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UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Application of versatile Acetic Acid Bacteria to innovative bioprocesses

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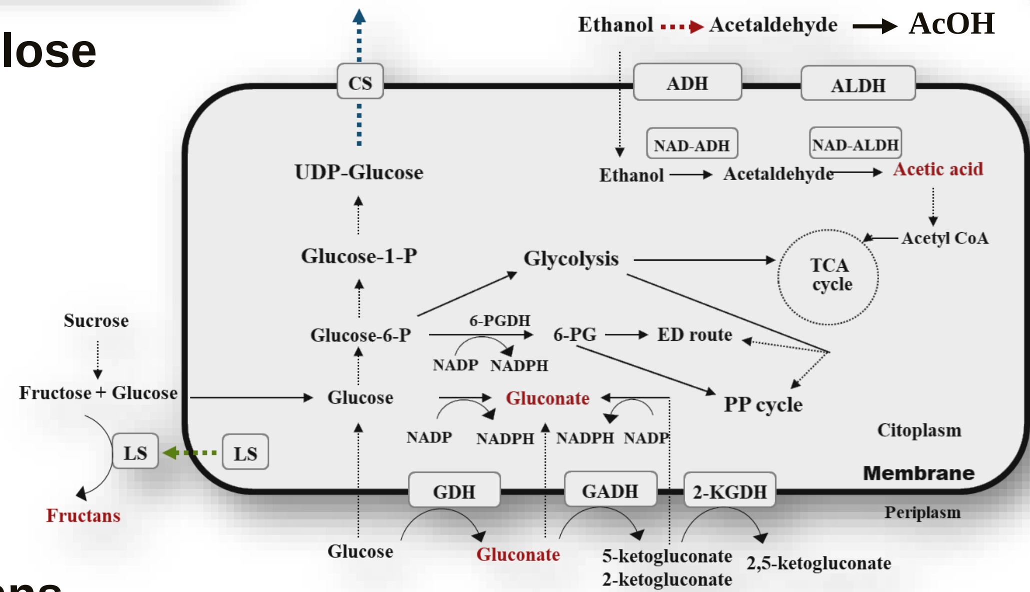
Ph.D. Workshop in Agri-Food Sciences, Technologies and Bio-Technologies
1 December 2017 , Reggio Emilia (RE) Italy

