TECHNISCHE UNIVERSITÄT MÜNCHEN

TUM School of Life Sciences

Lehrstuhl für Technische Mikrobiologie

Characterization and exploitation of glucan production of lactic acid bacteria in plant-based food fermentations

Jonas Schmid

Vollständiger Abdruck der von der promotionsführenden Einrichtung TUM School of Life Sciences der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktor der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender: Prof. Dr. J. Philipp Benz

Prüfer der Dissertation:

1. Prof. Dr. Rudi F. Vogel
2. Prof. Dr.-Ing. Peter Eisner

Die Dissertation wurde am 29.01.2021 bei der Technischen Universität München eingereicht und durch die promotionsführende Einrichtung TUM School of Life Sciences der Technischen Universität München am 30.03.2021 angenommen.

Danksagung

Die vorliegen Arbeit entstand im Rahmen eines Projekts (AiF 18794 N), welches aus Haushaltsmitteln des BMWi über die AiF-Forschungsvereinigung „Industrievereinigung für Lebensmitteltechnologie und Verpackung e.V.“ gefördert wurde.

Mein persönlicher Dank gilt:

Insbesondere meinem Doktorvater Herrn Prof. Dr. Rudi F. Vogel für die Möglichkeit an seinem Lehrstuhl meine Dissertation durchführen zu können sowie für die vielfältige Unterstützung und Betreuung in allen Bereichen zu jeder Tages und Nachtzeit.

Prof. Dr. Peter Eisner für die Begutachtung der Arbeit und Prof. Dr. Philipp Benz für die Übernahme des Prüfungsvorsitzes.

Dr. Frank Jakob für die äußerst wertvolle und umfassende Unterstützung sowie freundschaftliche Betreuung.

Prof. Dr. Matthias Ehrmann, Prof. Dr. Ludwig Niessen, Dr. Andreas Geißler, Dr. Jürgen Behr, Angela Seppeur, unseren technischen Assistentinnen und allen weiteren Kollegen am Lehrstuhl für jegliche fachliche und organisatorische Unterstützung.

Dr. Andrea Hickisch, Prof. Dr. Daniel Wefers und Prof. Dr. Petra Först für die unkomplizierte und schnelle Zusammenarbeit.

Meiner gesamten Familie, insbesondere Julia sowie meinen Eltern Rupert und Eva, welche mich während meiner gesamten Promotionszeit bedingungslos unterstützt, ausgehalten und aufgerichtet haben. Vielen lieben Dank euch allen!

INDEX

INDEX i
ABBREVIATIONS iv
1. Introduction 1
1.1. Bacterial polysaccharides 1
1.1.1. Homopolysaccharides by lactic acid bacteria 1
1.1.2. Heteropolysaccharides by lactic acid bacteria 3
1.1.3. Function of EPS 4
1.2. Biotechnological utilization of EPS 4
1.2.1. Dextran and gluco-oligosaccharides 5
1.2.2. Rheology of polysaccharides 6
1.3. Application of lactic acid bacteria 7
1.4. Adaption of lactic acid bacteria 7
1.5. Plant-derived dairy alternatives 8
1.6. Motivation, Hypotheses and Approaches 9
2. Material and Methods 11
2.1. Bacteria, media and culture conditions 11
2.1.1. Strains and culture conditions 11
2.1.2. Strain verification by MALDI-TOF-MS 13
2.2. Bacterial growth dynamics and EPS production 13
2.2.1. Screening of EPS formation on mMRS agar plates 13
2.2.2. Determination of colony forming units 13
2.2.3. Formation of EPS in liquid mMRS 14
2.2.4. Determination of general growth parameters 14
2.2.5. Determination of biogenic amine formation 14
2.2.6. Effect of sugar type on dextran yield of *L. hordei* TMW 1.1822 15
2.2.7. Production of dextran in buffer 16
2.2.8. LPI-fermentations 18
2.2.9. Isolation of EPS 18
2.2.10. Hydrolysis of EPS 19
2.3. Analytical Methods 19
2.3.1. HPLC-RI analysis 19
2.3.2. AF4-MALS-UV analysis for determination of molecular structure and size 19
2.3.3. HPAEC-PAD analysis for determination of glucose oligosaccharides 20
2.3.4. Rheological analysis 20

2.3.5. Chemical structure analysis 21
2.4. Molecular biological techniques 22
2.4.1. SDS-PAGE for dextransucrase verification 22
2.4.2. Bradford assay for protein quantification 23
2.4.3. DNA isolation 23
2.5. Genomics 23
2.5.1. Genome sequencing and annotation 23
2.5.2. Genome acquisition 24
2.6. Statistical analysis and data visualization 24
3. Results 25
3.1. Selection of EPS-forming strains as starter cultures for LPI-fermentations 25
3.1.1. Screening for EPS production 25
3.1.2. Determination of general growth kinetics in mMRS 26
3.1.3. Evaluation of growth on LPI substrate (IVV) and 1st strain selection 27
3.1.4. Genomic preconditions of selected strains 29
3.1.5. Determination of monomer composition and EPS type 30
3.1.6. Investigation of isolated HoPS by AF4-MALS-UV 30
3.1.7. 2nd strain selection 31
3.2. Evaluation of LPI-fermentations by *P. claussenii* TMW 2.340 wt/mut 32
3.3. Investigation of dextran formation by *L. hordei* TMW 1.1822 33
3.3.1. Dextran production in mMRS and LPI-medium 34
3.3.2. Dextran production in buffer with different pH values 35
3.3.3. AF4-MALS-UV analysis of dextran from different pH values 39
3.3.4. Methylation analysis 42
3.3.5. Sucrose and maltose influence on dextran formation 42
3.3.6. HPAEC-PAD analysis of the gluco-oligosaccharide produced via maltose acceptor reaction 45
3.3.7. Analysis of rheological properties of dextran of different molecular weight and size 47
3.4. Analysis of rheological properties of LPI-fermentations 53
4. Discussion 57
4.1. Selection of starter cultures 58
4.1.1. Adaption to cereal/plant fermentations 58
4.2. *Pediococcus claussenii* TMW 2.340 60
4.2.1. ß-glucan formation 60
4.2.2. Impact as starter culture for LPI-fermentation 61
4.3. *Liquorilactobacillus hordei* TMW 1.1822 62

4.3.1. Mechanism of dextran formation and structure....62
4.3.1.1. Influence of pH on the dextransucrase release and dextran amounts....62
4.3.1.2. Influence of sucrose on the dextransucrase release and dextran amounts....64
4.3.1.3. Influence of maltose on the dextransucrase specificity....65
4.3.1.4. Influence of pH on the structural properties of the dextran....66
4.3.2. Rheological and structural properties of different dextran fractions....67
4.3.3. Impact as starter culture for LPI-fermentation....69
4.4. Conclusion....70
5. Summary....71
6. Zusammenfassung....73
7. References....75
8. Appendix....93
8.1. Figures and tables....93
8.2. List of publications....99

ABBREVIATIONS

%	percentage
°C	degree Celsius
γ	shear strain/deformation
$\dot{\gamma}$	shear rate
η	viscosity zero
η_s	viscosity of solvent
η_{sp}	specific viscosity
η_0	zero shear viscosity
η_∞	infinite viscosity
µg	microgram
µl	microliter
ω	angular frequency
aa	amino acids
AAB	acidic acid bacteria
abs.	absolute
AF4	asymmetric flow field flow fractionation
CFU	colony forming unit
Da	dalton
dH_2O	deionized water
dn/dc	refractive index increment
EPS	exopolysaccharide
fru	fructose
g	gramm
G'	shear storage modulus
G''	shear loss modulus
GH	glycosyl hydrolase
glc	glucose
gtf	glycosyltransferase
h	hour
HePS	heteropolysaccharide
HoPS	homopolysaccharide

HPAEC	high performance anion exchange chromatography
HPLC	high performance liquid chromatrography
IVV	Institut für Verfahrenstechnik und Verpackung
kDa	kilodalton
L	liter
L.	*Liquorilactobacillus*
LAB	lactic acid bacteria
LPI	lupin protein isolate
LS	light scattering
LVE	linear viscoelastic
M	molarity
mal	maltose
MALDI-TOF-MS	matrix assisted laser desorption ionization time of flight mass spectrometry
MALS	multi angel light scattering
mg	milligram
min	minute
mL	milliliter
mM	millimolar
mol	millimole
mMRS	modified de Man, Rogosa and Sharpe medium
M_W	molecular weight (weight average)
MWCO	molecular weight cut off
nm	nanometer
OD	optical density
PAD	pulsed amperometric detection
PCA	perchloric acid
PDI	polydispersity index
P.	*Pediococcus*
RI	refractive index
rms	root mean square radius
rpm	rotations per minute
RT	room temperature

R_w	radius (weight average)
s	second
seq	sequence
sp.	species
suc	sucrose
Tagatose-6P	Tagatose-6-Phosphate
TCA	trichloroacetic acid
TFA	trifluoroacetic acid
TMW	Technische Mikrobiologie Weihenstephan
UV	ultraviolett
w/v	weight by volume
w/w	weight by weight

1. INTRODUCTION

1.1. Bacterial polysaccharides

The formation of polysaccharides and exopolysaccharides (EPS) is a basic characteristic of the growth and metabolism of various bacterial species, especially lactic acid bacteria (LAB) and is studied extensively for decades in several research works (Cerning, 1990; Misra et al., 2021; Sutherland, 1979; van Geel-Schutten et al., 1998). Intracellularly, the polysaccharides are part of numerous essential cell components and functions, such as cell wall-associated peptidoglycans, lipopolysaccharides and teichoic acids and may serve as stored carbon source (Delcour et al., 1999; Misra et al., 2021). The extracellularly secreted or produced, high molecular weight polysaccharides are the EPS. Depending on their monomeric composition, the bacterial EPS are classified into two categories. The homopolysaccharides (HoPS) are composed of a single type of sugar monomer, and the heteropolysaccharides (HePS) are consisting of multiple monomeric types (De Vuyst et al., 1999; Monsan et al., 2001). Both, HoPS and HePS can occur either extracellularly or cell wall anchored, whereas both conditions may apply for the same polymer produced by one strain, e.g. β-glucan (Sutherland, 1979).

1.1.1. Homopolysaccharides by lactic acid bacteria

HoPS like dextran, levan or inulin are commonly synthesized by extracellularly released enzymes, belonging to the glycosyl hydrolase (GH) family (CAZy database) (Cantarel et al., 2009; Notararigo et al., 2013; Torino et al., 2015). The different active or passive release mechanisms of extracellular sucrases of gram-positive bacteria are only partly known since there are representative sucrases and species, which lack signal peptides or cell wall anchor motifs (Bechtner et al., 2019). The enzymes preferentially utilize the disaccharide sucrose as a donor for the respective sugar monomers (glucose or fructose) and as an energy source due to the energy-rich glycosidic bond. In the following, the reaction mechanisms of the α-glucan biosynthesis as well as fructan-forming sucrases are described.

Synthesis of α-glucan by glucansucrase

The α – glucan forming glucansucrases originate from enzymes of the α-amylase type, which are commonly present among plant-associated bacteria, and belong to the GH 70 family (Van Hijum et al., 2006). The synthesis of α-glucans and the reaction mechanism of the respective glucansucrases was subject of numerous scientific discussions and was elucidated progressively in detail by Robyt et al. (1974), Sidebotham (1974), Mooser et al. (1989), Moulis et al. (2006), Vujičić-Žagar et al. (2010) and Leemhuis et al. (2013). The cleavage of the

glycosidic bond of the sucrose is accomplished by one active site and results in the formation of a covalently bound glycosyl-enzyme intermediate, while fructose is released. Subsequently the glycosyl moiety may be transferred to three different acceptor substrates, whereas the non-reducing end of a growing glucan chain is the only possible acceptor for the polymerization reaction of a long chained α-glucan. Additionally, the orientation of the accepting glucose molecule may determine the linkage type at position *O6*, *O4*, *O3* and *O2*, respectively (Moulis et al., 2006; Vujičić-Žagar et al., 2010). In the case of the transfer to another short-chained glucan, such as maltose or isomaltose, the reaction is called acceptor reaction (Koepsell et al., 1953; Paul et al., 1986), whereas the sole hydrolysis reaction is defined by the transfer to a water molecule (Mooser et al., 1989). The acceptor reaction may strongly compete with the appropriate polymerization process, which ultimately results in the formation of low molecular weight polymers (Böker et al., 1994; Heincke et al., 1999). Dextran, which is predominantly discussed in this work and one of the most prominent α-glucans, is composed of α-(1→6)-linked glucose monomers (backbone), water-soluble and naturally uncharged. The extracellular dextransucrases (EC 2.4.1.5) are exclusively expressed by LAB and depending on the catalytic domain, the dextran can be branched at positions *O2*, *O3* or *O4* (Bechtner et al., 2019; Notararigo et al., 2013). Figure 1 illustrates the complexity within a small dextran fragment, despite the comparatively limited variables.

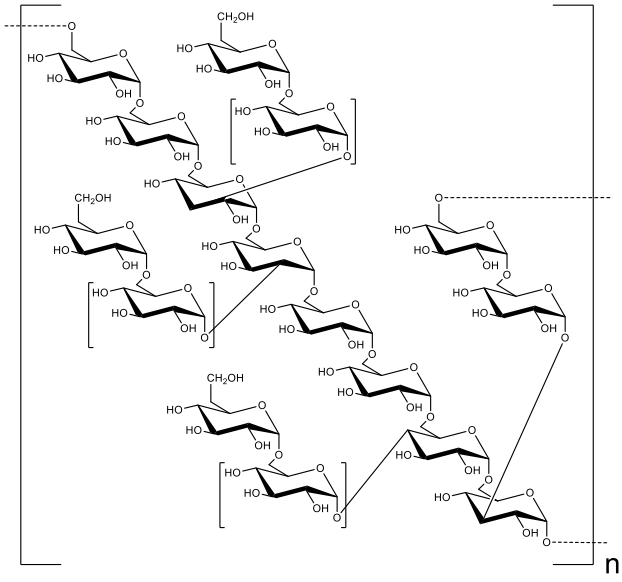


Figure 1: Structural fragment of dextran with possible side branching at position *O2, O3* and *O4*.

Synthesis of fructan by fructansucrase

In contrast to the glucansucrases, the extracellular levan- or inulosucrases are related to the sucrose-hydrolyzing invertases and belong to the GH 68 family (Pons et al., 2004; Van Hijum et al., 2006). Two main types of fructans are known, the β-(2→6)-linked levan and the β-(2→1)-linked inulin (Han, 1990). The basic reaction mechanism is comparable to the glucansucrases, whereas the fructose moiety is transferred to the growing fructan polymer, whilst the release of glucose and the three transfer possibilities which ultimately lead to polymerization, hydrolysis or the acceptor reaction (Ozimek et al., 2006; Pijning et al., 2011; Raga-Carbajal et al., 2018).

Synthesis of β-glucan

In comparison to the synthesis of the HoPS, β-glucan formation is mediated by an intracellular reaction mechanism, which involves a glycosyltransferase (family) 2 as a key enzyme. The mechanism is suggested to be comparable to the synthesis of HePS, including activated sugar nucleotides UDP-glucose and UDP-galactose as mentioned later (Karnezis et al., 2000; Torino et al., 2015; Werning et al., 2006).

Structure and composition of HoPS

The molecular weight, branching and therefore structure of HoPS may vary drastically, and is influenced by extrinsic factors like the pH value, sucrose and other sugars, salt concentrations, reaction temperature, as well as enzyme and product concentrations (Hundschell et al., 2019; Jakob et al., 2020; Jakob et al., 2013; Kim et al., 2003; Otts et al., 1988; Prechtl, Wefers, et al., 2018a; Sarwat et al., 2008; Ua-Arak et al., 2017). Therefore, the chain length and mass of known HoPS range from predominantly short chained HoPS like inulin (down to fructo-oligosaccharides backbone) to dextran with up to molecular weights above 1 x 10^8 Da (Anwar et al., 2010; Prechtl, Wefers, et al., 2018a)

1.1.2. Heteropolysaccharides by lactic acid bacteria

In contrast to HoPS, the synthesis of the more complex HePS is distinctly different. The intracellular synthesis is mediated by several different glycosyltransferases and takes place at the cell membrane (De Vuyst et al., 1999; Jolly et al., 2001). At first, the respective monosaccharides are activated by the formation of sugar nucleotides, which are further assembled to a growing oligosaccharide precursor. After the first elongation of this repeating unit, with subsequent transportation outside the cell by potentially three different pathways, the polymerization is finalized extracellularly (De Vuyst et al., 1999; Schmid et al., 2015; Zeidan et

al., 2017). Due to the great variability regarding the monosaccharide repeating units, the branching frequencies and the linkage type, the diversity of HePS produced by LAB is enormous (Zeidan et al., 2017). Overall, the molecular size of the HePS and amounts are distinctly smaller if compared to the high molecular weight HoPS like levan and dextran, but due to their high complexity their functional properties may be even greater (Prechtl, Wefers, et al., 2018b; Remus et al., 2012).

1.1.3. Function of EPS

The diversity of ecological niches inhabited by EPS-producing bacteria results in a broad spectrum of potentially functional roles of EPS, which are not completely revealed and therefore part of many studies. They are directly involved in diverse mechanisms, which are connected to the environment and the reaction to extrinsic factors. On the one hand, they facilitate the biofilm formation and colonization by the enhancement of surface adhesion (Augimeri et al., 2015; Dogsa et al., 2013; Fels et al., 2018; Flemming et al., 2010; Koczan et al., 2009; Laue et al., 2006; Leemhuis et al., 2013; Limoli et al., 2015; D. Xu et al., 2018; Zhu et al., 2009). On the other hand, they may protect the bacterium against osmotic stress, desiccation, antibiotics and other toxic molecules (ethanol, metal ions) as well as phages and enzymes (Badel et al., 2011; Donot et al., 2012; Sutherland, 1979; Zannini et al., 2016). For strains, which additionally possess EPS-degrading enzymes, they may as well serve as a backup carbon source during starvation. Furthermore, the interaction between single bacterial cells as well as a potential host organism are influenced through cell wall-anchored polysaccharides such as β-glucan or HePS, which may result in microbial mediated immunomodulation and anti-tumor effects (Ciszek-Lenda et al., 2011; Kulicke et al., 1997; Ruas-Madiedo et al., 2002). Accordingly, the functional aspects of immunological masking, evasion of the immune system and the enhanced adhesion in human as well as plant hosts are tough pivotal parts of pathogenic virulence factors (de Pinto et al., 2003; Killiny et al., 2013; Rudolph et al., 1994).

1.2. Biotechnological utilization of EPS

The use and application of bacterial EPS in numerous industrial fields is of growing importance and commercial interest because they are endowed with a broad structural diversity and concomitant manifold properties (De Vuyst et al., 2001; Duboc et al., 2001; Esa et al., 2014; Freitas et al., 2011; Harutoshi, 2013; Kambourova et al., 2015; N. Kaur et al., 2002). The functionalities of EPS are attributable to their specific structure-function relationship and the

macromolecular interactions between the single polysaccharide chains as well as with the surrounding matrix and other bacterial cells (De Kruif et al., 2001; De Vuyst et al., 1999; Hundschell et al., 2019; Jakob et al., 2013; Mozzi et al., 2006; Tirtaatmadja et al., 2001). Therefore, the molecular structure of the used polysaccharide, which is defined by its size, branching and monomer composition is the decisive factor for the appropriate application in any biotechnological field (Hundschell et al., 2020; Jakob et al., 2013; Rühmkorf et al., 2012; Zarour et al., 2017). In the food and cosmetic industry, EPS may replace commonly used synthetically produced additives as emulsifiers and stabilizers because of their water binding capacity or rather hydrocolloid and physico-chemical properties (Jolly et al., 2002; Sajna et al., 2015; Torino et al., 2015). Furthermore, EPS can play a future role in medical applications as antioxidants and anti-tumor agents, as part of drug delivery/nano particle systems, as blood plasma volume expander, and, the non-digestible short chained EPS, as possible prebiotics (Anwar et al., 2008; Baldwin et al., 2010; Barclay et al., 2019; Biedrzycka et al., 2004; Bowman, 1953; Li et al., 2020; Nair et al., 2019; Ryan et al., 2015; Silva et al., 2014). Due to their involvement in the biofilm formation, they are further utilized in research fields regarding tissue engineering and the production of biofuel (Lévesque et al., 2005; Pan et al., 2014; Rimondo et al., 2019; Smith et al., 2016; Veerubhotla et al., 2018). In analytical applications they can serve as matrices for the stationary phase in size-exclusion columns and separation gels as well as biological detoxicating/complexing agents in heavy metal polluted environments (Gupta et al., 2017; Porath et al., 1959; Sheng et al., 2014).

Since the structural and compositional possibilities of bacterial polysaccharides exceed the generally observed variety of synthetic polymers, which are utilized in numerous technological and industrial fields, the elucidation of strain-specific polysaccharide formation as well as the potential of controlled production of tailor-made EPS may provide new insights and possibilities for the replacement of synthetic polymers by the application of bacterial EPS.

1.2.1. Dextran and gluco-oligosaccharides

One of the most prominent bacterial EPS used in industrial applications is dextran. It is exploited in addition to the traditional composition in several food fermentations like sourdough, panettone and fruit juices (De Belder, 2003; Decock et al., 2005; Kothari et al., 2015; Monsan et al., 2001). Since the extreme variations in possible chain length and therefore molecular weights of dextrans contribute to differences regarding their functional properties, the manufacture of tailor-made dextrans may be obtained via the distinct control of the production conditions of one dextransucrase. By contrast, the structures and sizes of other commercially used plant-derived hydrocolloids such as cellulose or starch are comparably fixed and

therefore needed to be synthetically modified to obtain varying hydrocolloid and physico-chemical properties (Chen et al., 2018; L. Kaur et al., 2016; Murray, 2009; Saha et al., 2010). Additionally, the formation and use of diverse short-chain gluco-oligosaccharides that exhibit a low degree of polymerization, which are metabolized by various probiotic bacteria (Ejby et al., 2016; Mäkeläinen et al., 2009) is of increasing importance, due to the uprising consumers interest and demand in possible health benefits and prebiotic traits by naturally produced oligosaccharides (Davani-Davari et al., 2019; Mano et al., 2017; Oliveira et al., 2011). Therefore, the impact of the acceptor reaction of the dextransucrase was of particular interest.

1.2.2. Rheology of polysaccharides

The rheological flow behavior of liquid polymer systems can be divided into three major categories. (1) The shear-thinning (pseudoplastic) behavior, which applies for many polysaccharides/polymers in aqueous solution, (2) the shear-thickening (dilatant) behavior of e.g. starch solutions, and (3) liquids of Newtonian behavior like water or low concentrated polymer suspensions (Graessley, 1974; Lapasin et al., 1995; Mezger, 2011). Furthermore, the polysaccharides typically exhibit three spatial conditions if different but progressive shear rates are applied. At low shear rates the polysaccharides temporarily form completely entangled networks and behave like Newtonian solutions, where no changes of viscosity occur (zero shear viscosity η_0). With increasing shear rate, the tangled polysaccharides are loosened, which results in a decreased viscosity and the common shear-thinning behavior, since the polymers are aligned alongside the direction of shear. At high shear rates the entangled networks are destroyed, resulting in a low but infinite viscosity (η_∞) (Graessley, 1974; Lapasin et al., 1995; Mezger, 2011). The polysaccharide concentration as well as the type and molecular weight contribute to the specific flow behavior of the applied polysaccharide (Kasapis et al., 1994; Morris et al., 1981; Tirtaatmadja et al., 2001; Zarour et al., 2017). Polysaccharidic molecules of bigger size/weight and high complexity predominantly exhibit greater differences and possibilities regarding their specific flow behavior. The influence of polymer concentration can be further elucidated by the determination of the critical molecule density, the so-called overlap concentration (C^*), where the specific spatial distribution of the single polysaccharide chains within the solution allows their sufficient interaction and entanglement (Graessley, 1974; Morris et al., 1981). Accordingly, at concentrations underneath C^*, the distance between the scattered molecules is too far for any interaction. For the determination of C^*, the specific viscosity of the dextran fractions at multiple low polysaccharide concentrations must be determined during the zero shear viscosity phase at the Newtonian plateau and therefore before C^* is reached (Kulicke et al., 2004; Mezger, 2011).

Furthermore, the yield point (γ_y), determines the limit of the linear viscoelastic (LVE) region and the destruction of the samples structure, whereas the flow point (γ_f) is the crossover of the loss modulus G'' and storage modulus G' and thus the point where the sample flows since the viscous portion dominates (Kulicke et al., 2004; Mezger, 2011).

1.3. Application of lactic acid bacteria

LAB are traditionally used in multiple industrial fields throughout centuries. The acidification of food products and the concomitant preservation plays a major role in the manufacturing of many fermented food products. Therefore, they are used as starter cultures in meat, vegetable and dairy fermentations (Duboc et al., 2001; Hugas et al., 1997; Leroy et al., 2004). Additionally, the application of LAB is closely connected to their capability of producing EPS and thus are comparable to the application fields of EPS, as mentioned before. For example, the dairy industry takes advantage of EPS-producing LAB starter cultures to optimize structural properties of dairy products (Duboc et al., 2001; Jolly et al., 2002; Tabibloghmany et al., 2014) and to synthesize short-chain prebiotic oligosaccharides *in situ* (Duboc et al., 2001; Jolly et al., 2002; Tabibloghmany et al., 2014). But LAB are also utilized in biotechnological processes like green biorefineries (Lübeck et al., 2019) and as cell factories for the production of chemicals (Sauer et al., 2017). Besides these positive aspects, LAB are also contaminants in fermentations as well as industrial facilities. For example, the spoilage of beer during the brewing process or through whole biofilms within the production chain and dispensing equipment, is, amongst other factors, caused by LAB and may also involve EPS formation (Rodríguez-Saavedra et al., 2020; Suzuki, 2011; Z. Xu et al., 2020). Since LAB are specialized on various habitats and therefore exhibit numerous metabolic strategies, the search for LAB with the desired adaptions is of importance.

1.4. Adaption of lactic acid bacteria

The appearance of LAB in various habitats is predominantly defined by their adaption to nutrient rich but stressful environments, which are common in plant and dairy fermentations. Due to the general overwhelming availability of specific proteins, sugars, amino acids (aa) etc. in these ecological niches, the different metabolic capabilities of the adapted LAB may both be highly rudimentary and advanced, respectively (K. Makarova et al., 2006; K. S. Makarova et al., 2007; Papadimitriou et al., 2016). The simplification of the metabolic traits, due to the ubiquitous polypeptide and amino acid availability led to the loss of unnecessary biological pathways and induced numerous aa auxotrophies, and, in turn, the maintenance and

expression of diverse peptide transporters belonging to the ATP-binding cassette (ABC) superfamily as well as several peptidases. Because of the high protein concentrations in the dairy milieu, these adaptions are more distinct among strains associated to this environment (Ayad et al., 1999; Liu et al., 2010). Furthermore, the carbohydrate utilization and transport of dairy LAB is heavily reduced, due to the predominant occurrence and metabolization of lactose and its galactose moieties via the Leloir and Tagatose-6-Phosphate (Tagatose-6P) pathway (de Vos et al., 1994; Iskandar et al., 2019; Kandler, 1983). In contrast the complexity and variety of plant-derived simple mono- and disaccharides like arabinose and cellobiose up to polysaccharides like cellulose and xylan may force plant-associated LAB to hold multiple sugar transporting and utilizing systems (Siezen et al., 2008). These systems include proteins of ABC superfamily, the major facilitator (MFS) superfamily, the glycoside-pentoside-hexuronide (GPH) superfamily and the phosphotransferase system (PTS) superfamily (Papadimitriou et al., 2016). Therefore, the metabolic strategies and adaptation to the cereal environment might play an essential role in the selection of appropriate starter cultures for the fermentation of such substrates, if conventionally manufactured products wanted to be replaced or enhanced by alternative products, which cannot be produced with the established bacteria and traditional starting substrates.

1.5. Plant-derived dairy alternatives

The production of plant-derived dairy alternatives started with the exploitation of soy-based alternatives and was introduced in China, probably centuries ago. Therefore, dairy alternatives based on soy are widely spread and traditionally used in East Asia (Shurtleff et al., 1979). A broad spectrum of products based on genetically engineered soy varieties is, thus, commercially available and commonly used in East Asia and other countries (Eapen, 2008). Due to the continuing skepticism regarding the agricultural utilization of genetically modified plants among European consumers, the need for indigenous plants with similar processing characteristics is high (Bader et al., 2011; Eapen, 2008; Mäkinen et al., 2016). Hence, lupins gained interest as a considerable alternative to soy, as they are comparatively simple to cultivate in the European area, exhibiting low demands regarding nutritional and climate conditions (Trinick, 1977). Additionally, the symbiotic relationship of *Leguminosae*, the family of lupin, with nitrogen fixating bacteria belonging the genus *Rhizobium*, provides an improvement of soil quality for subsequent cultivations on the same field (Lodwig et al., 2003; Peoples et al., 1995). The potential health benefits of lupin proteins, due to their blood glucose-lowering effect and positive influence on the lipid metabolism in human, may further enhance the utilization and consumer acceptance (Bähr et al., 2013; Bertoglio et al., 2011; Bettzieche

et al., 2008; Lovati et al., 2012). Since the raw lupin seed/flour contains antinutritional compounds, the raw material has to be further processed to obtain high and pure protein concentrations. The techniques for purification of the lupin protein are versatile and more or less economically reasonable. Therefore, the isolation method through an alkaline extraction with subsequent isoelectric participation of the lupin protein was used as described by Wäsche et al. (2001), which results in the isolation of a lupin protein isolate (LPI) of considerably high grade of purity (Fritsch et al., 2015; Wäsche et al., 2001). There are various studies and products concerning lupin-based dairy alternatives, focusing on the exploitation of different lupin varieties and preprocessing techniques, however, knowledge on appropriate EPS-producing starters for LPI-based fermentations is scarce. (Gugger et al., 2016; Hickisch et al., 2016; Jiménez-Martínez et al., 2003; Snowden et al., 2004). This is certainly necessary, as starters play an important role in the formation of the typical physico-chemical properties of dairy products such as yoghurt that are essential for consumer acceptance. While products based on lupin protein are on the market, their sensorial and structural qualities are limited and can only be reached by the additives. Since these characteristics can alternatively be achieved by EPS-producing LAB, the investigation of new EPS-producing starters adapted to plant-based (food) fermentations is the reasonable consequence (De Vuyst et al., 1999; Fritsch et al., 2015; Mende et al., 2016).

1.6. Motivation, Hypotheses and Approaches

The increasing consumer and health demands of plant-based food products as a replacement of fermentation products, which are traditionally realized using substrates originating from animals, are well known across the advanced modern societies. The key challenge of an economic, sustainable and effective production of such alternative food fermentations is the selection of appropriate starter cultures. Since the starting substrate potentially holds different and possibly more stringent properties compared to the traditional substrates, the enhancing effect of metabolic side products, which are brought into the fermentation by the applied bacteria, are of even greater importance. Furthermore, this *in situ* production prevents labeling of the product (clean labelled), which as well affects the consumer behavior and sales. Since the fermentation of cereal- or legume-derived proteins is of course decisively different from traditional dairy fermentations, the conventionally and extensively used dairy starter cultures are unlikely suitable in this context. Also, existing fermented plant-derived dairy alternatives frequently lack attractive sensorial properties, namely with respect to structure and mouthfeel. Furthermore, the extremely variable properties of bacterial EPS may possess further potential, especially trough the distinctly controlled production of unique tailor-made EPS. Therefore, the

focus of this work was the enhanced fermentation of a lupin protein isolate (LPI) medium with exopolysaccharide-producing lactic acid bacteria to manufacture a yoghurt alternative based on plant protein and to additionally gain new insights into the intrinsic structural effects of EPS and its formation process. Considering these set of ideas, the following working hypotheses were composed:

- EPS-producing LAB, which grow on LPI can be found.
- The selected strains enhance the structural properties of the yoghurt alternative.
- The effect decisively relies on the formation of EPS.
- The structural composition of EPS is targetable by controlled changes during the production process.
- These distinct structural varieties lead to exploitable changes in the functional properties of the EPS.

From these conducted working hypotheses, the following practical approaches were derived:

- Screening for EPS-producing LAB strains predominantly originating from plant (fermentations).
- Evaluation of their growth behavior and EPS production in competitive LPI-fermentations and standard medium.
- Characterization of the macromolecular structure of the EPS of most promising strains by AF4-MALS-UV.
- Identification of extrinsic factors influencing the EPS formation by applying different experimental setups and subsequent analytical methods (HPLC, AF4-MALS-UV)
- Exploitation of these influencing factors for the controlled production of tailor-made EPS fractions for subsequent investigations.
- Determination of physico-chemical properties of sole EPS solutions and of LPI-fermentations via rheological measurements.

2. MATERIAL AND METHODS

2.1. Bacteria, media and culture conditions

2.1.1. Strains and culture conditions

At the begin of the study 27 strains, listed in Table 1, were cultivated on mMRS and were tested for their growth on the LPI substrate by the project partner of the Fraunhofer Institut für Verfahrenstechnik und Verpackung (IVV) in Freising. Further investigations were carried out using only the highlighted (*****) strains. The strains were obtained from the in-house strain collection of the Chair of Technical Microbiology and had been reported to produce varying amounts/types of EPS. Table 2 and Table 3 depict the components of the standard mMRS as described before (De Man et al., 1960). Formerly, the species *Levilactobacillus, Latilactobacillus, Limosilactobacillus, Liquorilactobacillus, Lactiplantibacillus, Fructilactobacillus* had all been summed up within the genus *Lactobacillus* and were recently taxonomically rearranged by Zheng et al. (2020).

Table 1: List of initial strain selection with species, TMW number / alternative ID, source of isolation and growth temperature.

Species	TMW/alternative IDs	Isolation source	Growth temperature [°C]
***Lactobacillus johnsonii** **	1.142	porcine colon	37
Levilactobacillus brevis	1.2112	beer	30
Levilactobacillus brevis	1.2113	beer	30
***Latilactobacillus curvatus** **	1.1928	raw sausage	30
Limosilactobacillus frumenti	1.103	sourdough	37
Limosilactobacillus frumenti	1.666/DSM 13145T	sourdough	37
Lentilactobacillus hilgardii	1.1828	water kefir	30
***Liquorilactobacillus hordei** **	1.1822	water kefir	30
***Lactiplantibacillus plantarum subsp. plantarum** **	1.1478	honey	30
***Lactiplantibacillus plantarum subsp. plantarum** **	1.617	sourdough	30
***Lactiplantibacillus plantarum subsp. plantarum** **	1.64		30
Lactiplantibacillus plantarum subsp. plantarum	1.1308		30
Limosilactobacillus pontis	1.675	sourdough	37
Limosilactobacillus pontis	1.1115	sourdough	37
Limosilactobacillus pontis	1.1468	sourdough	37
Limosilactobacillus reuteri	1.106	sourdough	37
Limosilactobacillus reuteri	1.693/DSM 20016	human intestine	37
Limosilactobacillus reuteri	1.977	duck colon	30
Limosilactobacillus reuteri	1.272	sourdough	30
Latilactobacillus sakei subsp. sakei*	1.411	sauerkraut	30
Fructilactobacillus sanfranciscensis	1.54/LTH1729	sourdough	30
Leuconostoc citreum	2.1194	water kefir	30

Leuconostoc gelidum ssp. gasicomitatum	2.1619	cattle meat	30
Lc. mesenteroides	2.1073	water kefir	30
Pediococcus claussenii wt*	2.340/ATCC BAA-344	beer	30
Pediococcus claussenii mut*	2.340	beer	30
Weissella confusa	1.934	sourdough	30

***** strains selected after first screening for further investigations

Table 2: Composition of standard mMRS medium.

