Institute of Food Science and Biotechnology/Dept. of Food Microbiology and Hygiene

Selection and Characterization of Exopolysaccharide Producing Lactic Acid Bacteria for the Stabilization of Fruit Preparations

Dor Zipori

Stabilization of Fruit Preparations – Microbes instead of Chemicals?

Intermediate products in the food industry

Uniform distribution of fruit pieces → use of additives in form of **hydrocolloids**

Fig. 1: Commercial strawberry fruit preparation [1]

Modified starches

Amidated pectin
(E440ii)

Xanthan
(E 415)

Hydroxypropyl Methyl Cellulose
(E464)

Stabilization of Fruit Preparations – Microbes instead of Chemicals?

Intermediate products in the food industry

Uniform distribution of fruit pieces → use of additives in form of **hydrocolloids**

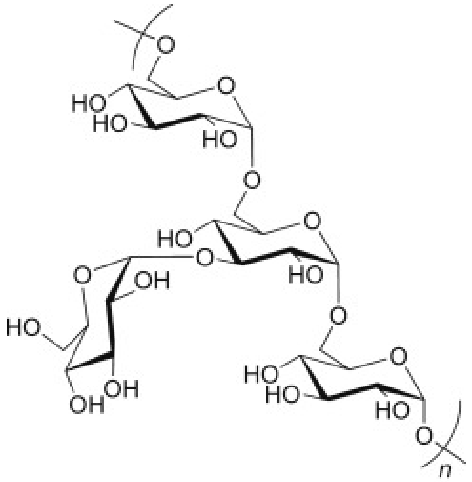
Fruit components

Fruit preparation

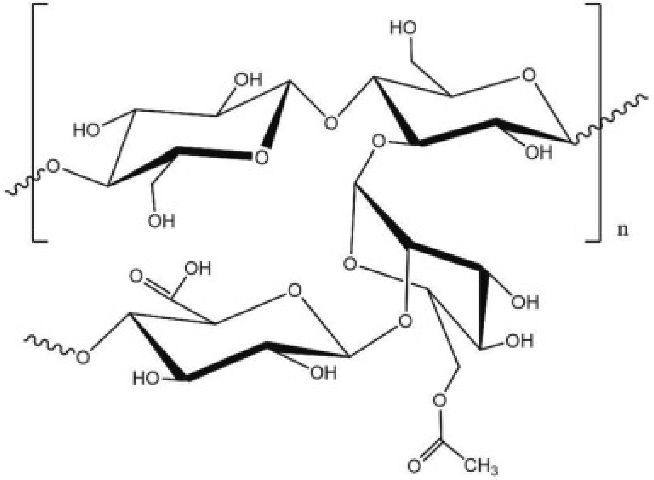
Fermentation with exopolysaccharide - forming lactic acid bacteria (LAB)

Exopolysaccharides (EPS): Texture Enhancer for Novel Foods

- Sugar polymers
- Release to the surrounding environment or bound onto the cell surface
- Biological function: survival in harsh environments
- In form of **Homopolysaccharides** (HoPS) or **Heteropolysaccharides** (HePS)

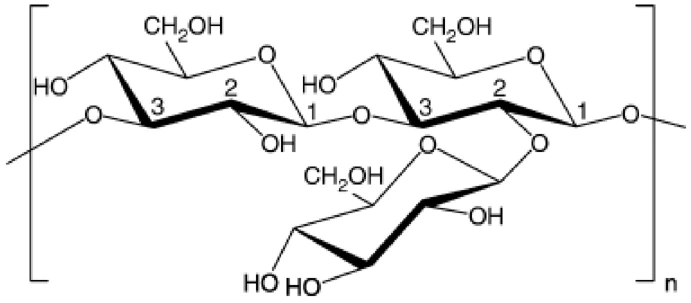


Dextran
(HoPS)

