prehrambeno biotehnološki fakultet | faculty of food technology and biotechnology

Sveučilište u Zagrebu
University of Zagreb

Understanding of metabolic flexibility of lactic acid bacteria under severe impertinence

Anita Slavica

International Society of Biocatalysis and Agricultural Biotechnology (http://www.isbab.org)

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and

President Jei-Fu Shaw of the National Chung Hsing University (NCHU), Taichung, Taiwan

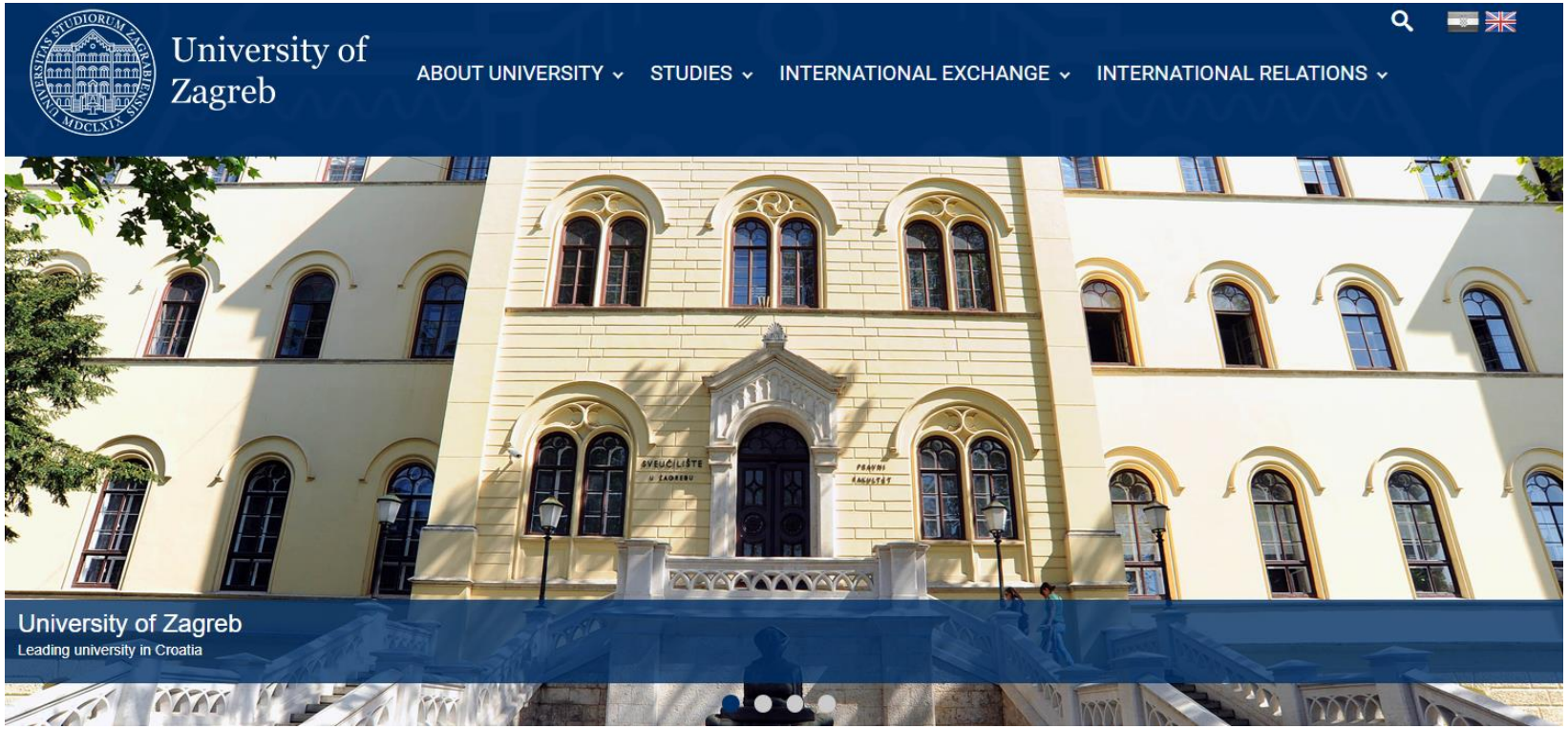
Professor Milan Čertík, PhD, Slovak University of Technology, Slovak Republic,

Chair of the 18thISBAB PS

Thank you for your kind invitation!

. University of Zagreb (https://www.unizg.hr); the oldest university in Southeast Europe established in 1669

. University of Zagreb Faculty of Food Technology and Biotechnology (http://www.pbf.unizg.hr/)



. overwiev

. stressors

.. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531

... proteolitic, amylolytic and fermentative activity

... production of different ratios of D-/L-lactic acid by three *Lactobacillus* sp.

(*L. amylovors* DSM 20531, *L. rhamnosus* DSM 20021, and L. *coryniformis* subsp. *torquens* DSM 20004)

... doors of opportunity - manufacturing of high-value chemicals from sustainably produced D-/L-lactic acid

.. effect of dissolved oxygen concentration on acetate production by *L. coryniformis* subsp. *torquens* DSM 20004

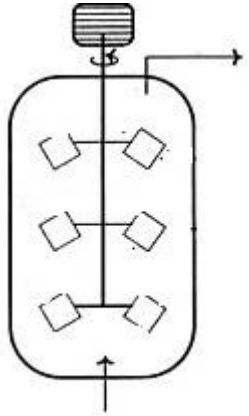
.. encapsulation of *L. amylovors* DSM 20531 with polymeric substrate - towards integrated process for ethyl lactate production

.. production of α-D-glucosylglycerol by *Leuconostoc mesenteroides* LMG 7954 under osmotic stress

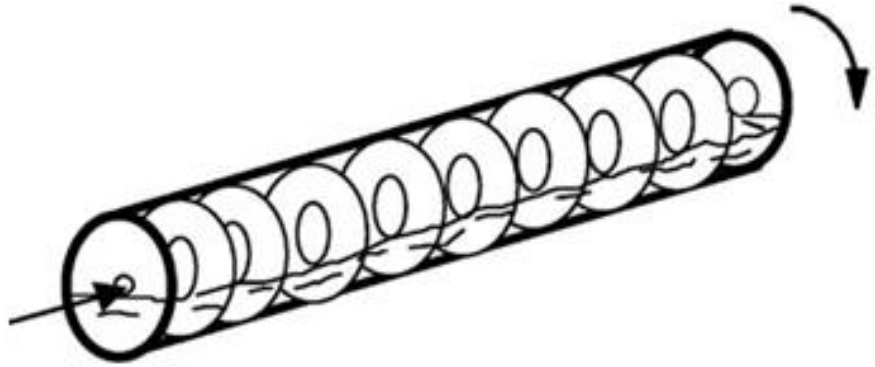
future plans

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531

polenta



STB



HRTB

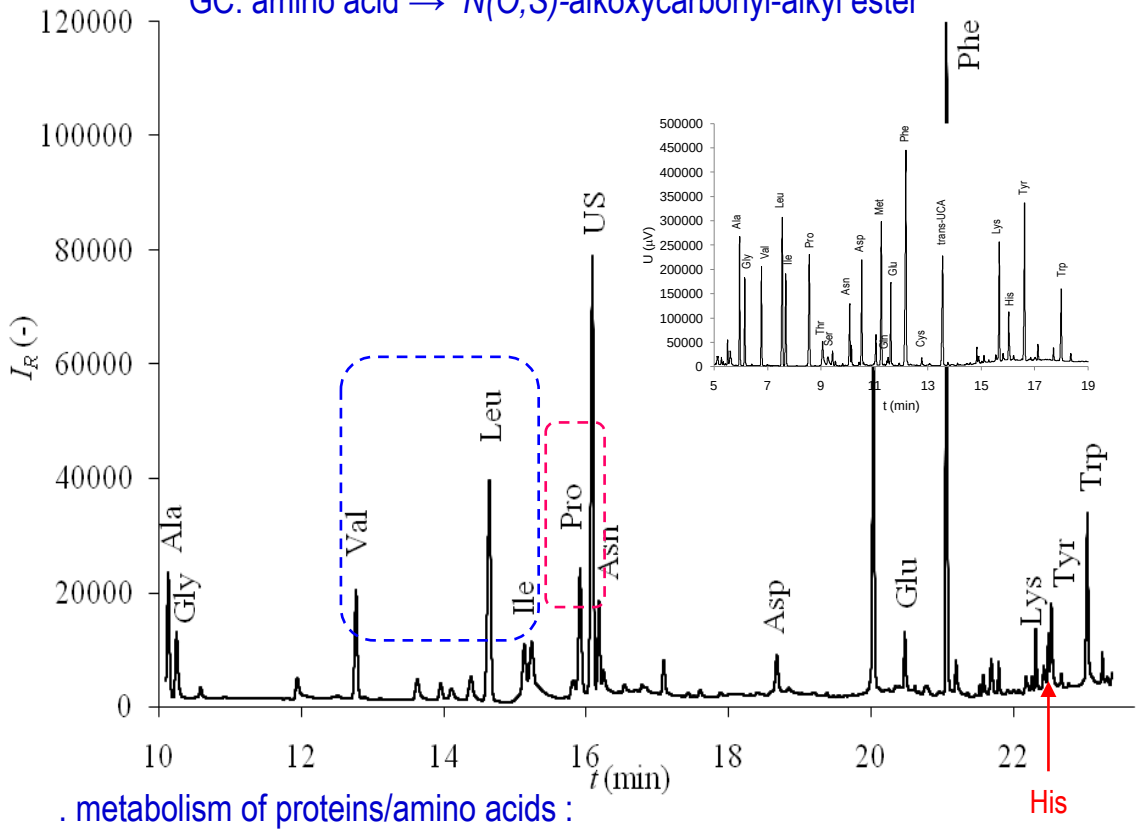
cultivations:
- . batch
- . fed-batch
- . semicontinuous
- . continuous

unpublished data

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: proteolytic activity

polenta

GC: amino acid → *N(O,S)*-alkoxycarbonyl-alkyl ester



. metabolism of proteins/amino acids :