Compound	Supplier	Final concentration
Peptone from casein	Roth, Germany	10 g/L
Yeast extract	Roth, Germany	5 g/L
Meat extract	Merck Millipore, USA	5 g/L
K_2HPO_4 • 3 H_2O	Merck Millipore, USA	4 g/L
KH_2PO_4	Merck Millipore, USA	2.6 g/L
NH_4Cl	Roth, Germany	3 g/L
Tween80	Gerbu, Germany	1 g/L
Cysteine-HCl	Roth, Germany	0.5 g/L
(Agar)	Roth, Germany	15 g/L
Vitamin mix *		1 mL/L

* components of vitamin mix are depicted in Table 3

Table 3: Composition of vitamin mix used for mMRS medium.

Compound	Supplier	Final concentration
$MgSO_4$ • 7 H_2O	Merck Millipore, USA	0.2 g/L
$MnSO_4$ • H_2O	Merck Millipore, USA	0.038 g/L
Thiamine	Merck Millipore, USA	0.2 mg/mL
Folic acid	Merck Millipore, USA	0.2 mg/mL

The pH of the mMRS was adjusted to 6.2 using 2 to 6 M HCl and the medium was autoclaved at 121 °C for 20 min. The sugars were autoclaved separately to avoid the Maillard reactions and were added after he the sterilization step as well as the filtered (0.2 µm nylon filters, Phenomenex, Germany) vitamin mix. The strains were cultivated in closed vessels under static conditions and the optimum temperature (Table 1). If not specifically stated otherwise, the main cultures of the used medium were always inoculated with 4 %(v/v) of 30 h grown pre-cultures diluted to an OD_{590} of 2.0. The applied sugars for the different experimental setups are further mentioned in the sections 2.2.4, 2.2.1, 2.2.3, 2.2.5 and 2.2.7.

2.1.2. Strain verification by MALDI-TOF-MS

For the verification of the strains on species level, single colonies from mMRS plates were analyzed by matrix assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS). One loop or colony of the cell material was transferred into 300 µL dH_2O and ethanol was added to inactivate the bacteria (70 % v/v end concentration), followed by a protein extraction with formic acid and acetonitrile in water (35:50 % v/v). Finally, 1 µL of the solution was transferred onto a stainless-steel target and overlaid with 1 µL α-cyano-4-hydroxycinnamic (HCCA) matrix (Bruker Daltonics, Germany). The spectra were generated using a Microflex LT MALDI-TOF-MS (Bruker Daltonics, Germany) equipped with a nitrogen laser r (λ=337nm) within a linear positive ion detection mode under the control of Biotyper Automation Control 3.0 (Bruker Daltonics, Germany). The mass spectra of 240 laser shots were recorded to obtain one sum spectrum according to Kern et al. (2013), which was further compared to the database of Bruker.

2.2. Bacterial growth dynamics and EPS production

2.2.1. Screening of EPS formation on mMRS agar plates

The first determination of the EPS production was performed for all strains on mMRS agar plates containing 50 g/L sucrose and 20 g/L fructose, glucose and maltose each. The plates were incubated for 48 – 72 h at the respective temperature (Table 1) and visually screened for EPS formation.

2.2.2. Determination of colony forming units

The determination of viable cell counts in CFU/mL was achieved by the distribution of 100 µL of appropriate culture dilutions (0.9 % NaCl) on mMRS agar plates using sterile glass beads (2.7 mm, Roth, Germany). The plates were at least incubated for 48 h at the respective growth temperature.

2.2.3. Formation of EPS in liquid mMRS

The production of EPS was performed in 50 mL vessels (Sarstedt, Germany) with 15 mL of mMRS containing either 50 g/L sucrose or 50 g/L glucose or 20 g/L each glucose, fructose, maltose at the respective incubation temperature for 24 h. EPS was isolated and quantified as described in section 2.2.9.

2.2.4. Determination of general growth parameters

To determine the maximum growth rate and lag phase all strains were cultivated in 96-well microtiter plates (Sarstedt, Germany). 200 µL of mMRS containing 5 g/L fructose, glucose and maltose each, was inoculated with 48 h precultures to obtain a starting OD_{590} of 0.05 and covered with 100 µL sterile paraffin oil (Roth, Germany). The plates were incubated at their respective growth temperature (Table 1) for 72 h. The cell density was recorded at a wavelength of 590 nm every 30 min after a 30 sec shaking step with a microplate reader (SPECTROstar Nano, BMG LABTECH, Germany. The obtained data was analyzed using RStudio (R, 2013; RStudio, 2013) and the package *grofit* (Kahm et al., 2010), where μ_{max} represents the maximum increase of OD_{590} within the exponential growth phase.

For the detailed screening of *P. claussenii* TMW 2.340 wt/mut the EPS production, CFU and pH decrease at the timepoints 0, 4, 8, 14, 19, 24 h in 45 mL of mMRS with 10 g/L each glucose + maltose was recorded.

2.2.5. Determination of biogenic amine formation

To determine the potential formation of biogenic amines by the selected strains, a screening on decarboxylase agar plates (pH 5.2) was performed (Bover-Cid et al., 1999). The strains were cultivated at their respective growth temperature for 24-48 h on plates supplemented with the compounds listed in Table 4. The potential decarboxylation of the provided amino acids was indicated by the alkalization of the medium and the eventual change of color from yellow to blue.

Table 4: Composition of decarboxylation medium.

Compound	Supplier	Final concentration
Tryptone	Roth, Germany	5 g/L
Yeast extract	Roth, Germany	5 g/L
Meat extract	Merck Millipore, USA	5 g/L
NaCl	Merck Millipore, USA	2.5 g/L
Glucose*	Merck Millipore, USA	0.5 g/L
Tween80	Gerbu, Germany	1 g/L
K_2HPO_4 • 3 H_2O	Merck Millipore, USA	0.2 g/L
Ammonium citrate	Roth, Germany	2 g/L
$MgSO_4$ • 7 H_2O	Merck Millipore, USA	0.2 g/L
$MnSO_4$ • H_2O	Merck Millipore, USA	0.05 g/L
$FeSO_4$	Merck Millipore, USA	0.04 g/L
$CaCO_3$	Merck Millipore, USA	0.1 g/L
Thiamine*	Merck Millipore, USA	0.01 g/L
Bromocresol purple	Roth, Germany	0.06 g/L
Agar	Roth, Germany	20 g/L
Amino acids**		10 g/L

*added after autoclaving **arginine, histidine, lysine, ornithine, phenylalanine, tyrosine

2.2.6. Effect of sugar type on dextran yield of *L. hordei* TMW 1.1822

To determine the sugar influence on the dextran yields of *L. hordei* TMW 1.1822, the strain was cultivated in 45 mL mMRS with 1: 50 g/L sucrose, 2: 25 g/L each sucrose and glucose 3: 25 g/L each sucrose and fructose, 4: 25 g/L sucrose and 8.3 g/L each glucose + fructose + maltose 5: 25 g/L each sucrose + maltose. After 48 h at 30 °C the CFU as well as the pH were recorded and the dextran formation via isolation (2.2.9) determined.

For a more detailed screening of growth behavior, *L. hordei* TMW 1.1822 was cultivated in mMRS with 10 g/L each glucose + fructose and 19 g/L sole sucrose for 24 h with monitoring of pH, CFU and dextran yields after 0, 4, 8, 14, 19, 24 h. The sugar amounts were adjusted to obtain equal molarities.

Due to the obtained results, the sugar influence was further investigated in an experimental setup, which is described in detail in the second part of section 2.2.7.

2.2.7. Production of dextran in buffer

Figure 2 depicts the procedure for the recovery of solutions containing the dextransucrase of *L. hordei* TMW 1.1822. At first, 45 mL main cultures were incubated for 18 h at 30 °C to obtain cells in the mid-exponential growth phase (Figure 10). The grown main culture (53 mL) was split in 7.5 mL tubes. Cells were then removed by centrifugation at 10000 x g for 10 and dissolved in equal volumes (7.5 mL) of 0.1 M citrate-phosphate buffers containing 0.1 M sucrose (Han, 1990). After 3.0 h of incubation at 30 °C the cells were removed by centrifugation (10000 x g, 10 min) and sterile filtration (0.2 µm nylon filters, Phenomenex, Germany). To reach the final dextran production pH, the pH of the acidified buffer was re-adjusted to the desired value by adding 7.5 mL of buffer solution of calculated pH values according to McIlvaine (1921). The release of the enzyme for 3.0 h in presence of 0.1 M sucrose was either performed at pH values from 3.5 to 6.5 (setup A) or at pH 4.5 (setup B) for all samples. Subsequently, the EPS were synthesized at 30 °C for 24 h at pH values of 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5. Additionally, one intermediate condition was analyzed, where the cells were just removed after the dextran production and therefore uncontrolled pH decrease occurred during the dextran synthesis (UC, not listed in Figure 2). Furthermore, a more detailed setup was carried out, with pH values from 3.6 to 4.8 (0.2 steps). Enzymatic reactions were stopped by adding two volumes (15 mL) of chilled ethanol for following dextran isolation or by shock freezing (- 20 °C) in case of subsequent HPLC analysis.

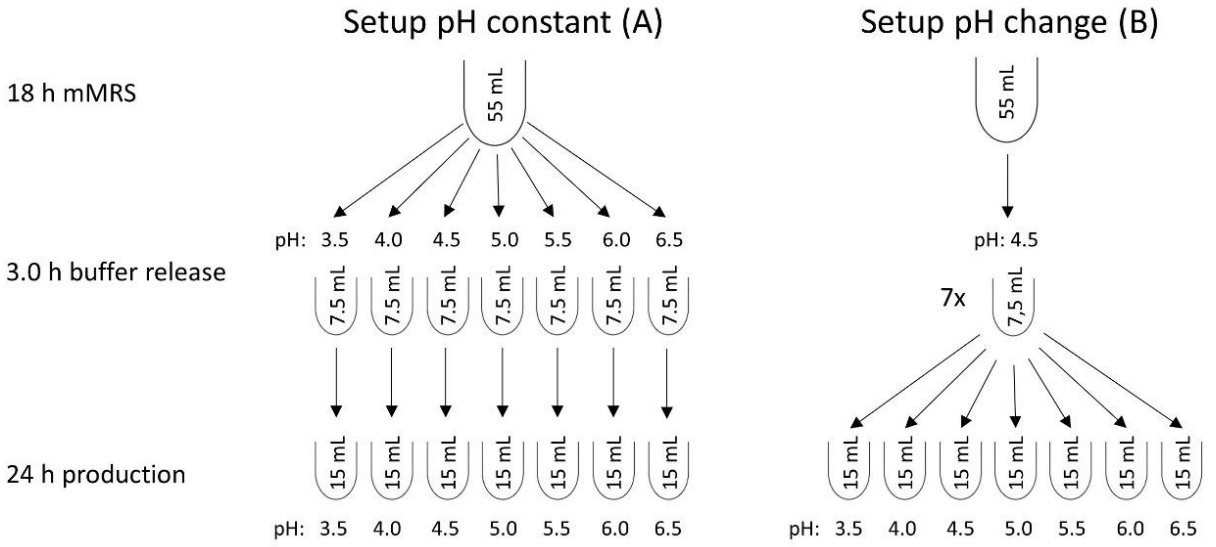


Figure 2: Overview of the experimental setups used to characterize dextran production at different pH. Cells were grown in 53 mL mMRS medium (without sucrose) for 18 h, split up to 7.5 mL samples, centrifuged and dissolved in 7,5 mL 0.1 M citrate-phosphate buffer (0.1 M sucrose) of the desired pH values, respectively. In setup (A), dextransucrase containing supernatants were collected at pH 3.5 to 6.5, while in setup (B) the release pH for all samples was kept constant at pH 4.5. After 3.0 h of incubation in these buffers, the cells were removed by centrifugation and the supernatants were sterile filtered. 7.5 mL buffer of the required pH to reach the final production pH were added, followed by 24 h of incubation and subsequent dextran quantification.

For the determination of the sucrose and maltose influence on the dextran product and dextransucrase productivity in buffer another experimental setup was chosen, which is shown in Figure 3. Therefore, the mMRS of the main cultures was further supplemented either with 10 g/L each glucose and fructose (glc + fru) or 5 g/L each glucose + fructose and 9.5 g/L sucrose (glc + fru + suc) or 19 g/L sucrose (suc). Amounts were adjusted to obtain equal molarities. The cells were cultivated in 15.5 mL mMRS and after 18 h the supernatants were removed by centrifugation at 10000 x g for 10 minutes followed by the addition of 7.5 mL 0.1 M citrate-phosphate buffer pH 4.5 containing either 0.1 M sucrose or 0.1 M sucrose + maltose each. After 3 h at 30 °C the cells were removed by centrifugation (10000 x g, 10 min) and sterile filtration (0.2 µm nylon filters, Phenomenex, Germany), followed by the second addition of 7.5 mL of the respective buffer and the dextran production with for 24 h at 30 °C. Additionally, cells of mMRS ± sucrose after 24 h of incubation were lysed with 10 µg/mL lysozyme at 37 °C over night, with subsequent dextran production at pH 4.5 in 0.1 M citrate-phosphate buffer containing 0.1 M sucrose. Again, the enzymatic reactions were stopped by adding two volumes (15 mL) of chilled ethanol for following dextran isolation or by shock freezing (- 20 °C) in case of subsequent HPLC and HPAEC-PAD analysis.

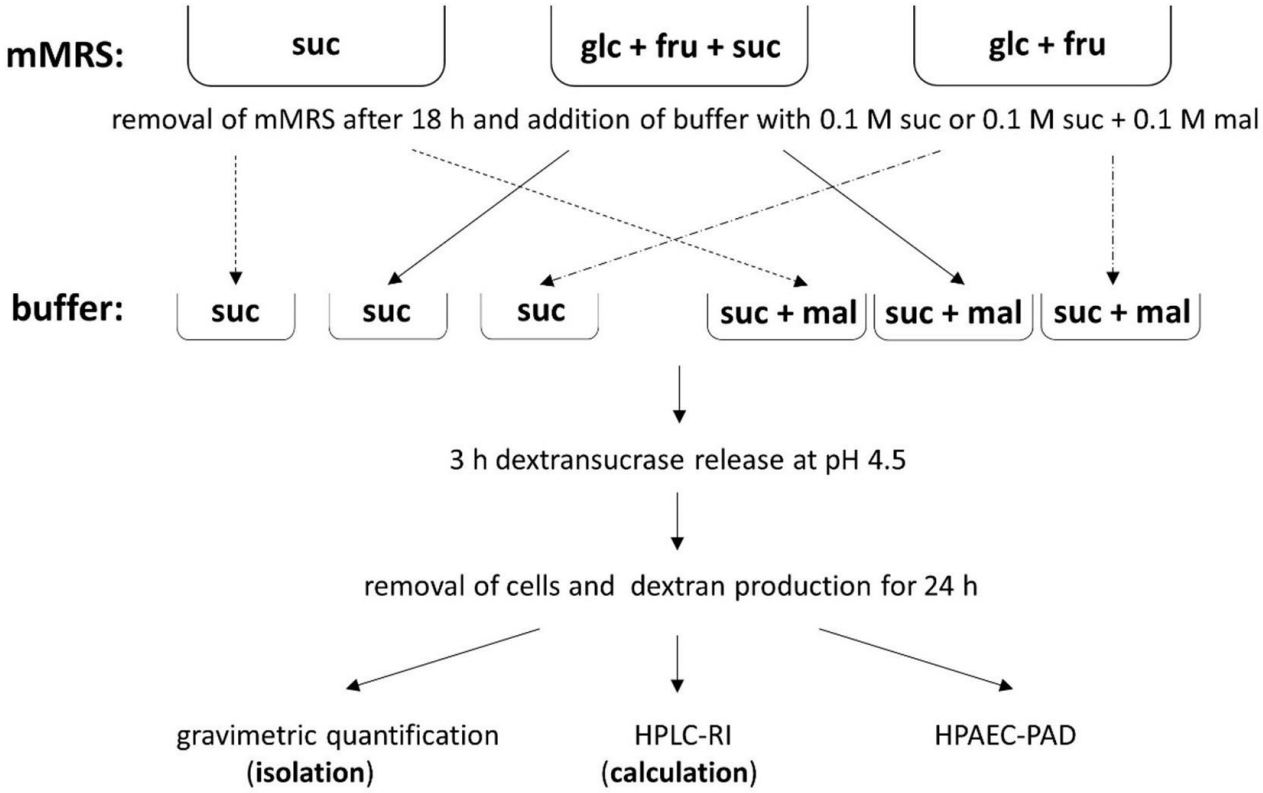


Figure 3: Overview of the experimental setups used to characterize dextran production dependent of sucrose and maltose. Cells were cultivated in 15.5 mL medium containing sole sucrose (suc), glucose + fructose + sucrose (glc + fru + suc) and glucose + fructose (glc + fru) with adjusted molarities for 18 h, centrifuged and dissolved in 7.5 mL 0.1 M citrate-phosphate buffer pH 4.5 either supplemented with 0.1 M sucrose (suc) or 0.1 M sucrose and 0.1 M maltose (suc + mal). After 3 h of incubation, the cells were removed by centrifugation and sterile filtration. 7.5 mL of the respective buffer was added and the cell free supernatants were incubated for 24 h, followed by dextran isolation, HPLC-RI and HPAEC-PAD measurements.

2.2.8. LPI-fermentations

The LPI-medium was provided by the IVV Freising and the protein of the sweet blue lupin (*Lupinus angustifolius, Leguminosae* family) was obtained according to the procedure described by Wäsche et al. (2001). For homogenization and pasteurization of the LPI dispersion, the ingredients depicted in Table 5 were processed using a processing plant for laboratory scale. The LPI-fermentations at the IVV were performed in 50 mL glasses for 24 h at the respective growth temperature and sugar (glucose or sucrose) of the inoculated strain. For evaluation of the grown bacteria via MALDI-TOF-MS, CFU plates were provided. After 24 h the glasses were stored at 4°C overnight before further usage for the tasting test.

The self-performed LPI-fermentations were carried out at the respective growth temperature of the investigated bacteria under static conditions for 24 h. *L. hordei* TMW 1.822 was cultivated in 45 mL LPI-medium containing either 10 g/L each glucose + fructose or 19 g/L sole sucrose and *P. claussenii* TMW 2.340 wt/mut in LPI-medium supplemented with 10 g/L each maltose + glucose, which were inoculated with ~ 10^7 cells. The CFU, pH CFU and dextran yields were determined after 0, 4, 8, 14, 19, 24 h of incubation. Furthermore, samples (triplicates) were stored overnight at 4 °C, after they reached the intended pH of 4.5, for subsequent rheological analysis (2.3.4).

Table 5: Composition of the lupin protein isolate (LPI) medium provided by the IVV Freising.

Compound	
Lupin protein isolate	4.6 %
Sugar	5.6 %
Cocos fat	3.8 %
NaCl	0.05%
Tap H_2O	86.2 %

2.2.9. Isolation of EPS

The standard EPS isolation of α-glucans and fructans was performed as described before (Korakli et al., 2001) with an initial centrifugation step (10000 x g, 30 mins) to remove solid material, followed by a precipitation step with 2 volumes of chilled ethanol for 24 h at 4 °C. To loosen cell bound polysaccharide, prior to the cell removal and the ethanol addition, the β-glucan and HePS samples were incubated at 60 °C for 2 h and vortexed extensively. The precipitated EPS was further dissolved in dH_2O and dialyzed against dH_2O at 4 °C for 48 h using a dialysis tubing with a molecular weight cut off (MWCO) of 3500 Da (SERVAPOR, SERVA Electrophoresis GmbH, Germany). Finally, the EPS solutions were lyophilized

(FreezeZone™, Labconco, USA), gravimetrically quantified with a laboratory balance (LA 310 S, Sartorius, Germany) and stored at room temperature (RT).

2.2.10. Hydrolysis of EPS

The hydrolysis of HoPS prior to the HPLC analysis was accomplished by dissolving 10 mg lyophilized HoPS in 995 or 930 µL of dH_2O and the addition of either 5 µ µL perchloric acid (PCA, 70 % w/w) for the soft hydrolysis of fructans or 70 µL PCA for the β- and α-glucan hydrolysis. Subsequently, the solutions were incubated for 4 h at 100 °C, centrifuged and after filtration (0.22 µm nylon filters, Phenomenex, USA) further subjected to HPLC analysis (2.3.1). To hydrolyze the HePS, 15 mg of freeze-dried EPS was dissolved in 930 µL of dH_2O and 70 µL PCA was added. The hydrolysis was performed for 6 h at 100 °C, followed by the procedure described for the HoPS.

2.3. Analytical Methods

2.3.1. HPLC-RI analysis

For the determination and quantification of monosaccharides and organic acids, high pressure liquid chromatography (HPLC) (Dionex Ultimate 3000, Thermo Fisher Scientific, USA) coupled to refractive index (RI) detection (ERC Refractomax 521, Thermo Fisher Scientific, USA) was performed. Sugar separations were achieved by the Rezex RPM-Monosaccharide Pb^{2+} column (Phenomenex Ltd., Germany) using water as mobile phase. For acid separation a Rezex ROA-Organic acid H^+ column with 2.5 mM H_2SO_4 was used. Both columns operated at a flow rate of 0.6 mL/min at 85 °C. The organic acid samples were treated with 50 µL perchloric acid (70 % w/w) per 1 mL of sample and incubated over night at 4 °C, followed by a centrifugation step to remove any precipitate. All samples were filtered (0.22 µm nylon filters, Phenomenex, USA) prior to the injection of 20 µL (AS 50 autosampler, Thermo Fisher Scientific, USA). The sugars and acids were identified and quantified using appropriate standard solutions and subsequently generated calibration curves with the Chromeleon™ software (v. 6.8, Dionex, Germany).

2.3.2. AF4-MALS-UV analysis for determination of molecular structure and size

The characterization of molecular weights and rms radii of the isolated dextrans was accomplished by asymmetric flow field-flow fractionation (AF4) coupled with multi-angle laser

light scattering (MALS) (Dawn Heleos II, Wyatt Technology, Germany) analysis and UV detection (Dionex Ultimate 3000, Thermo Fisher Scientific, USA). The freeze-dried dextran was at first redissolved in 0.05 M $NaNO_3$ (which also served as the eluent) at a final concentration of 0.1 mg/mL. To remove any remaining unsolved particles, the samples were centrifuged at 10000 x g for 2 min. 100 µl of the respective sample were then injected into the separation channel, equipped with a 10 kDa cellulose membrane. Separation was performed using a detector-flow rate of 1 mL/min and a crossflow gradient of 3 to 0.1 mL/min over 15 min, followed by 15 min of a steady cross flow of 0.1 mL/min. All chromatograms were analyzed with the software ASTRA 6.1 (Wyatt Technologies, Germany) using a dn/dc value of 0.1423 mL/g (Yuryev et al., 2007) and model of best fit degree integrated in the ASTRA software, which was the Berry model for the dextran of *L. hordei* TMW 1.1822. The distributions of rms radii were calculated in the particle mode, whereas the molar mass distributions were determined by using the respective UV concentration signals of the dextrans at 400 nm (Bechtner et al., 2019; Prechtl, Wefers, et al., 2018a; Ua-Arak et al., 2017; D. Xu et al., 2018). The specific extinction coefficients of dextrans were determined in standard 96 microtiter plates at a wavelength of 400 nm (SPECTROstar, BMG LABTECH) according to the Beer-Lambert law.

2.3.3. HPAEC-PAD analysis for determination of glucose oligosaccharides

For identification of short-chain gluco-oligosaccharides produced by dextransucrases in the presence of maltose, high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (ICS5000, Thermo Fisher Scientific, USA) on a CarboPac PA20 column (Thermo Fisher Scientific, USA) was performed. The separation was accomplished at a flow rate of 0.5 mL/min and an isocratic elution with 150 mM NaOH (Merck Millipore, USA) for 80 min and final flushing step with 200 mM NaOH and 1 M sodium acetate (Merck Millipore, USA) for 20 min. For identification and quantification, external sugar standards were used (Carbosynth, Switzerland) and the calibration curves were generated with the Chromeleon™ software (v. 6.8, Dionex, Germany).

2.3.4. Rheological analysis

2.3.4.1. Concentric cylinder and cone plate system

For elucidation of the basic rheological properties of the LPI-fermentations and the produced dextrans, flow curves were recorded, and the capability of gel formation was studied using the

rheometer Physica MCR 501 (Anton Paar, Austria). To dissolve the dextran in dH_2O, samples were vortexed for 10 minutes, heated in a water bath at 55 °C for 45 minutes and centrifuged (10000 x g, 10 min) to remove air bubbles.

The measurements with the concentric cylinder geometry CC 27-SS, to determine the viscosity of the dextran solutions, were performed at 20 °C at a shearing rate ranging from 0.1 1/s to 1000 1/s and *vis versa*. The shear rate was increased linear and 15 measurements were recorded each lasting 10 seconds. For the determination of the viscosity and C^*, multiple concentrations ranging from 0.25 to 7.5 % were measured.

The oscillatory tests, including the amplitude (strain) and frequency sweep, were performed with the cone-plate geometry CP 25-1. The LVE region and the yield (τ_y) as well as flow point (τ_f) were gained by the amplitude sweep tests at constant 1 rad/s with stress ranging from 0.1 to 100 %. Subsequently, frequency sweep tests at angular frequencies of 0.1 rad/s to 100 rad/s and constant 1.0 % were conducted.

Concentrations of highest differences and interest were picked for visualization, resulting in 7.5, 5, 2.5 and 1 % w/v for the rotational tests (concentric cylinder) and 7.5, 10 and 12.5 % w/v for the amplitude and frequency sweep tests (cone plate).

The LP- fermentations were measured with the concentric cylinder geometry at 20 °C with shearing rates of 0.1 1/s to 500 1/s and *vis versa*. The shear rate was increased linear and 47 measurements were recorded, with a holding phase of 100 s at 500 1/s. The strain sweep tests were performed at constant 10 rad/s with stress ranging from 0.1 to 100 %, followed by frequency sweep tests at angular frequencies of 0.1 rad/s to 100 rad/s and constant 0.05 % strain.

2.3.5. Chemical structure analysis

2.3.5.1. Methylation analysis

The methylation analysis was performed by Dr. Daniel Wefers at the Department of Food Chemistry, which is part of the Karlsruhe Institute of Technology (KIT) as described in the literature by Fels et al. (2018). Briefly, the dextran samples were dissolved in dimethyl sulfoxide and methylated with powdered sodium hydroxide as well as methyl iodide. The methylated polysaccharide was further extracted using dichloromethane, washed and dried. To hydrolyze the polysaccharide, 2 M trifluoracetic acid (TFA) was added and the sample was incubated for 90 min at 121 °C. After the evaporation of the TFA, the partially methylated monosaccharides were reduced by using sodium borodeuteride. The reaction was terminated with glacial acetic

acid, followed by the addition of 1-methylimidazole and acetic anhydride for acetylation. Identification of the obtained partially methylated alditol acetates (PMAA) was accomplished with a GLC-MS system (GC-2010 Plus and GC-MS-QP2010 Ultra, Shimadzu, Japan) equipped with a DB-5MS column (Agilent Technologies, Canada) and semiquantitative analysis GC-FID under following conditions: 140 °C initial column temperature for 2 min; ramped at 1 °C/min up to 180 °C and held for 5 min; ramped at 10 °C/min up to 300 °C and held for 5 min. Helium was used as the carrier gas at a rate of 40 cm/sec. The split injection to the mass spectra was conducted at 250 °C at a ratio of 30:1. For the GC-FDI analysis only the split ratio was changed to 10:1 at a FID temperature of 240 °C and nitrogen as makeup gas. All analyses were performed in duplicates and the molar response factor according to Sweet et al. (1975) was used to calculate the portions of the PMAAs.

2.4. Molecular biological techniques

2.4.1. SDS-PAGE for dextransucrase verification

The protein compositions of the buffer supernatants (2.2.7) was analyzed via vertical dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a Mini-PROTEAN® Tetra Cell Electrophoresis System (Bio-Rad laboratories, USA) equipped with a 12 % (w/v) separation gel and a 4 % (w/v) stacking gel. All samples were diluted in 2x Laemmli buffer (Sigma-Aldrich, USA) and denatured (90 °C, 10 min) prior to the application on the gel. All steps were carried out at RT if not stated otherwise. The initial separation was performed at 80 V, followed by 100 V for approximately 120 min via a Pack 3000 power adapter (Bio-Rad laboratories, USA). To visualize the separated proteins, a silver staining as described by Blum et al. (1987) was conducted. For the identification and size determination of the SDS-PAGE lane corresponding to the dextransucrase, an activity staining was performed. After the electrophoresis, the SDS gel was washed three times for 10 min with sodium acetate buffer (20 mM, 0.3 mM $CaCl_2$, 0.1 % Tween 80, pH 5.4) at 4 °C, followed by incubation step (30 °C, overnight) in the same buffer but supplemented with 5 % (w/v) sucrose. Afterwards, the gel was washed 30 min with an aqueous solution containing 10 % (v/v) acetic acid and 50 % (v/v) methanol and subsequently for 30 min in dH_2O. The oxidation of the formed dextran was accomplished by incubating the gel in a periodic acid solution (1 % (w/v) periodic acid (≥ 99.0 %, Sigma-Aldrich, USA) and 3 % (v/v) acetic acid) for 45 min. The gel was washed once again with dH_2O for 1 h and the dextran staining was finalized by Schiff´s reagent (Sigma-Aldrich, USA) until discrete pink bands were visible. The washed gels (dH_2O, 5 min) were then photographed.

2.4.2. Bradford assay for protein quantification

Total protein concentrations of the buffer supernatants were determined by the Coomassie/Bradford Protein Assay Kit (Thermo Fisher Scientific, USA). The assay was performed in standard 96 microtiter plates according to the instructions of the manufacturer.

2.4.3. DNA isolation

DNA isolation was performed using the E.Z.N.A Bacterial DNA Kit (Omega Bio-Tek, USA) with increased lysozyme incubation of at least 24 h. The remaining steps were performed according to the manufacturer's protocol.

2.5. Genomics

2.5.1. Genome sequencing and annotation

Multiple genomes of the strains of the second selection, were already available except for TMW 1.142, 1.64 and 1.617. Whole genome shotgun sequences were obtained by employing the Illumina MiSeq sequencing technology and for the assembly of the raw reads, SPAdes v. 3.9 was used. For the subsequent annotation the Prokaryotic Genome Annotation Pipeline of NCBI and Rapid Annotation Subsystem Technology of RAST was used. Additionally, all annotations of interest were further identified via an in-house pipeline using the database of The Institute of Genomic Research (TIGR). The final cross check of genes and annotations of interest was performed via blast against the NCBI databases. To further identify potential extracellular peptidases the MEROPS database as well as the signal peptide prediction tools SignalIP and Phobius were used.

2.5.2. Genome acquisition

The strains with their corresponding accession numbers are listed in Table 6. Locus tags of interest are mentioned separately in the respective context.

Table 6: List of used strains with corresponding accession number of NCBI.

Species	TMW	Accession number
Lactobacillus johnsonii	1.142	SAMN14342666
Latilactobacillus curvatus	1.1928	SAMN09650818
Liquorilactobacillus hordei	1.1822	SAMN06052353
Lactiplantibacillus plantarum	1.1478	CP021932
Lactiplantibacillus plantarum	1.617	SAMN14342838
Lactiplantibacillus plantarum	1.64	SAMN14342905
Latilactobacillus sakei	1.411	QOSE00000000
Pediococcus claussenii	2.340	SAMN02604240

2.6. Statistical analysis and data visualization

Data and graphics were either processed and evaluated with OriginPro software (v. 9.7, OriginLab Corporation, USA) or SigmaPlot software (v. 12.5, Systat Software GmbH, Germany) by one-way ANOVA. Differences in the means were considered as significant for $p < 0.05$.

3. RESULTS

3.1. Selection of EPS-forming strains as starter cultures for LPI-fermentations

The initial selection of 27 different LAB strains as starting cultures was primarily done according to the possibility of growth advantages of LAB, which are adapted to cereal fermentations. Furthermore, the selection had to represent a broad spectrum of EPS and species. Therefore, the selection contained representatives of 16 different LAB species and potentially various EPS types with α-glucan, β-glucan, fructan and heteropolysaccharides.

3.1.1. Screening for EPS production

The first screenings of the EPS production on simple agar plates and, for subsequent isolation, in liquid medium after 48 h of incubation are depicted in Table 7. After the isolation from mMRS cultures, sizeable EPS amounts were determined for all investigated strains except for 1.64, 1.675 and 2.340 mut. Since HePS and capsular polysaccharides are isolated insufficiently via the standard isolation procedure, these strains were still used for further investigations.

Table 7: Results of EPS screening on agar plates and via gravimetrical quantification after EPS isolation from liquid cultures of selected LAB strains. +++ refers to strong, ++ to medium and + to weak EPS formation.

Species	TMW	Plate	Amount EPS [g/L]
Lactobacillus johnsonii	1.142	+	0,941
Levilactobacillus brevis	1.2112	+++	1,173
Levilactobacillus brevis	1.2113	+++	1,053
Latilactobacillus curvatus	1.1928	+++	1,467
Limosilactobacillus frumenti	1.103	-	0,247
Limosilactobacillus frumenti	1.666	+	0,605
Lentilactobacillus hilgardii	1.1828	+++	4,787
Liquorilactobacillus hordei	1.1822	+++	4,760
Lactiplantibacillus plantarum	1.1478	+	0,993
Lactiplantibacillus plantarum	1.617	+	0,713
Lactiplantibacillus plantarum	1.64	++	0,088
Lactiplantibacillus plantarum	1.1308	+	0,071
Limosilactobacillus pontis	1.675	-	0,007
Limosilactobacillus pontis	1.1115	+	0,540
Limosilactobacillus pontis	1.1468	+	0,540
Limosilactobacillus reuteri	1.106	+++	3,240
Limosilactobacillus reuteri	1.693	+	0,467
Limosilactobacillus reuteri	1.977	++	1,267
Limosilactobacillus reuteri	1.272	+	0,453
Latilactobacillus sakei	1.411	++	1,967

Fructilactobacillus sanfranciscensis	1.54	+	1,633
Leuconostoc citreum	2.1194	+	1,247
Leuconostoc gelidum	2.1619	+	0,753
Leuconostoc mesenteroides	2.1073	++	2,593
Pediococcus claussenii wt	2.340	+	0,787
Pediococcus claussenii mut	2.340	-	0,047
Weissella confusa	1.934	+++	4,360

3.1.2. Determination of general growth kinetics in mMRS

The statistical analysis (R: *grofit* package) of the growth experiment in 96-well microtiter plates resulted in the depicted values of the lag phase and μ_{max} (Figure 4). Due to the strong EPS and slime formation of TMW 1.2112 and 1.2113, these strains were excluded from this experiment. The strains TMW 1.103, 1.666, 1.675 and 1.1115 showed delayed growth behavior with extended lag-phases and low values for μ_{max} compared to the other strains.