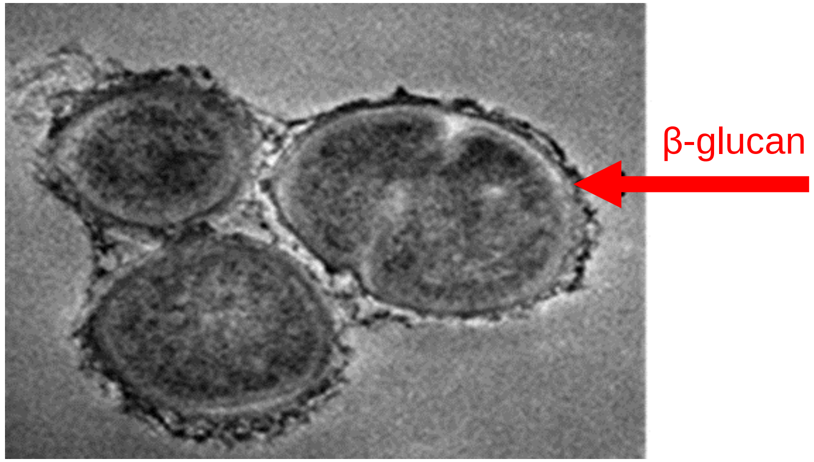


Xanthan
(HePS)

β-D-Glucan: Hidden Potential for Food Applications



Microbial O2-substituted (1,3)-β-D-glucan



β-glucan network around *Pediococcus parvulus* [3]

“Ropiness” in beer [4]

β-D-Glucan: Hidden Potential for Food Applications

Prebiotics properties

High viscosity

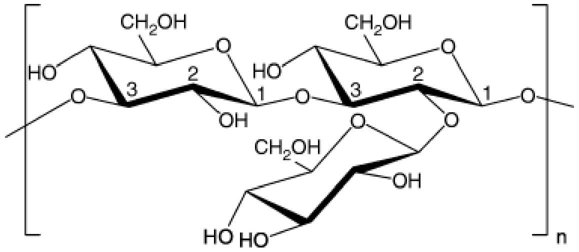
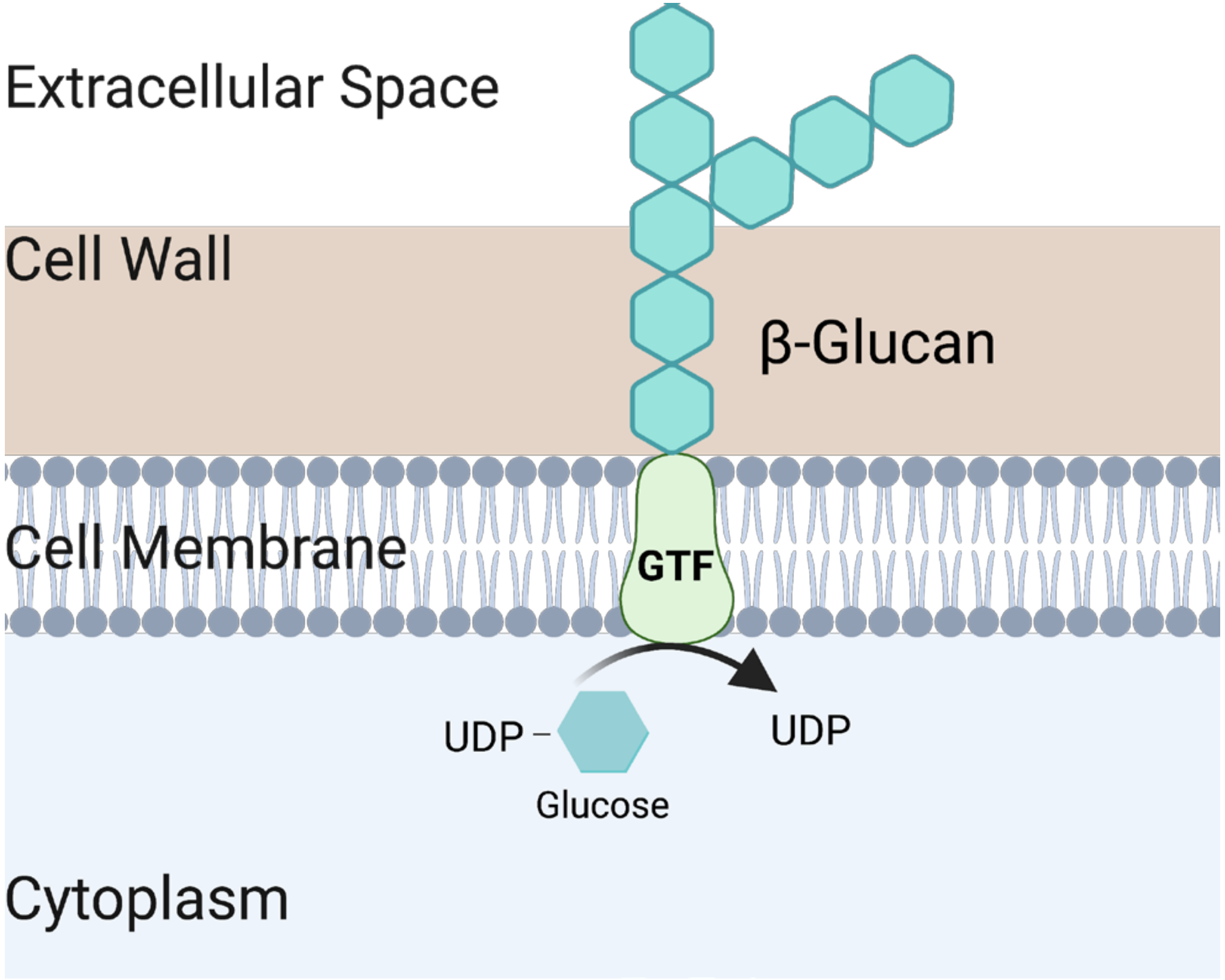