Versatility of Acetic Acid Bacteria

Cellulose

New vinegars

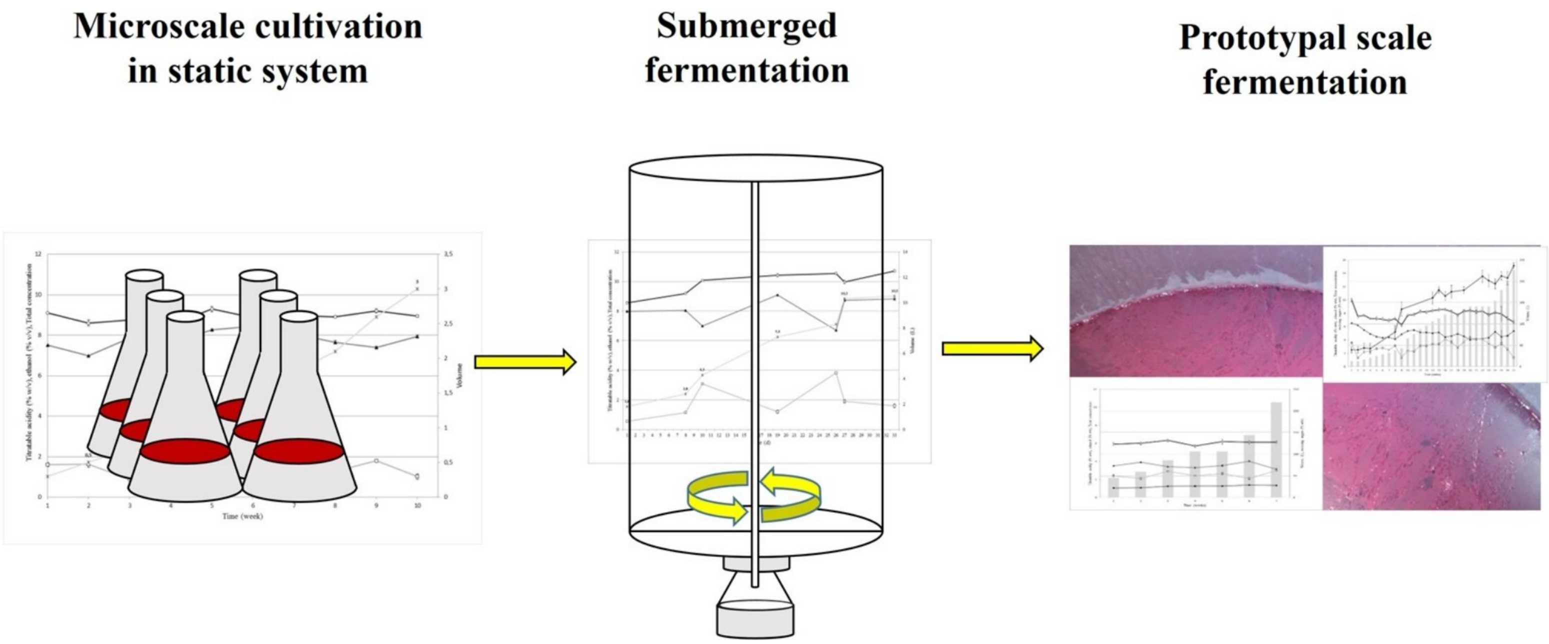


Bioactive attractants

Fructans

Part 1

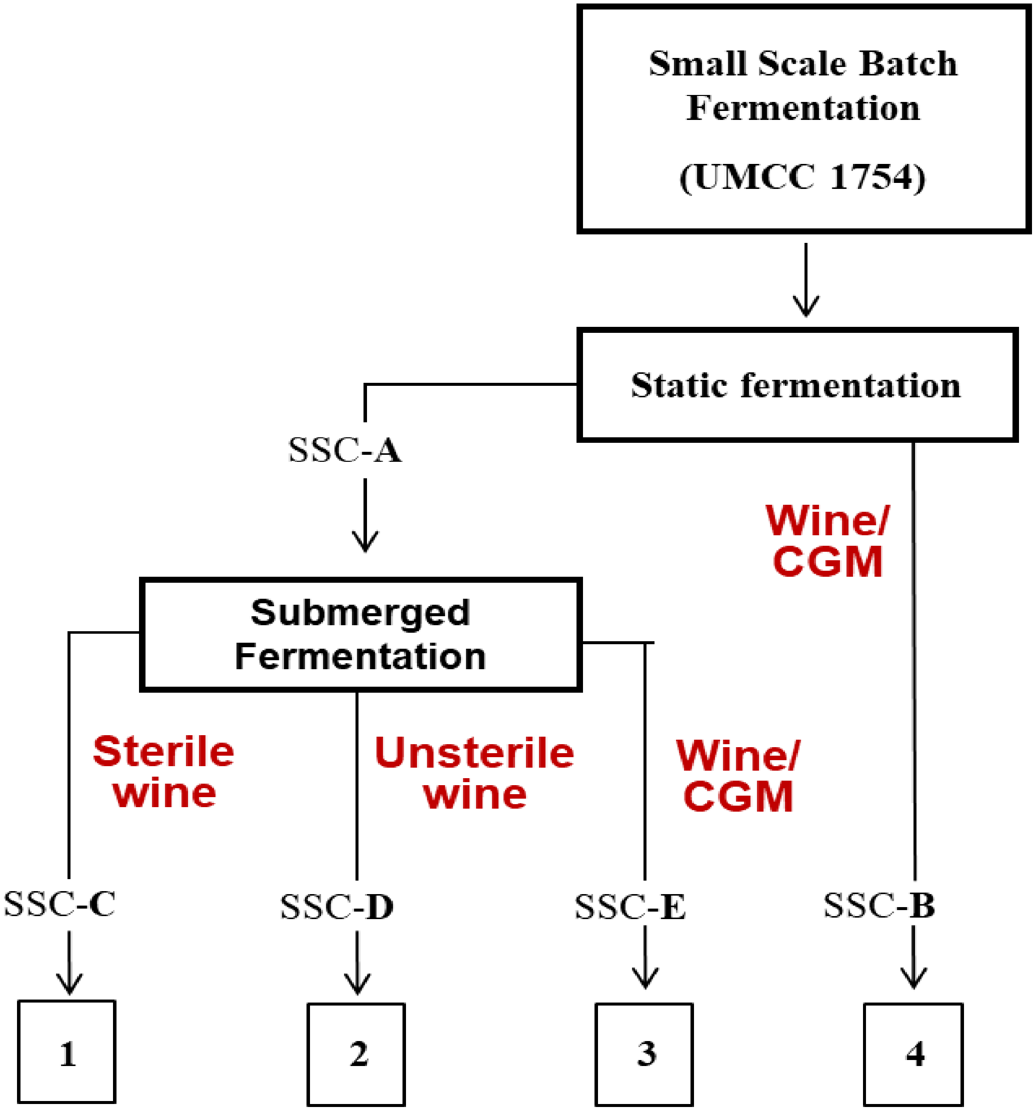
Feasible acetic acid fermentations of alcoholic and sugary substrates in combined operation mode

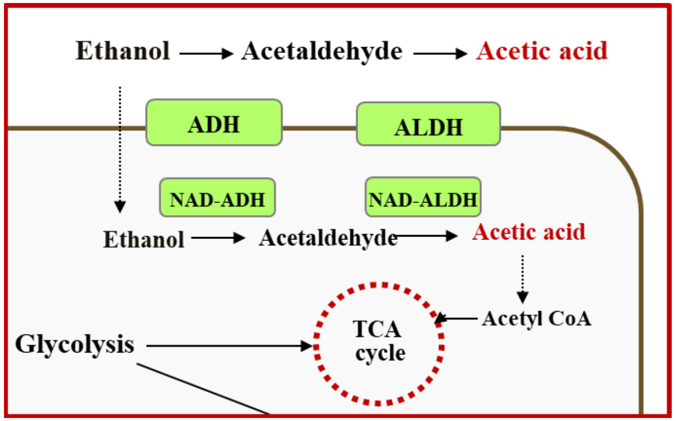


Published on:

Process Biochemistry 51, 1129-1139, 2016

Implementation of a combined system (static and submerged)





A. pasteurianus UMCC 1754

- High acetic acid production rate
- Efficient start-up and persistence
- Phenotypic stability
- No production of undesired products
- Low nutritional needs