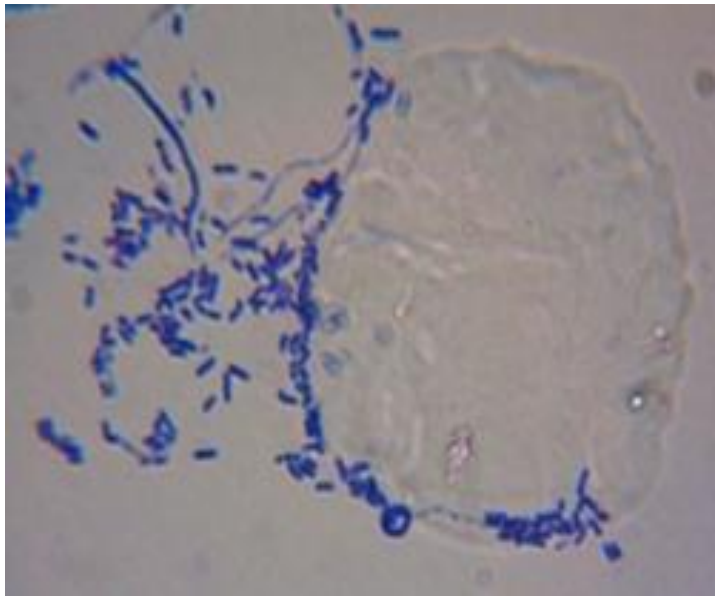
accumulation of Pro (osmotic stress);

His concentration below detection level;

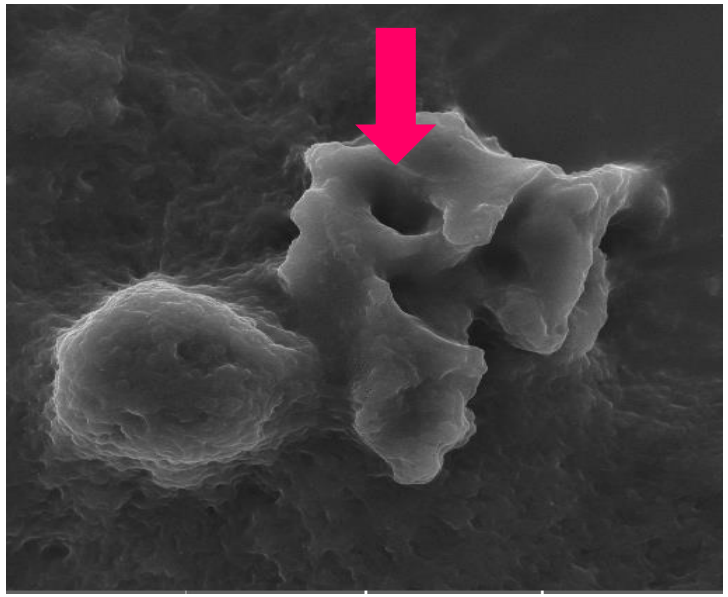
very low concentration of branched-chain amino acids.

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: amylolytic activity

polenta



microscopic image of *L. amylovorus* DSM 20531 attached to starch granules



SEM image of starch granules obtained during hydrolysis by *L. amylovorus* DSM 20531 amylases

unpublished data

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: amylolytic activity

polenta

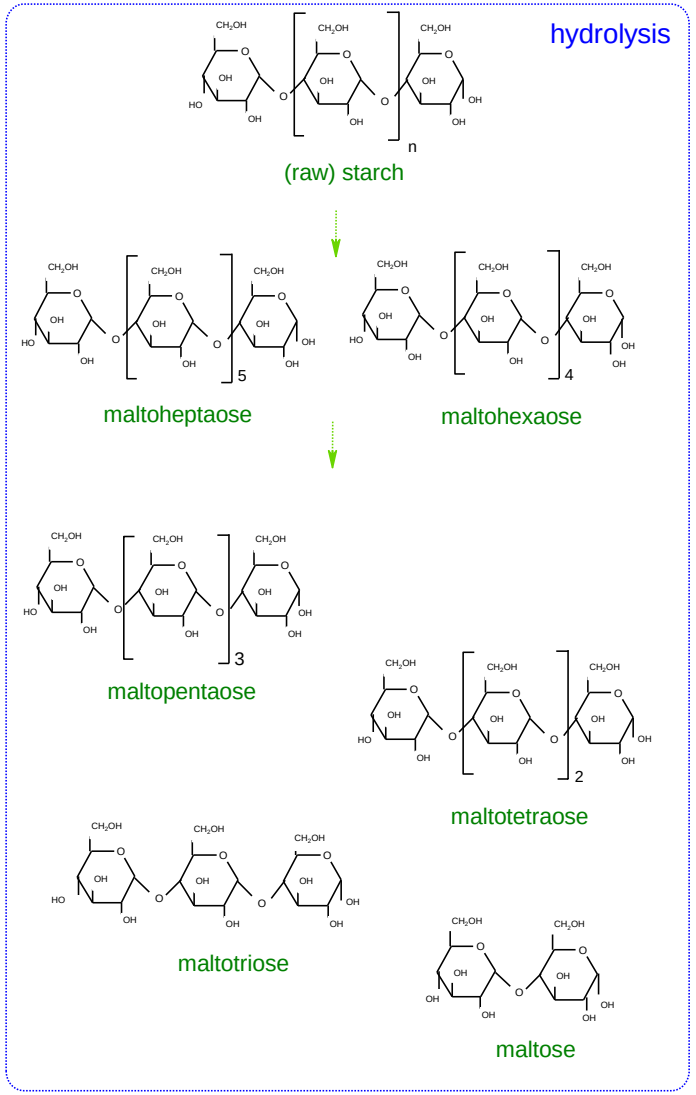
. complete hydrolysis of linear and branched chains of (raw) starch to maltooligosaccharides and maltose

unpublished data

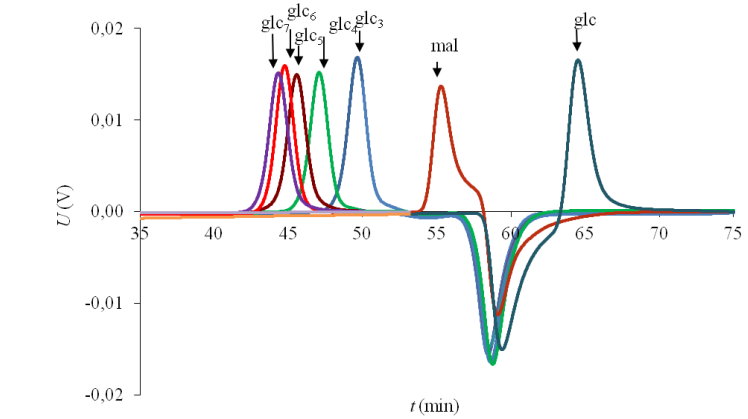
. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: amylolytic activity

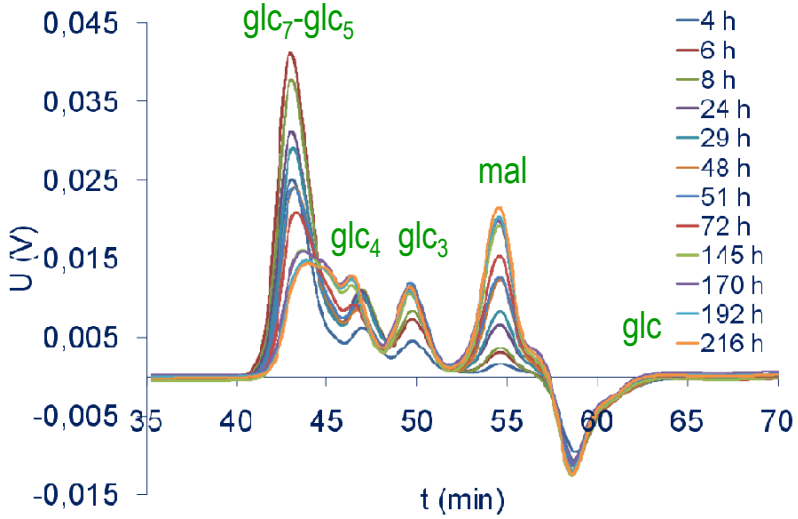
. complete hydrolysis of linear and branched chains of (raw) starch to maltooligosaccharides and maltose