Fig. 2 Microbial O2-substituted (1,3)-β-D-glucan

Heat and acid stability

Multiple sugar precursors

Mechanisms of β-glucan synthesis



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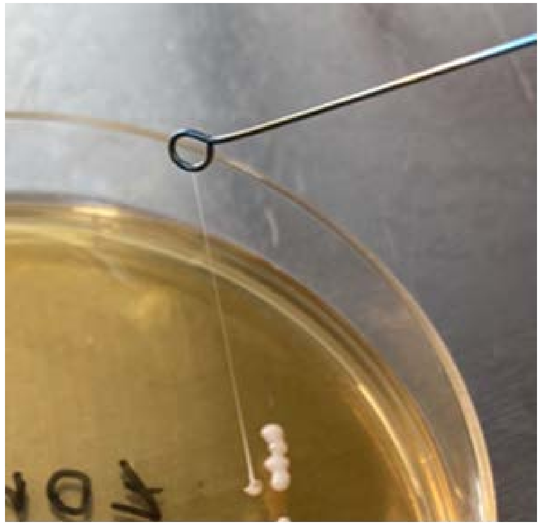
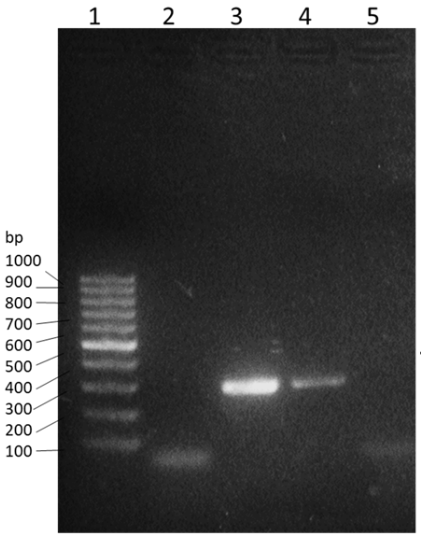
Aim and Research Objectives