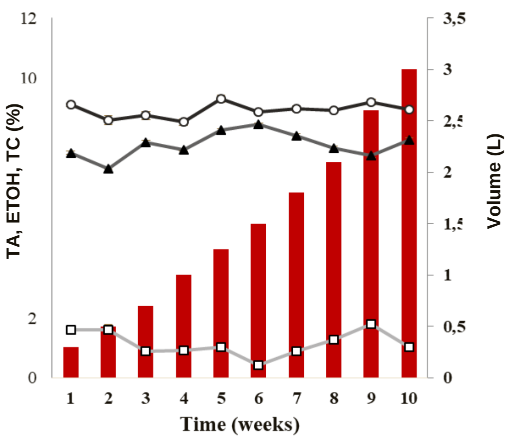
4 DIFFERENT VINEGARS

Selected Starter Cultures at laboratory scale

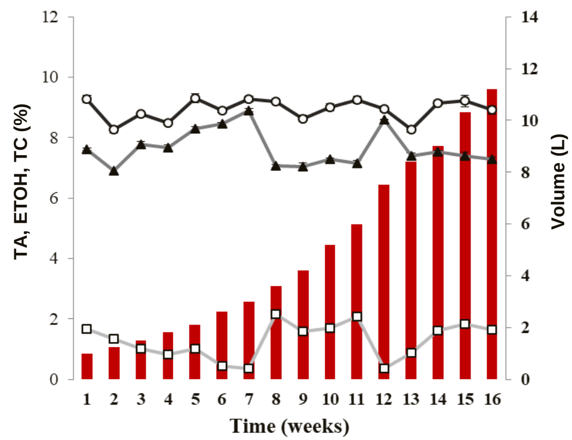
STATIC SYSTEM

TA: Titratable acidity; **EtOH:** Ethanol; **TC:** Total concentration

SSC-A



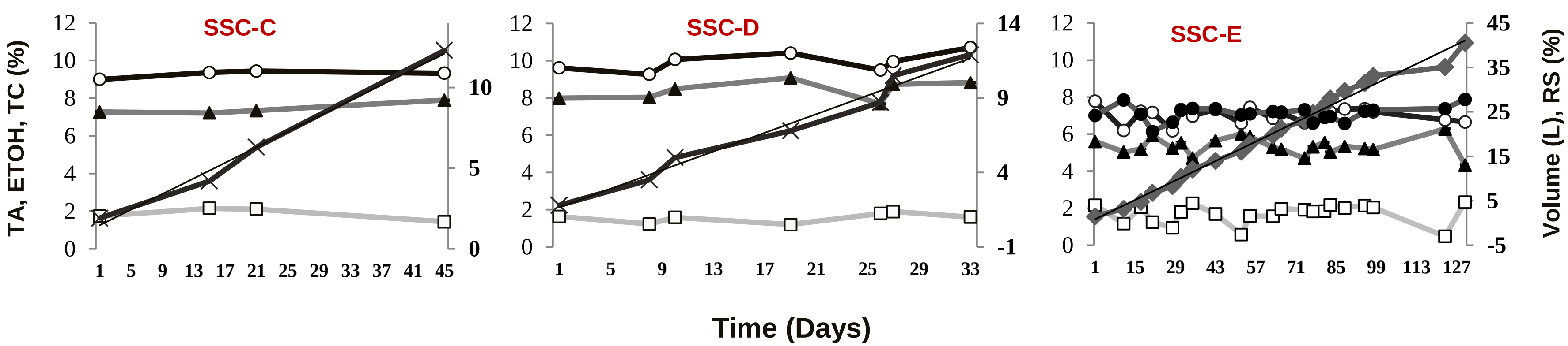
SSC-B



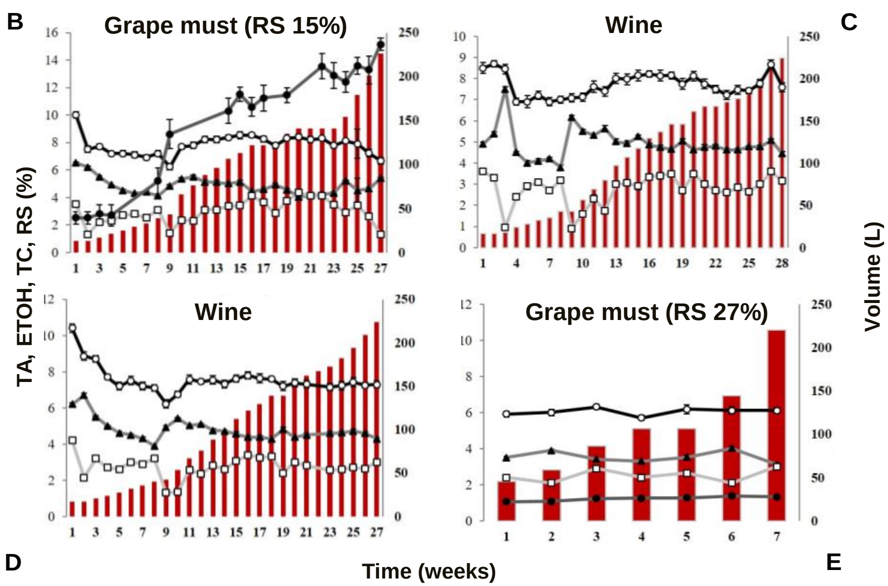
- SSC-A to start-up submerged fermentation
- SSC-B to prototype scale
- Slow fermentation