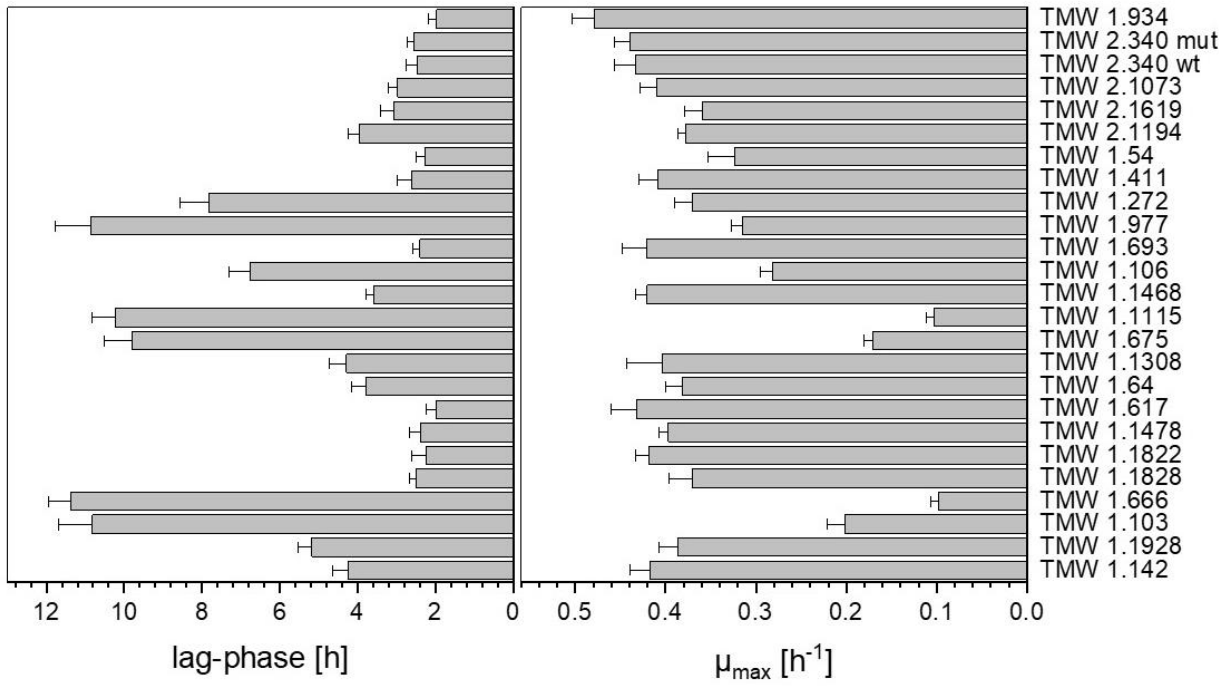


Figure 4: Growth curve parameters: Duration of lag-phase (left) and maximum growth rate μ_{max} (right) of the selected strains in mMRS supplemented with glucose + fructose and incubated for 48 h. Data are expressed with mean ± SD of three biological replicates.

3.1.3. Evaluation of growth on LPI substrate (IVV) and 1st strain selection

The evaluations of the general consumer acceptance of the pilot LPI-fermentations were performed by project partner at the IVV in Freising. To exclude any contamination and to verify the inoculated strain, LPI-fermentation samples were plated on mMRS and at least 30 single colonies from 3 different plates were measured via MALDI-TOF-MS. As depicted in Figure 5, the fermentations with TMW 1.666, 1.1828, 1.675, 1.1115, 1.977 and 2.1619 were not sufficient since contaminants from different species were detectable.

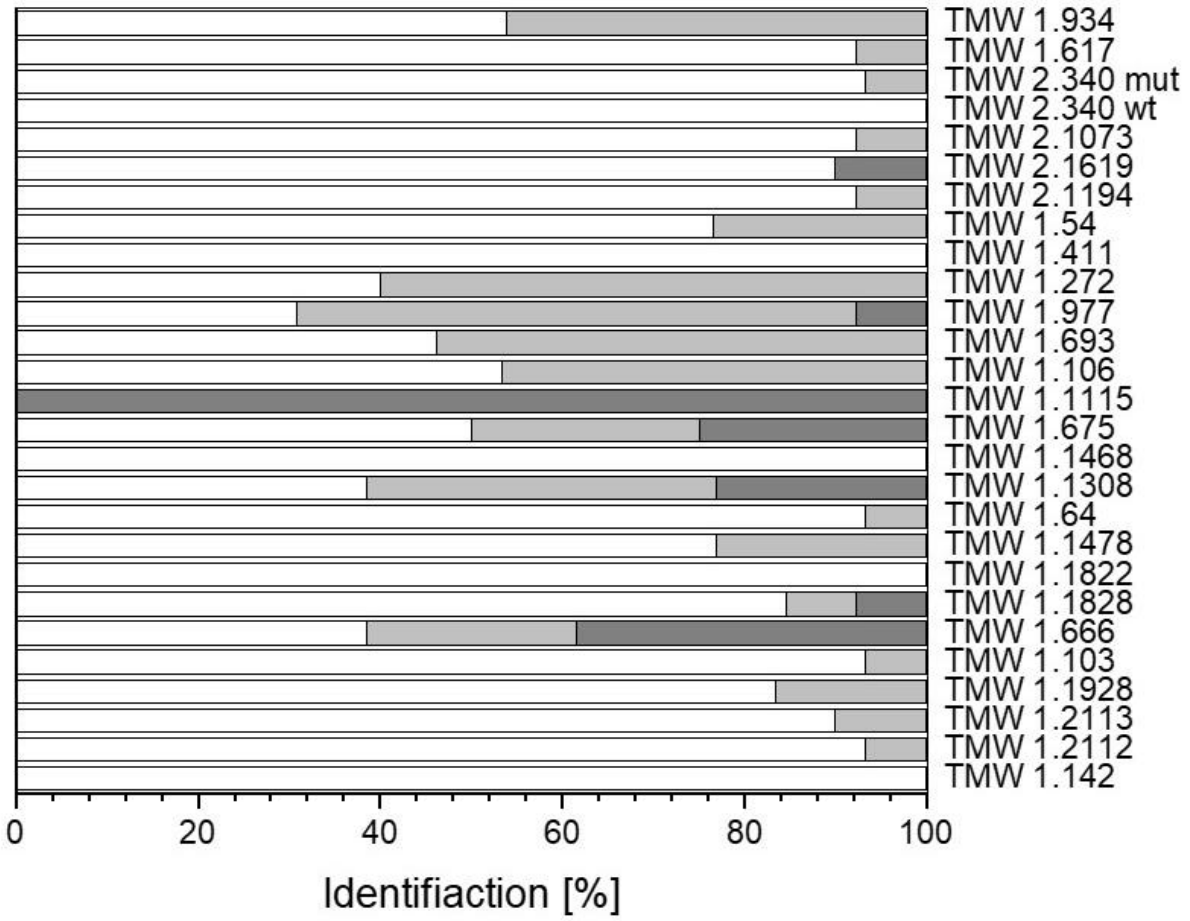


Figure 5: Results of the identification via MADLI-TOF-MS of single colonies derived from the plated pilot LPI-fermentations at the IVV. White correspondes to an appropoate idnetifiaction on species level of the inoculated strain with a socre > 2, light grey score < 2 and dark grey to a bacetrial contaminant of different species.

The pilot LPI-fermentations were performed at the IVV by Dr. Andrea Hickisch. Figure 6 depicts the overall popularity and the firmness of the LPI-fermentations since they represent the most significant parameters of the evaluation parameters. The subject groups evaluated the fermentations with respect to 10 different parameters and assigned scores from 0 (negative) to 10 (positive). The strains of the fermentations which provided almost equal or better results (black squares) as the commercially available lupin product (red square) and proved to show significant growth in mMRS (Figure 4) and assertiveness in the LPI-fermentations (Figure 5) within the first 24 h of incubation, were selected for further investigations (Table 1*). The most promising strain regarding the overall assigned scores was *P. claussenii* TMW 2.340 wt.

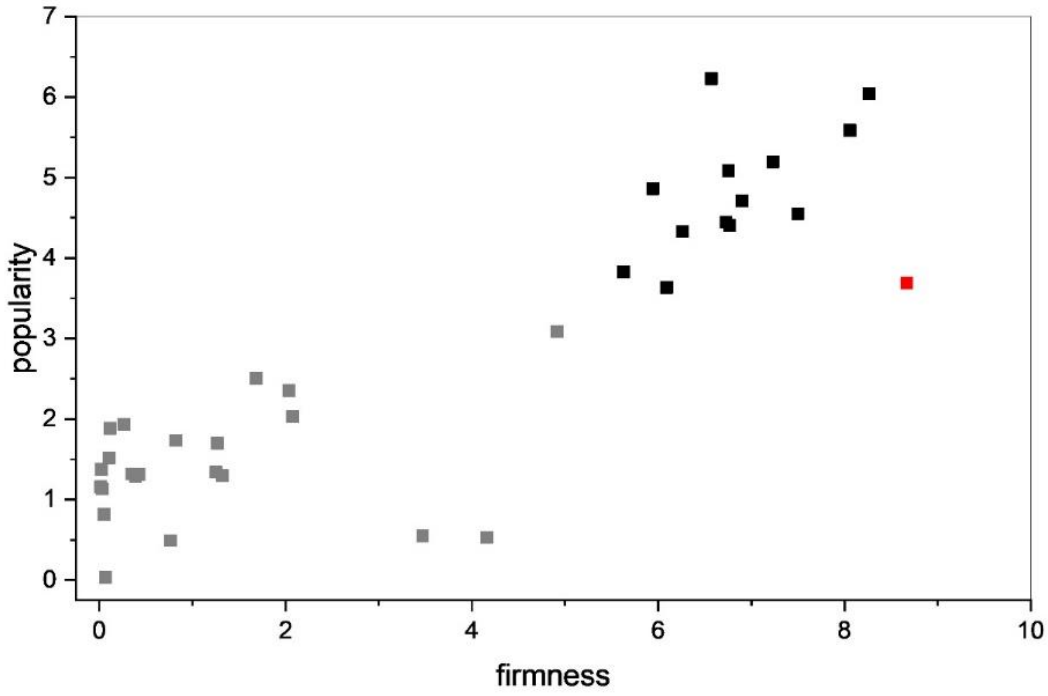


Figure 6: Popularity and firmness of the pilot LPI-fermentations with the 27 selected strains as evaluated by a tasting experiment at the IVV. Scores of 1 to 10 where 10 represents the most positive evaluation score. Strains referring to the black squares were used for further investigations. Red square represent a commercially available lupin yoghurt alternative.

3.1.4. Genomic preconditions of selected strains

In Table 8 an overview the predictively most relevant metabolic characteristics is given, which can further play decisive roles in the establishment of these strains as appropriate starter cultures for alternative plant fermentations. The detailed list with all single genes, enzymes and corresponding locus tags can be found in Appendix 5. Due to the negative aspects of biogenic amines in food products, the functionality of the amino acid decarboxylases was additionally controlled by the growth in decarboxylase medium, which resulted in no color changes to blue and therefore no formation of biogenic amines (data not shown) (Spano et al., 2010).

Table 8: List of the presence or cumulative number of genes/gene clusters of a selection of potentially relevant metabolic traits for the adaption to plant fermentations and for further usage of the pre-selected strains, respectively. + refers to presence and – to absence of a single gene or functionality of complete pathway with multiple genes involved.

	1.1822	2.340	1.142	1.1928	1.1478	1.617	1.64	1.411
Dextran-sucrase	**+**	-	-	**+**	-	-	-	**+**
Levansucrase/ inulosucrase	-	-	**+**	-	-	**+**	-	-
Glycosyltransferase 2	-	**+**	-	-	-	-	-	-
HePS-cluster	-	-	-	-	**+**	-	**+**	-
Tagatose-6-phosphate pathway*	-	-	-	-	-	-	-	-
Xylose utilization	-	-	**+**	-	**+**	**+**	**+**	-
Cellobiose utilization	**+**	**+**	**+**	**+**	**+**	-	-	**+**
Arabinose utilization	**+**	-	**+**	-	-	**+**	**+**	**+**
Ribose transporter	-	-	**+**	**+**	**+**	**+**	**+**	**+**
ß-glucoside transporter	6	0	1	1	2	2	3	1
(6-p-) ß-glucosidases	12	2	2	2	4	4	4	0
Peptidase ** (extracellular)	5	10	3	7	12	10	10	6
Peptide transporter	13	6	7	7	9	5	9	5
aa-decarboxylase	1	0	1	1	1	1	1	0
α-amylase α-glucosidase	**+**	**+**	**+**	-	**+**	**+**	**+**	**+**

* lack of 1 or more enzymes required for the complete pathway

** prediction via identification of signal peptide

3.1.5. Determination of monomer composition and EPS type

The EPS types of the respective strains were determined by sugar monomer analysis, and the genomic equipment (Table 8). In Table 9 the monomer composition and the genomic bases of the respective EPS are listed.

Table 9: Sugar monomer composition, EPS type and corresponding locus tags of the specific enzymes/cluster of selected strains determined via HPLC-RI and genome analysis.

Species	TMW	Sugar monomer	EPS type	locus tag
Lactobacillus johnsonii	1.142	fructose	levan/inulin	G9276_00805
Latilactobacillus curvatus	1.1928	glucose	dextran	DT351_11209
Liquorilactobacillus hordei	1.1822	glucose	dextran	BSQ49_11535
Lactiplantibacillus plantarum	1.1478	glucose, rhamnose, galactose	HePS	CEB41_05155 - 05245
Lactiplantibacillus plantarum	1.617	fructose	levan/inulin	G9277_01110
Lactiplantibacillus plantarum	1.64	glucose, rhamnose, galactose	HePS	G9279_11825 - 11870
Latilactobacillus sakei	1.411	glucose	dextran	DT321_09485
Pediococcus claussenii wt	2.340	glucose	β-glucan	PECL_RS09485

3.1.6. Investigation of isolated HoPS by AF4-MALS-UV

To further evaluate the strains of greatest interest and potential, AF4-MALS-UV measurements of the isolated EPS were performed. The HePS are not depicted since the minor isolated amounts as well as their low solubility led to deficient results. The fructans of TMW 1.142 and 1.617 exhibited the smallest radii with 44 and 52.1 nm, respectively. The dextran producers TMW 1.1822, 1.1928 and 1.411 showed a wide range of radii with 76.6, 167.8 and 183.1 nm. The chromatogram of TMW 2.340 serves only as an illustration of the insufficient measurements of the β-glucan since the light scattering signal at 90° was not representative for most of the 18 detectors.

Table 10: Determined RMS radii of EPS produced by the selected strains via AF4-MALS-UV.

Species	TMW	R_W [nm]
Lactobacillus johnsonii	1.142	44 ± 1.01
Latilactobacillus curvatus	1.1928	167.8 ± 1.17
Liquorilactobacillus hordei	1.1822	76.6 ± 0.08
Lactiplantibacillus plantarum	1.617	52.1 ± 0.10
Latilactobacillus sakei	1.411	183.1 ± 0.73
Pediococcus claussenii wt	2.340	134.0 ± 42.48

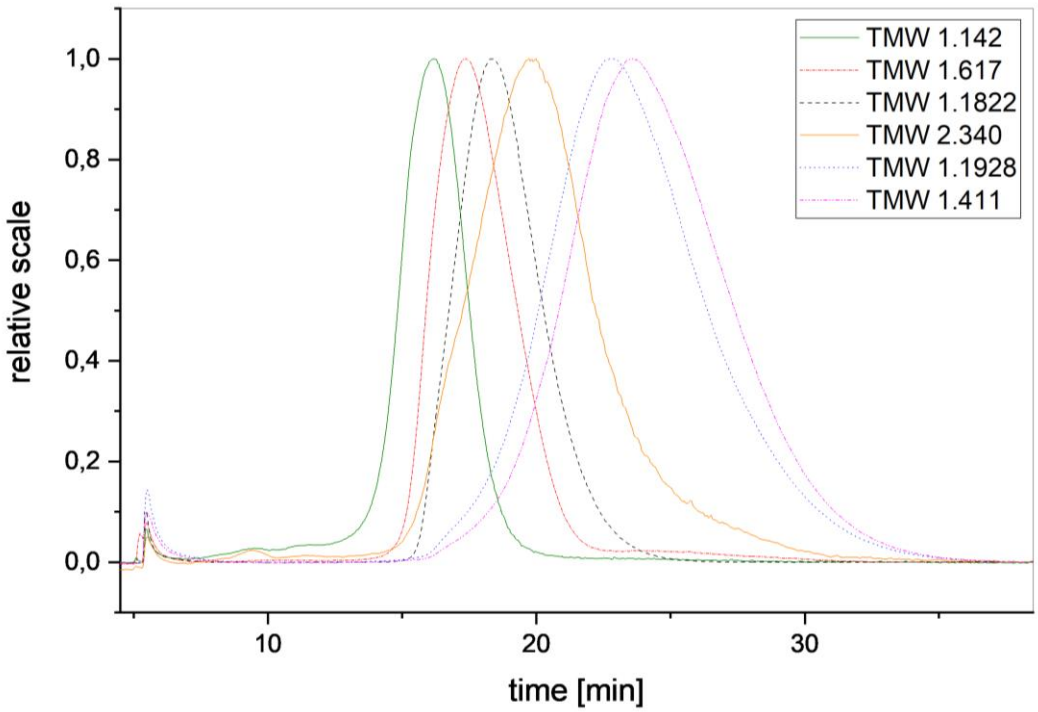


Figure 7: Overview of AF4 chromatograms (light scattering signal at 90°) of isolated EPS of strains selected for further investigation.

3.1.7. 2nd strain selection

For more detailed investigations, the strain selection had to be further diminished. On the basis of the obtained results in 3.1.1, 3.1.3, 3.1.2, 3.1.4, 3.1.6 and the genomic investigation, the strains *L. hordei* TMW 1.1822 and *P. claussenii* TMW 2.340 were selected.

3.2. Evaluation of LPI-fermentations by *P. claussenii* TMW 2.340 wt/mut

In Figure 8 and Figure 9, the growth behavior of *P. claussenii* TMW 2.340 wt/mut in 45 mL either mMRS or LPI-medium containing glucose and maltose are shown. The determined ß-glucan amounts, cell counts and pH values differed significantly between the two media, whereas wt and mut showed no significant differences while comparing the same media conditions. The intended pH value of 4.5 was reached after ~ 20 h of incubation.

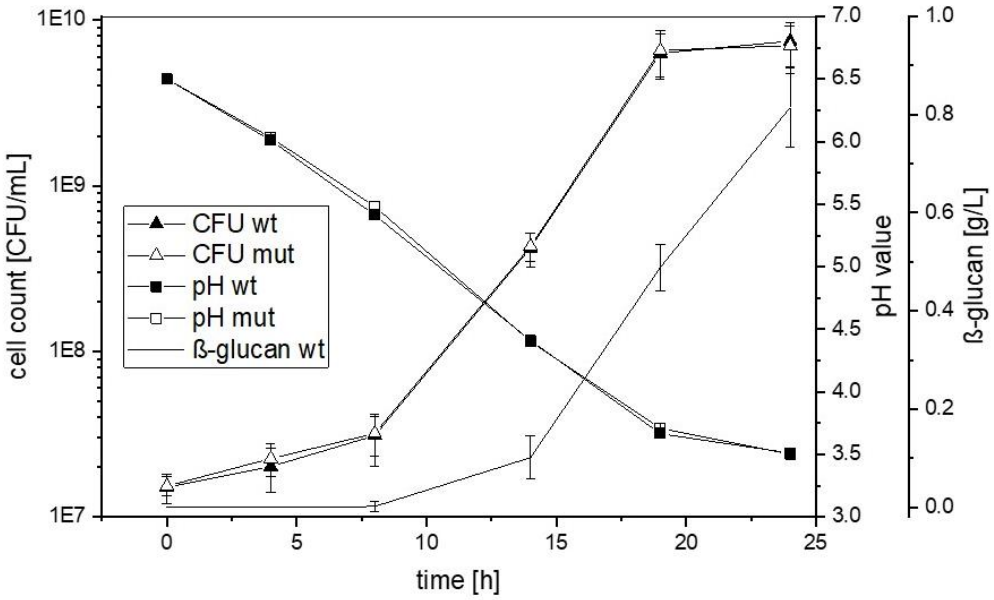


Figure 8: Monitoring of the growth parameters cell count (CFU), pH and ß-glucan formation of *P. claussenii* TMW 2.340 wt and mut over 24 h in mMRS supplemented with glucose + maltose.

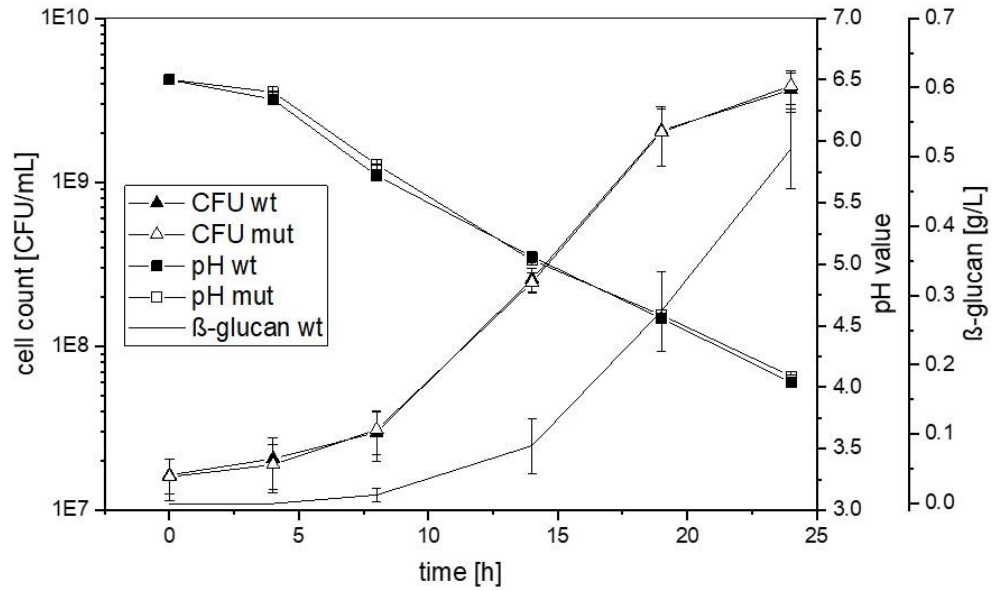


Figure 9: Monitoring of the growth parameters cell count (CFU), pH and ß-glucan formation of *P. claussenii* TMW 2.340 wt and mut over 24 h in LPI-medium supplemented with glucose + maltose.

3.3. Investigation of dextran formation by *L. hordei* TMW 1.1822

To study and evaluate the dextran formed by *L. hordei* TMW 1.1822, different experiments were performed. At first, the growth behavior in mMRS and LPI-medium with different carbon sources was determined (3.3.1). The second step included the dextran production with cell-free, but dextransucrase-containing buffer supernatants to investigate the effect of different pH values on the dextransucrase release, productivity and product specificity (3.3.2). As LAB are naturally subjected to pH fluctuations, which occur during their growth, this might be a crucial factor influencing the release of the dextransucrase as well as its activity implying the complexity of natural dextran formation which may consequently lead to the formation of different dextrans by one single dextransucrase type and bacterial strain. Furthermore, this may enable the improved and controlled formation of potentially tailor-made dextran fractions. In the third part different structural and rheological analyses of the dextran derived from the buffer experiments were performed, to reveal variations (3.3.3, 3.3.4, 3.3.7). At last, the influence of sucrose and maltose on the dextran structure and isolated yields was studied (3.3.5, 3.3.6).

3.3.1. Dextran production in mMRS and LPI-medium

Figure 10 and Figure 11 illustrate the growth behavior in 45 mL either mMRS or LPI-medium containing sucrose or glucose + fructose over 24 h. For both cultivation media the general growth and acidification were highly similar for the approaches containing either sucrose or glucose + fructose. The determined dextran amounts, the cell counts and the pH values in identical volumes of mMRS and LPI-medium differed significantly. In presence of sucrose the dextran yields of the LPI fermentations averaged approximately 2.7 g/L whereas the ones of the mMRS cultures reached 4.6 g/L. The desired pH of 4.5 for the LPI-fermentation was reached after ~ 23 h of incubation. Overall, the pH drop was delayed, and the cell counts were decreased for the LPI-fermentations.

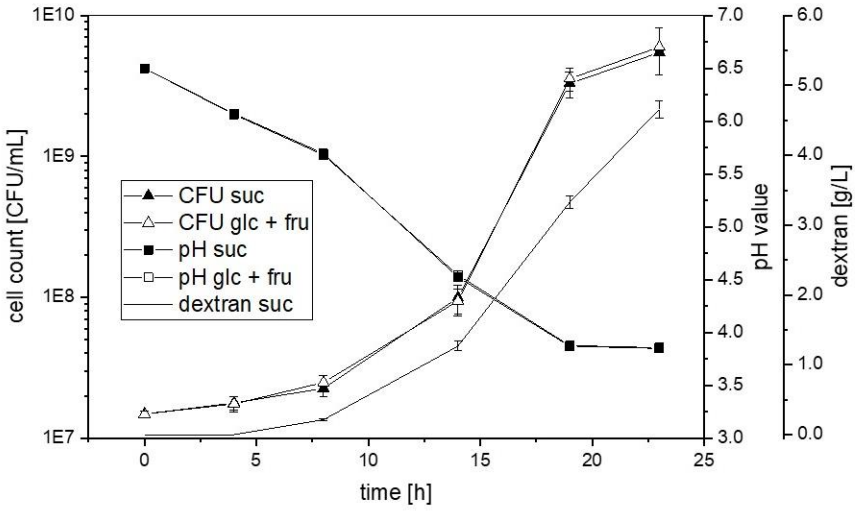


Figure 10: Monitoring of the growth parameters cell count (CFU), pH and dextran formation of *L. hordei* TMW 1.1822 over 24 h in mMRS with glucose / fructose or sole sucrose.

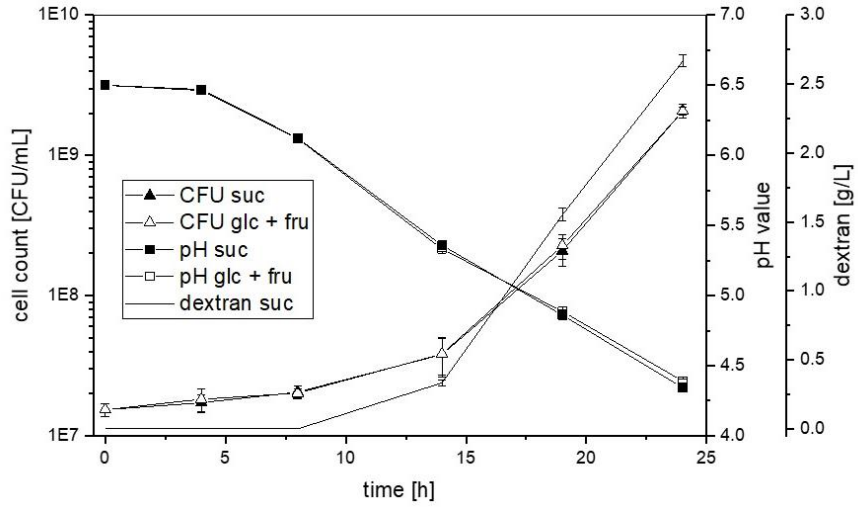


Figure 11: Monitoring of the growth parameters cell count (CFU), pH and dextran formation of *L. hordei* TMW 1.1822 over 24 h in LPI-fermentation with glucose / fructose or sole sucrose.

3.3.2. Dextran production in buffer with different pH values

The high molecular weight dextrans were gained via two experimental setups, which are depicted and described in section 2.2.7 and Figure 2. These setups were picked to reveal potential influences of the pH value on the polymerization of the dextran as well as the release process and functionality of the dextransucrase. Since TMW 1.1822 exclusively releases its dextransucrase in the presence of sucrose, the cells were pre-cultured in mMRS and subsequently resuspended und incubated in sucrose supplemented citrate-phosphate buffers of different initial pH values. The pH variation was either solely applied on the polymerization (setup B) or additionally on the preceding dextransucrase release (setup A). If the pH was only varied once (setup B), the dextransucrase release was performed under a pH of 4.5. After 3 h at 30 °C the respective viable cells, pH values as well as the released and consumed sugars were determined (Table 11 and Table 12). Afterwards, the cells were removed and the dextransucrase containing supernatants were utilized to produce dextran for 24 h at different pH values. According to McIlvaine (1921) and Appendix 4, the respective pH was adjusted to the final and desired values and at the end of the experiment, the sugar concentrations were once again determined (Table 12). The dextrans obtained thereby were subsequently quantified by weighing of the isolated dextran and the calculation of the total dextran amounts in g/L by the equation

$$c\,(transglycosylated\ glc) \times 162.16\ \frac{g}{mol}\ (molar\ mass\ of\ glc\ in\ dextran),$$

since the transglycosylated glucose corresponded to the difference of the quantified free glucose and fructose in the samples.

Furthermore, the identification of the dextransucrase and evaluation of its activity was accomplished by SDS-PAGE. The gels in Figure 12 confirmed the presence of the dextransucrase of an, via the genomic data, predicted size of 120 kDa with visually similar band intensities for all conditions. The activity staining on the left part furthermore approved the functionality of the dextransucrase under the tested pH values. Since setup B refers to the simple setup with a consistent initial release pH of 4.5, the SDS-PAGE was only performed for setup A.

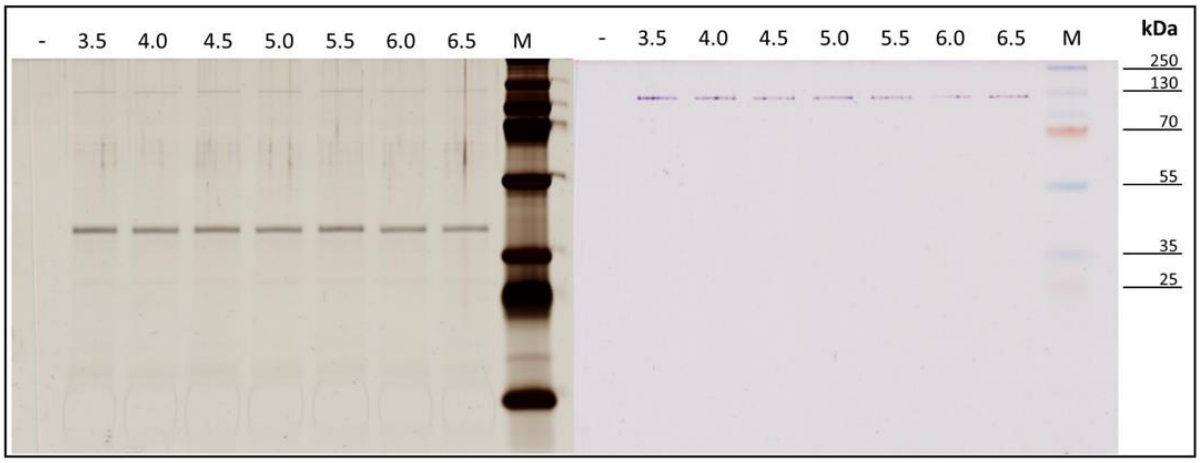


Figure 12: Silver (left) and activity (right) stained SDS-gels of the buffer supernatants obtained after 3 h of incubation of TMW 1.1822 at different pH as depicted in Figure 2 (2.2.7); negative control (-), marker (M).

In Table 11 and Table 12 the important parameters of all tested conditions and samples are listed. In order to ensure clarity in the tables and Figure 13, the corresponding statistical values are listed in Appendix 1. To exclude the possibility of subsequent variations due to enhanced cell disruptions during the dextransucrase release, cell counts (CFU) before and after the first 3h in buffer were recorded. Prior to the buffer addition the CFU averaged 9.71 ± 0.25 log10CFU/mL, with similar values of viable cells for all 7 pH values after the 3 h of incubation. As explained before for the SDS-PAGE, the cell counts as well as the protein concentrations were not determined for setup B. The protein concentrations were determined by the Bradford-assay and showed no significant variations among the tested conditions. The pH values after 3 h as well as the fructose concentrations after 3 and 27 h varied as expected. Whereas the concentrations of liberated free glucose, which reflects the hydrolysis activity of the dextransucrase, after 3 and 27 h remained steady at approximately 5 mM and 12 mM, respectively.

Table 11: Log10 CFU/mL, pH and protein concentrations of the buffer supernatants of the experimental setup shown in Figure 2 (2.2.7). The protein concentrations were determined by the Bradford-assay. Data are expressed with mean ± SD of three biological replicates, respectively.

	Smp.* / pH	Log10 CFU/mL 3h	pH 3h	Protein [µg/mL]
Setup A	3.5	9.69 ± 0.17	3.37 ± 0.01	16.38 ± 2.11
	4	9.61 ± 0.03	3.88 ± 0.01	16.39 ± 1.87
	4.5	9.63 ± 0.13	4.38 ± 0.00	16.40 ± 1.90
	5	9.64 ± 0.09	4.77 ± 0.01	16.27 ± 1.98
	5.5	9.71 ± 0.10	5.25 ± 0.01	15.83 ± 2.02
	6	9.62 ± 0.10	5.73 ± 0.01	17.12 ± 2.00
	6.5	9.67 ± 0.05	6.21 ± 0.00	16.60 ± 1.88
Setup B	3.5		4.37 ± 0.01	
	4		4.37 ± 0.01	
	4.5		4.37 ± 0.01	
	5		4.37 ± 0.01	
	5.5		4.37 ± 0.01	
	6		4.37 ± 0.01	
	6.5		4.37 ± 0.01	

* Smp. = sample

Table 12: Glucose, fructose concentrations after 3 h of dextransucrase release. For calculation of the totally produced dextran amounts, glucose and fructose concentrations were additionally determined at the end of dextran production (27 h). Sugar concentrations were determined according to 2.3.1. Data are expressed with mean ± SD of three biological replicates, respectively.

	Smp.* / pH	Glucose 3h [mM]	Fructose 3h [mM]	Glucose 27h [mM]	Fructose 27h [mM]
Setup A	3.5	4.84 ± 0.02	4.90 ± 0.07	12.00 ± 0.29	14.69 ± 0.04
	4	4.91 ± 0.08	8.41 ± 0.19	12.07 ± 0.43	30.04 ± 1.00
	4.5	5.03 ± 0.05	8.50 ± 0.32	12.18 ± 1.02	29.86 ± 0.07
	5	5.04 ± 0.03	7.77 ± 0.36	12.16 ± 1.29	25.74 ± 0.52
	5.5	4.72 ± 0.13	7.07 ± 0.30	11.34 ± 0.72	20.88 ± 1.73
	6	5.22 ± 0.13	7.27 ± 0.11	11.77 ± 0.62	19.81 ± 0.75
	6.5	4.89 ± 0.03	5.83 ± 0.20	11.78 ± 0.02	15.21 ± 0.51
Setup B	3.5	4.84 ± 0.07	8.40 ± 0.14	11.86 ± 0.32	17.94 ± 0.35
	4	4.93 ± 0.02	8.58 ± 0.22	12.04 ± 0.37	30.04 ± 0.51
	4.5	5.03 ± 0.10	8.44 ± 0.54	12.35 ± 0.26	30.49 ± 0.85
	5	5.16 ± 0.06	8.28 ± 0.46	12.40 ± 0.97	28.66 ± 0.73
	5.5	4.99 ± 0.13	8.56 ± 0.57	11.64 ± 0.82	26.99 ± 0.37
	6	4.93 ± 0.09	7.90 ± 0.41	12.06 ± 0.69	23.71 ± 1.09
	6.5	4.71 ± 0.08	8.27 ± 0.87	11.79 ± 0.20	18.24 ± 0.22

* Smp. = sample

The isolated dextran amounts as well as the predictively produced total amounts calculated by the aforementioned equation are displayed in Figure 13. In both setups the maximum dextran amounts were isolated at pH 4.0 to 4.5, averaging ~ 1.70 g/L. The lowest amounts of 0.35 to 0.40 g/L were obtained at pH 3.5 and 6.5. Outside of the depicted pH range, no dextran formation occurred and therefore no isolation. In setup B the isolated dextran amounts were significantly higher at pH 5.5 and 6.0, whereas the produced amounts were similar at the remaining pH values. The loss of the dextran during the applied isolation procedure accounts for approximately 40 % in between the pH values of 4.0 and 6.0 in setup A and B, since the greatest calculated amounts averaged ~ 2.35 g/L. However, at pH 3.5 and 6.5, these differences in the calculated and isolated amounts of dextran were not detectable. Comparable tendencies were further recorded with the same experimental setup, but pH steps of only 0.2 over a pH range of 3.6 to 4.8 (Appendix 2).