. mixture of maltooligosaccharides - a platform mixture to be used in numerous enzymatic and whole-cell catalyzed reactions



IEX-HPLC





unpublished data

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: proteolytic, amylolytic and fermentative activity

polenta → D-/L-lactic acid solution

unpublished data

. a high initial concentration of polymeric substrates - case of *Lactobacillus amylovorus* DSM 20531: ~~proteolytic~~, amylolytic and fermentative activity

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polenta → D-/L-lactic acid solution

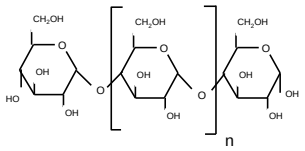
I. Inkret, I. Jakša

D. Kolanović

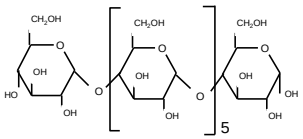
D. Kostelac

D. Švec

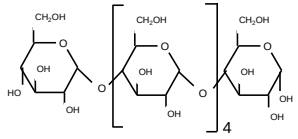
hydrolysis



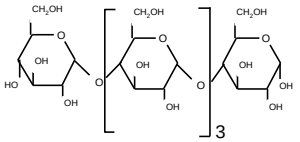
(raw) starch



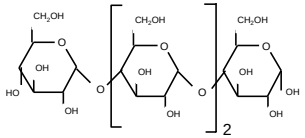
maltoheptaose



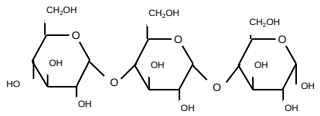
maltohexaose



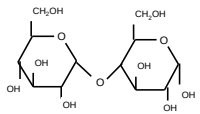
maltopentaose



maltotetraose

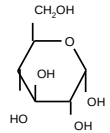


maltotriose

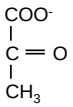


maltose

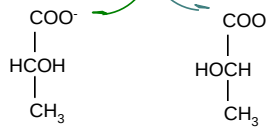
fermentation



glucose



pyruvate



D-lactate L-lactate

A. Trontel

I. Dusper

V. Tadić

A. Polenus

unpublished data

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$$\left(\frac{d\gamma_{glc}}{dt}\right)_{s_5 f} = \left(\frac{d\gamma_{glc}}{dt}\right)_h + \left(\frac{d\gamma_{glc}}{dt}\right)_f$$

$$\left(\frac{d\gamma_{mal}}{dt}\right)_{s_5 f} = \left(\frac{d\gamma_{mal}}{dt}\right)_h + \left(\frac{d\gamma_{mal}}{dt}\right)_f$$

$$\left(\frac{d\gamma_{glc}}{dt}\right)_h = r_{glc_7/glc+glc_6} + r_{glc_6/glc+glc_5} + r_{glc_5/glc+glc_4} + r_{glc_4/glc+glc_3}$$

$$\left(\frac{d\gamma_{mal}}{dt}\right)_h = r_{glc_7/mal+glc_5} + r_{glc_6/mal+glc_4} + r_{glc_5/mal+glc_3} + r_{glc_4/mal+mal}$$

$$\left(\frac{d\gamma_{glc_3}}{dt}\right)_h = r_{škrob/glc_3} + r_{glc_{\rangle 7}/glc_3} + r_{glc_7/glc_3+glc_4} + r_{glc_6/glc_3+glc_3} + r_{glc_5/mal+glc_3} + r_{glc_4/glc+glc_3}$$

$$\left(\frac{d\gamma_{glc_4}}{dt}\right)_h = r_{škrob/glc_4} + r_{glc_{\rangle 7}/glc_4} + r_{glc_7/glc_3+glc_4} + r_{glc_6/mal+glc_4} + r_{glc_5/glc+glc_4} - r_{glc_4/mal+mal} - r_{glc_4/glc+glc_3}$$

$$\left(\frac{d\gamma_{glc_5}}{dt}\right)_h = r_{škrob/glc_5} + r_{glc_{\rangle 7}/glc_5} + r_{glc_7/mal+glc_5} + r_{glc_6/glc+glc_5} - r_{glc_5/mal+glc_3} - r_{glc_5/glc+glc_4}$$

$$\left(\frac{d\gamma_{glc_6}}{dt}\right)_h = r_{škrob/glc_6} + r_{glc_{\rangle 7}/glc_6} + r_{glc_7/glc+glc_6} - r_{glc_6/glc_3+glc_3} - r_{glc_6/mal+glc_4} - r_{glc_6/glc+glc_5}$$

$$\left(\frac{d\gamma_{glc_7}}{dt}\right)_h = r_{škrob/glc_7} + r_{glc_{\rangle 7}/glc_7} - r_{glc_7/glc+glc_6} - r_{glc_7/mal+glc_5} - r_{glc7/glc_3+glc_4}$$

$$\left(\frac{d\gamma_{glc_{5-7}}}{dt}\right)_h = \left(\frac{d\gamma_{glc_5}}{dt}\right)_h + \left(\frac{d\gamma_{glc_6}}{dt}\right)_h + \left(\frac{d\gamma_{glc_7}}{dt}\right)_h$$

$$\left(\frac{d\gamma_{glc_{\rangle 7}}}{dt}\right)_h = r_{škrob/glc_{\rangle 7}} - r_{glc_{\rangle 7}/glc_n} \qquad \left(\frac{d\gamma_{škrob}}{dt}\right)_h = -r_{škrob/glc_{\rangle 7}} - r_{škrob/glc_n}$$

$$r_{škrob/glc_{\rangle 7}} = k_{škrob,glc_{\rangle 7}} \cdot \gamma_{škrob}$$

$$r_{škrob/glc_n} = \sum_3^7 k_{škrob,glc_n} \cdot \gamma_{škrob}$$

$$r_{glc_{\rangle 7}/glc_n} = \sum_{n=3}^{7} k_{glc_{\rangle 7},glc_n} \cdot \gamma_{glc_{\rangle 7}}$$

$$r_{glc_7/glc_n+glc_{7-n}} = \sum_{n=1}^{3} k_{glc_7,glc_n+glc_{7-n}} \cdot \gamma_{glc_7}$$

$$r_{glc_6/glc_n+glc_{6-n}} = \sum_{n=1}^{3} k_{glc_6,glc_n+glc_{6-n}} \cdot \gamma_{glc_6}$$

$$r_{glc_5/glc_n+glc_{5-n}} = \sum_{n=1}^{2} k_{glc_5,glc_n+glc_{5-n}} \cdot \gamma_{glc_5}$$

$$r_{glc_4/glc_n+glc_{4-n}} = \sum_{n=1}^{2} k_{glc_4,glc_n+glc_{4-n}} \cdot \gamma_{glc_4}$$

unpublished data

$$\left(\frac{d\gamma_{glcNH}}{dt}\right)_{s_5 f} = \left(\frac{d\gamma_{glc}}{dt}\right)_{s_5 f} + \left(\frac{d\gamma_{mal}}{dt}\right)_{s_5 f} + \left(\frac{d\gamma_{glc_3}}{dt}\right)_h + \left(\frac{d\gamma_{glc_4}}{dt}\right)_h + \left(\frac{d\gamma_{glc_{5-7}}}{dt}\right)_h + \left(\frac{d\gamma_{glc_{>7}}}{dt}\right)_h + \left(\frac{d\gamma_{škrob}}{dt}\right)_h$$

$$\left(\frac{d\gamma_{glc}}{dt}\right)_f = -\frac{1}{Y_{X_1/glc}} \cdot \frac{d\gamma_{X_1}}{dt} - m_s \cdot \gamma_{X_1}$$

$$\left(\frac{d\gamma_{mal}}{dt}\right)_f = -\frac{1}{Y_{X_1/mal}} \cdot \frac{d\gamma_{X_1}}{dt} - m_s \cdot \gamma_{X_1}$$

$$\frac{d\gamma_{UH}}{dt} = -\frac{1}{Y_{X/UH}} \cdot \frac{d\gamma_X}{dt} - m_S \cdot \gamma_X$$

$$\frac{d\gamma_X}{dt} = \mu \cdot \gamma_X - k_D \cdot \gamma_X$$

$$\mu = \mu_m \cdot \frac{\gamma_{UH}}{K_{UH} + \gamma_{UH}} \cdot \frac{K_i}{K_i + \gamma_{MK}}$$

$$k_D = k_{D_0} \cdot (1 + C \cdot \gamma_{MK})$$

$$\frac{d\gamma_{MK}}{dt} = \alpha_{MK} \cdot \frac{d\gamma_X}{dt} + \beta_{MK} \cdot \gamma_X$$