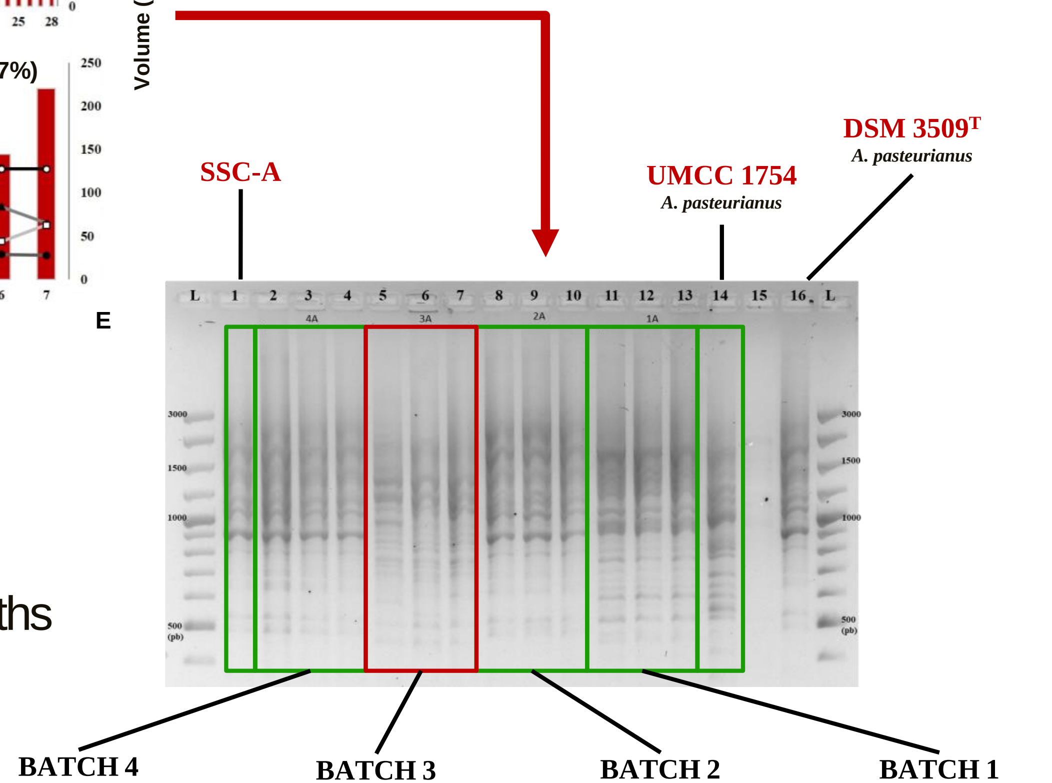
- SSC-C, D wine
- SSC E grape must
- Fast fermentation

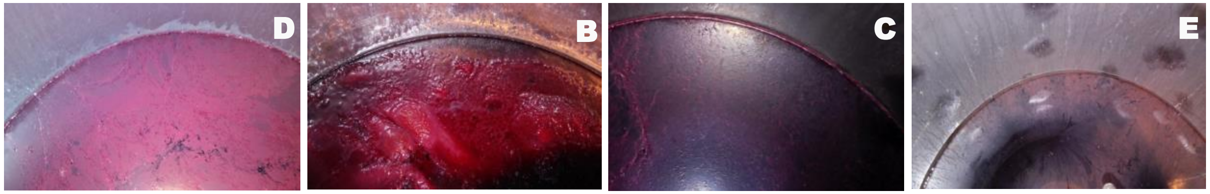
SUBMERGED SYSTEM



Part 1 Cultures performance at prototypal scale







SCALE-UP

Over 200L of vinegar/batch in 6 months

BATCH B-E: Wine

BATCH C-D: CGM

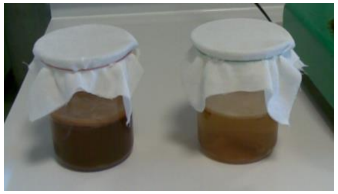
Conclusions

Combined fermentation by *A. pasteurianus* UMCC 1754 yielded viable SSCs at both laboratory and prototype scales

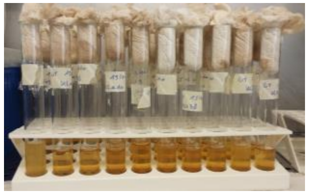
Process stability in static, submerged and prototype-scale confirmed the feasibility of using SSCs in industrial vinegar fermentations

Part 2

Increased production of bacterial cellulose as starting point for scaled-up applications



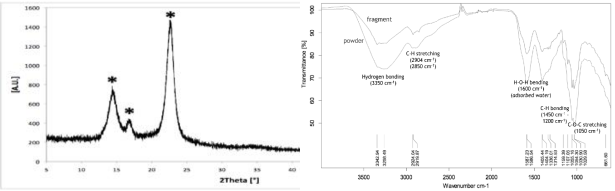
ISOLATION



QUALITATIVE TEST

QUANTITATIVE TEST

MEDIUM OPTIMIZATION



STRUCTURAL CHARACTERIZATION

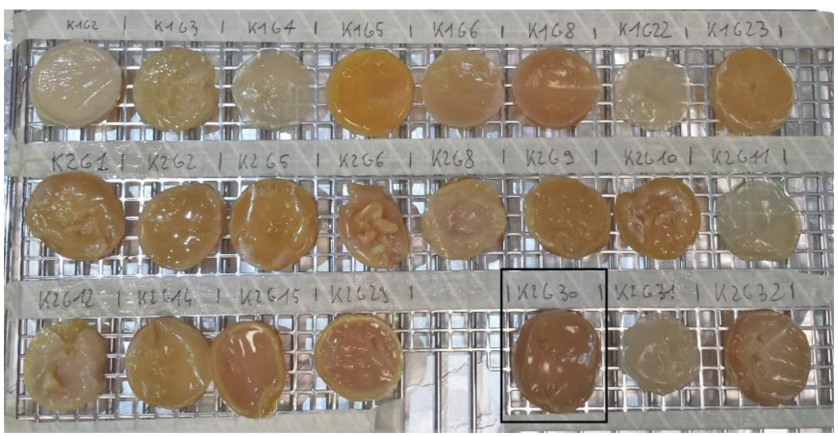
Published on:

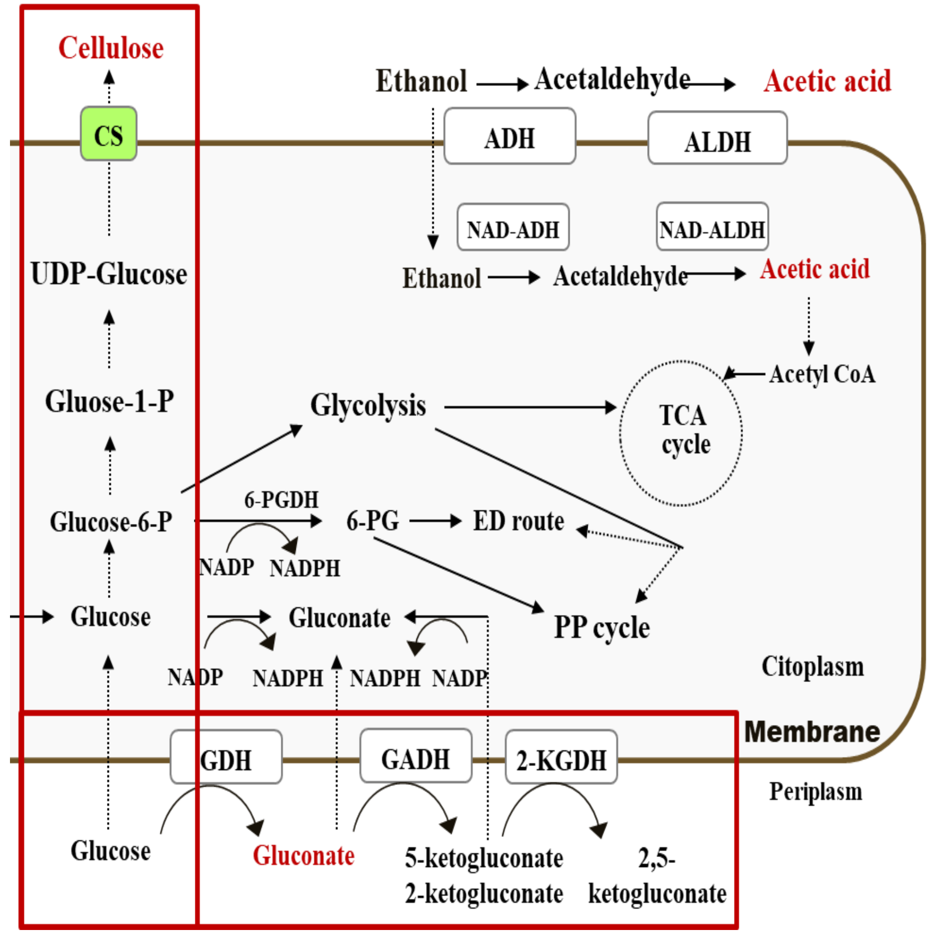
Applied Microbiology and Biotechnology 101, 2017

Bacterial cellulose synthesis

- D-glucose with β(1-4) glycosidic bond
- Synthetized by cellulose synthase (CS)

- High purity
- High degree of crystallinity
- High W.A.R. and resistance to tensile strength