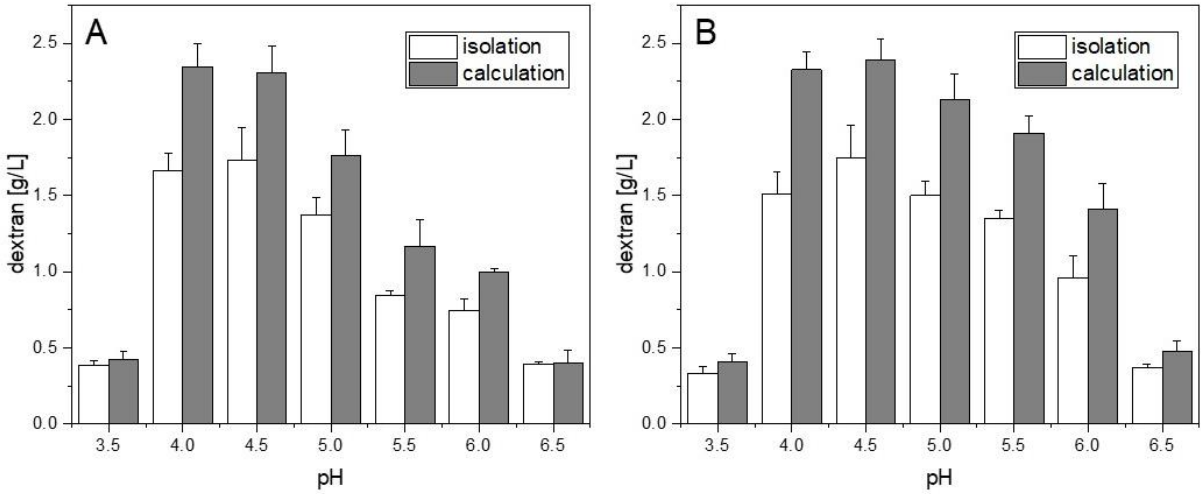


Figure 13: Dextran amounts produced in the experimental setup shown in Figure 2 (2.2.7). Bars indicate the produced dextran amounts in g/L, which were either determined gravimetrically (isolated dextran) or via calculation of the totally produced amount using the calculated transglycosylation activity (24 h). (A) refers to setup A, (B) to setup B. Data are expressed with mean ± SD of three biological replicates.

3.3.3. AF4-MALS-UV analysis of dextran from different pH values

The dextran produced at pH values ranging from 3.5 to 6.5 were further analyzed by AF4-MALS-UV to determine and investigate their rms radii, molar masses and basic spatial structure. Both setups exhibited similar tendencies, as the rms radii and molar masses increased with rising pH – values over a range of ~ 71.4 ± 0.8 to 93.9 ± 0.3 nm and ~ 9.43 ± 0.1 x 10^7 to 1.97 ± 0.2 x 10^8 Da at the same extent except for pH 6.0 as depicted in Figure 14 A and B. At pH 6.5 the averaged molar masses and rms radii decreased at both experimental setups. Figure 15 exemplifies the average distribution of dextrans from 3 representative technical replicates of the experimental setup A. For reason of clarity, only the dextrans from pH 3.5, 6.0 and 6.5 are depicted. The dextrans of greatest averaged molar masses and rms radii from pH 6.0 were restrained for ± 1 min longer and the dextran from pH 6.5 only differs by ± 7.5 sec compared to the dextran from pH 3.5 (Figure 15 A). Thus, according to the separation principle of the AF4 channel, the hydrodynamic volumes of the investigated dextrans increased with their molecular weight and rms radii. Again, similar observations were made for the rms radii with the same experimental setup, but pH steps of only 0.2 over a range of 3.6 to 4.8 (Appendix 3, data not shown).

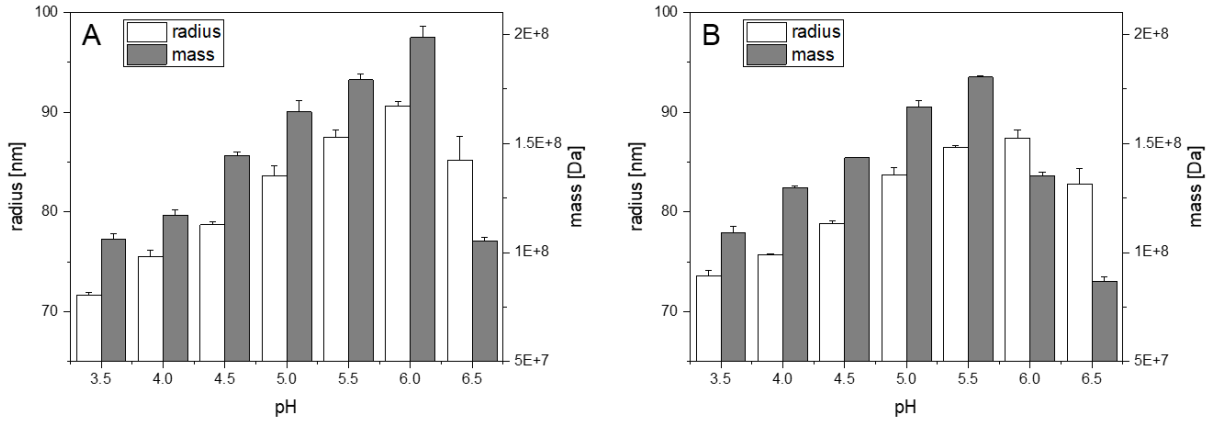


Figure 14: Averaged molar masses [Da] and rms radii [nm] of dextrans produced in experimental setups depicted in Figure 2 (2.2.7). (A) refers to setup A, (B) to setup B. Data are expressed with mean ± SD of three biological replicates.

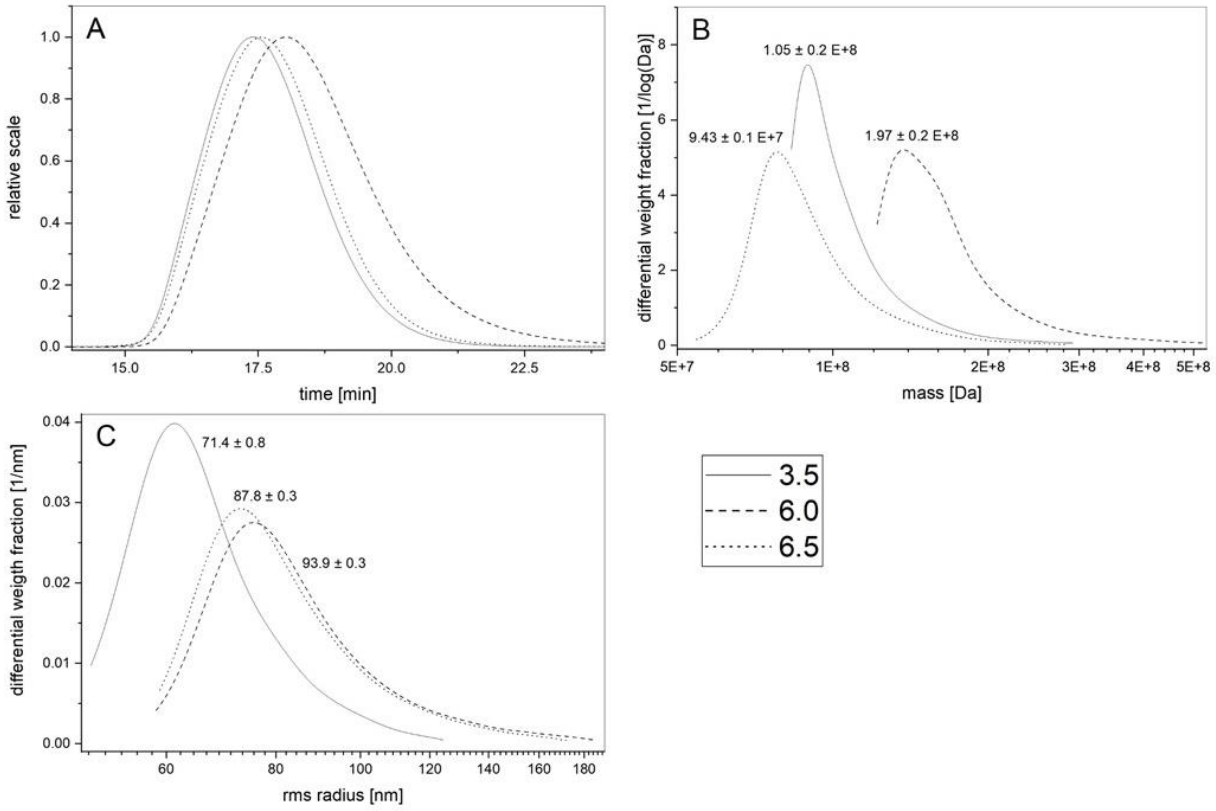


Figure 15: AF4-MALS-UV analysis of representative dextrans isolated at pH 3.5, 6.0 and 6.5 of experimental setup A depicted in Figure 2 (2.2.7) (A): AF4 chromatograms (light scattering signal at 90°). (B): Differential weight fractions of the molar masses with corresponding M_w ± SD of three technical replicates. (C): Differential weight fractions of rms radii with corresponding R_w ± SD of three technical replicates.

For further rheological investigations, the experimental setup depicted in Figure 2 was simplified, but upscaled and one additional condition (UC) was implemented. The dextran formation was performed at constant pH values of 4.0 and 5.5, since the dextransucrase produced acceptable dextran amounts at these conditions and the isolated dextrans exhibited big differences in their molecular weights and rms radii. The UC (uncontrolled) dextran was produced with TMW 1.1822 cells in the established buffer system without pH control. Furthermore, dextran isolated from a LPI-fermentation was also investigated by AF4-MALS-UV (Figure 16). The results for the dextrans from pH 4.0 and 5.5 were similar to the above mentioned, with bigger values of 94.3 ± 0.95 and 1.86 ± 0.02 x 10^8 Da for pH 5.5 compared to the smaller ones of 73.5 nm ± 0.7 nm and 1.09 ± 0.04 x 10^8 Da for pH 4.0 (Table 13). The UC dextran (77.3 nm ± 1.0 and 1.40 ± 0.02 x 10^8 Da) as well as the dextran from the LPI-fermentation (83.0 nm ± 0.5 and 1.57 ± 0.02 x 10^8 Da) showed rms radii and molar masses in between pH 5.5 and 4.0. The quotients of $M_w \backslash M_n$ and $R_w \backslash R$ which both represent the index of polydispersity for the entire polymer fraction were within the same range for all samples. Furthermore, the slopes of the conformation plots were also in comparatively similar ranges (0.74 ± 0.1 to 0.79 ± 0.2).

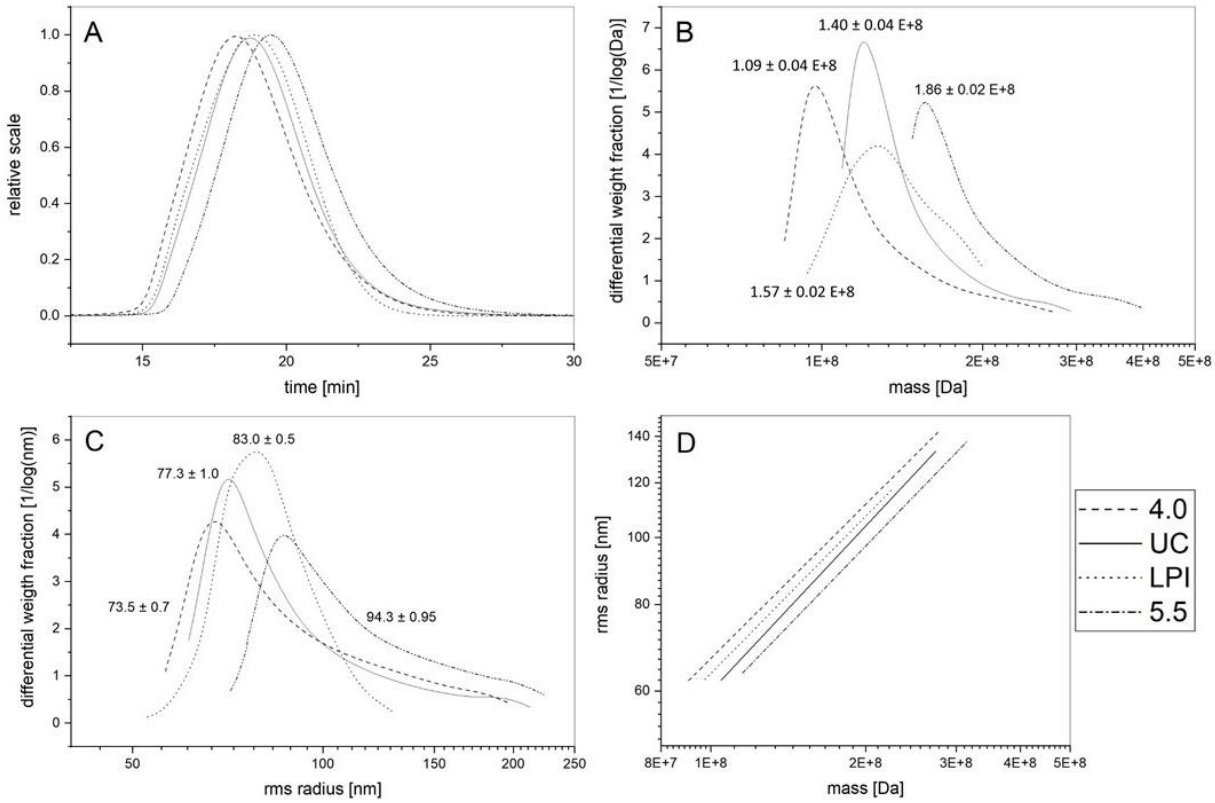


Figure 16: AF4-MALS-UV analysis of representative dextrans isolated at pH 4.0, 5.5, UC and from a LPI-fermentation (A): AF4 chromatograms (light scattering signal at 90°). (B): Differential weight fractions of the molar masses with corresponding M_w ± SD of three technical replicates. (C): Differential weight fractions of RMS radii with corresponding R_w ± SD of three technical replicates. (D): Confirmation plot of rms radii against the molar masses.

Table 13: Averaged molar masses, rms radii and corresponding distribution quotients as well as the slope of the confirmation plots determined by AF4-MALS-UV. The dextrans were isolated at pH 4.0, 5.5, UC and from a LPI-fermentation of dextrans produced pH 4.0, 5.5.

	M_w [Da]	M_w/M_n (PDI)	R_w [nm]	R_w/R_n (PDI)	confirmation plot slope
pH 4.0	1.09 ± 0.04 x 10^8	1.10 ± 0.01	73.5 ± 0.7	1.13 ± 0.02	0.74 ± 0.1
pH 5.5	1.86 ± 0.02 x 10^8	1.09 ± 0.01	94.3 ± 0.9	1.11 ± 0.01	0.77 ± 0.2
UC	1.40 ± 0.02 x 10^8	1.08 ± 0.00	77.3 ± 1.0	1.12 ± 0.01	0.79 ± 0.2
LPI	1.56 ± 0.02 x 10^8	1.08 ± 0.00	83.0 ± 0.5	1.12 ± 0.00	0.75 ± 0.1

3.3.4. Methylation analysis

To finalize the structural elucidation, the dextran fractions of most interest were investigated via methylation analysis by Dr. Daniel Wefers at the Department of Food Chemistry, which is part of the Karlsruhe Institute of Technology (KIT). The analysis confirmed the presence of a dextran with a α-(1→6)-linked glucose backbone (1,6-Glc*p*) and side branching at the position *O3* (1,3,6-Glc*p*) to a low extent (Table 14). The variations in the portions of the glycosidic linkage types within the same samples were only marginal, which as well indicates comparable molecular and spatial/secondary structures for the investigated polysaccharides.

Table 14: Glycosidic linkage types (mol %) of *L. hordei* dextrans determined by methylation analysis. The dextrans were isolated at pH 5.5, 4.0, UC and from a LPI-fermentation.

Glycosidic bond	pH 5.5	UC	pH 4.0	LPI
t-Glc*p*	5.0 ± 0.0	4.9 ± 0.0	5.0 ± 0.02	5.7 ± 0.2
1,3-Glc*p*	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.1
1,6-Glc*p*	90.9 ± 0.0	90.6 ± 0.0	90.8 ± 0.3	88.8 ± 0.0
1,3,6-Glc*p*	2.8 ± 0.1	3.0 ± 0.1	2.6 ± 0.1	2.8 ± 0.2

t = terminal, *p* = pyranose. Numbers indicate the substitution position of the corresponding PMAA.

3.3.5. Sucrose and maltose influence on dextran formation

The incubation of *L. hordei* TMW 1.1822 with different sugar combinations in various above-mentioned experiments indicated influences of sucrose and maltose in pre and main cultures on the isolatable dextran yields, respectively. To identify and explore these effects and exclude potential influences due to variations regarding the general growth kinetics, strain TMW 1.1822 was cultivated in mMRS supplemented with different combinations of glucose, fructose, maltose and sucrose. After 48 h the CFU, OD_{590}, pH and dextran amounts were determined, resulting in significant differences only for the dextran yields, whereas the CFU, OD_{590}, and pH were similar at all tested conditions (Figure 17), which was in line with the described results for the cultivation in mMRS with sucrose or glucose/fructose in section 3.3.1 (Figure 10).

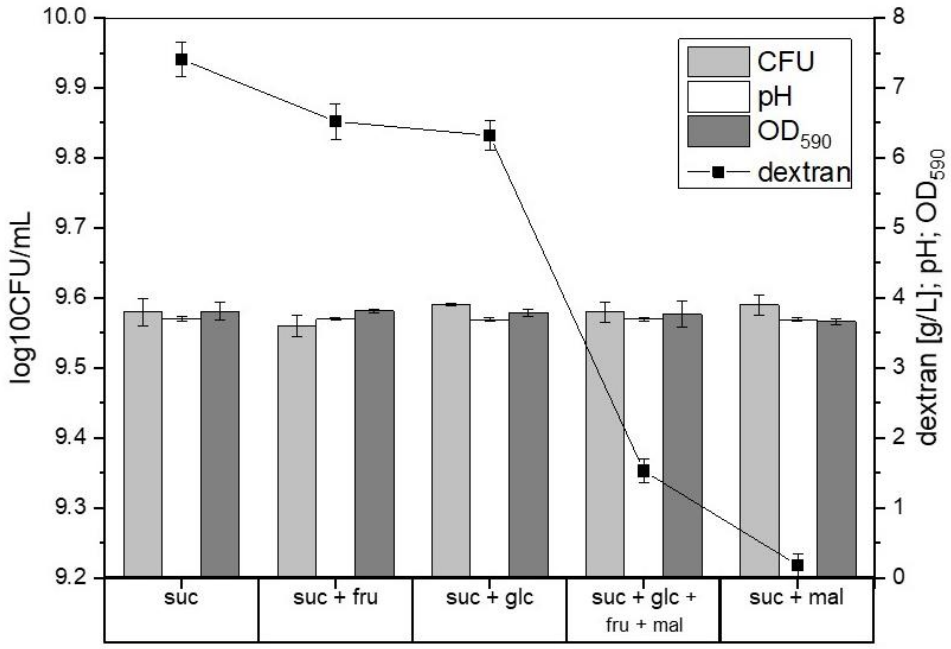


Figure 17: Overview of cell count (CFU), OD_{590}, pH and dextran amounts of TMW 1.1822 after 48 h incubation at 30 °C in mMRS with different sugar combinations.

Consequently, the method to produce dextran under controlled pH conditions in citrate-phosphate buffer was modified to study the evident influences of sucrose and maltose (Figure 3, 2.2.7). The pre-cultivation mMRS was either supplemented with sucrose as sole sugar source or with equal molarities of glucose and fructose. Subsequently the dextransucrase release as well as the dextran formation was performed either in the presence of sucrose (0.1 M) or sucrose and maltose (both 0.1 M). Again, the amounts were determined gravimetrically and by calculation via HPLC analysis.

For sucrose in the mMRS pre-cultivation, the dextran amounts obtained were considerably lower in comparison to those obtained under sucrose free conditions, as depicted in the left part of Figure 18. Furthermore, no significant variations in the formed dextran yields were observable if the cells had been pre-cultivated in mMRS supplemented with sucrose or the mixed sugar source glucose/fructose/sucrose.

In addition, the harvested cells obtained from mMRS ± sucrose were lysed, and these lysates were further used for dextran production in buffer. The isolated dextran amounts produced with the lysates form sucrose free pre-cultivation were significantly higher with 1.29 ± 0.014 g/L compared to 0.71 ± 0.056 g/L for the sucrose positive cells (Figure 19).

Furthermore, the right part of Figure 18 illustrates the drastically reduced amounts of isolatable dextran in the presence of maltose to less than 0.05 g/L independently of the pre-cultivation conditions. However, the overall activity of the dextransucrase was not reduced as compared to the corresponding experimental series performed without maltose. Since the concentrations

of the liberated glucose and free fructose were similar among the particularly comparable approaches (± maltose), the before mentioned (3.3.2) initial hydrolysis of sucrose prior to the transglycosylation step was again observable at all tested conditions.

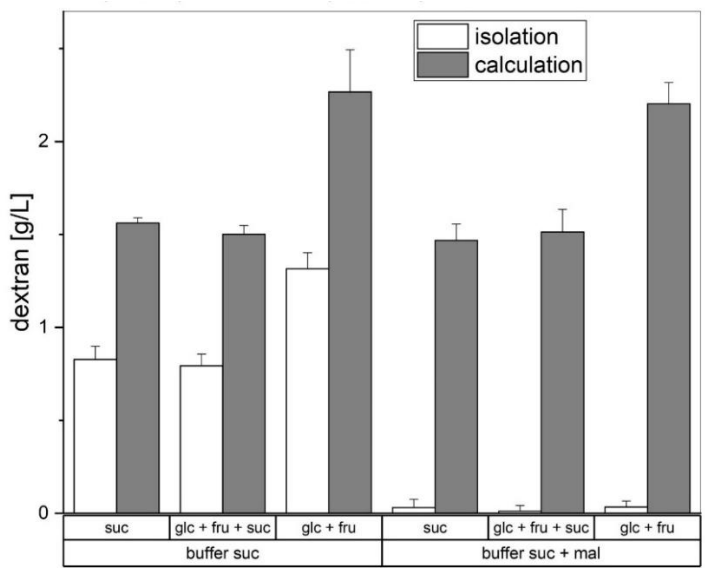


Figure 18: Dextran amounts produced in the experimental setup shown in Figure 3 (2.2.7). Bars indicate the produced dextran amounts in g/L, which were either determined gravimetrically (isolated dextran) or via calculation of the totally produced amount using the calculated transglycosylation activity (24 h). Data are expressed with mean ± SD of three biological replicates.

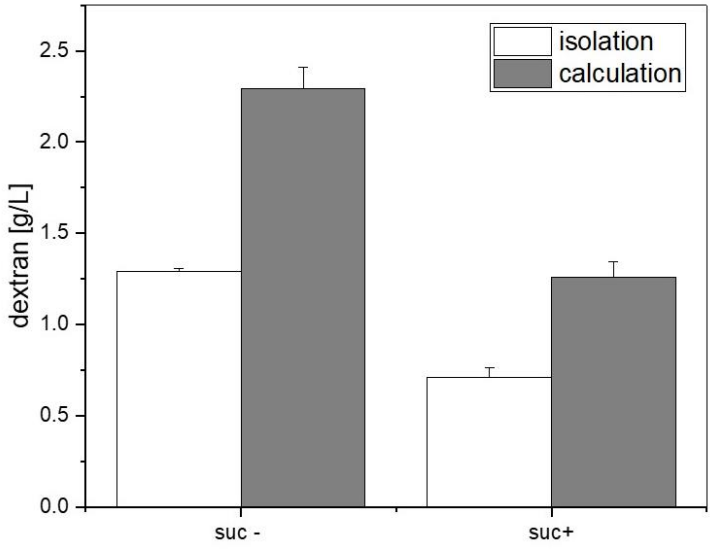


Figure 19: Dextran amounts produced in buffer by lysed cells after cultivation in mMRS containing either glucose, fructose and sucrose (suc +) or glucose and fructose (suc -). Bars indicate the produced dextran amounts in g/L, which were either determined gravimetrically (isolated dextran) or via calculation of the totally produced amount using the calculated transglycosylation activity (24 h). Data are expressed with mean ± SD of three biological replicates.

3.3.6. HPAEC-PAD analysis of the gluco-oligosaccharide produced via maltose acceptor reaction

To identify the gluco-oligosaccharides the diluted maltose supplemented sample supernatants of the experimental setup depicted in Figure 3 were further analyzed by HPAEC-PAD. In Figure 20 representative chromatograms of a standard sugar mixture containing glucose (1), fructose (2), sucrose (3), maltose (4), isomaltotetraose (5), panose (6), maltotriose (7) as well as a supernatant sample and an unused buffer control are depicted. Besides the peaks, which correspond to the sugar added to the production buffer (glc, fru, suc, mal), an additional peak (6) was detected in the supernatant. Corresponding to the determined retention times in the standard mixture, this peak was identified as panose, eluting after isomaltotetraose (5) and before maltotriose (7). The determined average concentration of the panose was ~ 15 mM, which is consistent with the reduction of maltose after 24 h (16 mM) and the calculated glucose contributing to the transglycosylation reaction (15 mM). Additional peaks at retention times after 80 min, which might refer to longer gluco-oligosaccharides were not detected. For the secure validation of the absence of high molecular weight dextran, default AF4-MALS-UV measurements of the untreated buffer supernatants were performed, which gave no valid results (data not shown).

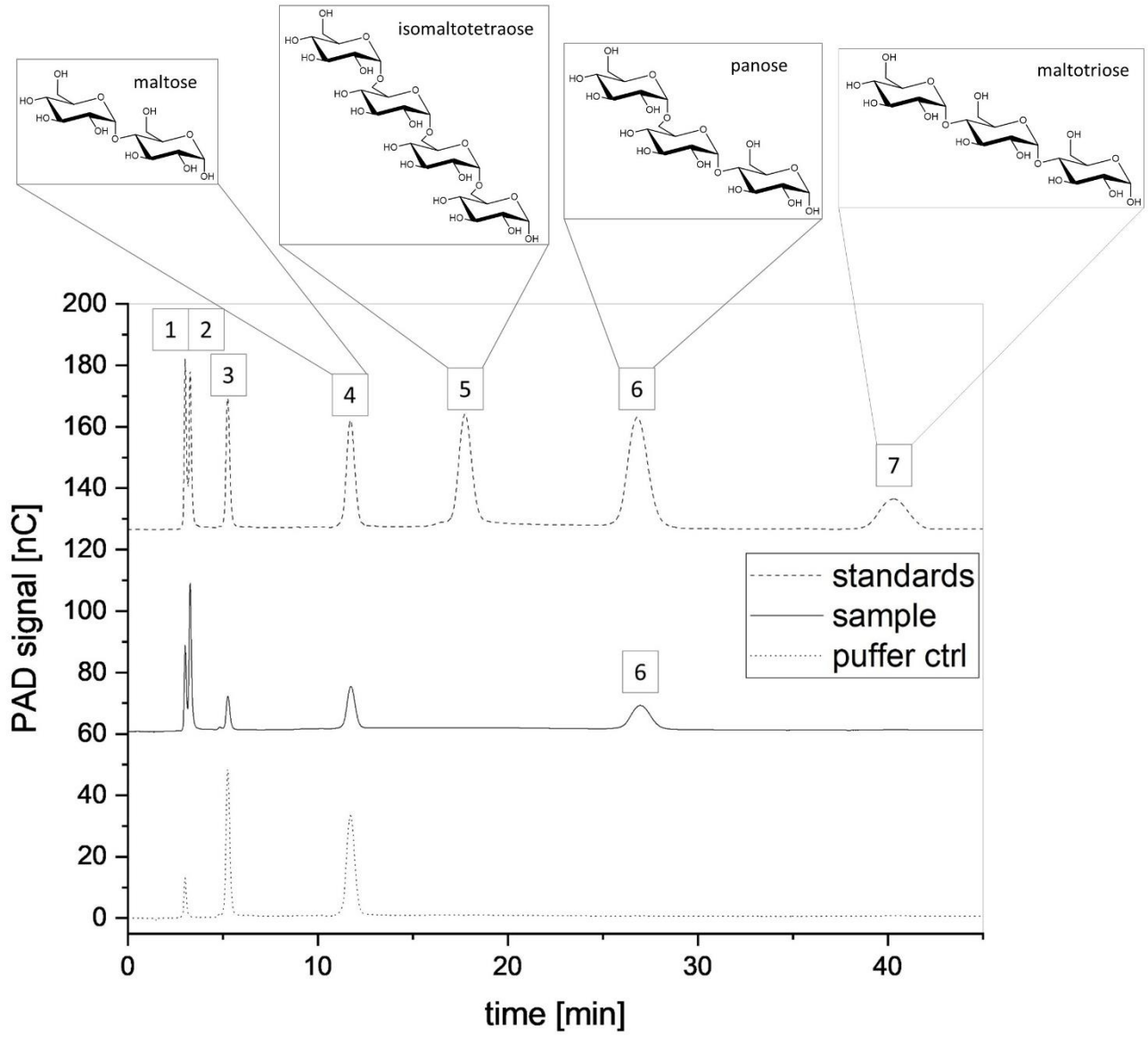


Figure 20: Representative HPAEC-PAD chromatograms of: (top) A standard mixture containing glucose (1), fructose (2), sucrose (3), maltose (4), isomaltotetraose (5), panose (6) and maltotriose (7). (middle) A diluted sample of the supernatant from the dextran production in buffer containing sucrose + maltose and (bottom) the sole buffer as control. The chromatogram only displays the retention interval of interest.

3.3.7. Analysis of rheological properties of dextran of different molecular weight and size

The high molecular weight dextrans generated at pH 4.0, 5.5 and UC in upscaled format (2.2.7, Figure 16) were further rheologically analyzed regarding their flow and viscoelastic behavior in aqueous solution. Figure 21 elucidates the visual differences of the basic gel forming capability of the predominantly smaller dextran fractions isolated at pH 4.0 and the bigger ones from pH 5.5. At 5 % and 12.5 % (w/v) dissolved in dH_2O the dextrans from pH 4.0 form a less viscous solution and decreased gel stability in comparison to the ones from pH 5.5.

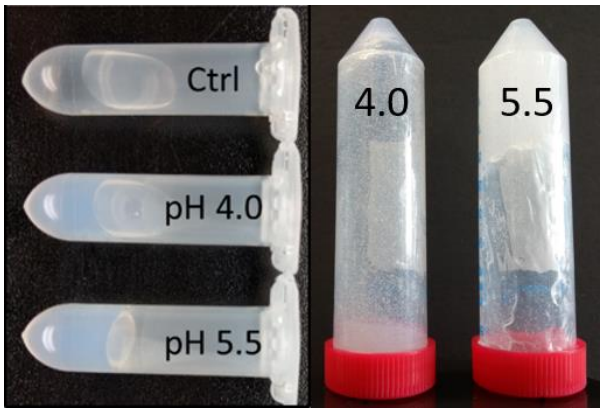


Figure 21: Photographs of dextrans produced at pH 5.5 and pH 4.0 dissolved in a concentration of 5% (w/v) (left) and 12.5 % (w/v) (right) in water; control (ctrl): pure water.

The generated flow curves are depicted in Figure 22 and represent the average of 3 technical replicates. The dextran from pH 5.5 exhibited shear-thinning behavior at the tested specific concentrations of 1.0, 2.5, 5.0 and 7.5 % (w/v) in dH_2O with the highest detected viscosity of approximately 459 Pa/s at a shear rate of 0.1/s and a concentration of 7.5 % (w/v) (Figure 22 A). In contrast, the dextran from pH 4.0 showed the comparatively lowest viscosities (~ 44 Pa/s at 0.1/s) at equally applied specific concentrations and no distinct viscosity at concentrations below 5 % (w/v) (Figure 22 C). The UC dextran was once again in between the two outer limits at equal test conditions with no apparent shear thinning behavior below 2.5 % (w/v) and a maximum viscosity of approximately 268 Pa/s at 0.1/s (Figure 22 B). For further oscillatory tests including amplitude and frequency sweeps, the concentrations 7.5, 10 and 12.5 % (w/v) were picked.

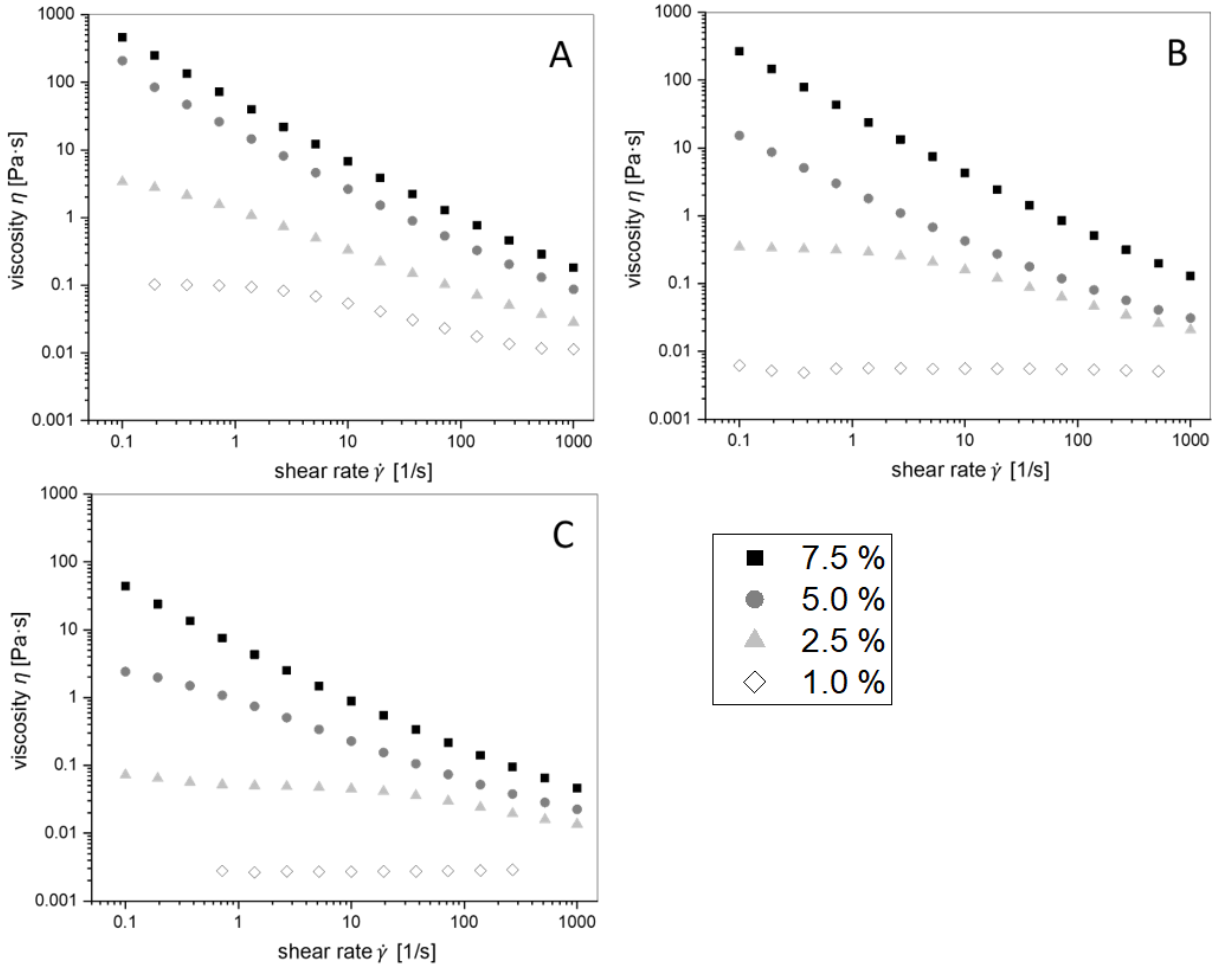


Figure 22: Concentration dependent viscosity at 20 °C versus the shear rate of dextrans produced at pH 5.5 (A), UC (B) and 4.0 (C).

