was utilized to explore the biodiversity of all isolated strains. Additionally, genetic differences of selected strains regarding adaptation to meat environment and marine environment were analyzed.

12.5.7.1 RAPD PCR fingerprinting

For evaluation of genomic similarities, polymerase chain reaction (PCR) was performed based on randomly amplified polymorphic DNA (RAPD) fingerprints. Thereby, colony material of all isolated *P.*

phosphoreum and *P. iliopiscarium* strains was picked using sterile toothpicks and suspended in 10 µl of deionized water. Afterwards, samples were homogenized by sonification for 5 min in an Ultra sonic water bath (Bandelin electronic, Berlin, Germany). Resulting suspensions were directly used as template. Amplification was done with primer M13V (5'-GTT TTC CCA GTC ACG AC-3', (Ehrmann *et al.*, 2003) and additionally with primer M14V (5'-CTG TCC AGT CAC GTC-3'), following the reaction mix depicted in table 6. Reagents were obtained from M.P Biomedicals, Irvine, USA. Reaction tubes (V=200 µl) were placed in a PCR cycler (Eppendorf AG, Hamburg, Germany) that run the amplification program shown in table 7.

Table 6 Reaction mix for PCR amplification. All reagents were carefully kept on ice. Utilized primers were M13V and M14V.

Reagent	Volume [µl]
$MgCl_2$ (25 mM)	10
10x buffer without $MgCl_2$	5
dNTPs (10 mM)	2
Taq-Polymerase (5U/µl)	0.3
Primer (100 µM)	0.5
Template (10-50 ng/ µl)	1
Deionized water	31.2
total	50

Table 7 Thermoprotocol for PCR amplification. After mixing the reaction mix it was immediately placed in the PCR cycler.

	Temperature [°C]	Time [min]
Denaturation	94	3
Annealing	40	5
Extension	72	5
	Repeat for 3 cycles	
Denaturation	94	1
Annealing	60	2
Extension	72	3
	Repeat for 32 cycles	
Final extension	72	5

PCR products were separated by agarose gel electrophoresis. The gel thereby contained 1.4% w/v agarose and 0.5x TBE buffer (45 mM Tris-HCl, 45 mM boric acid, 1 mM EDTA, freshly prepared from 250x stock solution). For separation,150 V power was applied for 2.5 h with an Electrophoresis Power Supply (EPS 300, Pharmacia Biotech, Uppsala, Sweden). Samples were mixed with 6x loading dye (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to the gel, together with Lambda DNA/EcoRI+HindIII ladder (Thermo Scientific, Hampshire, United Kingdom) as molecular weight marker and for normalization of the band patterns. Bands were visualized with dimidium bromide DNA staining and inspected with an UVT-28M transilluminator (Herolab, Wiesloch, Germany).

Obtained patterns were exported as image files to Bionumerics V7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium) for cluster analysis. Bands were thereby manually identified and normalized by means of the applied ladder. Hierarchical clustering was performed with the unweighted pair group method with arithmetic mean (UPGMA) and Dice's similarity coefficient (1% tolerance). The analysis was carried out three times per strain to ensure reliable clustering results.

12.5.7.2 Calculation of diversity indices

Calculation of the diversity indices and rarefaction analysis were supported by Dr. Maik Hilgarth and based on the RAPD PCR fingerprints on strain level. PAST software 3.25 (Hammer *et al.*, 2001) was used to calculate evenness (Simpson; (Simpson, 1949)), entropy (Shannon *et al.*, 1949) and richness (Chao1, (Chao, 1984)) of the operational taxonomic units (OTU, (Schloss and Handelsman, 2005)). Significant differences were accepted with $p < 0.05$. Calculation of the coverage of genotypes was performed with Good's coverage estimator (Good, 1953) applying the equation:

$$C = (1 - \left(\frac{N}{n}\right)) * 100$$

Thereby, N represents the OTUs that are found only once and n the total number of strains.

12.5.7.3 Comparative BADGE analysis

Genomic analysis was performed for *P. phosphoreum* TMW2.2134 and TMW2.2125 from meat and DSM15556^{T} from marine environment, as well as *P. carnosum* TMW2.2021^{T}, TMW2.2148 from meat and TMW2.2098 and TMW2.2186 from MAP salmon (compare table 5). DNA was obtained from concomitant work of Fuertes *et al*. (Fuertes-Perez *et al.*, 2021) and sequenced with whole genome shotgun sequencing and the MiSeq sequencing platform Illumina (Inc., San Diego, CA, USA). Sequences were annotated based on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP, (Angiuoli *et al.*, 2008)). Comparative analysis was carried out with BlAst Diagnostic Gene findEr (BADGE; (Behr *et al.*, 2016)) to identify unique open reading frames (ORF) as stated before by (Höll, 2018). MEGABLAST percent identity cut was thereby set to 85%, DC-MEGABLAST percent identity cut to 70% and blastp percent identity cut to 50%. Output was utilized for comparison on strain- and species-level.