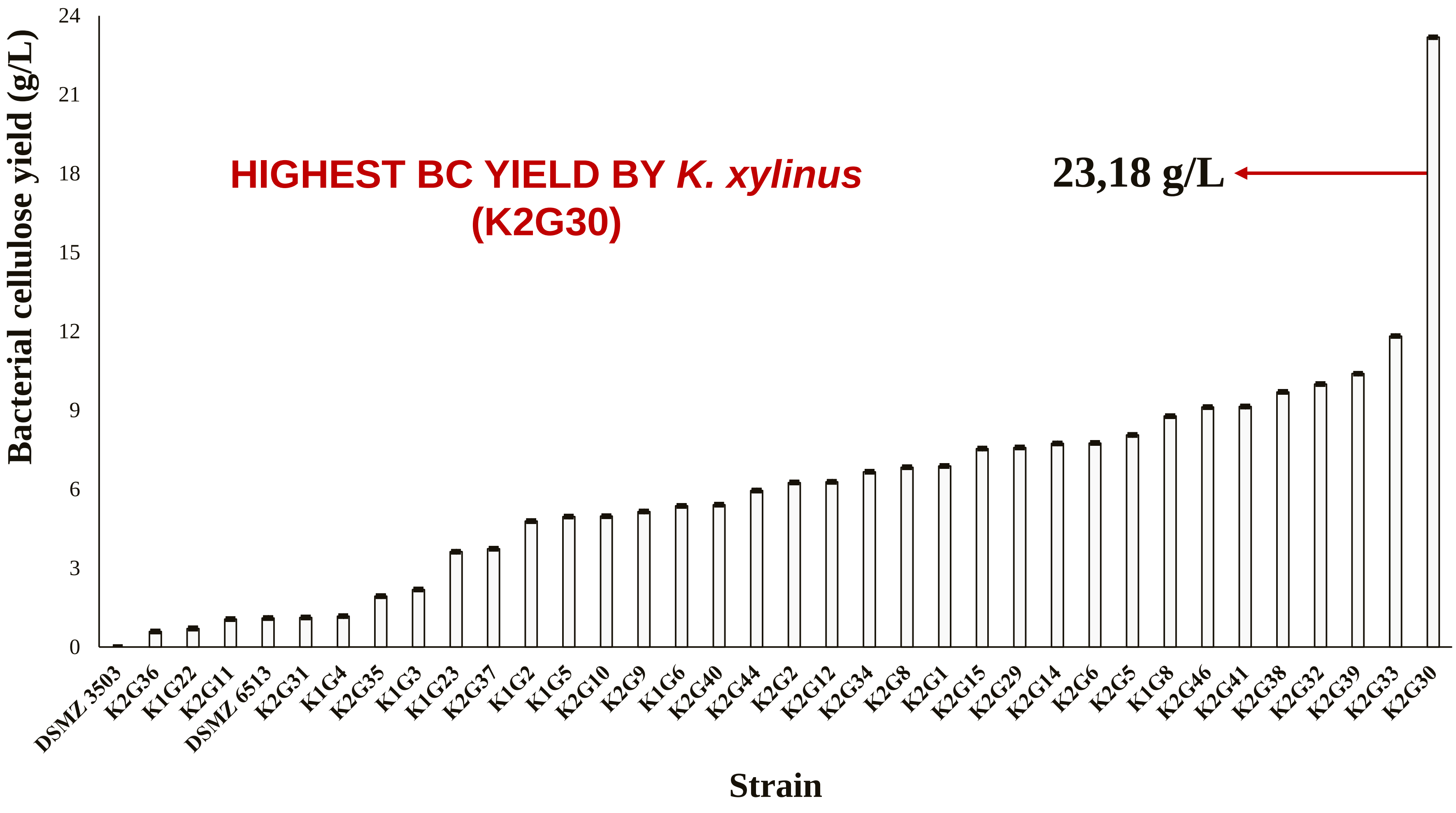




Part 2

Cellulose production

34 STRAINS SHOWED BC PRODUCTION



Part 2

Culture optimization

Strain K2G30

UMCC 2756 (*K. xylinus*)

FORMULATION OF AN ENHANCED BROTH

GET = GY- $CaCO_3$+ 1.4% ETOH

+

S/V RATIO OPTIMIZATION

0,23 cm^{-1}

HIGHER BC YIELD

LOWER BY-PRODUCTS FORMATION

Ethanol is an additional energy source so it allows glucose to be used mainly for BC synthesis

CARBON SOURCES CONSUMPTION AND BC PRODUCTION

Part 2 15 days of static cultivation in vessel (S/V 0.23 cm^{-1})

Broth	pH		Glu (g/L)		*EtOH (g/L)	G.A (g/L)	A.A (g/L)	BC g/L	BC/ cons sugar (g/g)
	Initial	**final**	**initial**	**final**	**final**				
GY	5.40	3.14	50.00 ± 0.03	15.73 ± 0.03	0.00	22.23 ± 0.05	0.00	13.25 ± 1.11	0,39
GET	6.37	**4.45**	50.00 ± 0.02	18.00 ± 0.08	2.20 ± 0.08	**11.46 ± 0.05**	0.67 ± 0.08	**19.64 ± 0.94**	**0,61**

OPTIMIZED CONDITIONS

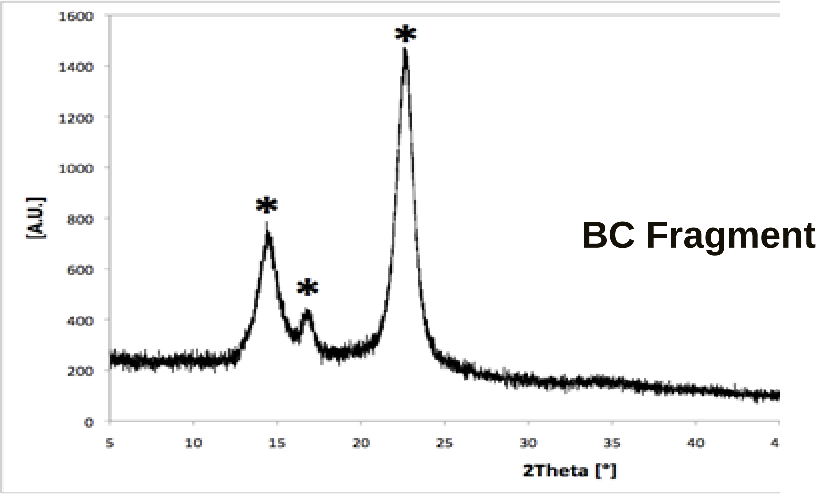
- **BC production increased of 30%**
- **Gluconic acid was ~ 50% lower**
- Final pH was higher
- g of BC produced per consumed sugar increased

***GET:** Initial Ethanol: 14%
Glu: glucose
EtOH: ethanol
GA: gluconic acid
A.A: acetic acid
BC: bacterial cellulose

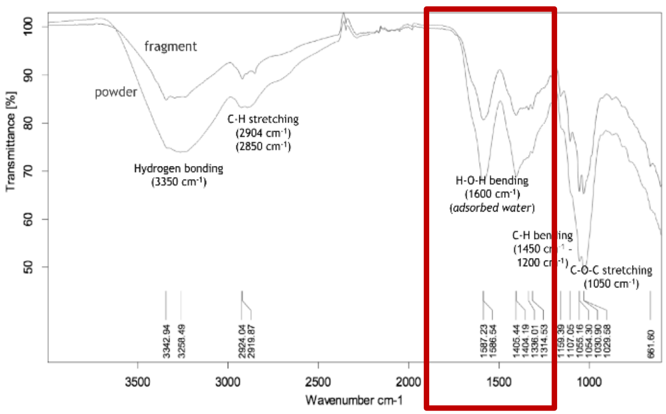
Part 2

Structural analysis of bacterial cellulose produced by K2G30

XRD

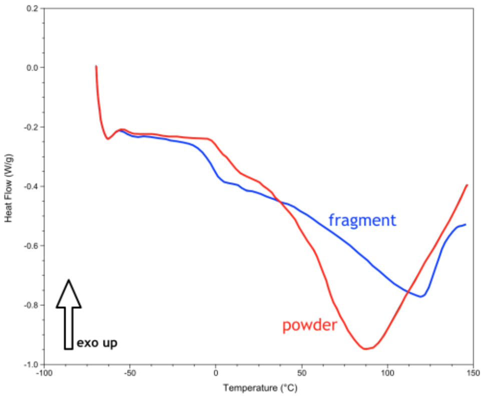


FT-IR



Absorbed water

DSC



- Typical peaks of BC with high degree of crystallinity
- D.o.c = > 80%
- Graphs are analogous and the peaks are typical for BC.
- Chemical bonds were not altered by the milling process
- Glass transition temperature (Tg) at about 0 °C.
- The mass loss (water removal) was 12.9%.
- W.A.R: 400%