- Establishing a screening method for the selection of β-glucan LAB strains
- Characterization of the selected LAB strains
- Development of technological approach for stabilization of fruit preparations using selected LAB strains

Screening and Selection of β-Glucan Producing LAB strains

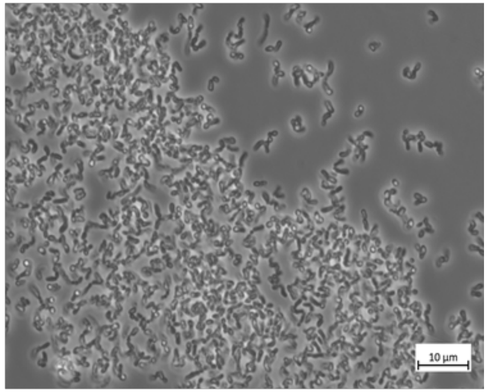


Loop-Test
EPS-formation



1: 100 bp DNA ladder
2: Non-template control
3: Pos. control
4. *L. paracasei* LTH 2407
5. Neg. control

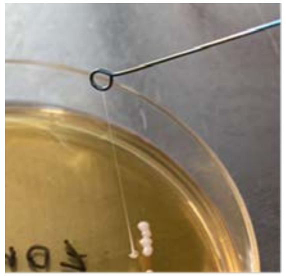
gtf **PCR**
Detection of β-glucan synthase-gene (*gtf-2*)



Agglutination Test
Detection of capsular β-glucan with specific antibodies

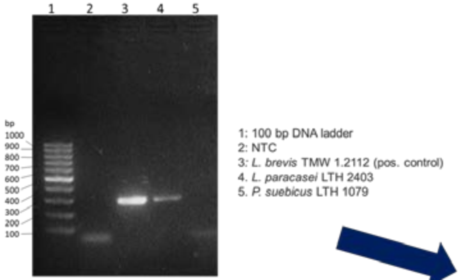
Selected LAB Strains for β-Glucan Production

- 247 LAB strains screened
  - *Pediococcus* (22) *Leuconostoc* (17), *Lactococcus* (2), *Oenococcus* (32), *Weissella* (4) and former *Lactobacillus* (170)



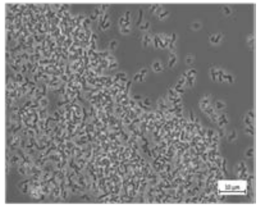
Loop-Test
EPS-formation

(+) 27 Strains



gtf PCR
Detection of β-glucan synthase-gene (*gtf-2*)

(+) 8 Strains



Agglutination Test
Detection of capsular β-glucan with specific antibodies

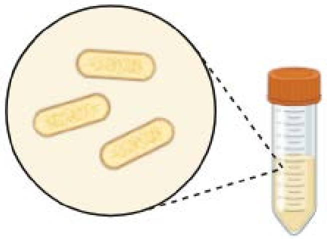
(+) 8 Strains

2 x *Pediococcus parvulus*
1 x *Pediococcus damnosus*
1 x *Pediococcus claussenii*
1 x *Lacticaseibacillus paracasei*
1 x *Levilactobacillus brevis*
2 x *Furfurilactobacillus rossiae*

P. parvulus LTH 1110
L. brevis TMW 1.2112

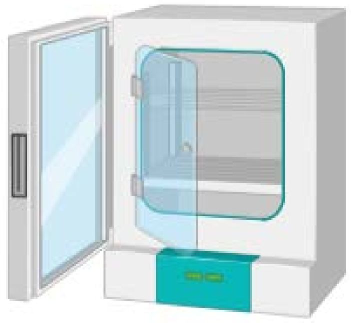
Stabilization of Fruit Preparations using β-Glucan-forming LAB