As mentioned in the introduction, to determine the critical overlap concentration (*C**) of the different dextran fractions the specific viscosity at low polymer concentration is required, which is calculated by the formula $\eta_{sp} = \frac{\eta - \eta_s}{\eta}$, where η_s is the viscosity of the of the sole solvent (dH_2O) and η the viscosity of the polysaccharide solution, during the zero shear viscosity phase (η_0) at the Newtonian plateau and therefore before *C** is reached. The logarithmic plot of the specific viscosity against the respective concentration, which is shown in Figure 23, results in a curve, which can be divided into two parts, whereas the slope of the first curve, which is calculated by the specific viscosities before the critical overlap concentration of the dextran fraction, is of interest. The pH 4.0 slope of 0.408 represents the lowest value and again, UC lies in between with 0.491 compared to 0.753 for pH 5.5. The estimated intersection point, which refers to *C** is ~ 0.80 % (w/v) for pH 4, ~ 0.86 % (w/v) for UC and ~ 0.64 for 5.5 % (w/v).

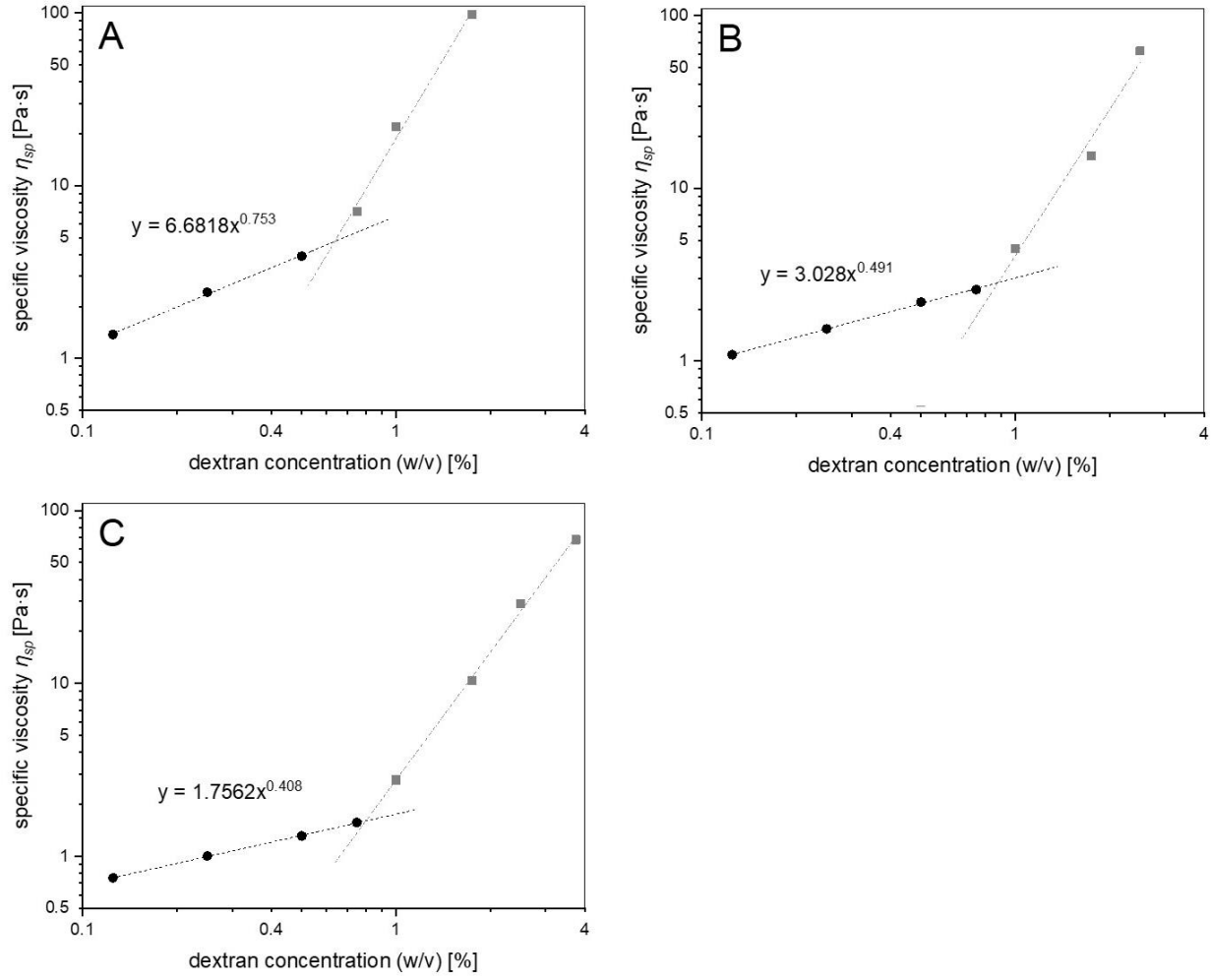


Figure 23: Specific viscosity versus dextran concentration of dextran produced at pH 5.5 (A), UC (B) and 4.0 (C).

To determine the upper limits of the linear viscoelastic (LVE) region, amplitude sweep tests were performed. Representative results are shown in Figure 24 where A refers to 12.5, B to 10 and C to 7,5 % (w/v). All samples exhibited gel like behavior with smaller loss moduli G'' compared to the storage moduli G' at the specific concentration of 12.5 % (w/v) over a consistently increasing strain range and constant frequencies of 1 rad/s (Figure 24 A). Dextran from pH 5.5 showed the highest values averaging ~ 2161 Pa (G') and ~ 142 Pa (G'') within the LVE region. At pH 4.0 the value did not exceed ~ 364 Pa (G') and ~ 49 Pa (G'') as well as ~ 821 Pa (G') and ~ 78 Pa (G'') for the intermediate dextran (UC), respectively. In Figure 24 B the reduced gel like behavior for the dextran from pH 4.0 at a concentration of 10 % (w/v) was evident with no intersection (γ_f) of G' and G'', followed by the same results for the UC dextran at 7.5 % (w/v) (Figure 24 C). Furthermore, the calculated yield (γ_y) and flow points (γ_f) were increasing with enhanced gel like behavior at higher concentrations as depicted in Table 15. Measurements of dextran from pH 4.0 at 7.5 % (w/v) were not possible due to its distinct fluid like behavior.

Table 15: Determined key parameters of the representative amplitude sweeps which are depicted in Figure 16 with the values of the deformation and storage modulus for the calculated yield and flow points. For clarity reasons all values are depicted without deviation.

	Yield point (γ_y)		**Flow point (γ_f)**	
	Deformation [%]	G' [Pa]	Deformation [%]	G' [Pa]
4.0 - 12.5 %	4.89	397.21	32.42	54.30
5.5 - 7.5 %	6.04	289.41	18.90	157.10
5.5 - 10 %	9.21	751.41	26.00	375.95
5.5 - 12.5 %	12.21	1949.67	47.73	646.64
UC - 10 %	4.11	287.90	16.33	129.65
UC - 12.5	5.91	768.86	44.02	182.51

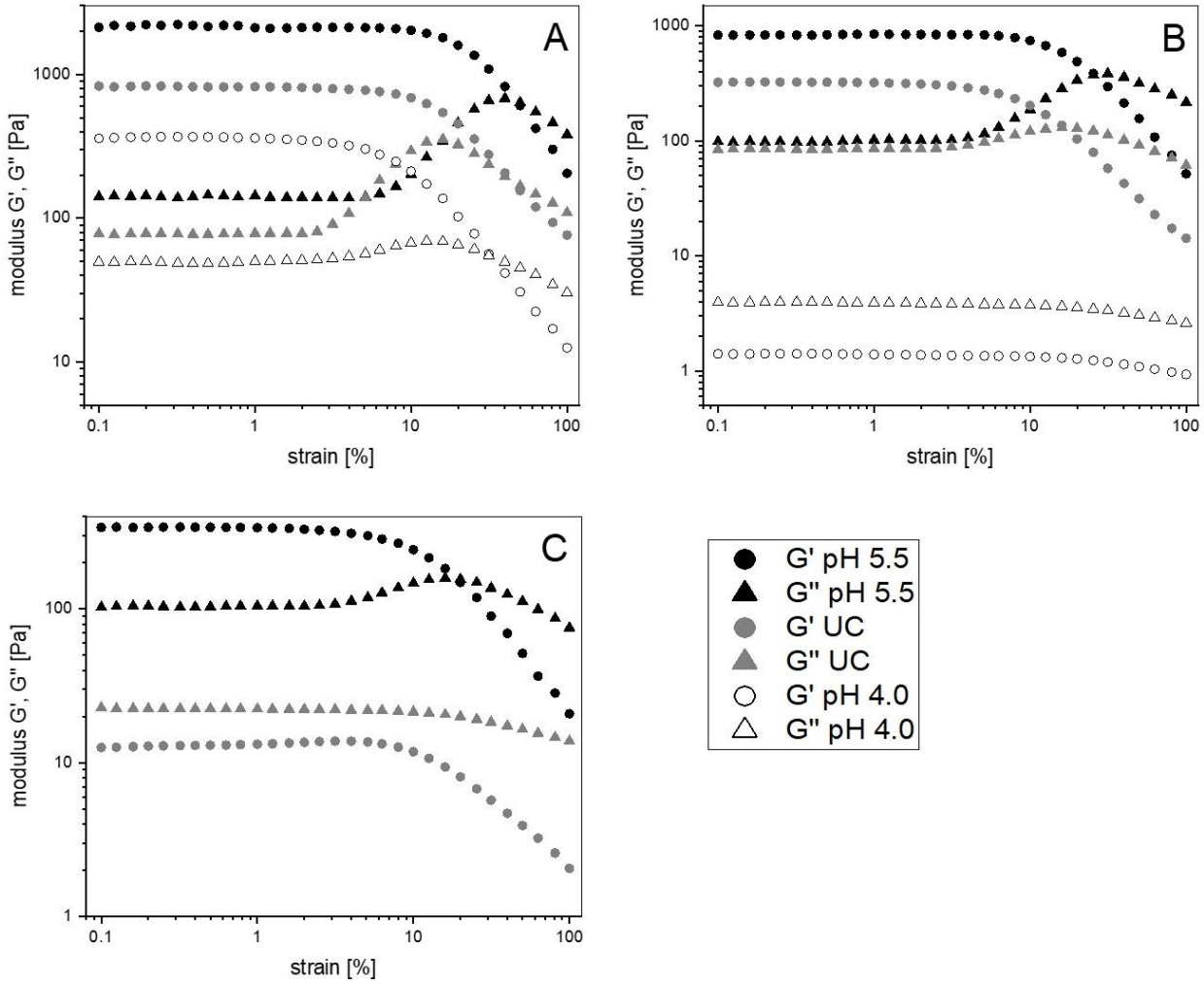


Figure 24: Amplitude sweep test with storage (G', circle) and loss (G", triangle) moduli versus the shear strain of dextrans produced at pH 5.5 (black), UC (grey) and 4.0 (white) at the concentrations 12.5 (A), 10 (B) and 7.5 % w/v (C).

For subsequent frequency sweep tests a constant strain of 1.0 % for the non-destructive deformation range was determined as most significant for all samples. In Figure 25 the results of the frequency sweeps are depicted, where A refers to the specific concentration of 12.5, B to 10 and C to 7.5 % (w/v). The dextrans from pH 5.5 and a concentration of 12.5 % (w/v) were defined by the highest shear storage modulus G' (~ 2376) and loss modulus G'' (~ 186), while G' exceeded G'' at all measured specific concentration for this dextran in contrast to dextrans from UC (Figure 25 C) and pH 4.0 (Figure 25 B).

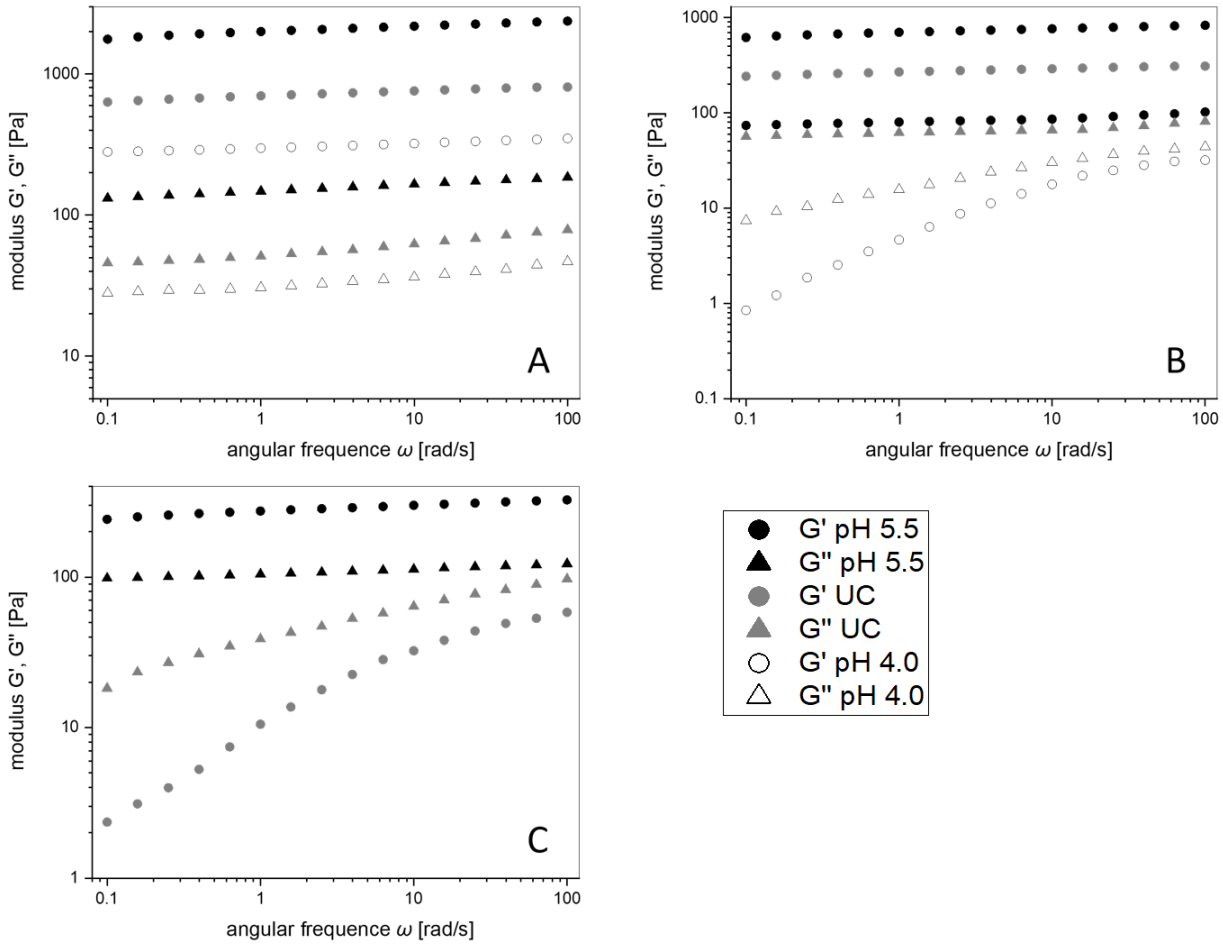


Figure 25: Frequency sweep test with storage G' and loss G'' moduli versus the angular frequency of dextrans produced at pH 5.5 (black) , UC (grey) and 4.0 (white) at the concentrations 12.5 (A), 10 (B) and 7.5 % w/v (C).

3.4. Analysis of rheological properties of LPI-fermentations

The last part of this work was the determination and comparison of the rheological properties of LPI-fermentations with *P. claussenii* TMW 2.340 (Figure 26 - 28 A) and *L. hordei* TMW 1.1822 (Figure 26 - 28 B) with or without the *in situ* formation of polysaccharides.. In section 3.3.1 the similar growth kinetics of TMW 1.1822 in LPI-medium with sucrose as the sole carbon source or glucose / fructose are shown. The growth curves of TMW 2.340 mut and wt are depicted in section 3.1.2 (mMRS) and 3.2 (LPI), which revealed no differences in their growth behavior for both conditions. Therefore, the differences in the rheological properties are not based on growth variations while comparing the EPS containing and EPS free fermentations. Additionally, a commercially available yoghurt (cows milk) was investigated (Figure 26 - 28 C). The depicted flow curves (Figure 26) revealed significant differences between the comparable EPS free controls (2.340 mut, 1.1822 glc + frc) and their respective EPS containing fermentations (2.340 wt, 1.1822 suc). The greatest area of hysteresis between the upward and downward flow curve was recorded for the yoghurt sample ~ 26851 Pa/s (Figure 26 C and Table 16). The EPS containing fermentations doubled the averaged areas of the EPS free samples with ~ 8804 (*L. hordei* TMW 1.1822) and ~ 11796 (*P. claussenii* TMW 2.340).

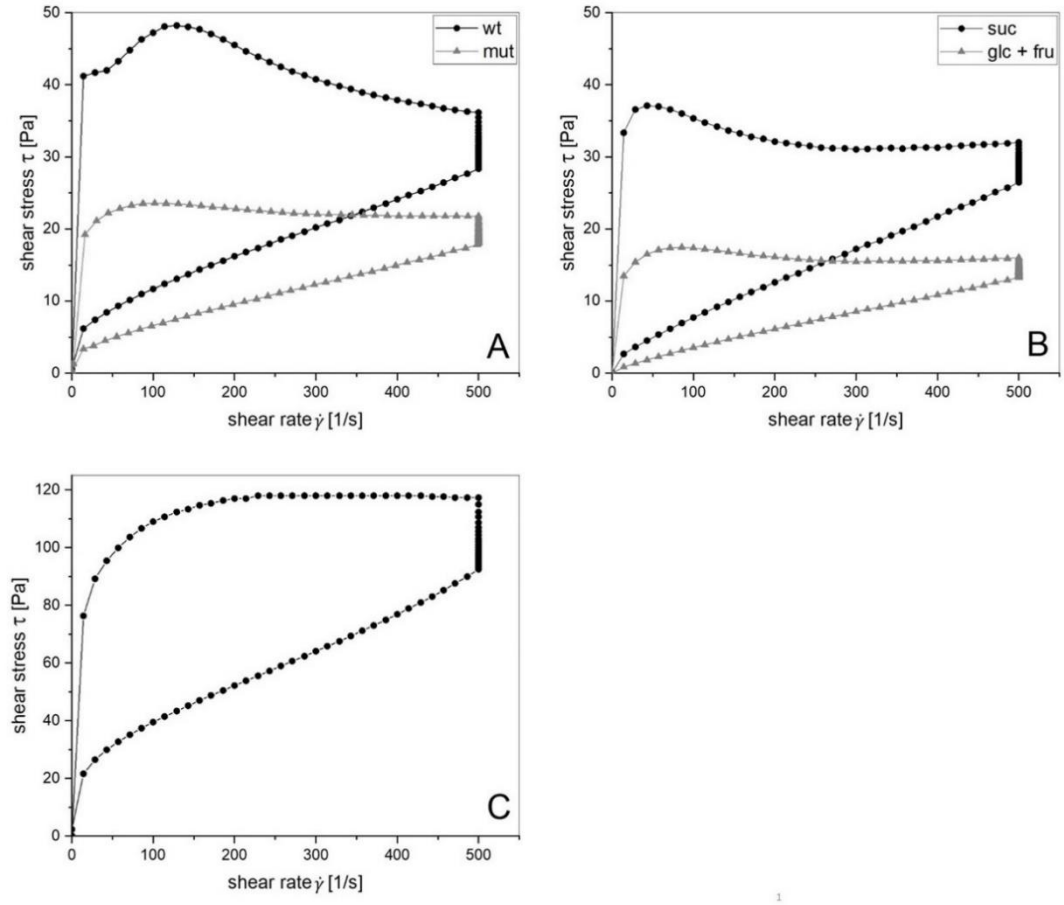


Figure 26: Upward and downward flow curves with hysteresis loop of the shear stress versus the shear rate of LPI-fermentation with TMW 2.340 wt and mut (A) LPI-fermentations with TMW 1.1822 with sucrose and glucose + fructose (B) commercially available milk yoghurt as control (C).

Representative results of amplitude sweep tests to determine of the upper limits of the linear viscoelastic (LVE) region are depicted in Figure 27. All tested samples exhibited gel like behavior with G'' < G', but interestingly, the EPS containing LPI-fermentation of TMW 2.340 showed higher values for the storage moduli (G') in comparison to the milk yoghurt control. Within the LVE region TMW 2.340 wt averaged 1291 Pa, TMW 1.1822 suc 757 Pa and the yoghurt control 708 Pa. The values of γ_y and γ_f increased with the *in situ* formation of EPS (Table 16).

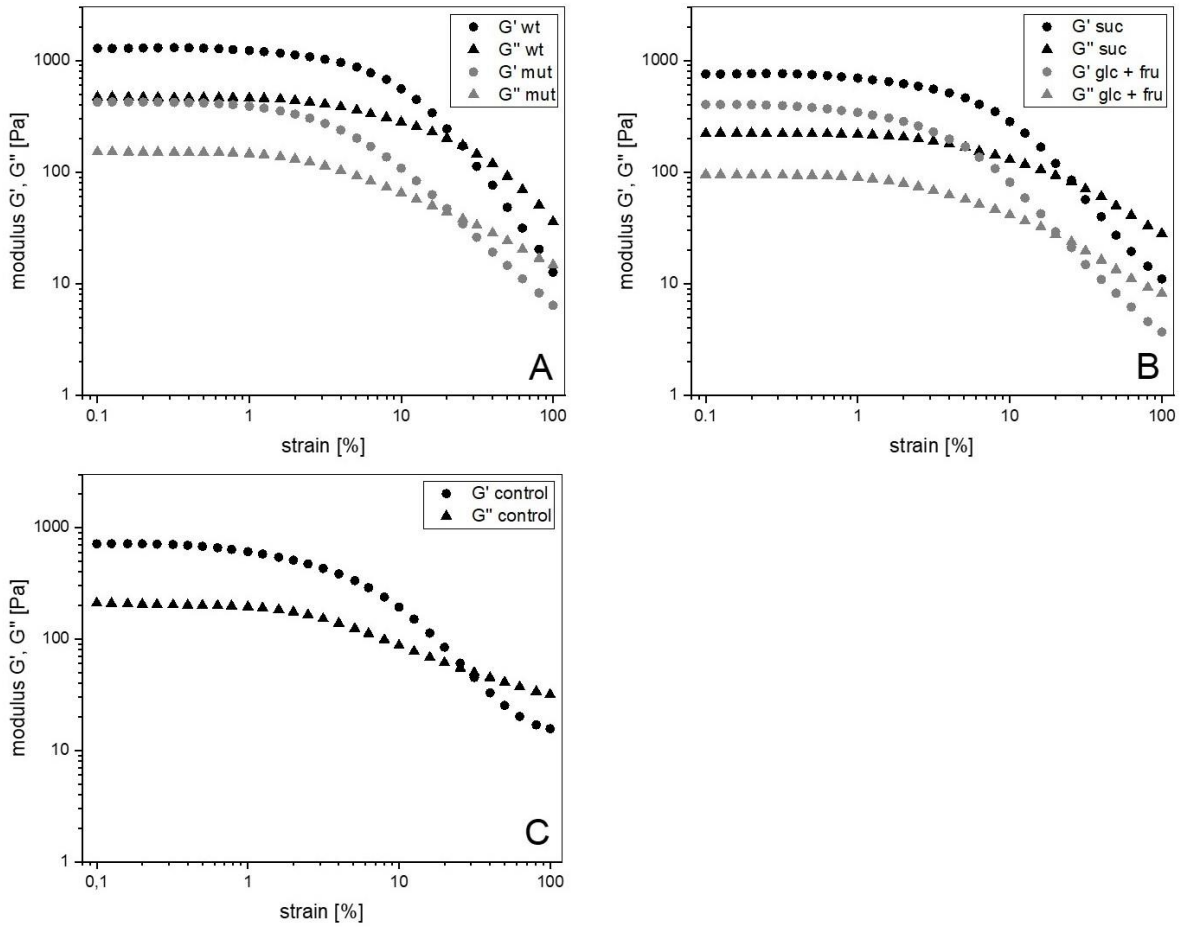


Figure 27: Strain sweep test with storage (G', circle) and loss (G'', triangle) moduli versus the shear strain of LPI-fermentations with TMW 2.340 wt and mut (A) LPI-fermentation with TMW 1.1822 with sucrose and glucose + fructose (B) commercially available yoghurt as control (C).

Table 16: Determined key parameters of the representative flow curves and amplitude sweeps which are depicted in Figure 26 and Figure 27 with the area of hysteresis as well as the values of the deformation and storage modulus for the calculated yield and flow points. For clarity reasons all values are depicted without deviation.

	Yield point (γ_y)		Flow point (γ_f)		Area of hysteresis
	Deformation [%]	G' [Pa]	Deformation [%]	G' [Pa]	[Pa/s]
2.340 wt	1.71	1101.06	25.06	176.21	11796
2.340 mut	1.33	370.05	22.06	41.50	5593
1.1822 suc	1.19	653.17	32.30	80.97	8804
1.1822 glc + fru	1.01	355.11	27.34	26.28	4301
yoghurt	1.09	601.61	28.31	52.36	26851

The subsequent frequency sweep tests at a permanent strain of 0.05 % are shown in Figure 28. Again, G'' < G' applied to all measured points and samples with maximum values of ~ 2213 Pa (2.340 wt), ~ 1417 Pa (1.1822 suc) and 996 Pa (yoghurt).

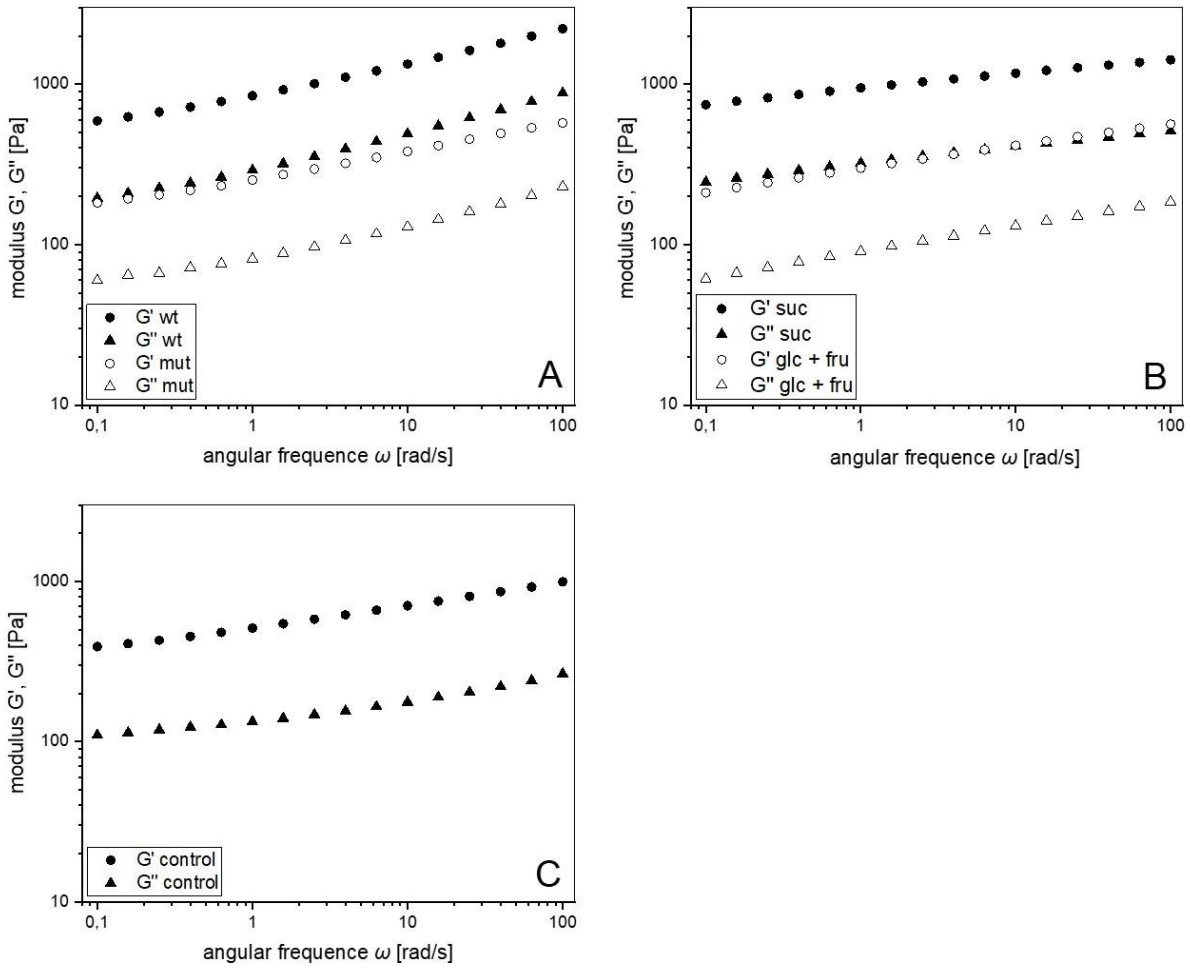


Figure 28: Frequency sweep test with storage (G', circle) and loss (G'', triangle) moduli versus the angular frequency of LPI-fermentations with TMW 2.340 wt and mut (A) LPI-fermentation with TMW 1.1822 with sucrose and glucose + fructose (B) commercially available yoghurt as control (C).

4. DISCUSSION

Vegan alternatives to dairy products are a widely seeked product category among, which has only arrived at a starting point in the development and exploitation of appropriate starter cultures with added value functions. One of these is the formation of EPSs, which enable structure design of fermented foods, as they can principally be tailor-made to exert different functions towards food structure and mouthfeel. This work demonstrates along the fermentation of LPI that specifically adapted EPS producing lactic acid bacteria can be exploited for a structural improvement of lupine-based vegan yoghurt alternatives and their EPSs can be tailored by deliberate variation of their macromolecular structure.

From this work the following theses can be derived, which delineate criteria for successful development of a novel category of starter cultures for the knowledge-based structuring of lupin-based yoghurt alternatives:

- The identification and selection of suitable EPS-producing LAB starters for the appropriate production of a yoghurt alternative based on lupin protein is possible.
- The selected strains *Pediococcus claussenii* TMW 2.340 and *Liquorilactobacillus hordei* TMW 1.1822 significantly enhance the structural properties of the LPI-fermentation product.
- The structural enhancement effect is predominantly induced by the formation of dextran by *L. hordei* and β-glucan by *P. claussenii*, respectively.
- The influencing factors of dextran macromolecular structure include sucrose/maltose presence, and pH value during the dextran formation process of *L. hordei* TMW 1.1822.
- Sucrose triggers the release of the constitutively present and intracellularly accumulated dextransucrase, which results in the reduced formation of dextran if the cells are in contact with sucrose prior to the intended dextran production/application step.
- In the presence of maltose, the (sole) formation of the trisaccharide panose is achieved, which is due to the acceptor reaction of the dextransucrase and therefore no polymerization of high molecular weight dextran occurs.
- Controlled fluctuations of the pH value enable the distinct production of various dextran fractions and allow the determination of the optimal polymerization conditions.
- Tailor-made dextrans of variable molecular size and weight exhibit different rheological properties.
- The metabolic adaption of both strains is reflected on genomic level and comprise favorable traits regarding their application in different cereal/plant fermentations.

4.1. Selection of starter cultures

The initial selection of potential starter strain was based by the in-house collection of EPS-producing strain of LAB and the isolation source. Within these, it was predominantly based on variety, to gather a broad spectrum of EPS-producing LAB from multiple origins. Strains, which are traditionally used in the dairy industry were not considered to match the appropriate properties and capabilities, which are needed for the growth on the LPI-substrate. The differences in the spectrum of carbohydrates, especially the lack of lactose, as well as in the available plant peptides are predictively limiting the growth of LAB during the fermentation, which are specifically adapted to the dairy environment. Furthermore, the galactose of the lactose serves as the major source for EPS production, since the HePS produced by numerous dairy starter cultures are predominantly composed of galactose (Bouzar et al., 1997). The first selection was performed comparing the general growth kinetics in mMRS, the assertiveness during the growth in LPI-medium and the obtained scores via the evaluation by a tasting group. The second and final selection was further taking the occurrences of investigable EPS into account leaving *P. claussenii* TMW 2.340 and *L. hordei* TMW 1.1822 as the most promising strains for detailed investigations. Both strains exhibited rapid growth dynamics on the LPI-substrate, which is a decisive factor regarding the elimination of potential contaminants in a highly up-scaled industrial production. TMW 2.340 wt achieved the most promising results regarding the evaluation of the LPI-fermentation. Additionally, the availability of a stable mutant of this strain, lacking the plasmid required for the β-glucan production was of special interest, as it could serve as an optimal control regarding the effects of β-glucan in LPI-fermentations, although the polysaccharide was not sufficiently isolable and, thus, not suitable for further investigations. TMW 1.1822 achieved better results regarding the evaluation of the LPI-fermentation, if compared to the other selected strains. Additionally, the strain showed good growth kinetics with sizable amounts of dextran that gave high-quality results during AF4-MALS-UV measurements.

4.1.1. Adaption to cereal/plant fermentations

The possibility of favorable adaptions of the two selected strains was further evaluated by the identification of genomic key features (Table 8 and Appendix 5). As adequately explained and only for clarification *L. hordei* TMW 1.1822 possesses a dextransucrase and *P. claussenii* TMW 2.340 the glycosyltransferase 2 encoding gene for EPS production. TMW 2.340 lacks a complete system for the specific uptake of lactose or galactose. However, as substrate specificity is difficult to determine solely from sequence analysis it could not be excluded that

the annotated permeases and phosphotransferase systems (PTS) are still capable of internalizing galactose and lactose. In strain TMW 1.1822 a PTS, which is specific for the transportation of lactose/cellobiose was found. For the further metabolization of lactose, the Tagatose-6-Phosphate (Tagatose-6P) pathway and/or the Leloir pathway are required, moreover both pathways are defined by 4 key enzymes (Iskandar et al., 2019; Kandler, 1983). (1) The Tagatose-6P pathway: galactose-6-phosphate isomerase, tagatose-6-phosphate kinase, tagatose-1,6-bisphosphate aldolase and tagatose-6-phopshate-4-epimerase. (2) The Leloir pathway: galactose mutarotase, galactose kinase, galactose-1-phosphate uridyltransferase and UDP-galactose 4-epimerase. Thus, the sole presence of the tagatose-1,6-bisphosphate aldolase in TMW 2.340 and the lack of all 4 Tagatose-6P pathway enzymes in TMW 1.1822 suggests a deficiency regarding the functionality of this pathway for both strains. Furthermore, of the enzymes belonging to the Leloir pathway, only the galactose mutarotase in both strains and the galactokinase in TMW 2.340 were found, resulting in a predictively incomplete Leloir pathway. However, the sole utilization of galactose might still be positive trait due to the presence of galactose in the family of *Leguminosae* as reported by Acosta et al. (1995). Furthermore, the presence of cellobiose transporters and several β-glucosidases of unknown specificity in both strains indicates the capability of metabolization of cellobiose and (potentially) other β-linked oligosaccharides/polysaccharides like xylan, which are commonly found on/in diverse plants and plant fermentations. Additionally, strain TMW 1.1822 possesses a protein belonging to the MFS superfamily (AraJ) and 6 unspecific β-glucoside transporters, which may facilitate the uptake of arabinose and plant associated β-glucosides like salicin and amygdalin. The utilization of starch, the polysaccharide predominantly found in many cereals, is accomplished by α-amylase which is present in strain TMW 2.340 and potentially by the several putative α-glucosidases of unknown specificity in both strains (Bechtner et al., 2020; Lynn et al., 1997). Both strains own multiple peptidases and peptide transporters, but the quantity of peptidases and peptide transporter may vary greatly among different LAB species, thus no appropriate reference is available (Christensen et al., 1999; Savijoki et al., 2006). The achieved results elucidated the adaption of *P. claussenii* TMW 2.340 and *L. hordei* TMW 1.1822 to certain plant specific characteristics and reduced functions connected to the dairy environment.