Conclusions

- **The highest yield was achieved by K2G30 by static cultivation among 34 studied strains**
- **Enhanced culture conditions increased BC production of 30%**
- **High-purity BC with high degree of crystallinity (80%) and WAR (400%)**

Selected strain was able to produce high amount of BC suitable for biomedical applications and food processing

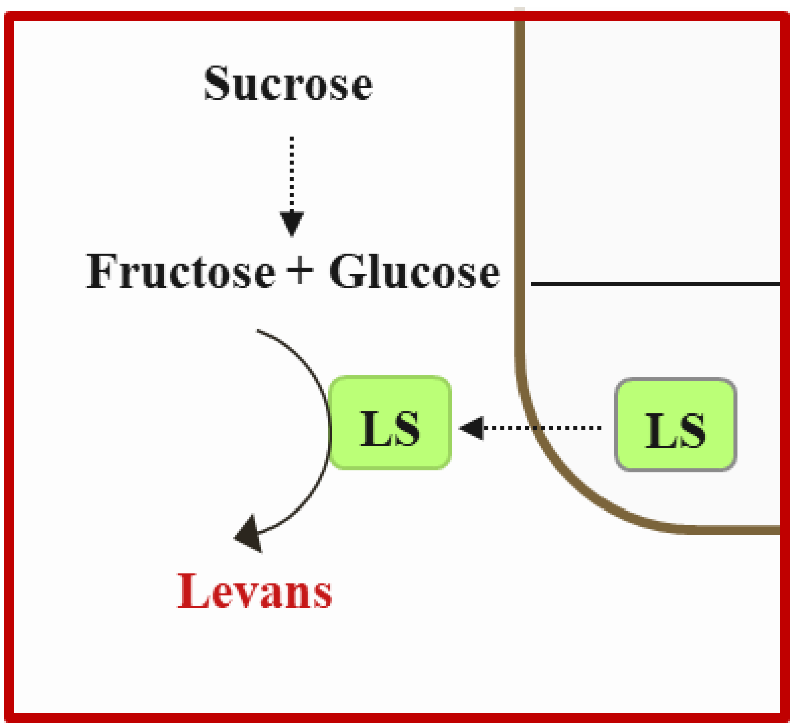
Part 3

Screening and selection of acetic acid bacteria for fructans production

Part 3

Background, aim and strategy

- Some AAB are levan producers β(2→6)
- Prebiotics, anti-cancer and ant-viral effect
- Thickening and gelling agents
- Biodegradable plastics, glues, cosmetics, textile coatings, detergents

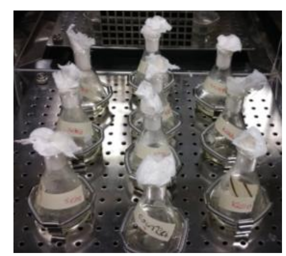


Some AAB are high fructan (Levan) producers

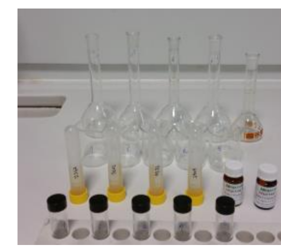
↓

Selection of strains to develop prebiotic beverages

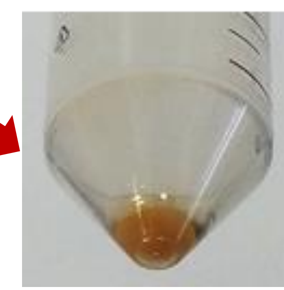
REVITALIZATION



FRUCTAN TEST



ASSAYS



EXTRACTION AND CHARACTERIZATION

MEDIUM OPTIMIZATION

Part 3

Identification of the screening conditions

Strain	Sucrose concentration	
	250 g/L	300 g/L
NBRC 101099^T	+	+
UMCC1754	+	weak
UMCC 1789	+	weak
DSM 3509^T	+	+
DSM 2343	+	+
DSM 2004^T	+	+
UMCC 2756	+	+

OSMOTOLERANCE SCREENING

- **Most AAB strains can grow at 300 g/L of sucrose**
- ***A. pasteurianus* strains prefer lower concentration**