- Approach: production of fermentate → replacement of hydrocolloid
- Fermentation of peach and pear purees (n=3)

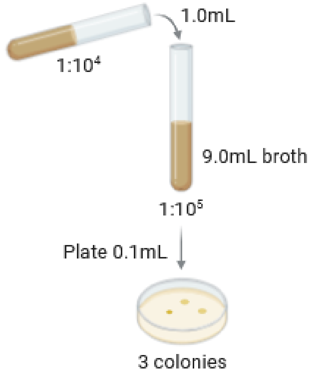


Inoculum
P. parvulus LTH 1110
or
L. brevis TMW 1.2112

Fermentation mixture
150 mL peach or pear puree
Initial bacterial count: ~10^7 cfu/mL
+ uninoculated control



Incubation
30 °C, 72 h

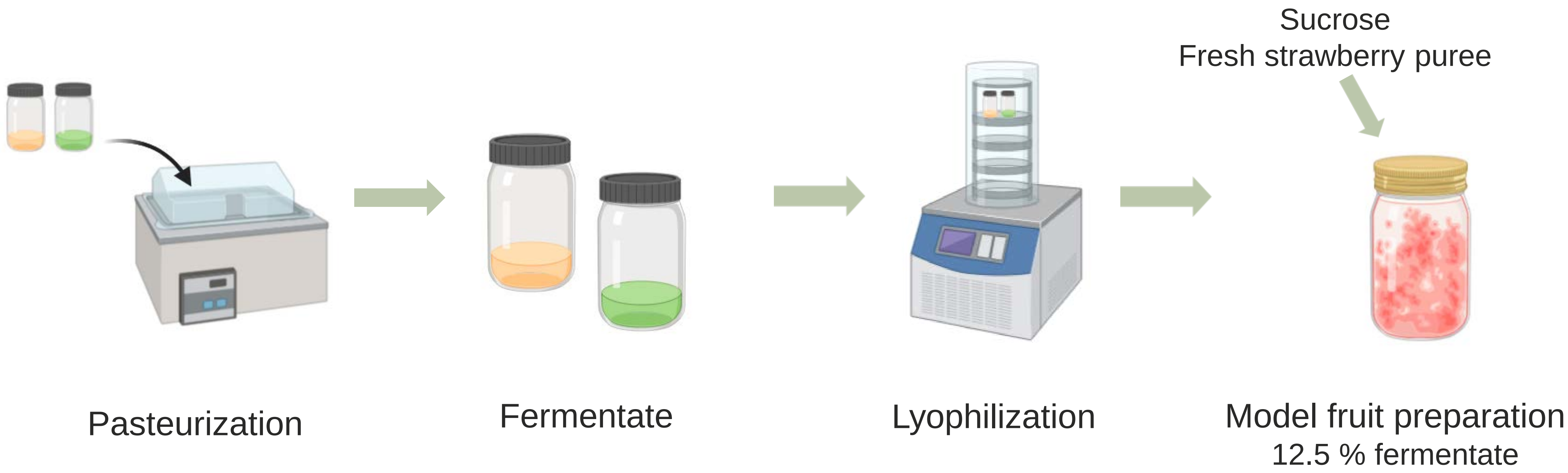


Viable count determination
and pH measurement
0 h and 72 h

(created using Biorender)