4.2. *Pediococcus claussenii* TMW 2.340

4.2.1. ß-glucan formation

The β-glucan formation of diverse LAB strains belonging to *Levilactobacillus brevis*, *P. ethanolidurans*, *P. parvulus*, *Lacticaseibacillus paracasei*, *L. mali*, *Lactiplantibacillus plantarum* and *Leuconostoc mesenteroides* is relying on the presence and expression of the glycosyltransferase gene, as shown by (Fraunhofer et al., 2018; Llamas-Arriba et al., 2018; Werning et al., 2006), which normally causes a ropy phenotype due to the slimy properties of the networks formed by these strains. Therefore, the appropriate determination of the molecular weight of the produced β-glucan was impossibly due to the increased insolubility of the EPS at higher concentrations in water, which is mandatory for the respective concentration series and the following calculation of the specific extinction coefficients. The roughly estimated radius of the β-glucan of *P. claussenii* TMW 2.340 was comparably larger than the investigated fructans and within the range of the measured dextrans (Table 10). Furthermore, a β-glucan deficient mutant, which misses the gtf-2 encoding plasmid #3, was available. Unfortunately, the stability of β-glucan formation of the wildtype strain was varying significantly within the upscaled LPI-fermentations at the IVV compared to the self-made 45 mL cultivation in mMRS and LPI-medium (data not shown). Due to the visual differences in their colony forms, plasmid deficient mutants were easily identified after the fermentations on the CFU plates, which were prepared for further MALDI-TOF-MS analysis. On the one hand the reduced availability of nutrients, except the carbon source, might have led to a plasmid loss encoding for proteins, which are not essential for the growth and survival under the applied harsh conditions (Carroll et al., 2018; San Millan et al., 2017). Furthermore, the inoculation of the different LPI-fermentations at the IVV during one experimental setup was accomplished by one provided main culture, which was cooled in between the different approaches, leading to additional cold stress. On the other hand, considering the potential function of the β-glucan as an enhancer for the fast distribution of the cells within the medium and the concomitant easier accessibility of any metabolically relevant substance, the plasmid might not be as important in the more viscous LPI-medium and is lost due to the lack of the stress parameter as reported for numerous functions like antibiotic resistances (MacLean et al., 2015; Wein et al., 2019). The possibility of losing the plasmid and therefore the *gtf* gene, might reduce or prevent the application of the strain, but the appropriate usage and further investigations concerning potential parameters that cause the reduced β-glucan production, might compensate or eradicate this effect.

4.2.2. Impact as starter culture for LPI-fermentation

P. claussenii TMW 2.340 wt/mut showed rapid growth in the LPI-medium and reached the desired pH value of 4.5 after ~ 20 h of fermentation without concomitant growth of other bacteria (Figure 5, Figure 8 and Figure 9). pH 4.5 was selected as the end pH since a post acidification effect occurs after the storage of the fermentations at 4°C due to the residual low metabolic activity of the inoculated starter (Donkor et al., 2006). For strain TMW 2.340 wt β-glucan formation was detectable, but the isolated amounts were significantly lower than in mMRS and not suitable for further investigations. The lower cell counts, due to the non-optimal medium conditions, combined with the high concentrations of protein might have caused the insufficient isolation and the concomitant impurity of the isolated EPS, which were inappropriate for further AF4-MALS-UV analysis. The most probable reason for the difficulty to isolate pure β-glucan is the fact that it forms a stable capsule around the cells, which is hard to remove. Still, very low amounts of β-glucan allow for the formation of cellular networks with structure forming impact on the fermentation product. Furthermore, the strain remains interesting because the formation of biogenic amines, that might cause severe health problems due to their potential toxicity, if critical amounts are present, was not detectable on decarboxylase agar plates (3.1.4) (Spano et al., 2010). The further reduction of the fermentation time may be feasible through increased inoculation size. To evaluate the LPI-fermentations in more detail rheological measurements were performed. A key parameter for the appropriate evaluation of the LPI-fermentations is the time phenomenon of thixotropy. It is defined by the change in viscosity when a shear force is applied, due to the breakdown of the gel network at critical shear rates, and the subsequent recovery of the viscosity with decreasing shear force. Thus, the hysteresis-area under the curves of shear rate vs shear stress is representing the degree of thixotropy (Benezech et al., 1994; Bourne, 2002). The obtained areas were significantly higher for the LPI-fermentations by the β-glucan producing wildtype, (Table 16 and Figure 26 A), which is in line with multiple reports about the higher degree of thixotropy and thus the enhanced structure of milk fermented by EPS-forming LAB (Amatayakul et al., 2006; Fajardo-Lira et al., 1997; Purohit et al., 2009). Furthermore, γ_y and γ_f increased with the *in situ* formation of EPS since the gel networks were more resistant to the applied stress compared to the EPS free fermentations, but both fermentation setups exhibited gel-like structures (Figure 27 A and Figure 28 A). The increased values of G' for *P. claussenii* TMW 2.340 wt, might be attributed to its strong production of a highly viscous biofilm. The parameters of the milk-fermentation control were out of reach, however as they only served as a control and rough benchmark, the differences are reasonable. Due to the comparable growth kinetics of the mutant and wildtype strain of TMW 2.340 in LPI-medium these results suggest

an enhancing effect on the structure by the *in situ* formation of β-glucan and ultimately represents an improved version of an existing and comparable product.

4.3. *Liquorilactobacillus hordei* TMW 1.1822

4.3.1. Mechanism of dextran formation and structure

The dextran formation relies on the release and activity of the dextransucrase (EC 2.4.1.5) (Côté et al., 1982; Naessens et al., 2005; Sidebotham, 1974). These dextransucrases are widely abundant among LAB, while their primary and secondary structures are highly variable (Bechtner et al., 2019; Leemhuis et al., 2013). This results in the production of dextrans varying in their molecular size, structure, branching and secondary structure, which ultimately results in differences concerning their properties and potential application (Netsopa et al., 2018; Sabatié et al., 1988; Vuillemin et al., 2018). However, as mentioned before, variations in the size and molecular weight of dextrans as well as the molecular structure are also influenceable by extracellular factors (Kim et al., 2003; Prechtl, Wefers, et al., 2018a; Sabatie et al., 1986). There are numerous studies about the sucrose-inducible expression of dextransucrases in LAB, as well as the influence of other sugars, which are structurally comparable to the preferred substrate sucrose, on the polymerization process (Dols et al., 1998; Paul et al., 1986; Prechtl, Janßen, et al., 2018; Robyt, 1995). Furthermore, a pH-dependence of the release mechanism and the dextran formation has been observed for dextransucrase and other EPS producing enzymes like the levansucrase (Jakob et al., 2020; Kim et al., 2003; Otts et al., 1988; Ua-Arak et al., 2017). Therefore, part of this study was to *inter alia* verify and elucidate the influence of sucrose and the environmental pH on the dextransucrase release, as well as the impact of sucrose, maltose and the pH regarding the amounts and structure of the synthesized dextran fractions. To study the sole influence of these extrinsic factors, the native dextransucrase was recovered in buffer and exposed to the different environmental conditions of interest.

4.3.1.1. Influence of pH on the dextransucrase release and dextran amounts

The pH influence on the dextran formation was investigated using two different buffer setups. In setup A, the pH was kept constant during the release and the formation process, whereas in setup B, pH 4.5 was chosen as release pH, followed by the adjustment to the same pH values as used in setup A (Figure 2). Maximum dextran amounts were observed at pH 4.0 and

4.5 for the setup A with constant pH values (Figure 13 A). These values are within the range of pH values reported for optimal activities of dextransucrases expressed by some other LAB (Naessens et al., 2005; Rühmkorf et al., 2013; Siddiqui et al., 2013). Since the respective dextransucrases were collected at multiple pH values, the quantified dextran amounts may have only significantly differed due to differences in the enzyme concentrations. Therefore, setup B was used to elucidate if the tendencies in regard to produced dextran amounts were driven by differing enzyme concentrations. Both setups revealed similar trends with decreased dextran amounts isolated and calculated via the determination of the free glucose amounts at pH 3.5 and pH 6.5 (Figure 13 A, B and Table 11). Furthermore, the glucose concentration was constant at all tested pH values in setup A and B with approximately 12 mM after 27 h of incubation (Table 12). Moreover, the overall protein concentrations of the buffer supernatants exhibited no significant differences in both setups as determined by a Bradford-assay (Table 11: ~ 16 µg/mL) and visually by the SDS-gel band intensity (Figure 12). These results strongly suggest the presence of similar dextransucrase concentrations for all tested conditions, considering that distinctly different concentrations of identical dextransucrases could not fall off in their transglycosylation or rather remain at the same level of hydrolysis activity at non-optimum pH values, respectively. However, at pH 5.0, 5.5 and 6.0 comparatively higher amounts of dextrans were quantified via isolation and calculation, if the dextransucrase recovery was performed at pH 4.5 in setup B. This implies a less reducing effect regarding the productivity and stability of the dextransucrase, due to the inevitable denaturation and the concomitant loss of the cumulative activity of hydrolysis and transglycosylation at non optimum pH, if it had been preliminarily recovered at its approximate optimum pH. The polymerization reaction can be divided into two parts, where the first includes the cleavage of sucrose, with the release of the fructose moiety and the eventual formation of the glycosyl-dextransucrase intermediate. This is followed by the transfer of the glucose to an acceptor saccharide, which represents the second part of the reaction (Leemhuis et al., 2013; Vujičić-Žagar et al., 2010). The constant concentrations of liberated glucose for all tested approaches indicate a hydrolysis reaction, which remains independently active despite the loss of the transglycosylation activity. However, the constant glucose concentrations may also be attributed to sucrose hydrolysis by a putative extracellular beta-fructosidase, that was present in significantly higher amounts after growth in sucrose-supplemented medium (Bechtner et al., 2020). However, it remains unknown whether this enzyme is also expressed within the experimental setup conducted here, while the predicted LPxTG cell wall anchor may leave the enzyme bound to the cells and, thus, widely removed from the cell-free buffers used for dextran formation. Furthermore, the obtained data revealed, that the standardized EPS isolation approach (Korakli et al., 2001; Notararigo et al., 2013; Torino et al., 2015) was responsible for the reduced isolation of considerable amounts of dextran. Since dextrans holding a polymerization grade of less than

20 single glucose units are lost during the ethanol precipitation and subsequent dialysis with a MWCO of 3.5 kDa of the samples, a more frequent occurrence of these short-chained dextrans could explain these differences. Hence, the dextran at the outer range is represented by fractions of small molecules. There are different factors, which might be responsible for the higher amounts of low molecular weight dextrans. 1st: The high and fast enzyme activities result in an imprecise polymerization process and the more frequent occurrence of small polysaccharides, which may even lead to more acceptor reactions with small oligosaccharides. 2nd: A faster degradation of the enzyme due to its higher activity or the shortened half-life time of the dextransucrase in the presence of dextran as described by Shukla et al. (2014) for *Pediococcus pentosaceus*. 3rd: The straitened reaction space and aggravated accessibility of the non – reducing acceptor site of already existing long-chained dextran molecules. Therefore, due to these possible factors the polymerization process is shortened and the transfer of glucose to smaller but accessible acceptor molecules like sucrose happens more frequently (Paul et al., 1986).

4.3.1.2. Influence of sucrose on the dextransucrase release and dextran amounts

As mentioned before, the formation of dextran is common among multiple LAB species, whereas the expression and release of the dextransucrase either occurs constitutively or is induced by the presence of different sugars (Bechtner et al., 2021; Bounaix et al., 2010; Dols et al., 1998; Kim et al., 1995). In this work, the buffer approach presented in Figure 3 was performed to elucidate the sucrose influence on the dextransucrase release and dextran amounts. The respective samples were, prior to the recovery and polymerization process in the sucrose containing buffer, either pre-cultivated with or without sucrose in mMRS. This revealed a significant reduction of isolated and calculated dextran amounts for the cells that were *ab initio* in contact with sucrose (Figure 18). Furthermore, the crude lysates of these cells were used to produce dextran in buffer, resulting in lower amounts for the cells recovered from mMRS pre-cultivations containing sucrose (Figure 19). Hence, the intracellular dextransucrase reservoir of strain TMW 1.1822 appeared to be partially dissipated by the early presence of sucrose, although its growth with or without sucrose in mMRS within the first 24 h was comparable (Figure 10). A reasonable way to boost native dextran production in *L. hordei* TMW 1.1822 may thus be to the exploitation of the constitutive expression and intracellular accumulation of the dextransucrase. The cells could then be used as storage source of the native dextransucrases that can be released by sucrose in minimal growth media without the aim of using the cells as growing metabolic machines.

4.3.1.3. Influence of maltose on the dextransucrase specificity

The intact dextransucrase activity in the presence of maltose, without the formation of high molecular weight dextran (Figure 17 and Figure 18) pointed to the formation of low molecular weight dextrans/gluco-oligosaccharides with weights underneath the MWCO of 3500 Da, which corresponds to a maximum of 20 glucose monomers. In simple growth tests (Figure 17), potential differences regarding the general growth behavior, estimated by the determined OD_{590}, pH and CFU after 24 h of dextran production in mMRS, could be excluded as potential factors, which might be responsible for the reduction of the dextran amounts due to lower dextransucrase concentrations in the presence of maltose. For further investigation of the formed products under constant conditions, dextran formation in buffer was performed once again. In both experiments (mMRS and buffer) a complete reduction of the dextran formation at a concentration ratio of 1:1 maltose sucrose was observable, with no differences regarding the growth behavior and therefore potential variations in the enzyme concentrations. The acceptor reaction with diverse sugars for the *Leuconostoc mesenteroides* dextransucrase was studied extensively by Robyt et al. (1978), Paul et al. (1986) and Heincke et al. (1999). They reported the highest affinity to form the acceptor reaction for the disaccharides maltose and isomaltose. For the dextransucrase of *L. hordei*, maltose was more favored as acceptor than sucrose or its glucose residue, which ultimately resulted in the significant inhibition of the elongation process of high molecular weight dextran. The sole formation of panose, as determined via HPAEC-PAD analysis (Figure 20), indicates, that this trisaccharide was no suitable acceptor for further glucose moieties. Panose is regarded to as prebiotic carbon source since various probiotic bacteria metabolize it, whereas it passes the endogenous digestion mechanisms of the human body unharmed (Ejby et al., 2016; Mäkeläinen et al., 2009). Therefore, the production of panose by the dextransucrase of *L. hordei* TMW 1.1822 *in situ* as well as *ex situ* could be optimized and increased by the simple concomitant addition of distinct concentrations of maltose during the dextran polymerization from sucrose. Furthermore as reported by Paul et al. (1986) and Koepsell et al. (1953) the sucrose/maltose ratio is a decisive factor, during the dextransucrase reaction, which may further lead to the controlled production of dextran molecules of distinct size, if other concentration ratios are used. However, manufacturers have to take into account that some fermented plant-based foods may intrinsically contain maltose as degradation product of starch, which then competes with the proper production of high molecular weight dextran (Dramićanin et al., 2018; Halford et al., 2011; Sánchez-Mata et al., 1998).

4.3.1.4. Influence of pH on the structural properties of the dextran

The elucidation of the molecular structure of the dextran fractions produced at different pH values was accomplished by AF4-MALS-UV and methylation analysis. The latter method reveals the positions and frequency of the glycosidic bonds within the analyzed EPS and therefore allows the distinct identification of the investigated EPS type. The AF4-MALS-UV is the most suitable method for the determination of the molecular size and weight/mass of water-soluble polysaccharides (Nilsson, 2013; Rübsam et al., 2012). Furthermore, the interpretation of the light scattering signals allows the estimation of spatial/secondary structures via different calculation models as well as the polydispersity of the sample. The separation principle of the AF4 channel relies on differences in hydrodynamic volumes of the analytes, resulting in prolonged restraint and therefore later retention times for the dextran fractions of high molecular weight and diffusion coefficient if compared to fractions of similar composition but lower mass (Nilsson, 2013; Rübsam et al., 2012). Therefore, as depicted in Figure 15 A the observed differences in the retention profiles pointed towards increased particles. The determined size and mass confirmed their continuous increase from pH 3.5 to 6.0 for setup A and 3.5 to 5.5 for setup B, which refers to the respective high molecular weight fractions. The dextran of the upscaled approach (Figure 16 A) showed the same tendencies, thus confirming the reproducibility and robustness of the chosen buffer approach and the dextransucrase activity. Furthermore, the dextran produced without pH control in buffer and within the LPI-fermentation displayed intermediate sizes due to the steady pH decrease during the formation process (Figure 16 A and Table 13). Despite the size variations, the polydispersity of all *L. hordei* TMW 1.1822 dextran samples showed only minor differences (Table 13), which points to similar distributions of particles sizes within the measured samples. Furthermore, the slopes of the confirmation plots, which represent the hydrodynamic coefficients (Figure 16 D and Table 13: 0.74 ± 0.1 to 0.79 ± 0.2) of the analyzed dextrans indicate similar random coil like structures for all 4 conditions, since the closer the slope value is to one, the more spherical the measured polymers are, and therefore changes in the structure of a measured polysaccharide lead to distinct changes in these slopes. As the models applied were evaluated by their fitting degree in the ASTRA software and were similar for the sphere and berry model and despite the fact that smaller sized fractions seem to be predominantly more spherical (data not shown), the spatial organization of this dextran may not be clearly definable by AF4-MALS-UV analysis. Alternatively differences in side-branching of dextran through the change of the polymerization conditions might contribute to the reduction of mass whilst the stability of radii. Kim et al. (2003) detected a loss of branching within the dextran through the change of temperature and sugar concentrations, but no influence of the pH value for dextran produced by *Leuconostoc mesenteroides*. The structural similarities were further confirmed by methylation analysis,

which only detected marginal differences in the side branching at position *O3* (Table 14). This relationship between sizes of sucrase-synthesized EPS and the fermentation pH coincides with the findings of levans synthesized by acetic acid bacteria (AAB) as well as for dextrans gained by the commercially used *Leuconostoc mesenteroides* strain B-512FMCM (Kim et al., 2003; Ua-Arak et al., 2017). Still the reasons for the pH-dependent mass increase of glucans and fructans still remain unclear. However, these findings indicate that the structural differences of the produced dextran fractions of *L. hordei* TMW 1.1822 in aqueous solutions are exclusively accountable on the chain length/molecular weight and thus, are the most decisive factor influencing concomitant variations within their physico-chemical properties, as reported for another uncharged water-soluble EPS (levan) (Hundschell et al., 2020; Hundschell et al., 2019). Overall, a proper and distinct pH control during the formation of dextran or other sucrase-synthesized EPS appears to be the key parameter for the recovery of tailor-made EPS, exhibiting versatile functionalities.

4.3.2. Rheological and structural properties of different dextran fractions

The dextran isolated at different pH values empirically showed distinct differences in their gelling properties at equally applied concentrations (Figure 21). Thus, dextran from pH 4.0, 5.5 and an intermediate without pH control where rheologically investigated in more detail. The viscosity of these dextrans increased with rising molecular size/weight, exhibiting shear-thinning behavior, despite their similar molecular structure and degree of branching as discussed in the previous section 4.3.1.4. As described in the introduction, the viscosity of entangled but unlinked single polymer chains is defined by three states η_0, η and η_∞ (Figure 29). At shear rates greater than 0.01 to 1 s^{-1} the molecules are partly orientated in shear direction and are progressively disentangling, resulting in the constant reduction of the viscosity until shear rates of 1000 to 10000 s^{-1}, where the polymer is fully orientated as well as disentangled and further decrease of viscosity is impossible (Lapasin et al., 1995; Mezger, 2011). The same entanglement principle applies on the critical concentration (C^*) where the molecules must overlap to interact (Graessley, 1974; Morris et al., 1981). Consequently, higher molecular masses increase and decrease the value of η_0 and C^*, respectively.

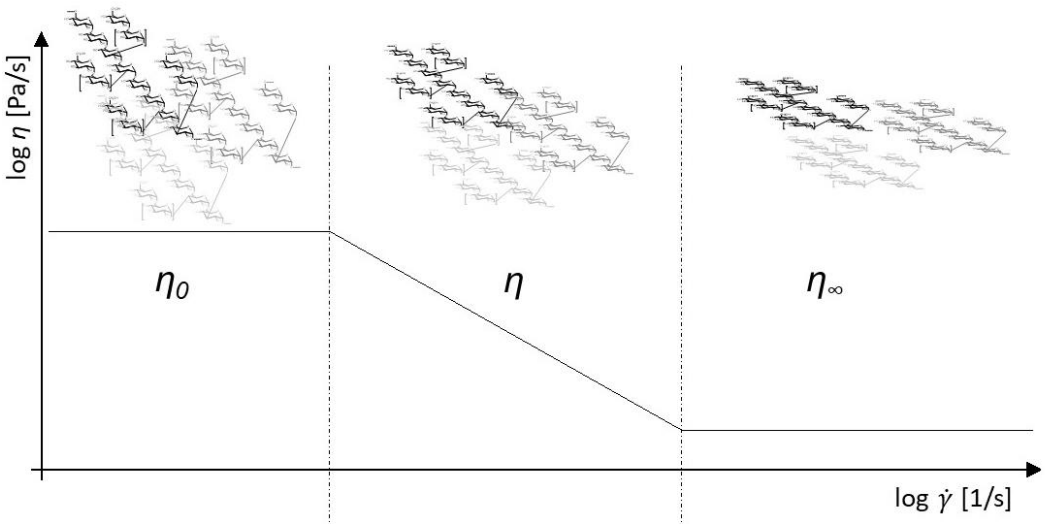


Figure 29: Exemplified viscosity function of a shear-thinning polymer with schematic entanglement of the dextran alongside increasing shear stress.

This shear-thinning/pseudoplastic behavior was reported for numerous dextrans synthesized by LAB, commercially available dextrans, levans from AAB as well as HePS from LAB and *Xanthomonas* (Galle et al., 2011; Hundschell et al., 2020; McCurdy et al., 1994; Petri, 2015). Comparable values regarding the flow curves were reported for linear dextrans of smaller and slightly higher molecular weights by Vuillemin et al. (2018) from *Oenococcus kitaharae*, Netsopa et al. (2018) from *Weissella confusa* and Zarour et al. (2017) from *Latilactobacillus sakei* and *Leuconostoc mesenteroides*. The increase in viscosity with rising molecular weights were observed by Vuillemin et al. (2018); Zarour et al. (2017) for dextrans exhibiting great size differences (40 x 10^6 Da vs 1 x 10^9 Da), while Zarour et al. (2017) investigated dextrans of different origin and *O3*/*O6* side branching frequencies, but smaller size differences (2 - 4 x 10^8 Da), where the rheological differences were not solely depending on the molecular mass. According to the flow curves at low dextran concentration (data not shown) the results suggested that the pseudoplastic behavior increases with the polymer concentration. Additionally, thixotropy was observable for all investigated dextrans at concentrations above 1 % (w/v) (data not shown), since the viscosity showed similar values with the *vice versa* decreasing shear rates after 20 s at the maximum (1000 s^{-1}), which was reported for other dextrans by X. Xu et al. (2009). The degree of thixotropy may be further investigated by the longer application of maximum shear rates. The determined critical overlap concentration *C** (Figure 23) was significantly higher for the smaller dextran fractions from pH 4 and UC (~ 0.86 % and ~ 0.80 % (w/v)) compared to the bigger ones from pH 5.5 (~ 0.64 % (w/v)). These results were consistent with investigated dextrans of comparable molecular weight in other studies, since the critical concentration is inversely proportional to the intrinsic viscosity at low dextran concentrations and therefore the molecular mass (Dong et al., 2001; Huggins, 1942; Kraemer, 1938). Furthermore, the amplitude and frequency sweep tests revealed gel-like behavior

above the respective critical concentrations of the different dextran fractions (Figure 24 and Figure 25), with increased G'' values as well as higher strain values at the flow/yield points (Table 15) and therefore a comparably more stable gel-like structure for the bigger dextran. As stated before, the differences of the investigated dextrans predominantly rely on chain length and therefore the molecular weight. This leaves the chain length as the most important influencing factor regarding the variations in the rheological behavior of the dextran produced by *L. hordei* TMW 1.1822 if the critical polymer concentration and size is exceeded. The bigger dextran molecules applied at representative and equal concentrations may have more possible contact points for the establishment of intermolecular hydrogen bonds resulting in more stable networks. Consequently, the of low molecular weight dextran are most likely insufficient for the formation of a stable network (Hundschell et al., 2020; Vuillemin et al., 2018). However, the rheological properties and key parameters of dextrans from different origins may vary significantly as reported in multiple other studies (Carrasco et al., 1989; Tirtaatmadja et al., 2001; X. Xu et al., 2009).

4.3.3. Impact as starter culture for LPI-fermentation

The fermentation of LPI-medium conducted by *L. hordei* TMW 1.1822 revealed rapid growth speed and the intended pH of 4.5 was reached after ~ 23 h without any bacterial contaminations (Figure 5, Figure 10 and Figure 11). Again, no biogenic amine production on decarboxylase agar plates was detectable (3.1.4). The dextran amounts gained by the LPI-fermentation supplemented with sucrose were (as mentioned before) comparatively lower due to the poorer growth conditions as in mMRS. The AF4-MALS-UV analysis of the EPS showed intermediate molecular weights, like the UC dextran from buffer, due to the constant pH decrease during the formation process (Figure 16 and Table 13). The rheological measurements revealed enhanced properties for the EPS-containing fermentations regarding the stability of the gel-structure as displayed by the differences in the hysteresis-area as well as γ_y and γ_f, although lower values were recorded if compared to TMW 2.340 wt (Table 16 and Figure 26 B). As already stated for TMW 2.340 since the growth behavior of TMW 1.1822 in the different LPI-media only varied regarding the EPS formation, the favorable structural effects were attributable to the *in situ* production of dextran.

4.4. Conclusion

The obtained results for *P. claussenii* TMW 2.340 and *L. hordei* TMW 1.1822, revealed the enhancing effect of both strains on the textural characteristics and potential consumer acceptance of LPI-fermentations. The improvement of the physico-chemical properties of these LPI-fermentation is attributable to the *in situ* formation of the EPS β-glucan by TMW 2.340 and dextran by TMW 1.1822, respectively. Due to the uncertainty of a potential plasmid loss in TMW 2.340, the dextran production of TMW 1.1822 is more reliable and, thus, this strain more reasonable for further application and investigations regarding LPI-fermentations. Furthermore, the results provide new insights into the release, functionality and product specificity of the dextransucrase expressed by *L. hordei* TMW 1.1822 under different pH and sugar conditions. The systematic buffer approach allows the controlled manufacture of tailor-made dextran with distinct molecular weights by applying different pH values, which vary regarding their rheological properties and could be transferred to other glucan- or fructansucrase expressing microbes. Due to the decreased sizes and amounts of dextran at pH values outside the optimum, the EPS production needs to be optimized towards the desired molecule size in addition to the final EPS yield. Since EPS-producing LAB are frequently used as starters for the improvement of structural properties of foods, the adequate pH control during the fermentation may permit the controlled *in situ* production of dextran exhibiting the desired physico-chemical e.g. thickening properties (Oleksy et al., 2018; Torino et al., 2015). By contrast, the comparatively fixed sizes and structures of commercially used plant-derived hydrocolloids such as starch need to be synthetically modified to exhibit different properties (Chen et al., 2018; L. Kaur et al., 2016; Murray, 2009; Saha et al., 2010). Moreover, the manufacturers have to label products containing these artificially added hydrocolloids, whereas highly variable dextran may be produced naturally from sucrose by food-grade LAB or the released dextransucrases yielding “clean-label” products (Duboc et al., 2001; Milani et al., 2012; Saha et al., 2010; Tieking et al., 2003; Torino et al., 2015). Furthermore, the dextransucrase of *L. hordei* TMW 1.1822 allows the manufacture of tailor-made dextran as well as the distinct production of panose, that may also be suitable for *ex situ* applications and may extend the common portfolio of commercially available dextran recovered from *Leuconostoc mesenteroides* B-512F (De Belder, 2003; Vuillemin et al., 2018). Additionally, due to the intrinsic functionalities of the EPS and the adaptions to plant fermentations, the application of the investigated strains might be transferable to other plant-based fermentations with different starting substrates but similar needs, regarding the enhancement of texture and optimization of fermentation procedure.

5. SUMMARY

The advancement of the traditional application of exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) and their decisive role in the food fermentation processes and beyond, enables novel product designs and fermentation strategies. Due to the increased consumer interest in alternative food as well as label free products, the industry is forced to exploit new approaches and techniques to keep up with these demands. However, any change or decrease of the products´ unique characteristics and properties might result in the rejection of these products by the consumer. Therefore, the elucidation of favorable strain-specific traits of traditional starter LAB for the standardized manufacturing of food alternatives and additives, is inevitable. One of these exploitable traits is the production of the EPS dextran, which may exhibit extreme variations regarding the molecular weight and the concomitant physico-chemical properties, resulting in numerous applications ranging from medicine and cosmetics to chromatography and foods. Since LAB are adapted to diverse ecological niches, the appropriate selection of LAB starters with desired metabolic characteristics for the fermentation of alternative food products is essential.

The screening of 27 potentially EPS-producing LAB strains, which were isolated from different sources, revealed 8 strains capable of growing on a plant-based protein isolate (LPI) substrate derived from the blue lupin (*Lupinus angustifolius*) together with a predominantly positive evaluation of these LPI-fermentations by a tasting group. These strains originated from sourdough, water kefir, sauerkraut but also raw sausages and produced different EPS types, namely α-glucan (dextran), β-glucan, fructan (inulin and levan) and heteropolysaccharides, which were determined by HPLC-RI measurements and genomic analysis.

The most promising strains *Lactobacillus hordei* TMW 1.1822 and *Pediococcus claussenii* TMW 2.340 producing dextran and β-glucan, respectively, were further analyzed in more detail. The investigation of the LPI-fermentations, supplemented with the respective sugar, revealed rapid growth kinetics, as both strains reached the desired pH of 4.5 within 24 h of incubation, as well as excellent overall evaluation scores by the testing group if compared to a commercially available yoghurt alternative based on lupin protein. Additionally, rheological measurements confirmed the enhancing effect of the used starters regarding the structure and gel stability. The comparison with similar but EPS free control fermentations, showed significantly increased viscosities and structural stabilities if β-glucan or dextran was produced.

To study the characteristics of the formation process and the structure of the dextran a buffer approach with the extracellular released and recovered dextransucrase, without the metabolically active cells, was performed. Via the controlled application of different pH values, the pH optimum of 5.0 to 5.5 for the dextransucrase activity and the pH independency of the

release mechanism could be determined, with dextran yields ranging from 0.35 g/L up to ~1.70 g/L. Furthermore, the buffer approach allowed the distinct and reproducible synthesis of dextran fractions, which were analyzed by AF4-MASL-UV and exhibited various molecular weights ranging from ~ 9.43 x 10^7 at pH 3.5 to 1.97 x 10^8 Da at pH 6.0 and 3.5. Moreover, these dextran fractions showed significant rheological differences, with lower viscosities and gel stability for the smaller dextran. Methylation analysis of the dextran fractions detected no differences regarding their structural composition, leaving the polymer chain length and the respective molecular weight as the sole factor influencing the physico-chemical properties of these polysaccharides.

Furthermore, the influence of sucrose and maltose on the dextransucrase activity was determined, which elucidated the constitutive expression and intracellular accumulation of the dextransucrase, despite the absence of sucrose and the induced release of the enzyme if sucrose is present. Moreover, initial contact with sucrose prior to the dextran production significantly reduced the obtained amounts. Whereas the presence of maltose during the formation process of dextran resulted in the synthesis of the trisaccharide panose due to the acceptor reaction with maltose and the reduction of the polysaccharide yields.

6. ZUSAMMENFASSUNG

Die Weiterentwicklung des traditionellen Einsatzes von Exopolysaccharid (EPS)-produzierenden Milchsäurebakterien (MSB) und ihre entscheidende Rolle in Lebensmittelfermentationen und darüber hinaus eröffnet neue Möglichkeiten für Fermentationsstrategien und zum Produktdesign. Aufgrund des zunehmenden Verbraucherinteresses bezüglich unkonventionellen Lebensmittelalternativen sowie deklarationsfreien Verpackungsaufschriften ist die Branche gezwungen, neue Ansätze und Techniken zu nutzen, um diesen Anforderungen gerecht zu werden. Jede Änderung oder Abnahme der spezifischen Eigenschaften und Charakteristiken der Produkte birgt jedoch die Gefahr, dass der Verbraucher diesen Produkten ablehnend gegenübersteht. Daher ist die Aufklärung vorteilhafter stammspezifischer Merkmale der traditionellen MSB-Starter für die standardisierte Herstellung von Lebensmittelalternativen und -zusätzen unvermeidlich. Eine dieser positiven Eigenschaften ist die Fähigkeit der Herstellung des EPS Dextran, welches extreme Schwankungen hinsichtlich des Molekulargewichts und der damit einhergehenden physikalisch-chemischen Eigenschaften aufweisen kann. Dadurch ergeben sich für Dextran zahlreiche Anwendungsgebiete, die von Medizin und Kosmetik über Chromatographie bis hin zu den Lebensmitteln reichen. Da MSB an verschiedene ökologische Nischen angepasst sind, ist die geeignete Auswahl von MSB Startern mit gewünschten Stoffwechseleigenschaften für die Fermentation alternativer Lebensmittelprodukte unerlässlich.

Ein Screening von 27 potenziell EPS-produzierenden MSB Stämmen, die aus unterschiedlichen Quellen isoliert wurden, ergab 8 Stämme, die auf einem pflanzlichen Proteinsubstrat (LPI) wachsen konnten, welches aus der blauen Lupine (*Lupinus angustifolius*) gewonnen wurde und deren LPI-Fermentationen von einer Verkostungsgruppe überwiegend positiv bewertet wurde. Die 8 Stämme wurden aus Sauerteig, Wasserkefir, Sauerkraut, aber auch Rohwürsten isoliert und produzierten verschiedenste EPS der Typen α-Glukan (Dextran), β-Glukan, Fruktan (Inulin und Levan) und Heteropolysaccharide, die durch HPLC-RI-Messungen und genomische Analyse bestimmt wurden.

Die vielversprechendsten Stämme *Liquorilactobacillus hordei* TMW 1.1822 und *Pediococcus claussenii* TMW 2.340 wurden für genauere Analysen ausgewählt. Die Untersuchung der LPI-Fermentationen, ergänzt mit dem jeweilig benötigten Zucker, ergab eine schnelle Wachstumskinetik, da beide Stämme innerhalb von 24 Stunden Inkubationszeit den gewünschten pH-Wert von 4,5 erreichten sowie hervorragende Gesamtbewertungen bei den durchgeführten Verkostung, im Vergleich zu handelsüblichen lupinenbasierten Joghurt-Alternativen. Zusätzlich bestätigten rheologische Messungen die Verbesserung der Struktur und der Gelstabilität durch die eingesetzten Starter. Der Vergleich mit den EPS-freien Kontrollfermentationen, welche ansonsten keine Unterschiede aufwiesen, zeigte eine

signifikante Erhöhung der Viskositäten und Gelstabilitäten, wenn *in situ* β-Glukan oder Dextran produziert wurde.