unpublished data

. production of different ratios of D-/L-lactic acid

substrate
glc, glucose
starch
cgs, corn grits
suc, sucrose
mal, maltose

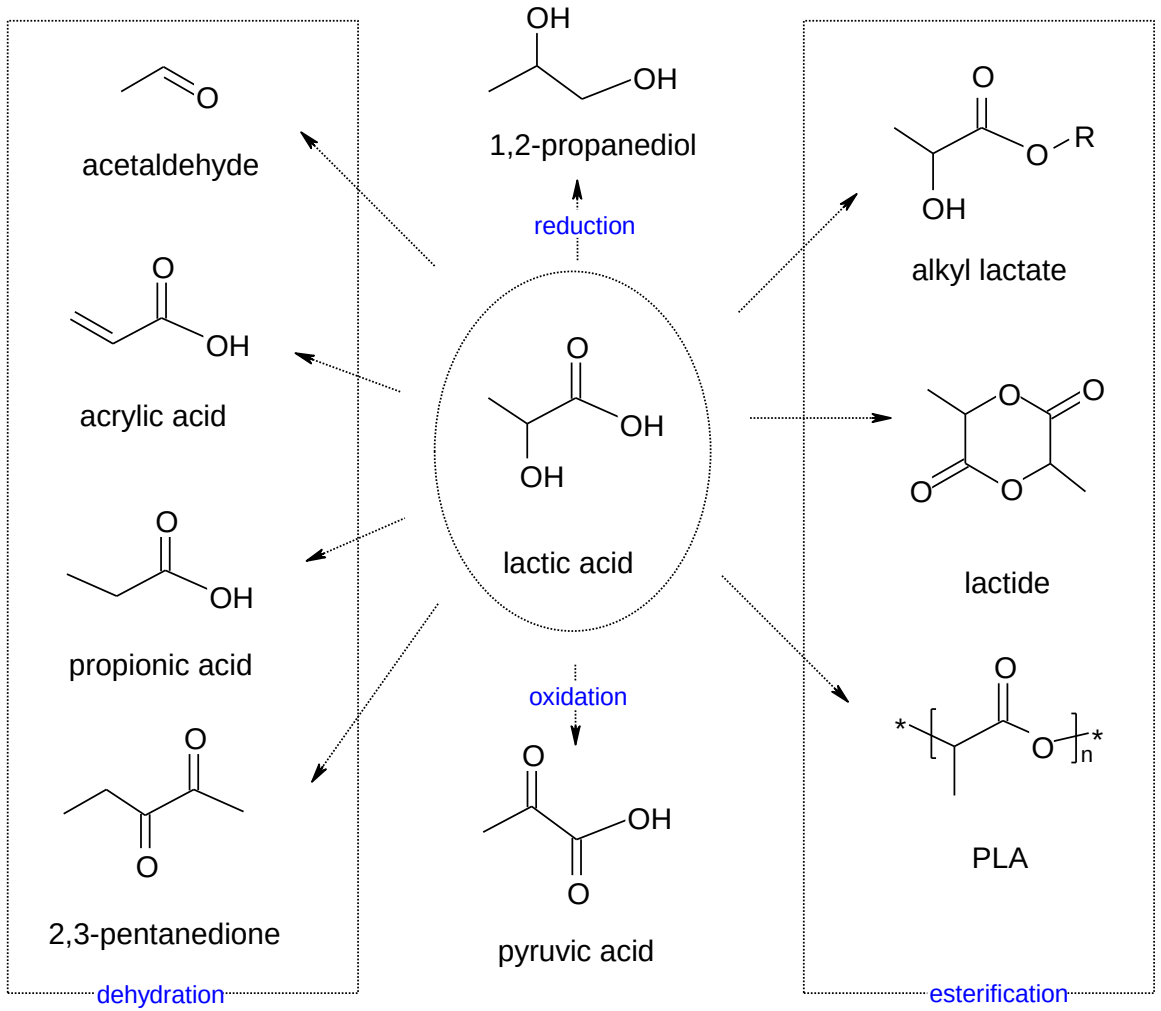
strains of lactic acid bacteria
L.a., *L. amylovorus* DSM 20531
L.r., *L. rhamnosus* DSM 20021
L.c.t., *L.coryniformis* subsp. *torquens* DSM 20004

bioreactor
STB, Stirred Tank Bioreactor
HRTB, Horizontal Rotating Tubular Bioreactor

product(s)
LA, lactic acid
AA, acetic acid

batch systems (carbon source$_{concentration}$ / LAB / temperature (˚C / bioreactor)	$w_{D\text{-}LA}$ (%)	$w_{L\text{-}LA}$ (%)	product(s)
glc$_{20}$/*L. r.*/40/STB	7.4	92.6	D-/**L**-LA
starch$_{10}$/*L. a.*+*L. r.*/40/STB **co-culture**	27.2	72.8	D-/**L**-LA
cgs$_{60}$/*L. a.*/20.5-34.7/HRTB	30.0	70.0	D-/**L**-LA
cgs$_{100}$/*L. a.*/45/STB	33.3	66.7	D-/**L**-LA
starch$_{10}$/*L. a.*/35/STB	43.7	56.3	D-/**L**-LA
suc$_{10}$/*L. a.*/40/STB	48.4	51.6	D-/**L**-LA
starch$_{10}$/*L. a.*/30/STB	49.1	50.9	D-/**L**-LA
starch$_{100}$/*L. a.*/45/STB	49.5	50.5	D-/**L**-LA
starch$_{10}$/*L. a.*/45/STB	49.7	50.3	D-/**L**-LA
glc$_{20}$/*L. a.*/40/STB	50.1	49.9	**D**-/L-LA
cgs$_{100}$/*L. a.*/45/STB	50.8	49.2	**D**-/L-LA
glc$_{10}$/*L. a.*/40/STB	50.8	49.2	**D**-/L-LA+AA
starch$_{10}$/*L. a.*/40/STB	52.0	48.0	**D**-/L-LA
mal$_{10}$/*L. a.*/40/STB	52.2	47.8	**D**-/L-LA+AA
starch$_{10}$/*L. a.*/50/STB	62.9	37.1	**D**-/L-LA
glc$_{20}$/*L. c. t.*/30/STB	100.0	0.0	**D**-LA+AA
glc$_{20}$/*L. c. t.*/30-50/STB	100.0	0.0	**D**-LA

unpublished data


acetaldehyde
1,2-propanediol
reduction
alkyl lactate
acrylic acid
lactic acid
lactide
propionic acid
oxidation
PLA
2,3-pentanedione
pyruvic acid
dehydration
esterification