Stabilization of Fruit Preparations using β-Glucan-forming LAB

- Approach: production of fermentate → replacement of hydrocolloid



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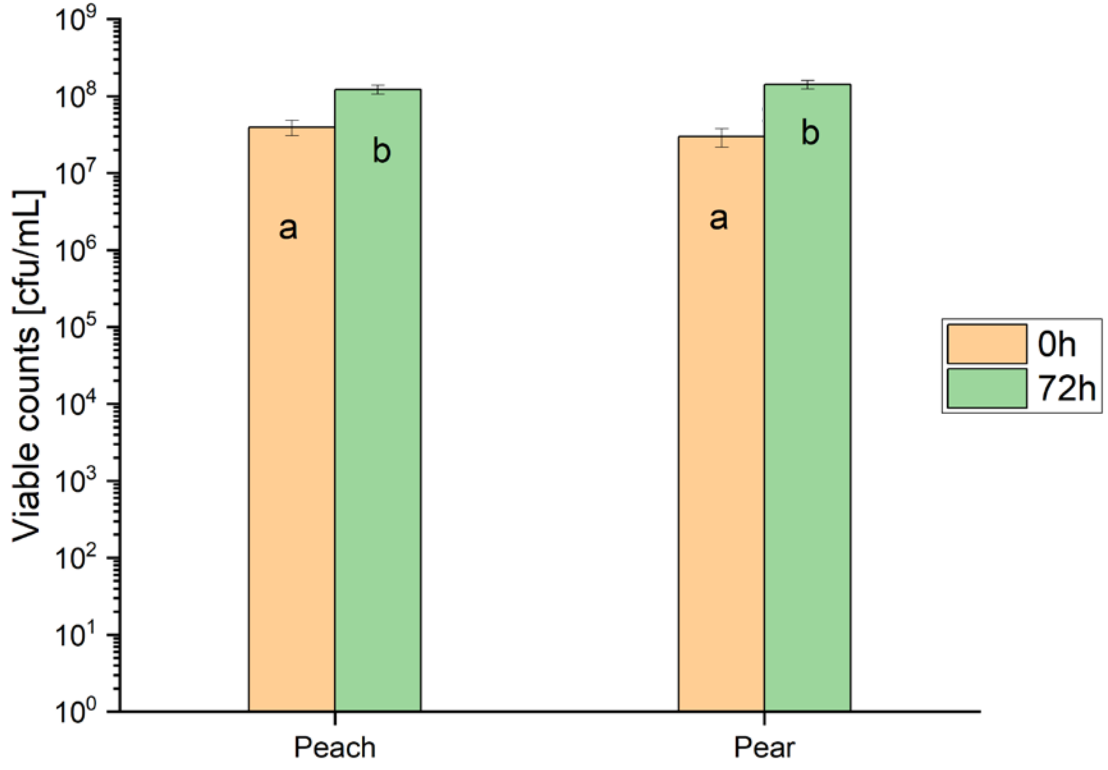
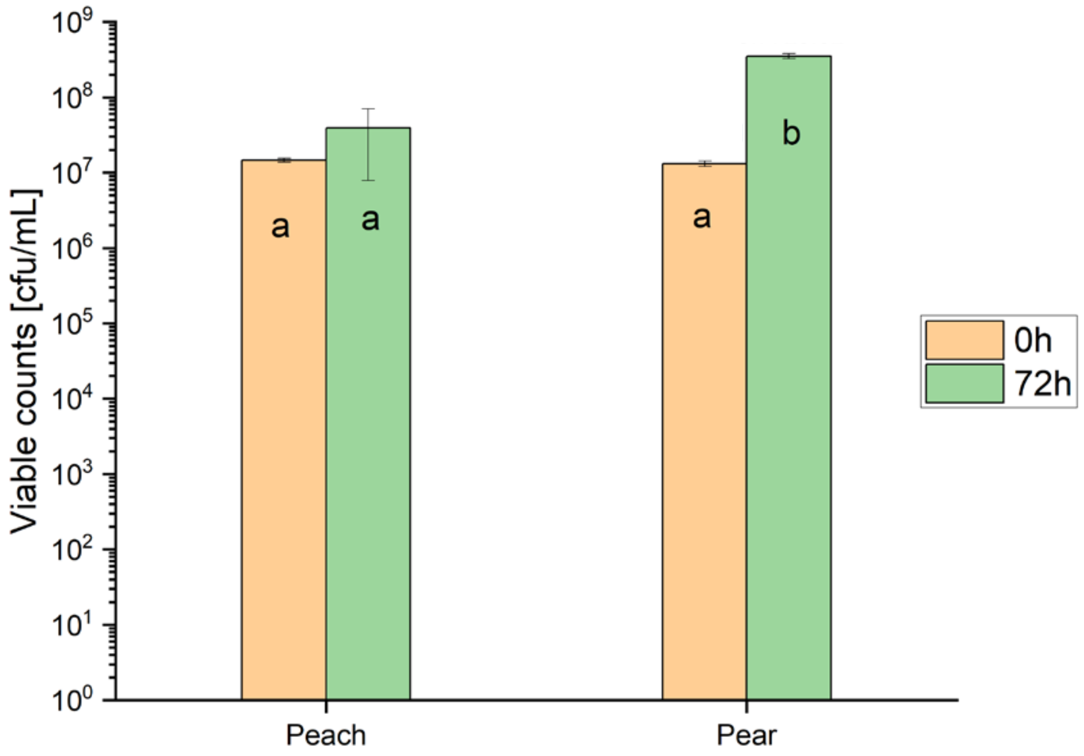
LAB Strains Exhibit Growth during Fruit Fermentation