Des Weiteren wurde, um die Eigenschaften des Bildungsprozesses und die Struktur des Dextrans zu untersuchen, ein experimenteller Ansatz im Puffer, welcher die extrazellulär freigesetzte und dadurch gewonnene native Dextransucrase ohne die metabolisch aktiven Zellen enthielt, durchgeführt. Durch den kontrollierten Einsatz unterschiedlicher pH-Werte konnte das pH-Optimum von 5,0 bis 5,5 für die Aktivität der Dextransucrase bestimmt und die pH-unabhängige Freisetzung des Enzyms gezeigt werden, wodurch EPS Ausbeuten von 0,35 bis ~ 1,70 g/l erzielt werden konnten. Darüber hinaus ermöglichte der Pufferansatz die eindeutige und reproduzierbare Synthese von unterschiedlichen Dextranfraktionen, die mittels AF4-MALS-UV analysiert wurden und verschiedene Molekulargewichte im Bereich von ~ 9,43 × 10^7 Da bei pH 3,5 bis 1,97 × 10^8 Da bei pH 6,0 und 3,5 aufwiesen. Diese Dextranfraktionen zeigten außerdem signifikante rheologische Unterschiede mit niedrigeren Viskositäten und Gelstabilitäten für das kleinere Dextran und dementsprechend erhöhten Werten für die größeren Fraktionen. Die Methylierungsanalyse der Dextranfraktionen ergab keine Unterschiede hinsichtlich ihrer strukturellen Zusammensetzung, was die Vermutung zulässt, dass die Polymerkettenlänge und das jeweilige Molekulargewicht der entscheidende Faktor ist, der die physikalisch-chemischen Eigenschaften dieses Polysaccharides beeinflusst.

In weiteren Experimenten wurde zudem der Einfluss von Saccharose und Maltose auf die Dextransucrase-Aktivität bestimmt, was die konstitutive Expression und intrazelluläre Akkumulation der Dextransucrase, ungeachtet der fehlenden Saccharose, und die induzierte Freisetzung des Enzyms unter Anwesenheit von Saccharose aufklärte. Darüber hinaus reduzierte der anfängliche Kontakt mit Saccharose vor der eigentlichen Dextranproduktion die erzielten EPS Mengen signifikant. Wohingegen die Anwesenheit von Maltose während der Dextranbildung aufgrund der Akzeptor-Reaktion der Dextransucrase mit Maltose zur Verringerung der Polysaccharidausbeuten bei gleichzeitiger Synthese des Trisaccharids Panose führte.

7. REFERENCES

Acosta, P. B., and Gross, K. C. (1995). Hidden sources of galactose in the environment. *European Journal of Pediatrics, 154*(2), 87-92. doi:10.1007/BF02143811

Amatayakul, T., Halmos, A. L., Sherkat, F., and Shah, N. P. (2006). Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. *International Dairy Journal, 16*(1), 40-51. doi:10.1016/j.idairyj.2005.01.004

Anwar, M. A., Kralj, S., Piqué, A. V., Leemhuis, H., van der Maarel, M. J. E. C., and Dijkhuizen, L. (2010). Inulin and levan synthesis by probiotic *Lactobacillus gasseri* strains: characterization of three novel fructansucrase enzymes and their fructan products. *Microbiology, 156*(4), 1264-1274. doi:10.1099/mic.0.036616-0

Anwar, M. A., Kralj, S., van der Maarel, M. J. E. C., and Dijkhuizen, L. (2008). The probiotic *Lactobacillus johnsonii* NCC 533 produces high-molecular-mass inulin from sucrose by using an inulosucrase enzyme. *Applied and Environmental Microbiology, 74*(11), 3426-3433. doi:10.1128/AEM.00377-08

Augimeri, R. V., Varley, A. J., and Strap, J. L. (2015). Establishing a role for bacterial cellulose in environmental interactions: Lessons learned from diverse biofilm-producing *Proteobacteria*. *Frontiers in Microbiology, 6*, 1282-1282. doi:10.3389/fmicb.2015.01282

Ayad, E. H. E., Verheul, A., de Jong, C., Wouters, J. T. M., and Smit, G. (1999). Flavour forming abilities and amino acid requirements of *Lactococcus lactis* strains isolated from artisanal and non-dairy origin. *International Dairy Journal, 9*(10), 725-735. doi:10.1016/S0958-6946(99)00140-5

Badel, S., Bernardi, T., and Michaud, P. (2011). New perspectives for lactobacilli exopolysaccharides. *Biotechnology Advances, 29*(1), 54-66. doi:10.1016/j.biotechadv.2010.08.011

Bader, S., Oviedo, J. P., Pickardt, C., and Eisner, P. (2011). Influence of different organic solvents on the functional and sensory properties of lupin (*Lupinus angustifolius* L.) proteins. *LWT - Food Science and Technology, 44*(6), 1396-1404. doi:10.1016/j.lwt.2011.01.007

Bähr, M., Fechner, A., Krämer, J., Kiehntopf, M., and Jahreis, G. (2013). Lupin protein positively affects plasma LDL cholesterol and LDL:HDL cholesterol ratio in hypercholesterolemic adults after four weeks of supplementation: a randomized, controlled crossover study. *Nutrition journal, 12*, 107. doi:10.1186/1475-2891-12-107

Baldwin, A. D., and Kiick, K. L. (2010). Polysaccharide-modified synthetic polymeric biomaterials. *Biopolymers, 94*(1), 128-140. doi:10.1002/bip.21334

Barclay, T. G., Day, C. M., Petrovsky, N., and Garg, S. (2019). Review of polysaccharide particle-based functional drug delivery. *Carbohydrate Polymers, 221*, 94-112. doi:10.1016/j.carbpol.2019.05.067

Bechtner, J., Hassler, V., Wefers, D., Vogel, R. F., and Jakob, F. (2021). Insights into extracellular dextran formation by *Liquorilactobacillus nagelii* TMW 1.1827 using secretomes obtained in the presence or absence of sucrose. *Enzyme and Microbial Technology, 143*, 109724. doi:10.1016/j.enzmictec.2020.109724

Bechtner, J., Ludwig, C., Kiening, M., Jakob, F., and Vogel, R. F. (2020). Living the sweet life: how *Liquorilactobacillus hordei* TMW 1.1822 changes its behavior in the presence of sucrose in comparison to glucose. *Foods, 9*(9). doi:10.3390/foods9091150

Bechtner, J., Wefers, D., Schmid, J., Vogel, R. F., and Jakob, F. (2019). Identification and comparison of two closely related dextransucrases released by water kefir borne *Lactobacillus hordei* TMW 1.1822 and *Lactobacillus nagelii* TMW 1.1827. *Microbiology, 165*(9), 956-966. doi:10.1099/mic.0.000825

Benezech, T., and Maingonnat, J. F. (1994). Characterization of the rheological properties of yoghurt - a review. *Journal of Food Engineering, 21*(4), 447-472. doi:10.1016/0260-8774(94)90066-3

Bertoglio, J. C., Calvo, M. A., Hancke, J. L., Burgos, R. A., Riva, A., Morazzoni, P., Ponzone, C., Magni, C., and Duranti, M. (2011). Hypoglycemic effect of lupin seed γ-conglutin in experimental animals and healthy human subjects. *Fitoterapia, 82*(7), 933-938. doi:10.1016/j.fitote.2011.05.007

Bettzieche, A., Brandsch, C., Schmidt, M., Weisse, K., Eder, K., and Stangl, G. I. (2008). Differing effect of protein isolates from different cultivars of blue lupin on plasma lipoproteins of hypercholesterolemic rats. *Bioscience, Biotechnolology and Biochemistry, 72*(12), 3114-3121. doi:10.1271/bbb.80221

Biedrzycka, E., and Bielecka, M. (2004). Prebiotic effectiveness of fructans of different degrees of polymerization. *Trends in Food Science & Technology, 15*(3), 170-175. doi:10.1016/j.tifs.2003.09.014

Blum, H., Beier, H., and Gross, H. J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *ELECTROPHORESIS, 8*(2), 93-99. doi:10.1002/elps.1150080203

Böker, M., Jördening, H.-J., and Buchholz, K. (1994). Kinetics of leucrose formation from sucrose by dextransucrase. *Biotechnology and Bioengineering, 43*(9), 856-864. doi:10.1002/bit.260430904

Bounaix, M.-S., Robert, H., Gabriel, V., Morel, S., Remaud-Siméon, M., Gabriel, B., and Fontagné-Faucher, C. (2010). Characterization of dextran-producing *Weissella* strains isolated from sourdoughs and evidence of constitutive dextransucrase expression. *FEMS Microbiology Letters, 311*(1), 18-26. doi:10.1111/j.1574-6968.2010.02067.x

Bourne, M. C. (2002) Food texture and viscosity - concept and measurement. 2nd ed. Academic Press, London.

Bouzar, F., Cerning, J., and Desmazeaud, M. (1997). Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yogurt production. *Journal of Dairy Science, 80*(10), 2310-2317. doi:10.3168/jds.S0022-0302(97)76181-2

Bover-Cid, S., and Holzapfel, W. H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology, 53*(1), 33-41. doi:10.1016/s0168-1605(99)00152-x

Bowman, H. W. (1953). Clinical evaluation of dextran as a plasma volume expander. *Journal of the American Medical Association, 153*(1), 24-26. doi:10.1001/jama.1953.02940180026009

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. (2009). The Carbohydrate-Active enZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research, 37*(Database issue), D233-238. doi:10.1093/nar/gkn663

Carrasco, F., Chornet, E., Overend, R. P., and Costa, J. (1989). A generalized correlation for the viscosity of dextrans in aqueous solutions as a function of temperature, concentration, and molecular weight at low shear rates. *Journal of Applied Polymer Science, 37*(8), 2087-2098. doi:10.1002/app.1989.070370801

Carroll, A. C., and Wong, A. (2018). Plasmid persistence: costs, benefits, and the plasmid paradox. *Canadian journal of microbiology, 64*(5), 293-304. doi:10.1139/cjm-2017-0609

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Reviews, 7*(1-2), 113-130.

Chen, Y.-F., Kaur, L., and Singh, J. (2018), Chapter 7 - chemical modification of starch, vol. 2. In: Starch in Food (M. Sjöö & L. Nilsson, eds.), pp. 283-321, Woodhead Publishing, Sawston, Cambridge.

Christensen, J. E., Dudley, E. G., Pederson, J. A., and Steele, J. L. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek, 76*(1), 217-246. doi:10.1023/A:1002001919720

Ciszek-Lenda, M., Nowak, B., Sróttek, M., Gamian, A., and Marcinkiewicz, J. (2011). Immunoregulatory potential of exopolysaccharide from *Lactobacillus rhamnosus* KL37: effects on the production of inflammatory mediators by mouse macrophages. *International Journal of Experimental Pathology, 92*(6), 382-391. doi:10.1111/j.1365-2613.2011.00788.x

Côté, G. L., and Robyt, J. F. (1982). Isolation and partial characterization of an extracellular glucansucrase from *Leuconostoc mesenteroides* NRRL B-1355 that synthesizes an alternating (1→6), (1→3)-α-d-glucan. *Carbohydrate Research, 101*(1), 57-74. doi:10.1016/S0008-6215(00)80795-8

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., and Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods, 8*(3). doi:10.3390/foods8030092

De Belder, A. N. (2003) Dextran. Handbook provided by Amersham Biosciences. ed.

De Kruif, C. G., and Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids, 15*(4–6), 555-563. doi:10.1016/S0268-005X(01)00076-5

De Man, J. C., Rogosa, M., and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology, 23*(1), 130-135. doi:10.1111/j.1365-2672.1960.tb00188.x

de Pinto, M. C., Lavermicocca, P., Evidente, A., Corsaro, M. M., Lazzaroni, S., and De Gara, L. (2003). Exopolysaccharides produced by plant pathogenic bacteria affect ascorbate metabolism in *Nicotiana tabacum*. *Plant and Cell Physiology, 44*(8), 803-810. doi:10.1093/pcp/pcg105

de Vos, W. M., and Vaughan, E. E. (1994). Genetics of lactose utilization in lactic acid bacteria. *FEMS Microbiology Reviews, 15*(2-3), 217-237. doi:10.1111/j.1574-6976.1994.tb00136.x

De Vuyst, L., De Vin, F., Vaningelgem, F., and Degeest, B. (2001). Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal, 11*(9), 687-707. doi:10.1016/S0958-6946(01)00114-5

De Vuyst, L., and Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews, 23*(2), 153-177. doi:10.1016/S0168-6445(98)00042-4

Decock, P., and Cappelle, S. (2005). Bread technology and sourdough technology. *Trends in Food Science & Technology, 16*(1), 113-120. doi:10.1016/j.tifs.2004.04.012

Delcour, J., Ferain, T., Deghorain, M., Palumbo, E., and Hols, P. (1999), The biosynthesis and functionality of the cell-wall of lactic acid bacteria. In: Lactic Acid Bacteria: Genetics, Metabolism and Applications: Proceedings of the Sixth Symposium on lactic acid bacteria: genetics, metabolism and applications, (W. N. Konings, O. P. Kuipers, & J. H. J. H. In 't Veld, eds.), pp. 159-184, Springer Netherlands, Dordrecht.

Dogsa, I., Brloznik, M., Stopar, D., and Mandic-Mulec, I. (2013). Exopolymer diversity and the role of levan in *Bacillus subtilis* biofilms. *PLoS One, 8*(4), e62044. doi:10.1371/journal.pone.0062044

Dols, M., Remaud-Simeon, M., Willemot, R. M., Vignon, M., and Monsan, P. (1998). Characterization of the different dextransucrase activities excreted in glucose, fructose, or sucrose medium by *Leuconostoc mesenteroides* NRRL B-1299. *Applied and Environmental Microbiology, 64*(4), 1298-1302. doi:10.1128/AEM.64.4.1298-1302.1998

Dong, Y., Qiu, W., Ruan, Y., Wu, Y., Wang, M., and Xu, C. (2001). Influence of molecular weight on critical concentration of chitosan/formic acid liquid crystalline solution. *Polymer Journal, 33*(5), 387-389. doi:10.1295/polymj.33.387

Donkor, O. N., Henriksson, A., Vasiljevic, T., and Shah, N. P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal, 16*(10), 1181-1189. doi:10.1016/j.idairyj.2005.10.008

Donot, F., Fontana, A., Baccou, J. C., and Schorr-Galindo, S. (2012). Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers, 87*(2), 951-962. doi:10.1016/j.carbpol.2011.08.083

Duboc, P., and Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal, 11*(9), 759-768. doi:10.1016/S0958-6946(01)00119-4

Eapen, S. (2008). Advances in development of transgenic pulse crops. *Biotechnology Advances, 26*(2), 162-168. doi:10.1016/j.biotechadv.2007.11.001

Ejby, M., Fredslund, F., Andersen, J. M., Vujičić Žagar, A., Henriksen, J. R., Andersen, T. L., Svensson, B., Slotboom, D. J., and Abou Hachem, M. (2016). An ATP binding cassette transporter mediates the uptake of α-(1,6)-linked dietary oligosaccharides in *Bifidobacterium* and correlates with competitive growth on these substrates. *The Journal of Biological Chemistry, 291*(38), 20220-20231. doi:10.1074/jbc.M116.746529

Esa, F., Tasirin, S. M., and Rahman, N. A. (2014). Overview of bacterial cellulose production and application. *Agriculture and Agricultural Science Procedia, 2*, 113-119. doi:10.1016/j.aaspro.2014.11.017

Fajardo-Lira, C., García-Garibay, M., Wacher-Rodarte, C., Farrés, A., and Marshall, V. M. (1997). Influence of water activity on the fermentation of yogurt made with extracellular polysaccharide-producing or non-producing starters. *International Dairy Journal, 7*(4), 279-281. doi:10.1016/S0958-6946(97)00011-3

Fels, L., Jakob, F., Vogel, R. F., and Wefers, D. (2018). Structural characterization of the exopolysaccharides from water kefir. *Carbohydrate Polymers, 189*, 296-303. doi:10.1016/j.carbpol.2018.02.037

Flemming, H.-C., and Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology, 8*(9), 623-633. doi:10.1038/nrmicro2415

Fraunhofer, M. E., Geissler, A. J., Wefers, D., Bunzel, M., Jakob, F., and Vogel, R. F. (2018). Characterization of β-glucan formation by *Lactobacillus brevis* TMW 1.2112 isolated from slimy spoiled beer. *International journal of biological macromolecules., 107*(Pt A), 874-881. doi:10.1016/j.ijbiomac.2017.09.063

Freitas, F., Alves, V. D., and Reis, M. A. M. (2011). Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends in Biotechnology, 29*(8), 388-398. doi:10.1016/j.tibtech.2011.03.008

Fritsch, C., Vogel, R. F., and Toelstede, S. (2015). Fermentation performance of lactic acid bacteria in different lupin substrates-influence and degradation ability of antinutritives and secondary plant metabolites. *Journal of Applied Microbiology, 119*(4), 1075-1088. doi:10.1111/jam.12908

Galle, S., Schwab, C., Arendt, E. K., and Ganzle, M. G. (2011). Structural and rheological characterisation of heteropolysaccharides produced by lactic acid bacteria in wheat and sorghum sourdough. *Food Microbiology, 28*(3), 547-553. doi:10.1016/j.fm.2010.11.006

Graessley, W. W. (1974), The entanglement concept in polymer rheology. In: Advances in Polymer Science, pp. 1-179, Springer Berlin Heidelberg.

Gugger, E. T., Galuska, P., and Tremaine, A. (2016). United states patent application 20160309732: legume-based dairy substitute and consumable food products incorporating same. General Mills, Inc. USA.

Gupta, P., and Diwan, B. (2017). Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. *Biotechnology Reports, 13*, 58-71. doi:10.1016/j.btre.2016.12.006

Han, Y. W. (1990). Microbial levan. *Advances in Applied Microbiology, 35*, 171-194. doi:10.1016/S0065-2164(08)70244-2

Harutoshi, T. (2013), Exopolysaccharides of lactic acid bacteria for food and colon health applications. In: Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes, (M. Kongo, ed.), IntechOpen.

Heincke, K., Demuth, B., Jördening, H.-J., and Buchholz, K. (1999). Kinetics of the dextransucrase acceptor reaction with maltose—experimental results and modeling. *Enzyme and Microbial Technology, 24*(8), 523-534. doi:10.1016/S0141-0229(98)00150-1

Hickisch, A., Beer, R., Vogel, R. F., and Toelstede, S. (2016). Influence of lupin-based milk alternative heat treatment and exopolysaccharide-producing lactic acid bacteria on the physical characteristics of lupin-based yogurt alternatives. *Food Research International, 84*, 180-188. doi:10.1016/j.foodres.2016.03.037

Hugas, M., and Monfort, J. M. (1997). Bacterial starter cultures for meat fermentation. *Food Chemistry, 59*(4), 547-554. doi:10.1016/S0308-8146(97)00005-8

Huggins, M. L. (1942). The viscosity of dilute solutions of long-chain molecules. IV. Dependence on concentration. *Journal of the American Chemical Society, 64*(11), 2716-2718. doi:10.1021/ja01263a056

Hundschell, C., Braun, A., Wefers, D., Vogel, R. F., and Jakob, F. (2020). Size-dependent variability in flow and viscoelastic behavior of levan roduced by *Gluconobacter albidus* TMW 2.1191. *Foods, 9*(2), 192. doi:10.3390/foods9020192

Hundschell, C., Jakob, F., and Wagemans, A. (2019). Molecular weight dependent structure and polymer density of the exopolysaccharide levan. *arXiv preprint* Retrieved from arxiv.org/abs/1909.07737

Iskandar, C. F., Cailliez-Grimal, C., Borges, F., and Revol-Junelles, A.-M. (2019). Review of lactose and galactose metabolism in lactic acid bacteria dedicated to expert genomic annotation. *Trends in Food Science & Technology, 88*, 121-132. doi:10.1016/j.tifs.2019.03.020

Jakob, F., Gebrande, C., Bichler, R. M., and Vogel, R. F. (2020). Insights into the pH-dependent, extracellular sucrose utilization and concomitant levan formation by

Gluconobacter albidus TMW 2.1191. *Antonie van Leeuwenhoek, 113*, 863-873. doi:10.1007/s10482-020-01397-3

Jakob, F., Pfaff, A., Novoa-Carballal, R., Rübsam, H., Becker, T., and Vogel, R. F. (2013). Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. *Carbohydrate Polymers, 92*(2), 1234-1242. doi:10.1016/j.carbpol.2012.10.054

Jiménez-Martínez, C., Hernández-Sánchez, H., and Dávila-Ortiz, G. (2003). Production of a yogurt-like product from *Lupinus campestris* seeds. *Journal of the Science of Food and Agriculture, 83*(6), 515-522. doi:10.1002/jsfa.1385

Jolly, L., and Stingele, F. (2001). Molecular organization and functionality of exopolysaccharide gene clusters in lactic acid bacteria. *International Dairy Journal, 11*(9), 733-745. doi:10.1016/S0958-6946(01)00117-0

Jolly, L., Vincent, S. J. F., Duboc, P., and Neeser, J.-R. (2002). Exploiting exopolysaccharides from lactic acid bacteria. *Antonie van Leeuwenhoek, 82*(1), 367-374. doi:10.1023/a:1020668523541

Kahm, M., Hasenbrink, G., Lichtenberg-Frate, H., Ludwig, J., and Kschischo, M. (2010). Grofit: fitting biological growth curves. *Nature Precedings*. doi:10.1038/npre.2010.4508.1

Kambourova, M., Oner, E. T., and Poli, A. (2015), Chapter 15 - Exopolysaccharides from prokaryotic microorganisms - promising sources for white biotechnology processes. In: Industrial Biorefineries & White Biotechnology, (A. Pandey, R. Höfer, M. Taherzadeh, K. M. Nampoothiri, & C. Larroche, eds.), pp. 523-554, Elsevier, Amsterdam, Netherlands.

Kandler, O. (1983). Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek, 49*(3), 209-224. doi:10.1007/bf00399499

Karnezis, T., McIntosh, M., Wardak, A. Z., Stanisich, V. A., Stone, B. A., and author_in_Japanese. (2000). The biosynthesis of ß-glycans. *Trends in Glycoscience and Glycotechnology, 12*(66), 211-227. doi:10.4052/tigg.12.211

Kasapis, S., Morris, E. R., Gross, M., and Rudolph, K. (1994). Solution properties of levan polysaccharide from *Pseudomonas syringae* pv. *phaseolicola*, and its possible primary role as a blocker of recognition during pathogenesis. *Carbohydrate Polymers, 23*(1), 55-64. doi:10.1016/0144-8617(94)90090-6

Kaur, L., and Singh, J. (2016), Starch: modified starches. In: Encyclopedia of food and health, (B. Caballero, P. M. Finglas, & F. Toldrá, eds.), pp. 152-159, Academic Press, Oxford, UK.

Kaur, N., and Gupta, A. K. (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences, 27*(7), 703-714. doi:10.1007/BF02708379

Kern, C. C., Usbeck, J. C., Vogel, R. F., and Behr, J. (2013). Optimization of matrix-assisted-laser-desorption-ionization-time-of-flight mass spectrometry for the identification of

bacterial contaminants in beverages. *Journal of Microbiological Methods, 93*(3), 185-191. doi:10.1016/j.mimet.2013.03.012

Killiny, N., Martinez, R. H., Dumenyo, C. K., Cooksey, D. A., and Almeida, R. P. (2013). The exopolysaccharide of *Xylella fastidiosa* is essential for biofilm formation, plant virulence, and vector transmission. *Molecular Plant Microbe Interactions, 26*(9), 1044-1053. doi:10.1094/mpmi-09-12-0211-r

Kim, D., and Robyt, J. F. (1995). Dextransucrase constitutive mutants of *Leuconostoc mesenteroides* B-1299. *Enzyme and Microbial Technology, 17*(12), 1050-1056. doi:10.1016/0141-0229(95)00039-9

Kim, D., Robyt, J. F., Lee, S.-Y., Lee, J.-H., and Kim, Y.-M. (2003). Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of *Leuconostoc mesenteroides* B-512FMCM dextransucrase. *Carbohydrate Research, 338*(11), 1183-1189. doi:10.1016/S0008-6215(03)00148-4

Koczan, J. M., McGrath, M. J., Zhao, Y., and Sundin, G. W. (2009). Contribution of *Erwinia amylovora* exopolysaccharides amylovoran and levan to biofilm formation: implications in pathogenicity. *Phytopathology, 99*(11), 1237-1244. doi:10.1094/PHYTO-99-11-1237

Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kazenko, A., Hoffman, C. A., Sharpe, E. S., and Jackson, R. W. (1953). Enzymatic synthesis of dextran; acceptor specificity and chain initiation. *The Journal of Biological Chemistry, 200*(2), 793-801.

Korakli, M., Rossmann, A., Gänzle, M. G., and Vogel, R. F. (2001). Sucrose metabolism and exopolysaccharide production in wheat and rye sourdoughs by *Lactobacillus sanfranciscensis*. *Journal of Agricultural and Food Chemistry, 49*(11), 5194-5200. doi:10.1021/jf0102517

Kothari, D., Das, D., Patel, S., and Goyal, A. (2015), Dextran and food application, vol. 1. In: Polysaccharides: bioactivity and biotechnology, (K. G. Ramawat & J.-M. Mérillon, eds.), pp. 735-752, Springer International Publishing, Cham, Switzerland.

Kraemer, E. O. (1938). Molecular weights of celluloses and cellulose derivates. *Industrial & Engineering Chemistry, 30*(10), 1200-1203. doi:10.1021/ie50346a023

Kulicke, W. M., and Clasen, C. (2004), Basic concepts. In: Viscosimetry of polymers and polyelectrolytes, pp. 5-11, Springer Berlin Heidelberg, Berlin, Heidelberg, Germany.

Kulicke, W. M., Lettau, A. I., and Thielking, H. (1997). Correlation between immunological activity, molar mass, and molecular structure of different (1-->3)-beta-D-glucans. *Carbohydrate Research, 297*(2), 135-143. doi:10.1016/s0008-6215(96)00273-x

Lapasin, R., and Pricl, S. (1995), Rheology of polysaccharide systems. In: Rheology of industrial polysaccharides: theory and applications, pp. 250-494, Springer US, Boston, USA.

Laue, H., Schenk, A., Li, H., Lambertsen, L., Neu, T. R., Molin, S., and Ullrich, M. S. (2006). Contribution of alginate and levan production to biofilm formation by *Pseudomonas syringae*. *Microbiology, 152*(10), 2909-2918. doi:10.1099/mic.0.28875-0

Leemhuis, H., Pijning, T., Dobruchowska, J. M., van Leeuwen, S. S., Kralj, S., Dijkstra, B. W., and Dijkhuizen, L. (2013). Glucansucrases: three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *Journal of Biotechnology, 163*(2), 250-272. doi:10.1016/j.jbiotec.2012.06.037

Leroy, F., and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology, 15*(2), 67-78. doi:10.1016/j.tifs.2003.09.004

Lévesque, S. G., Lim, R. M., and Shoichet, M. S. (2005). Macroporous interconnected dextran scaffolds of controlled porosity for tissue-engineering applications. *Biomaterials, 26*(35), 7436-7446. doi:10.1016/j.biomaterials.2005.05.054

Li, L.-L., Wang, Y.-T., Zhu, L.-M., Liu, Z.-Y., Ye, C.-Q., and Qin, S. (2020). Inulin with different degrees of polymerization protects against diet-induced endotoxemia and inflammation in association with gut microbiota regulation in mice. *Scientific Reports, 10*(1), 978. doi:10.1038/s41598-020-58048-w

Limoli, D. H., Jones, C. J., and Wozniak, D. J. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiology spectrum, 3*(3). doi:10.1128/microbiolspec.MB-0011-2014

Liu, M., Bayjanov, J. R., Renckens, B., Nauta, A., and Siezen, R. J. (2010). The proteolytic system of lactic acid bacteria revisited: a genomic comparison. *BMC Genomics, 11*, 36. doi:10.1186/1471-2164-11-36

Llamas-Arriba, M. G., Pérez-Ramos, A., Puertas, A. I., López, P., Dueñas, M. T., and Prieto, A. (2018). Characterization of *Pediococcus ethanolidurans* CUPV141: a β-D-glucan- and heteropolysaccharide-producing bacterium. *Frontiers in Microbiology, 9*, 2041. doi:10.3389/fmicb.2018.02041

Lodwig, E. M., Hosie, A. H., Bourdès, A., Findlay, K., Allaway, D., Karunakaran, R., Downie, J. A., and Poole, P. S. (2003). Amino-acid cycling drives nitrogen fixation in the legume-*Rhizobium* symbiosis. *Nature, 422*(6933), 722-726. doi:10.1038/nature01527

Lovati, M. R., Manzoni, C., Castiglioni, S., Parolari, A., Magni, C., and Duranti, M. (2012). Lupin seed γ-conglutin lowers blood glucose in hyperglycaemic rats and increases glucose consumption of HepG2 cells. *British Journal of Nutrition, 107*(1), 67-73. doi:10.1017/S0007114511002601

Lübeck, M., and Lübeck, P. S. (2019). Application of lactic acid bacteria in green biorefineries. *FEMS Microbiology Letters, 366*(3). doi:10.1093/femsle/fnz024

Lynn, A., Prentice, R. D. M., Cochrane, M. P., Cooper, A. M., Dale, F., Duffus, C. M., Ellis, R. P., Morrison, I. M., Paterson, L., Swanston, J. S., and Tiller, S. A. (1997), Cereal

starches. In: Cereals: novel uses and processes, (G. M. Campbell, C. Webb, & S. L. McKee, eds.), pp. 69-77, Springer US, Boston, USA.

MacLean, R. C., and San Millan, A. (2015). Microbial evolution: towards resolving the plasmid paradox. *Current Biology, 25*(17), R764-767. doi:10.1016/j.cub.2015.07.006

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N., Karamychev, V., Polouchine, N., Shakhova, V., Grigoriev, I., Lou, Y., Rohksar, D., Lucas, S., Huang, K., Goodstein, D. M., Hawkins, T., Plengvidhya, V., Welker, D., Hughes, J., Goh, Y., Benson, A., Baldwin, K., Lee, J. H., Díaz-Muñiz, I., Dosti, B., Smeianov, V., Wechter, W., Barabote, R., Lorca, G., Altermann, E., Barrangou, R., Ganesan, B., Xie, Y., Rawsthorne, H., Tamir, D., Parker, C., Breidt, F., Broadbent, J., Hutkins, R., O'Sullivan, D., Steele, J., Unlu, G., Saier, M., Klaenhammer, T., Richardson, P., Kozyavkin, S., Weimer, B., and Mills, D. (2006). Comparative genomics of the lactic acid bacteria. *Proceedings of the National Academy of Sciences 103*(42), 15611-15616. doi:10.1073/pnas.0607117103

Makarova, K. S., and Koonin, E. V. (2007). Evolutionary genomics of lactic acid bacteria. *Journal of Bacteriology, 189*(4), 1199-1208. doi:10.1128/JB.01351-06

Mäkeläinen, H., Hasselwander, O., Rautonen, N., and Ouwehand, A. C. (2009). Panose, a new prebiotic candidate. *Letters in Applied Microbiology, 49*(6), 666-672. doi:10.1111/j.1472-765X.2009.02698.x

Mäkinen, O. E., Wanhalinna, V., Zannini, E., and Arendt, E. K. (2016). Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition, 56*(3), 339-349. doi:10.1080/10408398.2012.761950

Mano, M. C. R., Neri-Numa, I. A., da Silva, J. B., Paulino, B. N., Pessoa, M. G., and Pastore, G. M. (2017). Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. *Applied Microbiology and Biotechnology*. doi:10.1007/s00253-017-8564-2

McCurdy, R. D., Goff, H. D., Stanley, D. W., and Stone, A. P. (1994). Rheological properties of dextran related to food applications. *Food Hydrocolloids, 8*(6), 609-623. doi:10.1016/S0268-005X(09)80068-4

McIlvaine, T. C. (1921). A buffer solution for colorimetric comparison. *Journal of Biological Chemistry, 49*(1), 183-186.

Mende, S., Rohm, H., and Jaros, D. (2016). Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products. *International Dairy Journal, 52*, 57-71. doi:10.1016/j.idairyj.2015.08.002

Mezger, T. G. (2011) The rheology handbook - for users of rotational and oscillatory rheometers. 3rd ed. Vincentz Network, Hannover, Germany.

Milani, J., and Maleki, G. (2012), Hydrocolloids in food industry. In: Food industrial processes, (B. Valdez, ed.), IntechOpen.

Misra, S., Sharma, V., and Srivastava, A. K. (2021), Bacterial polysaccharides: an overview. In: Polysaccharides: bioactivity and biotechnology, (K. G. Ramawat & J.-M. Mérillon, eds.), pp. 1-24, Springer International Publishing, Cham, Switzerland.

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R.-M., and Remaud-Siméon, M. (2001). Homopolysaccharides from lactic acid bacteria. *International Dairy Journal, 11*(9), 675-685. doi:10.1016/S0958-6946(01)00113-3

Mooser, G., and Iwaoka, K. R. (1989). Sucrose 6-alpha-D-glucosyltransferase from *Streptococcus sobrinus*: characterization of a glucosyl-enzyme complex. *Biochemistry, 28*(2), 443-449. doi:10.1021/bi00428a006

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., and Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers, 1*(1), 5-21. doi:10.1016/0144-8617(81)90011-4

Moulis, C., Joucla, G., Harrison, D., Fabre, E., Potocki-Veronese, G., Monsan, P., and Remaud-Simeon, M. (2006). Understanding the polymerization mechanism of glycoside-hydrolase family 70 glucansucrases. *Journal of Biological Chemistry, 281*(42), 31254-31267. doi:10.1074/jbc.M604850200

Mozzi, F., Vaningelgem, F., He´bert, E. M. a., Van der Meulen, R., Moreno, M. a. R. F., Font de Valdez, G., and De Vuyst, L. (2006). Diversity of heteropolysaccharide-producing lactic acid bacterium strains and their biopolymers. *Applied and Environmental Microbiology, 72*(6), 4431–4435. doi:10.1128/AEM.02780-05

Murray, J. C. F. (2009), 25 - Cellulosics, vol. 2. In: Handbook of hydrocolloids (G. O. Phillips & P. A. Williams, eds.), pp. 710-723, Woodhead Publishing, Sawston, Cambridge, UK.

Naessens, M., Cerdobbel, A., Soetaert, W., and Vandamme, E. J. (2005). *Leuconostoc* dextransucrase and dextran: production, properties and applications. *Journal of Chemical Technology & Biotechnology, 80*(8), 845-860. doi:doi:10.1002/jctb.1322

Nair, A. V., Raman, M., and Doble, M. (2019), Chapter 13 - Polysaccharide-based hydrogels for targeted drug delivery, vol. 1. In: Materials for Biomedical Engineering, (A.-M. Holban & A. M. Grumezescu, eds.), pp. 343-382, Elsevier, Amsterdam, Netherlands.

Netsopa, S., Niamsanit, S., Sakloetsakun, D., and Milintawisamai, N. (2018). Characterization and rheological behavior of dextran from *Weissella confusa International Journal of Polymer Science, 2018*(6), 1-10. doi:10.1155/2018/5790526

Nilsson, L. (2013). Separation and characterization of food macromolecules using field-flow fractionation: a review. *Food Hydrocolloids, 30*(1), 1-11. doi:10.1016/j.foodhyd.2012.04.007

Notararigo, S., Nácher-Vázquez, M., Ibarburu, I., Werning, M. L., de Palencia, P. F., Dueñas, M. T., Aznar, R., López, P., and Prieto, A. (2013). Comparative analysis of production and purification of homo- and hetero-polysaccharides produced by lactic acid bacteria. *Carbohydrate Polymers, 93*(1), 57-64. doi:10.1016/j.carbpol.2012.05.016

Oleksy, M., and Klewicka, E. (2018). Exopolysaccharides produced by *Lactobacillus* sp.: biosynthesis and applications. *Critical Reviews in Food Science and Nutrition, 58*(3), 450-462. doi:10.1080/10408398.2016.1187112

Oliveira, R. P. d. S., Perego, P., Oliveira, M. N. d., and Converti, A. (2011). Effect of inulin as prebiotic and synbiotic interactions between probiotics to improve fermented milk firmness. *Journal of Food Engineering, 107*(1), 36-40. doi:10.1016/j.jfoodeng.2011.06.005

Otts, D. R., and Day, D. F. (1988). Dextransucrase secretion in *Leuconostoc mesenteroides* depends on the presence of a transmembrane proton gradient. *Journal of bacteriology, 170*(11), 5006-5011.