12.5.7.4 Bioinformatic sequence analysis (BLAST)

Presence of selected genes associated with high pressure adaptation or osmotolerance was explored with the protein alignment function of Basic Local Alignment Search Tool (BLAST, (Altschul *et al.*, 1990; Camacho *et al.*, 2009)). Thereby, alignments were done with sequences of *P. profundum* strain SS9 (Nogi *et al.*, 1998), unless respective sequences were not available.

12.5.8 Transcriptomic characterization of bacteria

Transcriptomic analysis was performed to study the response of *P. phosphoreum* and *P. carnosum Ps. fragi* and *B. thermosphacta*. Thereby, RNA was isolated from photobacteria cultivated in presence and absence of the competitors. The isolation procedure followed the work of (Höll *et al.*, 2019).

12.5.8.1 Sample preparation

The surface of inoculated chicken breast fillets was rinsed with 5 ml RNAlater solution (ThermoFisher Scientific, Waltham, MA, USA) from both sides. The running off liquid was collected and used again for a second washing step. Afterwards, the collected liquid and any liquid that was present at the bottom of the polypropylene trays was transferred to 15 ml screw cap tubes and cooled down to 4°C. Loose meat fragments were thereby hold back and kept from distorting the samples. Cells were then obtained by centrifugation at 9000x g for 10 minutes. If samples could not be processed immediately, the supernatants were removed and cell pellets were stored at 4°C in closed screw cap tubes.

12.5.8.2 Isolation of RNA and sequencing

Chilled cell pellets were mixed with 100 µl RNAse free TE buffer, 50 mg/ml lysozyme (24,000 kU/mL, SERVA Electrophoresis GmbH, Heidelberg, Germany) and 20 mg/ml proteinase K solution (Qiagen, Hilden, Germany). The mixtures were transferred to 1.5 ml reaction tubes and incubated for 1 h at 37°C. Subsequent RNA isolation followed the provided protocol of the RNeasy Mini Kit (Qiagen, Hilden, Germany) with some modifications. Thereby, 700 ul lysis buffer containing 1% β-mercaptoethanol was added to each sample and samples were mixed vigorously. Afterwards, small-size glass beads were added and samples were shaked 5 times for 1 min at 4 m/s in a high-speed benchtop homogenizer (MP FastPrep-24, Thermo Fisher Scientific) to improve cell disruption and homogenization. After each homogenization cycle samples were kept on ice for 1 min. Samples were then centrifuged for 3 min at maximum speed and 760 µl of the supernatant was obtained. Supernatants were mixed with 590 µl of 70% ethanol and transferred in two steps to the column. Columns were centrifuged for 15 s at 10000x

g. DNA was digested directly on column as described in the provided protocol of the RNAse free DNase set of Qiagen (Qiagen, Hilden, Germany). Thereby, columns were washed with 350 ml of buffer solution and centrifuged for 15 s at 10000x g. Afterwards, 10 µl DNAse stock solution was mixed with 70 µl of buffer solution and applied to the column. Columns were incubated for 30 min at room temperature. Then, another 350 µl buffer solution were added, columns were incubated for 5 min at room temperature and subsequently centrifuged for 15 s at 10000x g. Columns were washed twice with 500 µl of a second buffer solution, centrifuged afterwards for 2 min at maximum speed and dried for 10 min at room temperature. Following elution was done with 50 µl of RNAse free water that was applied two times to the column. Samples were put on ice and stored at -80°C immediately after quality control. Quality of the obtained RNA was checked with a NanoDrop spectrophotometer (NanoDrop 1000 3.6.0 PeQLab Biotechnologie GmbH, Erlangen, Germany) and RNAse free water as blank measurement. Additionally, selected samples and all samples showing abnormalities were analyzed by PCR amplification. RNA was sent for sequencing to Eurofins Genomics GmbH (Konstanz, Germany). Sequencing was performed with Illumina HiSeq2500 method and mapping to respective reference genomes. Evaluation of obtained transcripts was done based on the provided values of the $\log_2$(counts per million) (logCPM, expression level) and the $\log_2$(fold change) (logFC, regulation). Regulations with $logFC \geq 2$ and $p < 0.05$ were included in the analysis.

13 Statutory declaration

I hereby declare that I wrote the present dissertation with the title

‘Provenance and integration of *Photobacterium phosphoreum* and *Photobacterium carnosum* into the spoilage consortium of modified atmosphere packaged meat.’

independently and used no other aids than those cited. In each individual case, I have clearly identified the source of the passages that are taken word for word or paraphrased from other works. Other contributions to this work in terms of collaboration and supervised student theses are clearly indicated and acknowledged in the “publications and presentations” section.