L. brevis TMW 1.2112



P. parvulus LTH 1110

Bacterial count of LAB strains and the pH values of peach and pear purees at the beginning and at end of the 72 h fermentation.

Bacterial count values with the same letters are not significantly different ($p \geq 0.05$)

Chemical Composition of Fruit Fermentates

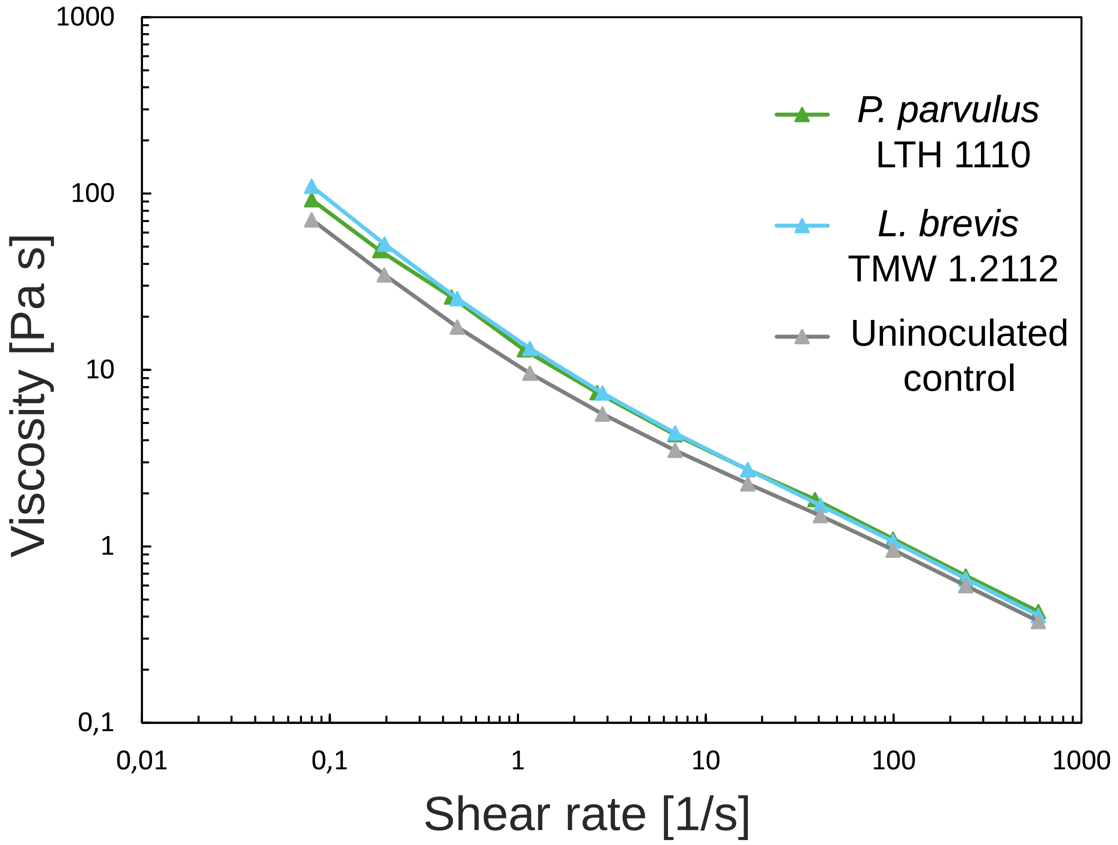
Content of free sugars, organic acids and ethanol in fermented peach puree [g/kg]