. production of different ratios of D-/L-lactic acid

substrate
glc, glucose
starch
cgs, corn grits
suc, sucrose
mal, maltose

strains of lactic acid bacteria
L.a., *L. amylovorus* DSM 20531
L.r., *L. rhamnosus* DSM 20021
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product(s)
LA, lactic acid
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	batch systems (carbon source$_{concentration}$ / LAB / temperature (˚C / bioreactor)	$w_{D\text{-}LA}$ (%)	$w_{L\text{-}LA}$ (%)	product(s)	
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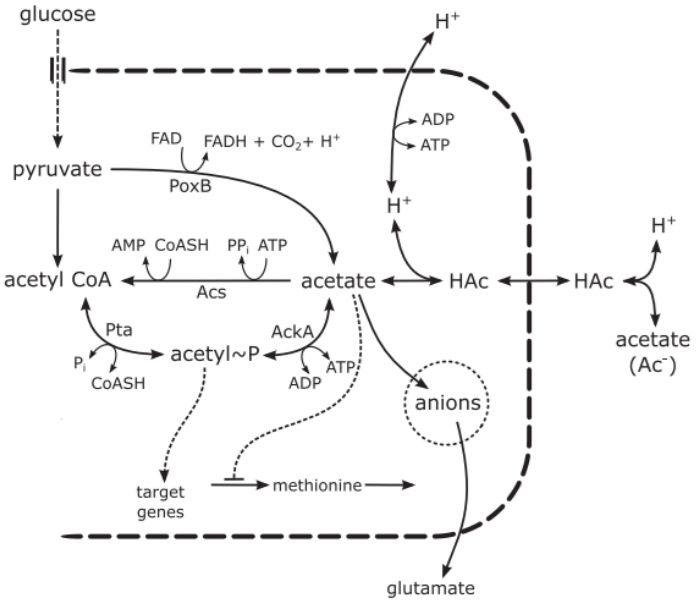
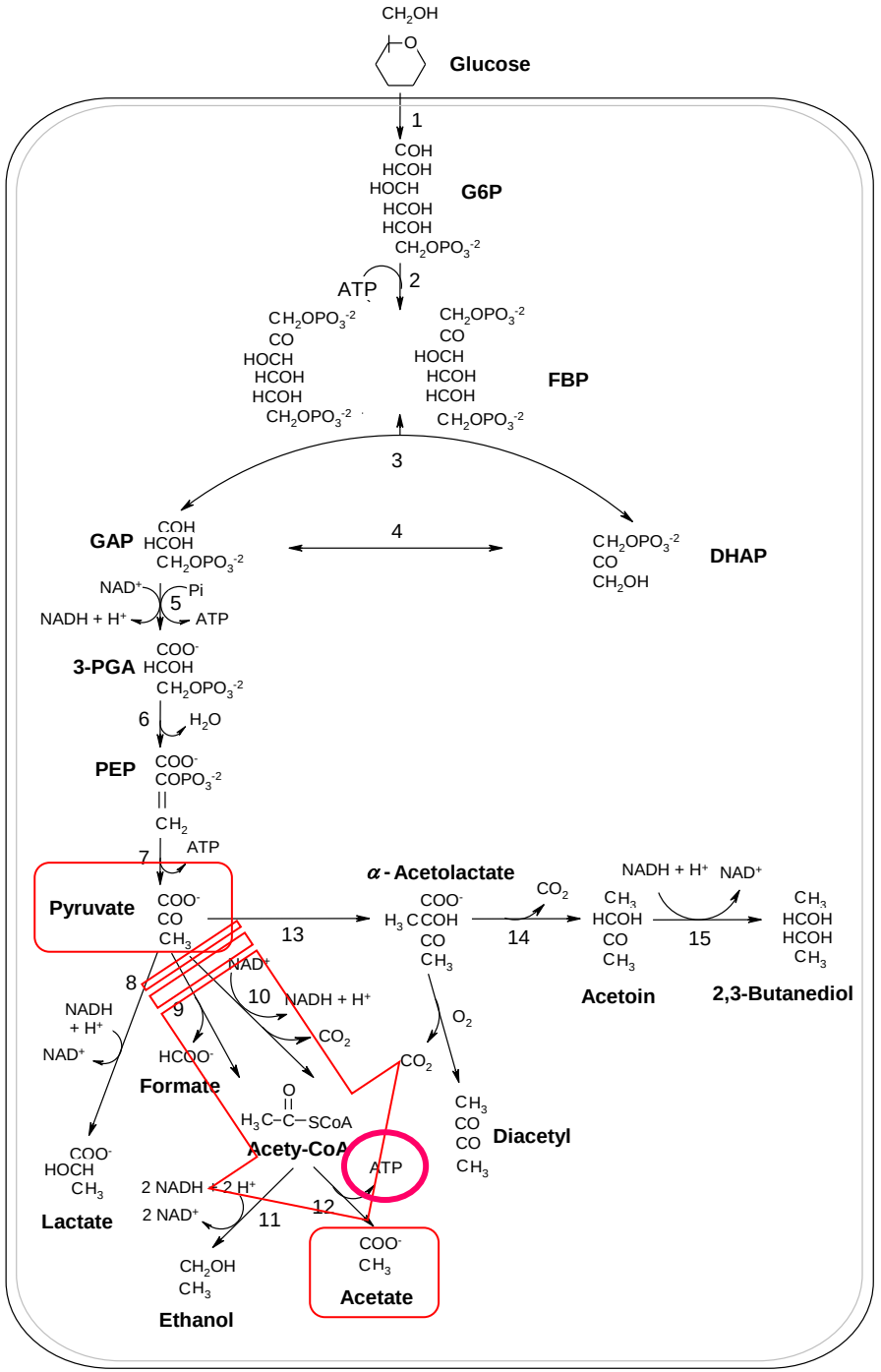
	cgs$_{60}$/*L. a.*/20.5-34.7/HRTB	30.0	70.0	D-/**L**-LA	
	cgs$_{100}$/*L. a.*/45/STB	33.3	66.7	D-/**L**-LA	
	starch$_{10}$/*L. a.*/35/STB	43.7	56.3	D-/**L**-LA	
	suc$_{10}$/*L. a.*/40/STB	48.4	51.6	D-/**L**-LA	
	starch$_{10}$/*L. a.*/30/STB	49.1	50.9	D-/**L**-LA	
	starch$_{100}$/*L. a.*/45/STB	49.5	50.5	D-/**L**-LA	
	starch$_{10}$/*L. a.*/45/STB	49.7	50.3	D-/**L**-LA	
	glc$_{20}$/*L. a.*/40/STB	50.1	49.9	**D**-/L-LA	
	cgs$_{100}$/*L. a.*/45/STB	50.8	49.2	**D**-/L-LA	
homofermentative	glc$_{10}$/*L. a.*/40/STB	50.8	49.2	**D**-/L-LA+AA	glc below 35.52 mM (6.40 g/L)
	starch$_{10}$/*L. a.*/40/STB	52.0	48.0	**D**-/L-LA	
	mal$_{10}$/*L. a.*/40/STB	52.2	47.8	**D**-/L-LA+AA	mal below 27.64 mM (9.46 g/L)
	starch$_{10}$/*L. a.*/50/STB	62.9	37.1	**D**-/L-LA	
facultatively heterofermentative	glc$_{20}$/*L. c. t.*/30/STB	100.0	0.0	**D**-LA+AA	glc below 38.41 mM (6.92 g/L)
	glc$_{20}$/*L. c. t.*/30-50/STB	100.0	0.0	**D**-LA	

Slavica et al., J Biotechnol, 2015

. acetate in the growth medium (glc_{20}) without growth inhibition

. acetate switch in *Escherichia coli*
. intracellular osmotic pressure
. synthesis of methionine
. pool of glutamate
. more organic acids in growth medium





Wolfe, Microbiol Mol Biol Rev. 2005 https://doi: 10.1128/MMBR.69.1.12-50.2005.
Pinhal S, Ropers D, Geiselmann J, de Jong H. 2019. J Bacteriol https://doi.org/10.1128/JB.00147-19.

. production of different ratios of D-/L-lactic acid

Acetate can be produced from:
(1) **pyruvate** in reactions catalyzed by pyruvate dehydrogenase, phosphotransacetylase and acetate kinase;
(2) **lactate** by the LDH-POX-ACK pathway;
and/or
(3) **amino acids**: Ser, Asp, Val, Leu and Ile.

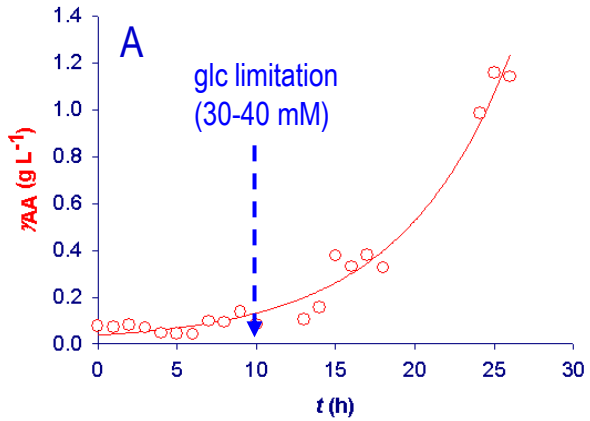
Drops in the concentration of three amino acids, **Asp, Val and Ile**, were connected with the start of the **acetate** production.
Further increase in the acetate concentration was extended in the **stationary phase** of *L. c. t.*
Molar ratios of utilized or produced **lactate**, **acetate** and **three amino acids** obtained in experiments do not correspond to stoichiometric ratios of metabolic pathways considered above.

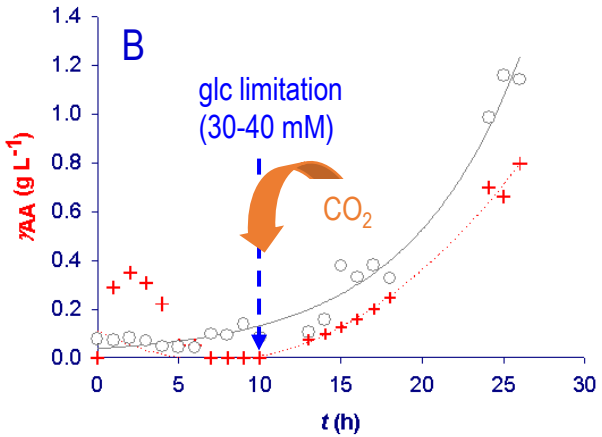
batch systems (carbon source$_{concentration}$ / LAB / temperature (˚C / bioreactor)	$w_{D\text{-}LA}$ (%)	$w_{L\text{-}LA}$ (%)	product(s)	
glc$_{20}$/*L. r.*/40/STB	7.4	92.6	D-/**L**-LA	
starch$_{10}$/*L. a.*+*L. r.*/40/STB **co-culture**	27.2	72.8	D-/**L**-LA	
cgs$_{60}$/*L. a.*/20.5-34.7/HRTB	30.0	70.0	D-/**L**-LA	
cgs$_{100}$/*L. a.*/45/STB	33.3	66.7	D-/**L**-LA	
starch$_{10}$/*L. a.*/35/STB	43.7	56.3	D-/**L**-LA	
suc$_{10}$/*L. a.*/40/STB	48.4	51.6	D-/**L**-LA	
starch$_{10}$/*L. a.*/30/STB	49.1	50.9	D-/**L**-LA	
starch$_{100}$/*L. a.*/45/STB	49.5	50.5	D-/**L**-LA	
starch$_{10}$/*L. a.*/45/STB	49.7	50.3	D-/**L**-LA	
glc$_{20}$/*L. a.*/40/STB	50.1	49.9	**D**-/L-LA	
cgs$_{100}$/*L. a.*/45/STB	50.8	49.2	**D**-/L-LA	
glc$_{10}$/*L. a.*/40/STB	50.8	49.2	**D**-/L-LA+AA	glc below 35.52 mM (6.40 g/L)
starch$_{10}$/*L. a.*/40/STB	52.0	48.0	**D**-/L-LA	
mal$_{10}$/*L. a.*/40/STB	52.2	47.8	**D**-/L-LA+AA	mal below 27.64 mM (9.46 g/L)
starch$_{10}$/*L. a.*/50/STB	62.9	37.1	**D**-/L-LA	
glc$_{20}$/*L. c. t.*/30/STB	100.0	0.0	**D**-LA+AA	glc below 38.41 mM (6.92 g/L)
glc$_{20}$/*L. c. t.*/30-50/STB	100.0	0.0	**D**-LA	