250 g/L MAXIMUM SUCROSE CONCENTRATION

SCREENING OF FRUCTAN PRODUCTION

STANDARD CONDITIONS

- **Sucrose concentration:** 70 g/L
- **Shaking speed:** 140 rpm

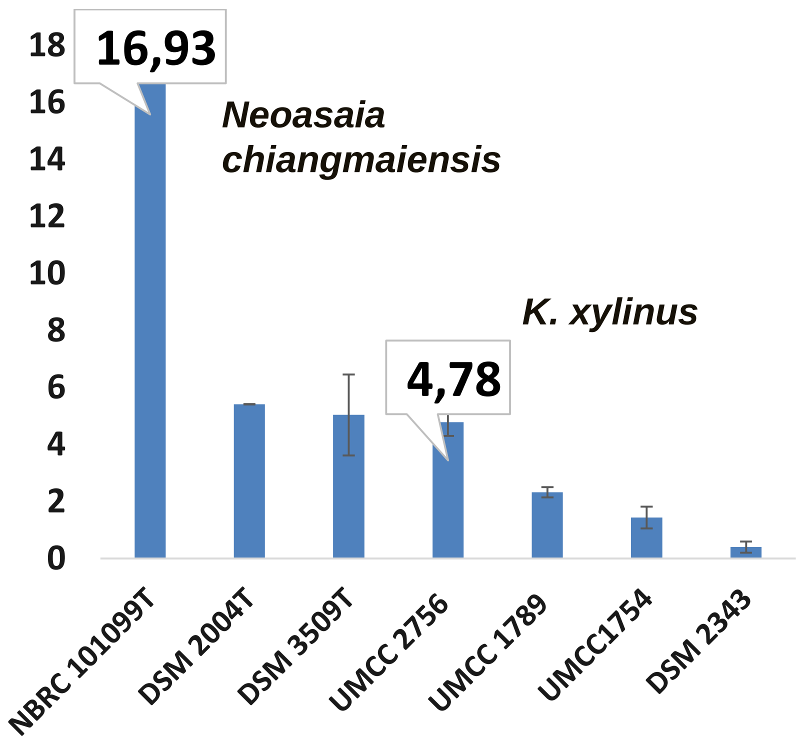
OPTIMIZED CONDITIONS

- **Sucrose concentration: 250 g/L**
- **Shaking speed: 200 rpm**

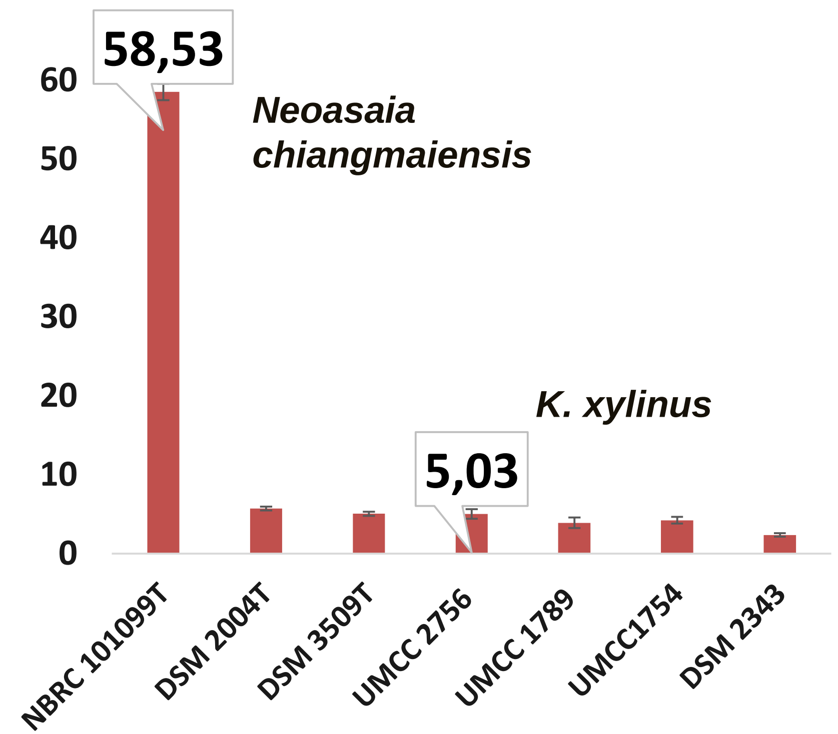
Part 3

Fructan production optimization

STANDARD CONDITIONS



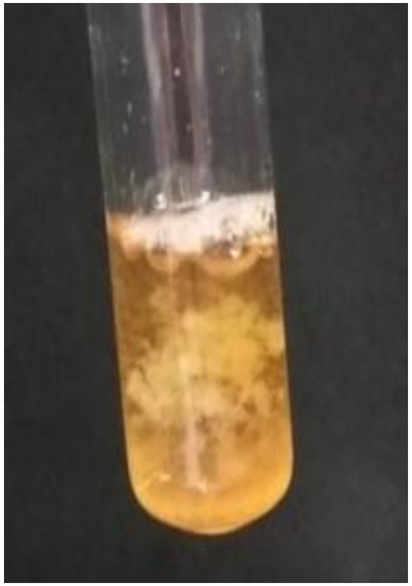
OPTIMIZED CONDITIONS



- ***Neoasaia chiangmaiensiss*** showed the highest fructan production in both conditions
- ***Acetobacter*** and ***K. xylinus* strains** produced about **5 g/L** in both conditions

CELLULOSE SCREENING AND EXTRACTION

Strain	QUALITATIVE BC PRODUCTION TEST	
	HS-G	HS-S
NBRC 101099^T	-	-
DSM 2343	-	-
DSM 2004^T	+	+
DSM 3509^T	-	-
UMCC1754	-	-
UMCC 1789	-	-
UMCC 2756	+	+



FRUCTANS EXTRACTION PROTOCOL

- Centrifugation
- + 2 Volume of cold ETOH
- Precipitation
- Centrifugation
- Levan-pellets collected
- Levan purification

FRUCTAN CHARACTERIZATION

Preliminary results showed a FT-IR spectra of pure levan

Fructans extracted by ethanol precipitation

Conclusions

Neoasaia chiangmaiensis **NBRC101099 showed the highest fructan production in both standard and optimized culture conditions**

Strains suitable for industrial production

Komagataeibacter **strains showed a considerable production of both fructans and cellulose**

K. xylinus **strain UMCC 2756 is suitable to develop functional foods and beverages**

General conclusions of the Ph.D. project

- High versatility of Acetic Acid Bacteria opens wide perspectives in both food and non food industry
- Possibility to develop new products
- Research on small scale is essential to improve the processes on large scale

Performing a further engineering of the investigated processes is the most suggested development, both in research and industrial scale

A special thanks to…

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