	Uninoculated control	*L. brevis* TMW 1.2112	*P. parvulus* LTH 1110
Substance			
Sorbitol	3.6 ± 0.4[a]	3.4 ± 0.2[a]	3.2 ± 0.2[a]
Mannitol	n.d.[a]	3.1 ± 0.3[b]	n.d.[a]
Glucose	21.7 ± 0.6[a]	20.1 ± 1.0[a]	20.4 ± 1.5[a]
Sucrose	26.3 ± 2.4[a]	25.7 ± 1.3[a]	25.7 ± 4.4[a]
Fructose	23.0 ± 1.9[a]	19.6 ± 1.4[a]	21.9 ± 2.9[a]
Total sugars	**74.6 ± 3.6**[a]	**72.0 ± 3.5**[a]	**71.3 ± 9.0**[a]
Acetate	0.07 ± 0.05[a]	0.84 ± 0.13[b]	0.08 ± 0.01[a]
Citrate	0.13 ± 0.03[a]	0.10 ± 0.04[a]	n.d.[a]
Malate	3.10 ± 0.10[a]	n.d.[b]	1.13 ± 0.60[c]
D-lactate	0.70 ± 0.24[a]	1.21 ± 0.28[b]	0.51 ± 0.03[a]
L-lactate	0.16 ± 0.27[a]	2.93 ± 0.36[b]	1.80 ± 0.39[c]
Ethanol	0.01 ± 0.01[a]	0.04 ± 0.01[a]	0.04 ± 0.01[a]

n.d.: below detection limit

Values in one row with no common letter are significantly different ($p \leq 0.05$)

Viscosity Increase of Fruit Preparation Through Fermentation



Flow curve of model fruit preparations containing lyophilized peach fermentates (n=9)

Viscosity of model fruit preparation with LAB - fermentates

Shear rate [1/s]	**Viscosity [Pa s]**		
	Uninoculated control	*L. brevis* TMW1.2112	*P. parvulus* LTH 1110
0.08	71.1 ± 15.0[a]	110.00 ± 12.0[b]	92.4 ± 28.0[b]
2.82	5.6 ± 0.7[a]	7.4 ± 0.6[b]	7.2 ± 1.9[b]
99.33	1.0 ± 0.1[a]	1.1 ± 0.0[a]	1.1 ± 0.2[a]

Values in one row with no common letter are significantly different ($p \leq 0.05$)

Viscosity increase through fermentation

Shear rate [1/s]	**Factor of viscosity change [-]**	
	$(\eta_{L.\ brevis} / \eta_{Control})$	$(\eta_{p.\ parvulus} / \eta_{Control})$
0.08	1.5	1.30
2.82	1.3	1.3
99.33	1.1	1.2

Summary and Final Discussion

- Eight β-D-glucan- forming strains selected for fruit fermentation
- Moderate growth of two LAB strains in the fruit matrix
- No significant change in sugar content through fermentation
- Significant involvement of malic acid metabolism
- Increase in viscosity through fermentation with selected LAB strains only in the case of peach fermentate

Strawberry fruit preparation containing peach fermentate of *L. brevis* TMW 1.2112

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Thank you for your attention!