Slavica et al., J Biotechnol, 2015

. effect of dissolved oxygen concentration on acetate production by *L. coryniformis* subsp. *torquens* DSM 20004

. STB (working volume of 10 L)
. glc_{20}
. 28˚C, pH 5.5 -6.1, 100 rpm
. D-LA + AA



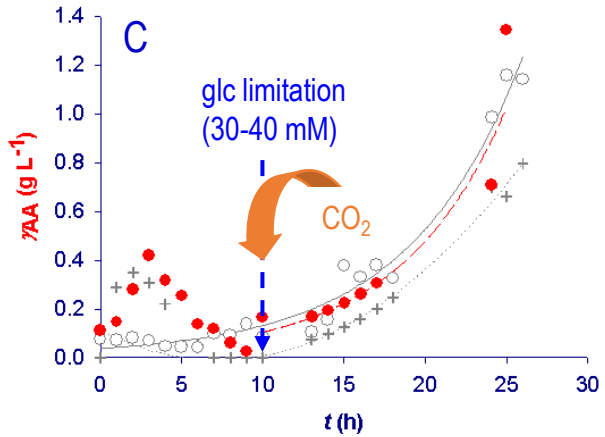


(A) p_{O2} = 80-100%; μ_{max} = 0.21 h^{-1}
(B) p_{O2} = 20-30%; μ_{max} = 0.17 h^{-1}
(C) p_{O2} = 0-10%; μ_{max} = 0.21 h^{-1}