Ozimek, L. K., Kralj, S., van der Maarel, M. J. E. C., and Dijkhuizen, L. (2006). The levansucrase and inulosucrase enzymes of *Lactobacillus reuteri* 121 catalyse processive and non-processive transglycosylation reactions. *Microbiology, 152*(4), 1187-1196. doi:doi:10.1099/mic.0.28484-0

Pan, J.-f., Yuan, L., Guo, C.-a., Geng, X.-h., Fei, T., Fan, W.-s., Li, S., Yuan, H.-f., Yan, Z.-q., and Mo, X.-m. (2014). Fabrication of modified dextran–gelatin in situ forming hydrogel and application in cartilage tissue engineering. *Journal of Materials Chemistry B, 2*(47), 8346-8360. doi:10.1039/C4TB01221F

Papadimitriou, K., Alegría, Á., Bron, P. A., de Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J. A., Linares, D. M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., and Kok, J. (2016). Stress physiology of lactic acid bacteria. *Microbiology and Molecular Biology Reviews, 80*(3), 837-890. doi:10.1128/mmbr.00076-15

Paul, F., Oriol, E., Auriol, D., and Monsan, P. (1986). Acceptor reaction of a highly purified dextransucrase with maltose and oligosaccharides. Application to the synthesis of controlled-molecular-weight dextrans. *Carbohydrate Research, 149*(2), 433-441. doi:10.1016/S0008-6215(00)90063-6

Peoples, M. B., Herridge, D. F., and Ladha, J. K. (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil, 174*(1), 3-28. doi:10.1007/BF00032239

Petri, D. F. S. (2015). Xanthan gum: a versatile biopolymer for biomedical and technological applications. *Journal of Applied Polymer Science, 132*(23). doi:10.1002/app.42035

Pijning, T., Anwar, M. A., Böger, M., Dobruchowska, J. M., Leemhuis, H., Kralj, S., Dijkhuizen, L., and Dijkstra, B. W. (2011). Crystal structure of inulosucrase from *Lactobacillus*: insights into the substrate specificity and product specificity of GH68 fructansucrases. *Journal of Molecular Biology, 412*(1), 80-93. doi:10.1016/j.jmb.2011.07.031

Pons, T., Naumoff, D. G., Martínez-Fleites, C., and Hernández, L. (2004). Three acidic residues are at the active site of a β-propeller architecture in glycoside hydrolase families 32, 43, 62, and 68. *Proteins: Structure, Function, and Bioinformatics, 54*(3), 424-432. doi:10.1002/prot.10604

Porath, J., and Flodin, P. E. R. (1959). Gel filtration: a method for desalting and group separation. *Nature, 183*(4676), 1657-1659. doi:10.1038/1831657a0

Prechtl, R. M., Janßen, D., Behr, J., Ludwig, C., Küster, B., Vogel, R. F., and Jakob, F. (2018). Sucrose-induced proteomic response and carbohydrate utilization of *Lactobacillus sakei* TMW 1.411 during dextran formation. *Frontiers in Microbiology, 9*, 2796. doi:10.3389/fmicb.2018.02796

Prechtl, R. M., Wefers, D., Jakob, F., and Vogel, R. F. (2018a). Cold and salt stress modulate amount, molecular and macromolecular structure of a *Lactobacillus sakei* dextran. *Food Hydrocolloids, 82*, 73-81. doi:10.1016/j.foodhyd.2018.04.003

Prechtl, R. M., Wefers, D., Jakob, F., and Vogel, R. F. (2018b). Structural characterization of the surface-associated heteropolysaccharide of *Lactobacillus plantarum* TMW 1.1478 and genetic analysis of its putative biosynthesis cluster. *Carbohydrate Polymers, 202*, 236-245. doi:10.1016/j.carbpol.2018.08.115

Purohit, D. H., Hassan, A. N., Bhatia, E., Zhang, X., and Dwivedi, C. (2009). Rheological, sensorial, and chemopreventive properties of milk fermented with exopolysaccharide-producing lactic cultures. *Journal of Dairy Science, 92*(3), 847-856. doi:10.3168/jds.2008-1256

R, C. T. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Raga-Carbajal, E., López-Munguía, A., Alvarez, L., and Olvera, C. (2018). Understanding the transfer reaction network behind the non-processive synthesis of low molecular weight levan catalyzed by *Bacillus subtilis* levansucrase. *Sci Rep, 8*(1), 15035. doi:10.1038/s41598-018-32872-7

Remus, D. M., van Kranenburg, R., van, S., II, Taverne, N., Bongers, R. S., Wels, M., Wells, J. M., Bron, P. A., and Kleerebezem, M. (2012). Impact of 4 *Lactobacillus plantarum* capsular polysaccharide clusters on surface glycan composition and host cell signaling. *Microb Cell Fact, 11*, 149. doi:10.1186/1475-2859-11-149

Rimondo, S., Perale, G., and Rossi, F. (2019), Polysaccharide-based scaffold for tissue-regeneration, vol. 1. In: Functional polysaccharides for biomedical applications, (S. Maiti & S. Jana, eds.), pp. 189-212, Woodhead Publishing, Sawston, Cambridge.

Robyt, J. F. (1995). Mechanisms in the glucansucrase synthesis of polysaccharides and oligosaccharides from sucrose. *Advances in Carbohydrate Chemistry and Biochemistry, 51*, 133-168. doi:10.1016/S0065-2318(08)60193-6

Robyt, J. F., Kimble, B. K., and Walseth, T. F. (1974). The mechanism of dextransucrase action: Direction of dextran biosynthesis. *Archives of Biochemistry and Biophysics, 165*(2), 634-640. doi:10.1016/0003-9861(74)90291-4

Robyt, J. F., and Walseth, T. F. (1978). The mechanism of acceptor reactions of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydrate Research, 61*(1), 433-445. doi:10.1016/S0008-6215(00)84503-6

Rodríguez-Saavedra, M., González de Llano, D., and Moreno-Arribas, M. V. (2020). Beer spoilage lactic acid bacteria from craft brewery microbiota: microbiological quality and food safety. *Food Research International, 138*, 109762. doi:10.1016/j.foodres.2020.109762

RStudio, T. (2013). RStudio: integrated development for R. RStudio, Inc., Boston. URL: http://www.rstudio.com/.

Ruas-Madiedo, P., Hugenholtz, J., and Zoon, P. (2002). An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal, 12*(2–3), 163-171. doi:10.1016/S0958-6946(01)00160-1

Rübsam, H., Krottenthaler, M., Gastl, M., and Becker, T. (2012). An overview of separation methods in starch analysis: the importance of size exclusion chromatography and field flow fractionation. *Starch - Stärke, 64*(9), 683-695. doi:10.1002/star.201100188

Rudolph, K. W. E., Gross, M., Ebrahim-Nesbat, F., Nöllenburg, M., Zomorodian, A., Wydra, K., Neugebauer, M., Hettwer, U., El-Shouny, W., Sonnenberg, B., and Klement, Z. (1994), The role of extracellular polysaccharides as virulence factors for phytopathogenic pseudomonads and xanthomonads. In: Molecular mechanisms of bacterial virulence, (C. I. Kado & J. H. Crosa, eds.), pp. 357-378, Springer Netherlands, Dordrecht.

Rühmkorf, C., Bork, C., Mischnick, P., Rübsam, H., Becker, T., and Vogel, R. F. (2013). Identification of *Lactobacillus curvatus* TMW 1.624 dextransucrase and comparative characterization with *Lactobacillus reuteri* TMW 1.106 and *Lactobacillus animalis* TMW 1.971 dextransucrases. *Food Microbiology, 34*(1), 52-61. doi:10.1016/j.fm.2012.11.002

Rühmkorf, C., Rübsam, H., Becker, T., Bork, C., Voiges, K., Mischnick, P., Brandt, M. J., and Vogel, R. F. (2012). Effect of structurally different microbial homoexopolysaccharides on the quality of gluten-free bread. *European Food Research and Technology, 235*(1), 139-146. doi:10.1007/s00217-012-1746-3

Ryan, P. M., Ross, R. P., Fitzgerald, G. F., Caplice, N. M., and Stanton, C. (2015). Sugar-coated: exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food & Function, 6*(3), 679-693. doi:10.1039/C4FO00529E

Sabatié, J., Choplin, L., Doublier, J. L., Arul, J., Paul, F., and Monsan, P. (1988). Rheology of native dextrans in relation to their primary structure. *Carbohydrate Polymers, 9*(4), 287-299. doi:10.1016/0144-8617(88)90047-1

Sabatie, J., Choplin, L., Paul, F., and Monsan, P. (1986). The effect of synthesis temperature on the rheological properties of native dextran. *Biotechnology Letters, 8*(6), 425-430. doi:10.1007/bf01026747

Saha, D., and Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. *Journal of Food Science and Technology, 47*(6), 587-597. doi:10.1007/s13197-010-0162-6

Sajna, K. V., Gottumukkala, L. D., Sukumaran, R. K., and Pandey, A. (2015), Chapter 18 - White biotechnology in cosmetics. In: Industrial biorefineries & white biotechnology, (A.

Pandey, R. Höfer, M. Taherzadeh, K. M. Nampoothiri, & C. Larroche, eds.), pp. 607-652, Elsevier, Amsterdam, Netherlands.

San Millan, A., and MacLean, R. C. (2017). Fitness costs of plasmids: a limit to plasmid transmission. *Microbiology Spectrum, 5*(5). doi:10.1128/microbiolspec.MTBP-0016-2017

Sarwat, F., Ul Qader, S. A., Aman, A., and Ahmed, N. (2008). Production & characterization of a unique dextran from an indigenous *Leuconostoc mesenteroides* CMG713. *International Journal of Biological Sciences, 4*(6), 379-386. doi:10.7150/ijbs.4.379

Sauer, M., Russmayer, H., Grabherr, R., Peterbauer, C. K., and Marx, H. (2017). The efficient clade: lactic acid bacteria for industrial chemical production. *Trends in Biotechnology, 35*(8), 756-769. doi:10.1016/j.tibtech.2017.05.002

Savijoki, K., Ingmer, H., and Varmanen, P. (2006). Proteolytic systems of lactic acid bacteria. *Applied Microbiology and Biotechnology, 71*(4), 394-406. doi:10.1007/s00253-006-0427-1

Schmid, J., Sieber, V., and Rehm, B. (2015). Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. *Frontiers in Microbiology, 6*, 496. doi:10.3389/fmicb.2015.00496

Sheng, Q., Su, X., Li, X., Ke, Y., and Liang, X. (2014). A dextran-bonded stationary phase for saccharide separation. *Journal of Chromatography A, 1345*, 57-67. doi:10.1016/j.chroma.2014.03.076

Shukla, R., and Goyal, A. (2014). Purified dextransucrase from *Pediococcus pentosaceus* CRAG3 as food additive. *Indian Journal of Experimental Biology, 52*(11), 1036-1044.

Shurtleff, W., and Aoyagi, A. (1979) The book of tofu: tofu and soymilk production. 2nd ed. Soyfoods Center, Lafayette, USA.

Siddiqui, N. N., Aman, A., and Qader, S. A. U. (2013). Mutational analysis and characterization of dextran synthesizing enzyme from wild and mutant strain of *Leuconostoc mesenteroides*. *Carbohydrate Polymers, 91*(1), 209-216. doi:10.1016/j.carbpol.2012.08.026

Sidebotham, R. L. (1974). Dextrans. *Advances in Carbohydrate Chemistry and Biochemistry, 30*, 371-444. doi:10.1016/S0065-2318(08)60268-1

Siezen, R. J., Starrenburg, M. J., Boekhorst, J., Renckens, B., Molenaar, D., and van Hylckama Vlieg, J. E. (2008). Genome-scale genotype-phenotype matching of two *Lactococcus lactis* isolates from plants identifies mechanisms of adaptation to the plant niche. *Applied and Environmental Microbiology, 74*(2), 424-436. doi:10.1128/aem.01850-07

Silva, A. K. A., Letourneur, D., and Chauvierre, C. (2014). Polysaccharide nanosystems for future progress in cardiovascular pathologies. *Theranostics, 4*(6), 579-591. doi:10.7150/thno.7688

Smith, A. M., Moxon, S., and Morris, G. A. (2016), 13 - Biopolymers as wound healing materials, vol. 2. In: Wound healing biomaterials, (M. S. Ågren, ed.), pp. 261-287, Woodhead Publishing, Sawston, Cambridge, UK.

Snowden, J., Sipsas, S., and St John, C. (2004). US20070154611 A1: Method to produce lupin protein-based dairy substitutes. The State of Western Australia Through its Department of Agriculture Australia.

Spano, G., Russo, P., Lonvaud-Funel, A., Lucas, P., Alexandre, H., Grandvalet, C., Coton, E., Coton, M., Barnavon, L., Bach, B., Rattray, F., Bunte, A., Magni, C., Ladero, V., Alvarez, M., Fernández, M., Lopez, P., de Palencia, P. F., Corbi, A., Trip, H., and Lolkema, J. S. (2010). Biogenic amines in fermented foods. *European Journal of Clinical Nutrition, 64 Suppl 3*, S95-100. doi:10.1038/ejcn.2010.218

Sutherland, I. W. (1979). Microbial exopolysaccharides. *Trends in Biochemical Sciences, 4*(3), 55-59. doi:10.1016/0968-0004(79)90262-7

Suzuki, K. (2011). 125th anniversary review: microbiological instability of beer caused by spoilage bacteria. *Journal of the Institute of Brewing, 117*(2), 131-155. doi:10.1002/j.2050-0416.2011.tb00454.x

Sweet, D. P., Shapiro, R. H., and Albersheim, P. (1975). Quantitative analysis by various glc response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Research, 40*(2), 217-225. doi:10.1016/S0008-6215(00)82604-X

Tabibloghmany, F. S., and Ehsandoost, E. (2014). An overview of healthy and functionality of exopolysaccharides produced by lactic acid bacteria in the dairy industry. *European Journal of Nutrition & Food Safety, 4*(2), 63-86. doi:10.9734/EJNFS/2014/6948

Tieking, M., Korakli, M., Ehrmann, M. A., Gänzle, M. G., and Vogel, R. F. (2003). In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology, 69*(2), 945-952. doi:10.1128/aem.69.2.945-952.2003

Tirtaatmadja, V., Dunstan, D. E., and Boger, D. V. (2001). Rheology of dextran solutions. *Journal of Non-Newtonian Fluid Mechanics, 97*(2), 295-301. doi:10.1016/S0377-0257(00)00226-3

Torino, M. I., Font de Valdez, G., and Mozzi, F. (2015). Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. *Frontiers in Microbiology, 6*, 834. doi:10.3389/fmicb.2015.00834

Trinick, M. J. (1977). Vesicular-arbuscular infection and soil phosphorus utilization in *Lupinus* spp. *New Phytologist, 78*(2), 297-304. doi:10.1111/j.1469-8137.1977.tb04833.x

Ua-Arak, T., Jakob, F., and Vogel, R. F. (2017). Fermentation pH modulates the size distributions and functional properties of *Gluconobacter albidus* TMW 2.1191 levan. *Frontiers in Microbiology, 8*, 807. doi:10.3389/fmicb.2017.00807

van Geel-Schutten, G. H., Flesch, F., ten Brink, B., Smith, M. R., and Dijkhuizen, L. (1998). Screening and characterization of *Lactobacillus* strains producing large amounts of exopolysaccharides. *Applied Microbiology and Biotechnology, 50*(6), 697-703. doi:10.1007/s002530051353

Van Hijum, S. A. F. T., Kralj, S., Ozimek, L. K., Dijkhuizen, L., and van Geel-Schutten, I. G. H. (2006). Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiology and Molecular Biology Reviews, 70*(1), 157-176. doi:10.1128/MMBR.70.1.157-176.2006

Veerubhotla, R., Varanasi, J. L., and Das, D. (2018), Chapter 12 - Biofilm formation within microbial fuel cells, vol. 1. In: Progress and recent trends in microbial fuel cells, (P. P. Kundu & K. Dutta, eds.), pp. 231-242, Elsevier, Amsterdam, Netherlands.

Vuillemin, M., Grimaud, F., Claverie, M., Rolland-Sabaté, A., Garnier, C., Lucas, P., Monsan, P., Dols-Lafargue, M., Remaud-Siméon, M., and Moulis, C. (2018). A dextran with unique rheological properties produced by the dextransucrase from *Oenococcus kitaharae* DSM 17330. *Carbohydrate Polymers, 179*, 10-18. doi:10.1016/j.carbpol.2017.09.056

Vujičić-Žagar, A., Pijning, T., Kralj, S., López, C. A., Eeuwema, W., Dijkhuizen, L., and Dijkstra, B. W. (2010). Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. *Proceedings of the National Academy of Sciences, 107*(50), 21406-21411. doi:10.1073/pnas.1007531107

Wäsche, A., Müller, K., and Knauf, U. (2001). New processing of lupin protein isolates and functional properties. *Food / Nahrung, 45*(6), 393-395. doi:10.1002/1521-3803(20011001)45:6<393::Aid-food393>3.0.Co;2-o

Wein, T., Hülter, N. F., Mizrahi, I., and Dagan, T. (2019). Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance. *Nature Communications, 10*(1), 2595. doi:10.1038/s41467-019-10600-7

Werning, M. L., Ibarburu, I., Dueñas, M. T., Irastorza, A., Navas, J., and López, P. (2006). *Pediococcus parvulus* gtf gene encoding the GTF glycosyltransferase and its application for specific PCR detection of beta-D-glucan-producing bacteria in foods and beverages. *Journal of Food Protection, 69*(1), 161-169. doi:10.4315/0362-028x-69.1.161

Xu, D., Fels, L., Wefers, D., Behr, J., Jakob, F., and Vogel, R. F. (2018). *Lactobacillus hordei* dextrans induce *Saccharomyces cerevisiae* aggregation and network formation on hydrophilic surfaces. *International Journal of Biological Macromolecules, 115*, 236-242. doi:10.1016/j.ijbiomac.2018.04.068

Xu, X., Li, H., Zhang, Z., and Qi, X. (2009). Hydrodynamic properties of aqueous dextran solutions. *Journal of Applied Polymer Science, 111*(3), 1523-1529. doi:10.1002/app.29043

Xu, Z., Luo, Y., Mao, Y., Peng, R., Chen, J., Soteyome, T., Bai, C., Chen, L., Liang, Y., Su, J., Wang, K., Liu, J., and Kjellerup, B. V. (2020). Spoilage lactic acid bacteria in the

brewing industry. *Journal of Microbiology and Biotechnology, 30*(7), 955-961. doi:10.4014/jmb.1908.08069

Yuryev, V., Tomasik, P., and Bertoft, E. (2007) Starch: achievements in understanding of structure and functionality. ed. Nova Science Publishers, New York, USA.

Zannini, E., Waters, D. M., Coffey, A., and Arendt, E. K. (2016). Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. *Applied Microbiology and Biotechnology, 100*(3), 1121-1135. doi:10.1007/s00253-015-7172-2

Zarour, K., Llamas, M. G., Prieto, A., Rúas-Madiedo, P., Dueñas, M. T., de Palencia, P. F., Aznar, R., Kihal, M., and López, P. (2017). Rheology and bioactivity of high molecular weight dextrans synthesised by lactic acid bacteria. *Carbohydrate Polymers, 174*, 646-657. doi:10.1016/j.carbpol.2017.06.113

Zeidan, A. A., Poulsen, V. K., Janzen, T., Buldo, P., Derkx, P. M. F., Øregaard, G., and Neves, A. R. (2017). Polysaccharide production by lactic acid bacteria: from genes to industrial applications. *FEMS Microbiology Reviews, 41*(Supp_1), 168-200. doi:10.1093/femsre/fux017

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'Toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., and Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology, 70*(4), 2782-2858. doi:10.1099/ijsem.0.004107

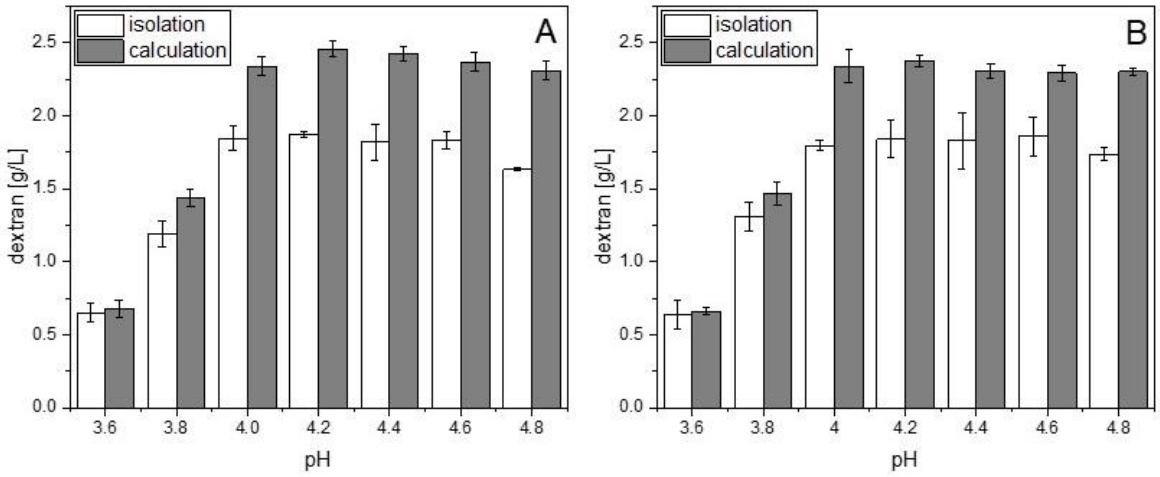
Zhu, M., Ajdić, D., Liu, Y., Lynch, D., Merritt, J., and Banas, J. A. (2009). Role of the *Streptococcus mutans* irvA gene in GbpC-independent, dextran-dependent aggregation and biofilm formation. *Applied and Environmental Microbiology, 75*(22), 7037-7043. doi:10.1128/aem.01015-09

8. APPENDIX

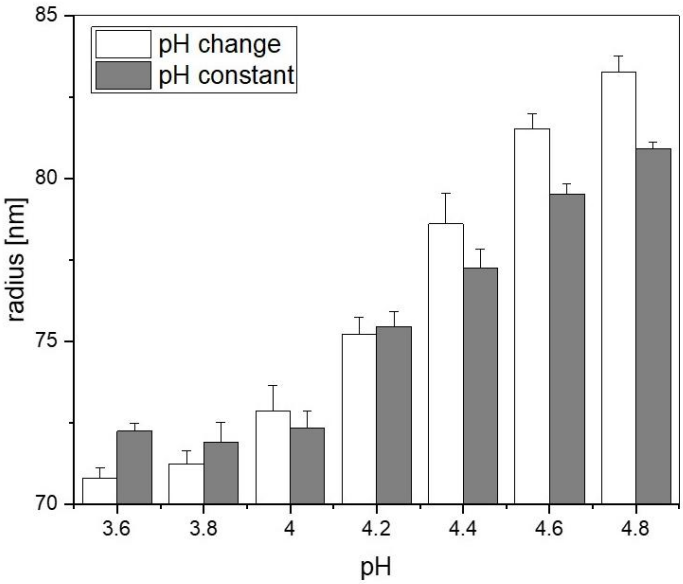
8.1. Figures and tables

		Setup A							Setup B						
	pH	3.5	4.0	4.5	5.0	5.5	6.0	6.5	3.5	4.0	4.5	5.0	5.5	6.0	6.5
Isolation	3.5	-	<0.001	<0.001	<0.001	<0.001	0.006		-	<0.001	<0.001	<0.001	0.022		
	4.0	<0.001	-				<0.001	<0.001	<0.001	-			<0.001	<0.001	<0.001
	4.5	<0.001		-		0.019	<0.001	<0.001	<0.001		-	0.015	<0.001	<0.001	<0.001
	5.0	<0.001			-		0.002	<0.001	<0.001		0.015	-	<0.001	<0.001	<0.001
	5.5	<0.001		0.019		-	0.023	<0.001	0.022	<0.001	<0.001	<0.001	-		0.025
	6.0	0.006	<0.001	<0.001	0.002	0.023	-	0.013		<0.001	<0.001	<0.001		-	
	6.5		<0.001	<0.001	<0.001	<0.001	0.013	-		<0.001	<0.001	<0.001	0.025		-
Calculation	3.5	-	<0.001	<0.001	<0.001	<0.001	<0.001		-	<0.001	<0.001	<0.001	<0.001	0.003	
	4.0	<0.001	-			<0.001	<0.001	<0.001	<0.001	-		0.002	<0.001	<0.001	<0.001
	4.5	<0.001		-		0.006	<0.001	<0.001	<0.001		-	0.003	<0.001	<0.001	<0.001
	5.0	<0.001			-		<0.001	<0.001	<0.001	0.002	0.003	-	0.001	<0.001	<0.001
	5.5	<0.001	<0.001	0.006		-	0.004	<0.001	<0.001	<0.001	<0.001	0.001	-		<0.001
	6.0	<0.001	<0.001	<0.001	<0.001	0.004	-	<0.001	0.003	<0.001	<0.001	<0.001		-	0.002
	6.5		<0.001	<0.001	<0.001	<0.001	<0.001	-		<0.001	<0.001	<0.001	<0.001	0.002	-
Glucose 27 h	3.5	-							-						
	4.0		-							-					
	4.5			-							-				
	5.0				-							-			
	5.5					-							-		
	6.0						-							-	
	6.5							-							-
Mass	3.5	-	0.013	<0.001	<0.001	<0.001	<0.001		-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	4.0	0.013	-	<0.001	<0.001	<0.001	<0.001	0.021	<0.001	-	<0.001	<0.001	<0.001	0.039	<0.001
	4.5	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	0.002	<0.001
	5.0	<0.001	<0.001	<0.001	-	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001
	5.5	<0.001	<0.001	<0.001	0.002	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001
	6.0	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	0.039	0.002	<0.001	<0.001	-	<0.001
	6.5		0.021	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-
Radius	3.5	-	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.042	<0.001	<0.001	<0.001	<0.001	<0.001
	4.0	0.007	-	0.026	<0.001	<0.001	<0.001	<0.001	0.042	-	0.002	<0.001	<0.001	<0.001	<0.001
	4.5	<0.001	0.026	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	-	<0.001	<0.001	<0.001	<0.001
	5.0	<0.001	<0.001	<0.001	-	0.008	<0.001		<0.001	<0.001	<0.001	-	0.007	<0.001	
	5.5	<0.001	<0.001	<0.001	0.008	-	0.030		<0.001	<0.001	<0.001	0.007	-		<0.001
	6.0	<0.001	<0.001	<0.001	<0.001	0.030	-	<0.001	<0.001	<0.001	<0.001	<0.001		-	<0.001
	6.5	<0.001	<0.001	<0.001			<0.001	-	<0.001	<0.001	<0.001		<0.001	<0.001	-
Bradford	3.5	-													
	4.0		-												
	4.5			-											
	5.0				-										
	5.5					-									
	6.0						-								
	6.5							-							

Appendix 1: P-values of all relevant and discussed samples from experimental setup depicted in Figure 2. Empty grey fields refer to p > 0.05.



Appendix 2: Dextran amounts produced according to the experimental setup shown in Figure 2 (2.2.7) with pH values ranging from 3.6 to 4.8. Bars indicate the produced dextran amounts in g/L, which were either determined gravimetrically (isolated dextran) or via calculation of the totally produced amount using the calculated transglycosylation activity (24 h). (A) refers to setup A, (B) to setup B. Data are expressed with mean ± SD of three biological replicates.



Appendix 3: Rms radii [nm] of dextrans produced according to the experimental setups depicted in Figure 2 (2.2.7) with pH values ranging from 3.6 to 4.8. (A) refers to setup A, (B) to setup B. Data are expressed with mean ± SD of three biological replicates.

Setup A					
	set values 7.5 mL start			values 7.5 mL after 3 h	
pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)	pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)
3.5	2.276	5.224	3.37	2.138	5.363
4.0	2.891	4.609	3.88	2.777	4.723
4.5	3.407	4.093	4.38	3.308	4.193
5.0	3.863	3.638	4.77	3.698	3.803
5.5	4.266	3.234	5.25	4.020	3.480
6.0	4.736	2.764	5.73	4.442	3.058
6.5	5.325	2.175	6.21	4.958	2.543
	set values in 15 mL			required buffers 7.5 mL	
pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)	pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)
3.5	4.553	10.448	3.6	2.415	5.085
4.0	5.783	9.218	4.1	3.006	4.494
4.5	6.814	8.186	4.6	3.506	3.994
5.0	7.725	7.275	5.2	4.028	3.473
5.5	8.531	6.469	5.8	4.511	2.989
6.0	9.473	5.528	6.3	5.031	2.469
6.5	10.650	4.350	6.7	5.693	1.808
Setup B					
	set values 7.5 mL start			values 7.5 mL after 3 h	
pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)	pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)
3.5	2.276	5.224	4.4	3.308	4.193
4.0	2.891	4.609	4.4	3.308	4.193
4.5	3.407	4.093	4.4	3.308	4.193
5.0	3.863	3.638	4.4	3.308	4.193
5.5	4.266	3.234	4.4	3.308	4.193
6.0	4.736	2.764	4.4	3.308	4.193
6.5	5.325	2.175	4.4	3.308	4.193
	set values in 15 mL			required buffers 7.5 mL	
pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)	pH	0.2 M Na2HPO4 (mL)	0.1 M citric acid (mL)
3.5	4.552	10.448	2.8	1.244	6.255
4.0	5.782	9.218	3.6	2.474	5.025
4.5	6.814	8.186	4.6	3.506	3.993
5.0	7.726	7.276	5.7	4.418	3.083
5.5	8.532	6.468	6.4	5.224	2.275
6.0	9.472	5.528	7.0	6.164	1.335
6.5	10.65	4.350	8.2	7.342	0.157

Appendix 4: Volumes of citrate and phosphate buffers added to the respective samples from experimental setup A and B to obtain the desired final pH value on the basis of McIlvaine (1921).

Appendix 5: List of genes/gene cluster with their respective locus tags of a selection of potentially relevant metabolic traits for the adaption to plant fermentations.

		1.1822 BSQ49_	**2.340 PECL_RS**	**1.142 G9276_**	**1.928 DT351_**	**1.1478 CEB41_**	**1.617 G9277_**	**1.64 G9279_**	**1.411 DT321_**
EPS	Dextransucrase	11535			11209				09485
	Levansucrase			00805			01110		
	Glycosyltransferase 2		09485						
	HePS-cluster					05155 - 05245		11825 - 11870	
Tagatose-6-phosphate pathway	Galactose-6-phosphate isomerase			07095 -07100	06605 -06610 01730 -01735				
	Tagatose-6-phosphate kinase				01740				
	Tagatose-1,6-bisphosphate aldolase		00195	06100					
	Tagatose-6-phopshate-4-epimerase								
Leloir pathway	galactose-mutarotase	06265	07215	01070	06555	15455, 03570, 07770	05690, 07025, 11520	00835, 06515, 09470	02515
	galactokinase		07945	01080	06570	15430	11545	09495	02500
	galactose-1-phosphate uridyltransferase								
	UDP-galactose 4-epimerase								

Carbohydrate utilization / transport	Cellobiose	01210, 01220, 08995, 09005	00665, 08195	06690, 06695, 06720, 07110	00915, 01750, 02695	01200, 06110, 12400, 13400, 13940			00340
	Xylose			05960		04320, 11900	09395, 14795	05180, 07205, 09115,	
	Arabinose	09875		07275			00100, 13735, 15560, 11590	02505, 08795, 09155	03945
	Ribose			08530	09795,09800	16105	00170	08725	06150
	ß-glucoside transporter	09270, 09575, 09740, 00775, 01110, 05795		04675	10810	00775, 14380	01070, 03660	06840, 13515, 03215	04280
	(6-p-) ß-glucosidases	00615, 01115, 01130, 01215, 04165, 06130, 08990, 09010, 09260, 09265, 09570, 09735	08625,00670	06685 07380	01515, 01530	01880, 12390, 12395, 13935	03230, 12330, 13400, 13405	04190, 04695, 04700, 14730	
Peptide transporter		00740, 01060, 02080, 02225, 02245, 02650, 05400, 05570, 06940, 09250, 10460, 11180, 11940	00405, 01365, 01680, 06865, 08205,	06415, 06420, 07635, 08105, 08220, 08935, 08955	01575, 02315, 04545, 06955, 07870, 07940, 09855	00080, 00385, 00825, 00830, 01805, 02240, 02360, 03350, 05525	01125, 05885, 10860, 12255, 16440	02535, 02820, 03265, 03270, 04110, 08450, 08615, 12520, 13820	00035, 02190, 04725, 09405, 09710
Extracellular peptidase*		01515, 07205, 07310, 10235, 11125	00075, 00815, 03960,04940, 06295, 09750, 07135, 07880, 08730, 09225	05065, 05205, 06395	01055, 01545, 05355, 05440, 09345, 09945, 11160	02255, 04345, 04420, 04880, 05435, 05595, 06135, 06200, 09770, 13725, 14030, 14250	03020, 03310, 03530, 09295, 09370, 10790, 10945, 11420, 13985, 13985	02165, 07230, 07305, 07735, 08365, 08520, 09750, 10315, 13385, 14650	03460, 06180, 07040, 07830, 08440, 09430
α-amylase α-glucosidase		01980, 09930 10580, 11585	08175, 08175, 08180, 01900	06830, 06870		00740, 00715, 00795, 00810, 10670, 14400, 16040	01035, 00090, 01010, 01085, 01100, 03665, 05065	03180, 03155, 03230, 03245, 08805, 08870, 13520	04285

aa-decarboxylases	Glutamate					15135	11810	
	Ornithine	00470	04280					
	Tyrosine			06540				
	Lysine							09755

* prediction via identification of signal peptide

8.2. List of publications

Parts of this thesis have been published, with Jonas Schmid as principal investigator and first author.

Peer-reviewed Journals

Schmid, J., Bechtner, J., Vogel, R. F., & Jakob, F. (2019). **A systematic approach to study the pH-dependent release, functionality and product specificity of dextransucrases**. *Microbial Cell Factories*, 18(1), 153.

Schmid, J., Wefers, D., Vogel, R. F., & Jakob, F. (2021). **Analysis of structural and functional differences of glucans produced by the natively released dextransucrase of *Liquorilactobacillus hordei* TMW 1.1822**. *Applied Biochemistry and Biotechnology,* **193**, 96–110.

Bechtner, J., Wefers, D., Schmid, J., Vogel, R. F., & Jakob, F. (2019). **Identification and comparison of two closely related dextransucrases released by water kefir borne *Lactobacillus hordei* TMW 1.1822 and *Lactobacillus nagelii* TMW 1.1827**. *Microbiology, 165*(9), 956-966.

Poster presentations

Schmid, J., Jakob, F., Vogel, R.F. (2017). **Characterization of exopolysaccharides of lactic acid bacteria to improve the texture of vegan yoghurt alternatives**. *LAB12*.

Schmid, J., Jakob, F., Vogel, R.F. (2019). **Characterization of the production and structure of dextran synthesized by *Lactobacillus hordei* TMW 1.1822 at different pH values**. *FEMS 2019.*