glc depletion ↓ **D-LA production** ↑

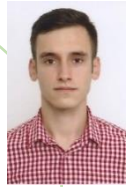
. reoxidation of NADH + H^+

· CO_2 → acety-CoA

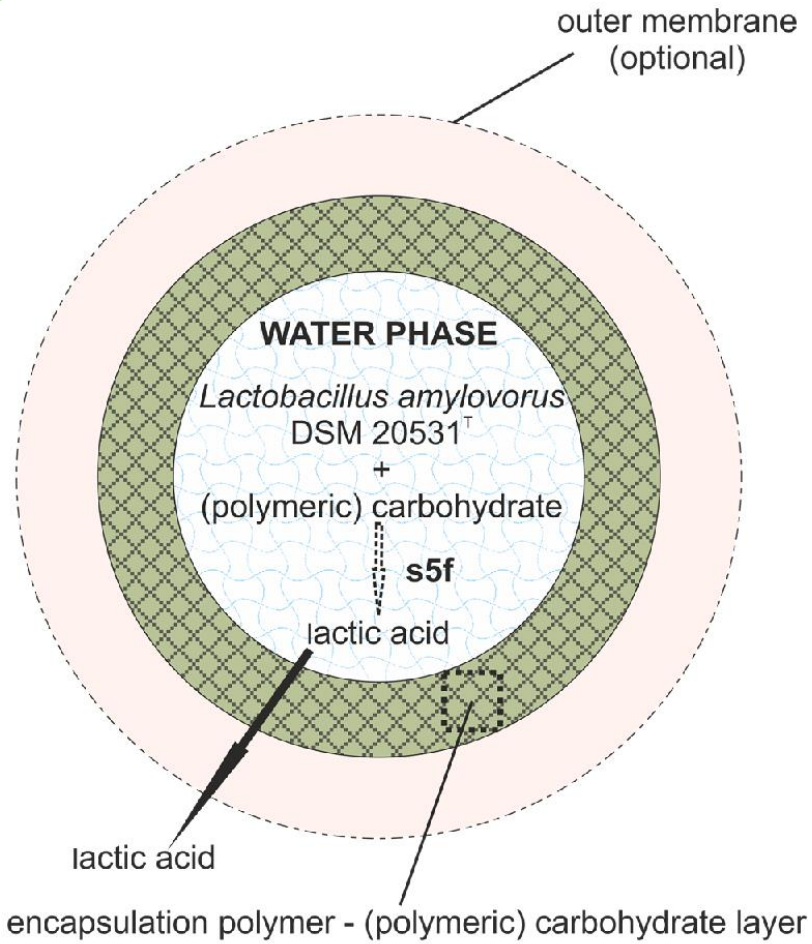


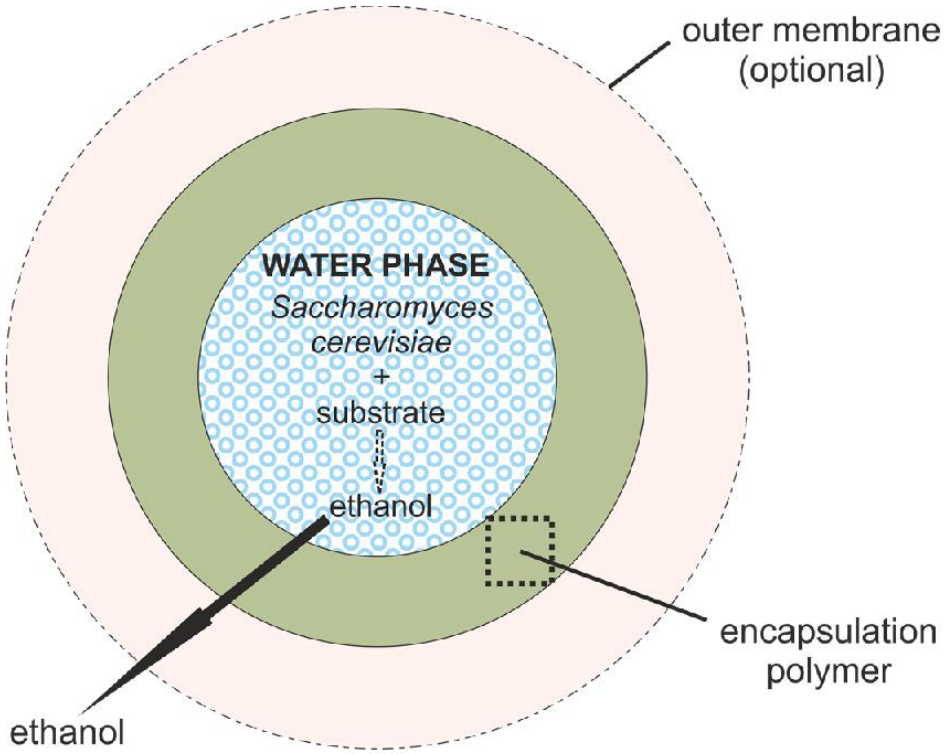
unpublished data

. encapsulation of *L. amylovors* DSM 20531 with polymeric substrate



B. Duić

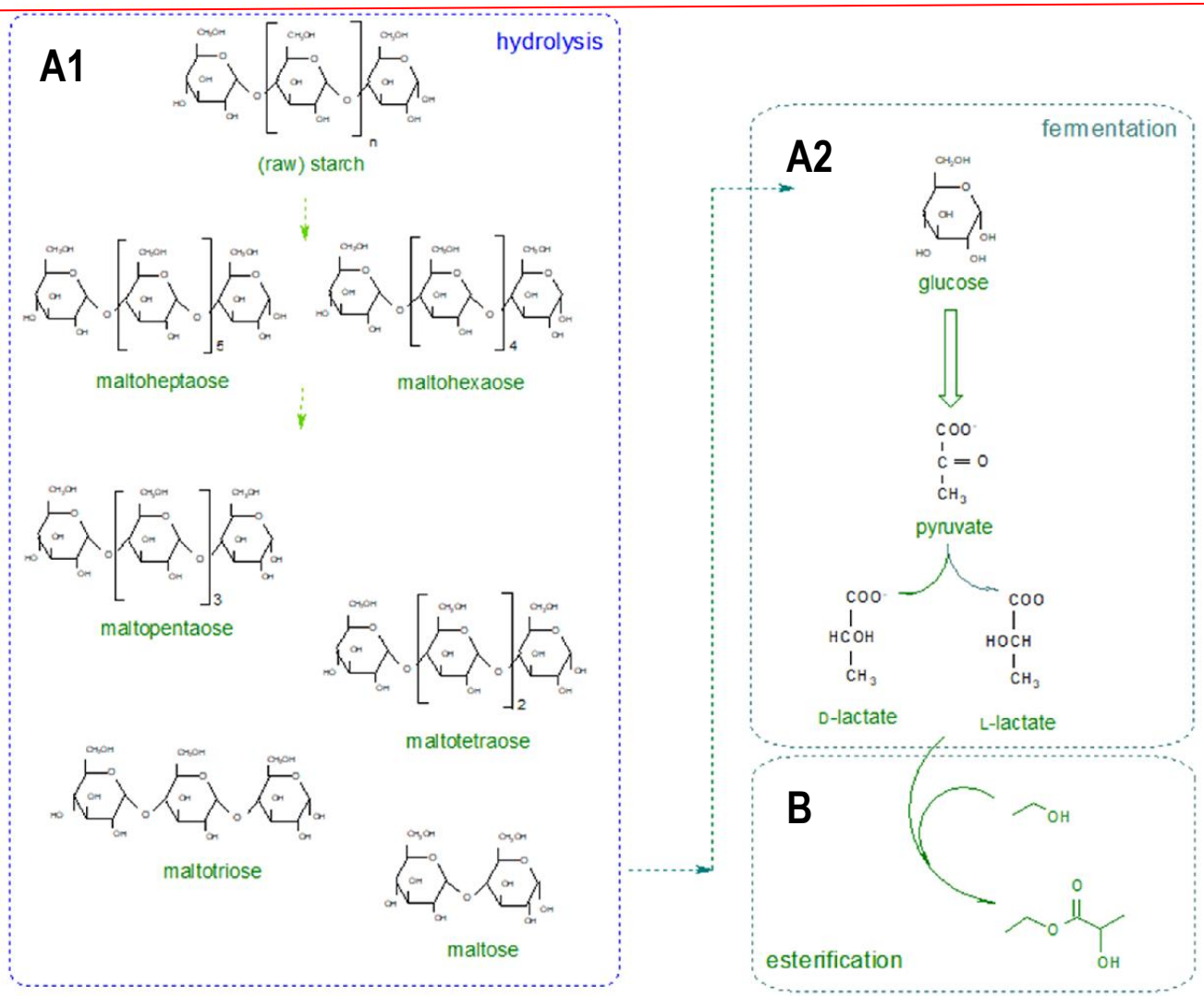


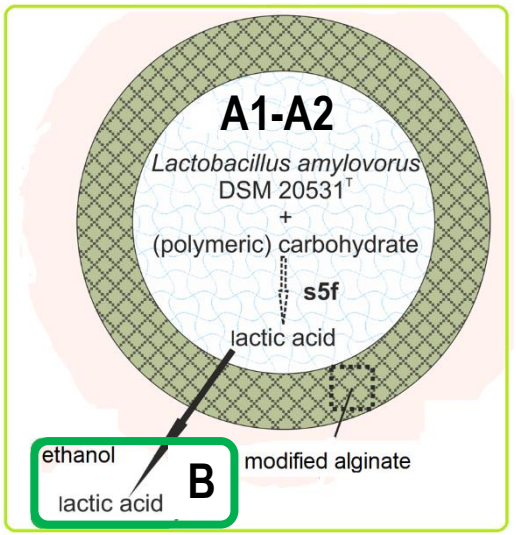


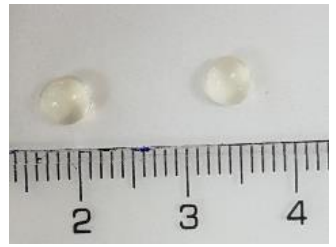
ORGANIC PHASE

unpublished data

. encapsulation of *L. amylovors* DSM 20531 with polymeric substrate - development of an integrated bioprocesses

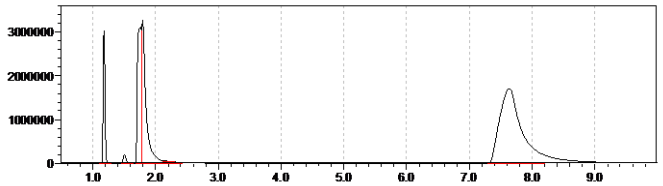


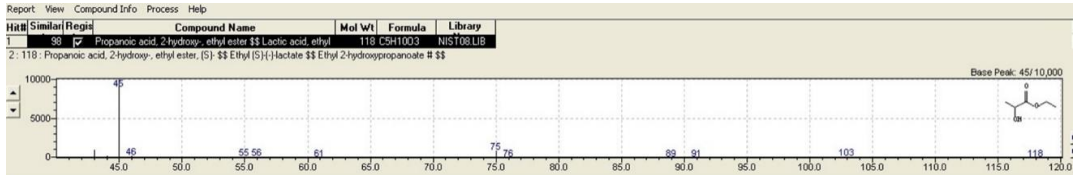




Leo Moguš

MS detection of ethyl lactate

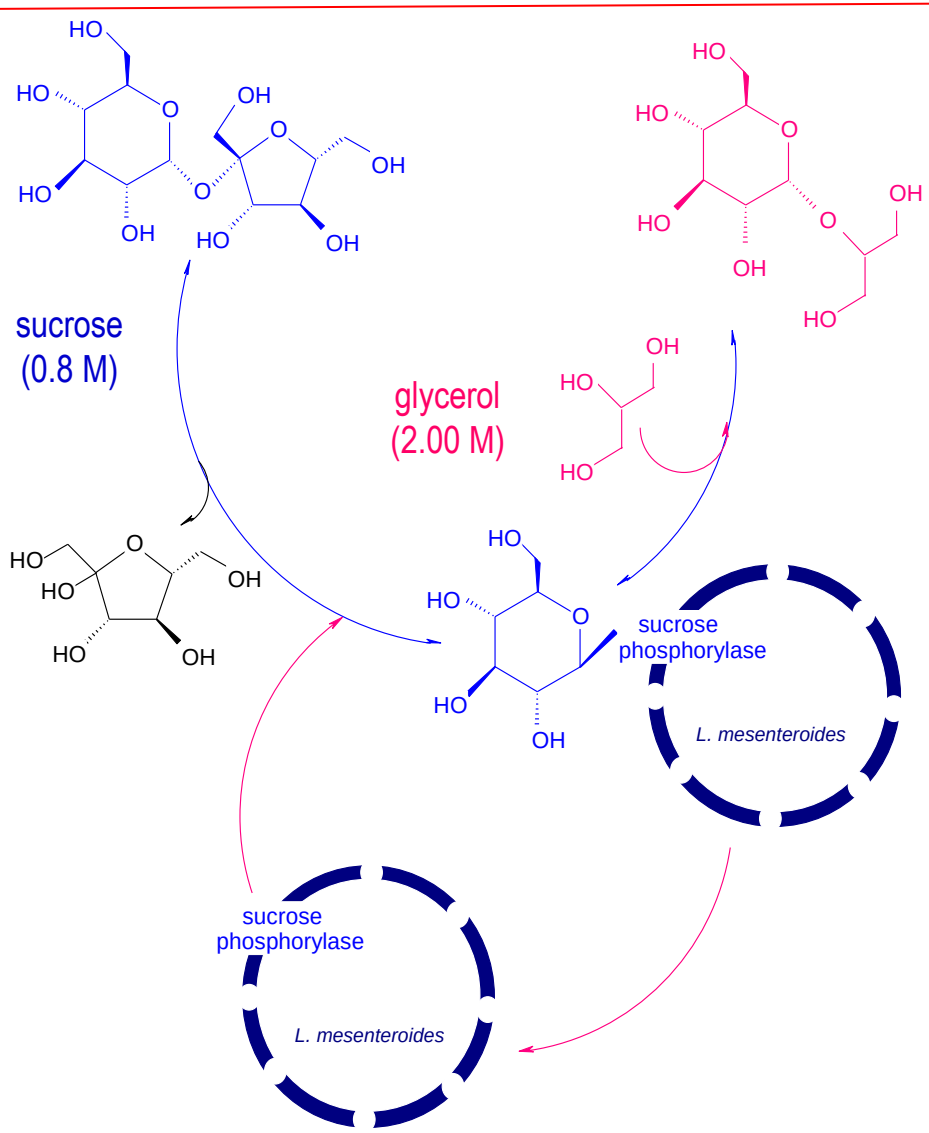




unpublished data

. *Leuconostoc mesenteroides* LMG 7954 whole-cell catalysed glycosylation

. ultrasound-assisted *L. mesenteroides* LMG 7954 permeabilization
. sucrose phosphporylase (EC 2.4.1.7)
. < 100 mM α-D-glucosylglycerol



α-D-glucosylglycerol

I. Vučenović

Iva Blašković
Ana-Marija Gruičić
Lucija Vrtodušić

unpublished data

Abstract

Yeast *Saccharomyces cerevisiae* is widely recognized as a versatile chassis for constructing microbial cell factories. However, producing chemicals from toxic, highly concentrated, or cell-impermeable substrates, or chemicals dependent on enzymatic reactions incompatible with the yeast's intracellular environment, remains challenging. In this study, we engineered a yeast microbial cell factory that, in a single-step reaction, produces 2-O-(α-D-glucopyranosyl)-sn-glycerol (glucosyl glycerol, αGG), a natural osmolyte used in the cosmetics and healthcare industries. For this purpose, we employed sucrose phosphorylase (SucP) from *Leuconostoc mesenteroides*, a glucosyl transferase that, in a low-water, glycerol-rich, phosphate-free environment, transfers the glucosyl moiety from sucrose to glycerol. To accomplish this, we utilised surface display methodology to engineer yeast cells that covalently bind SucP to their cell wall, thus converting their outer surface into a self-sustaining αGG-producing catalyst. To efficiently anchor SucP near its C-terminus, we developed a miniature Ccw12-tag, derived from the abundant yeast GPI-anchored cell wall protein Ccw12. This tag adds only 1.1 kDa to the enzyme of interest while enabling its covalent attachment to the cell wall. The C-terminally anchored SucP produced extracellularly 37.3 g l^{-1} (146 mM) of αGG in five days, while the host cells metabolised reaction by-products, thereby simplifying downstream processing.

Keywords: glucosyl glycerol, osmolyte, sucrose phosphorylase, *Saccharomyces cerevisiae*, surface display, Ccw12

Engineering *Saccharomyces cerevisiae* for the one-step production of natural osmolyte glucosyl glycerol through Ccw12-based surface display of sucrose phosphorylase from *Leuconostoc mesenteroides*
Journal of Biological Engineering, under revision

ACS Sustainable Chemistry & Engineering

pubs.acs.org/journal/ascecg

Research Article

Stability Increase of Phenolic Acid Decarboxylase by a Combination of Protein and Solvent Engineering Unlocks Applications at Elevated Temperatures

Kamela Myrtollari, Elia Calderini, Daniel Kracher, Tobias Schöngaßner, Stela Galušić, Anita Slavica, Andreas Taden, Daniel Mokos, Anna Schrüfer, Gregor Wirnsberger, Karl Gruber, Bastian Daniel,* and Robert Kourist*

Cite This: *ACS Sustainable Chem. Eng.* 2024, 12, 3575−3584

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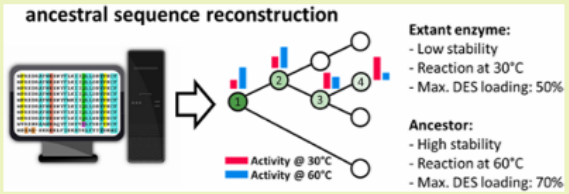
ABSTRACT: Enzymatic decarboxylation of biobased hydroxycinnamic acids gives access to phenolic styrenes for adhesive production. Phenolic acid decarboxylases are proficient enzymes that have been applied in aqueous systems, organic solvents, biphasic systems, and deep eutectic solvents, which makes stability a key feature. Stabilization of the enzyme would increase the total turnover number and thus reduce the energy consumption and waste accumulation associated with biocatalyst production. In this study, we used ancestral sequence reconstruction to generate thermostable decarboxylases. Investigation of a set of 16 ancestors resulted in the identification of a variant with an unfolding temperature of 78.1 °C and a half-life time of 45 h at 60 °C. Crystal structures were determined for three selected ancestors. Structural attributes were calculated to fit different regression models for predicting the thermal stability of variants that have not yet been experimentally explored. The models rely on hydrophobic clusters, salt bridges, hydrogen bonds, and surface properties and can identify more stable proteins out of a pool of candidates. Further stabilization was achieved by the application of mixtures of natural deep eutectic solvents and buffers. Our approach is a straightforward option for enhancing the industrial application of the decarboxylation process.



KEYWORDS: *biocatalysis, enzymatic decarboxylation, biobased polymers, deep eutectic solvents, ancestral sequence reconstruction*

. the workflow

WP1. Design and developing of connection between nonthermal processing techniques (high power ultrasound and pulsed electric field) with Internet of Things (IoT) hardware and software.

WP2. Optimisation of nonthermal IoT green extraction processes (NT-IoT-P) of proteins (RuBisCO): analytical and sustainable procedures.

WP3. Optimisation of electrospinning, electrospraying and encapsulation techniques of extracted proteins (RuBisCO): analytical procedures.

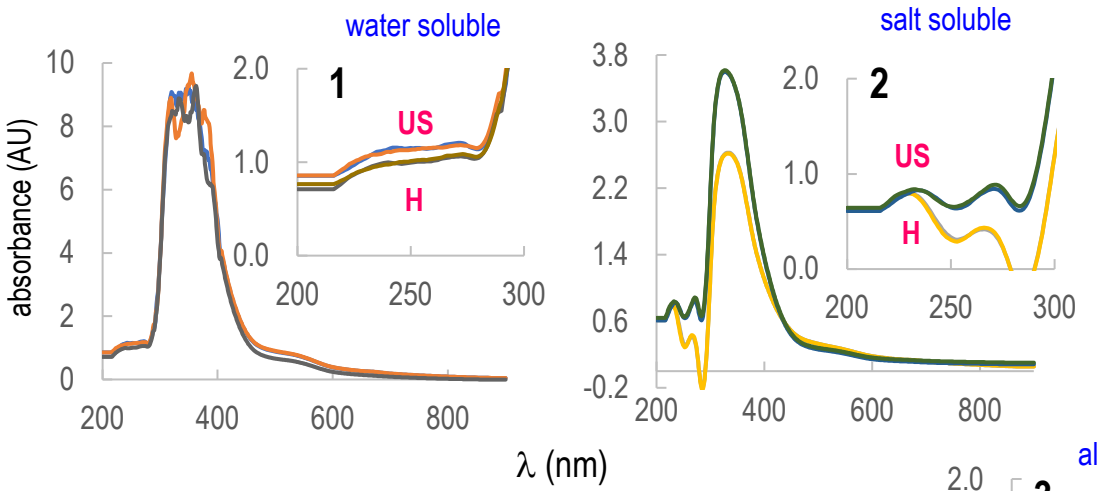
WP4. Developing and setting bioinformatic in-silico modeling in protein and peptide extraction.

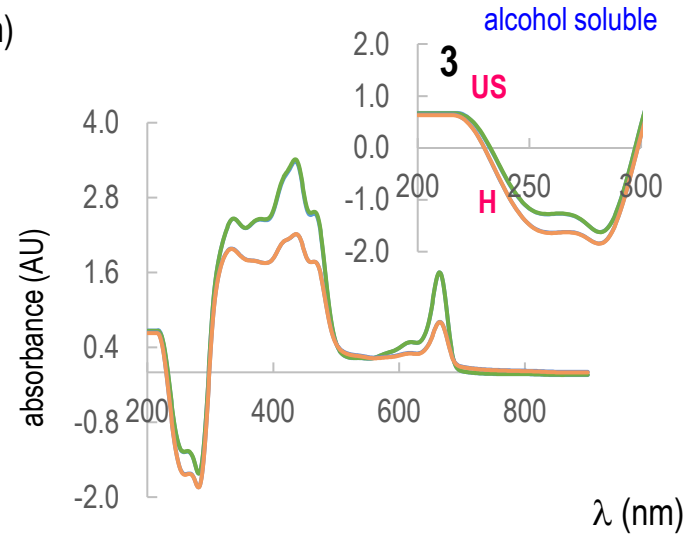
WP5. Economic and sustainable assessment on NT-IoT-P in green extraction of proteins (RuBisCO).

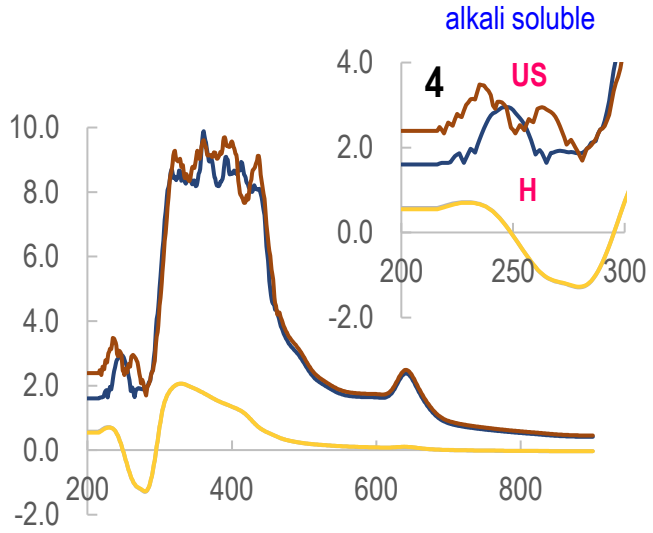
WP 6. Dissemination and knowledge transfer.

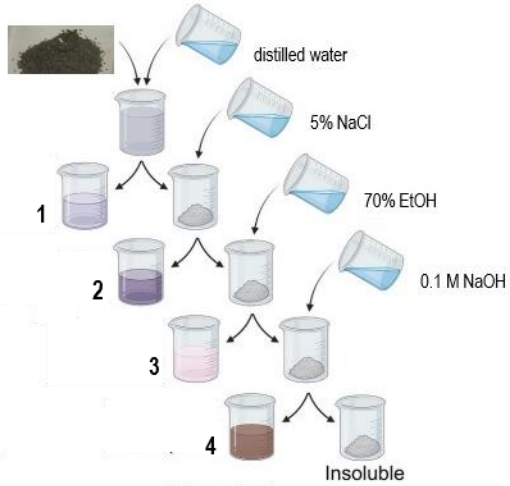
new challenges and opportunities - DEEP project

- heat (H)- vs ultrasound (US)-assisted Osborne fractionation of proteins (RuBISCo) and other bioactive compounds from Beta vulgaris L. (beetroot) leaves - preliminary data









acknowledgment

SCIENCE PASSION TECHNOLOGY TU Graz

Bernd Nidetzky, Univ.-Prof. Dipl.-Ing. Dr.techn. Dr.h.c

Univ.-Prof. Dr.rer.nat. Robert Kourist

HRZZ Croatian Science Foundation

Digitalisation of nonthermal Extraction of proteins from plant by-products and Electroforming as output Product (DEEP; IP-2022-10-2207)

Biotechnological application of incorporation of heterologous proteins into yeast cell walls (IP-2019-04-2891)

HRZZ Croatian Science Foundation

Ministry of Science, Education and Youth of the Republic of Croatia, Application of integrated bioprocesses in the production of lactic acid (058-0581990-1997)

Thank you for your kind attention!

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