

Uddannelses- og Forskningsstyrelsen

Food & Bio Cluster Denmark

FoodTure

## Using microbial diversity or complex communities for valorization of side-streams for food applications

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## Agenda

- Background and Motivation
- Isolation, adaptation and screening for riboflavin-producing lactic acid bacteria in plant based fermentation using droplet microfluidics:
  - Introduction
  - What is microfluidics ?
  - Results and discussion
  - From microfluidics application
- Acknowledgment

Taking a microbial-centric approach to plant-based foods

## The Planetary boundaries

Planetary boundaries: Guiding human development on a changing planet – science 2015

Earth beyond six of nine planetary boundaries – Science 2023



Food systems have the potential to nurture human health and support environmental sustainability, however our current trajectories threaten both the planet and the health of the humans.



## Sustainable go hand in hand with Healthy Diets



#### New definition WHO/FAO (2019)

"Sustainable Healthy Diets are dietary patterns that promote all dimensions of individuals' health and wellbeing; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable".



The EAT–Lancet Commission also addresses the need to feed a growing global population a healthy diet while also defining sustainable food systems that will minimise damage to our planet.

Food in the Anthropocene: the EAT-*Lancet* Commission on healthy diets from sustainable food systems – lancet 2019

Sufficient, safe and healthy food for all, always also in the future!



## What is NFICC ?

- Establishing a collection of bacterial and yeast strains (NFICC) from plants and other environmental microbiomes collected around **Denmark**.
- Identifying them by a variety of techniques
- Sequencing to understand what genes and metabolic pathways may be present and thus what compounds they will make.
- Characterising their actual fermentation properties

#### Lactobacillus bulcaricus → Lactobacillus danica





6.500 retension at surgeat Notes heating vignationer heating surgeating of p. 1. Sampling areas in Bugars and detection of p. 2007 start factoria. Bints amples were collected in the four regions (0). At each area, the number of plant amples containing Landbollub bulgaricus, Streptoreccus thermohaldo or both strats and the total sample number are shown, together with the detection rate (5) in the paretheses.

FEMS Microbiol Lett 269 (2007) 160-169







## Why look for new strains back in nature ?







### **NFICC** and the link to food ?



Anders Peter Wätjen et al. 2023



Sanne Kjærulf Todorov<sup>a</sup>, Frantiska Tomasikova<sup>b</sup>, Mikkel Hansen<sup>a</sup>, Radhakrishna Shetty<sup>a</sup>, Celia L. Jansen<sup>a</sup>, Charlotte Jacobsen<sup>a</sup>, Timothy John Hobley<sup>a</sup>, René Lametsch<sup>b,\*</sup>, Claus Heiner Bang-Berthelsen ª,

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#### JOURNAL ARTICLE

Development of a yoghurt alternative, based on plant-adapted lactic acid bacteria, soy drink and the liquid fraction of brewers' spent grain 🕮

Sanne Kjærulf Madsen, Camilla Priess, Anders Peter Wätjen, Süleyman Øzmerih, Mohammad Amin Mohammadifar, Claus Heiner Bang-Berthelsen 🐱

FEMS Microbiology Letters, Volume 368, Issue 15, August 2021, fnab093, https://doi.org/10.1093/femsle/fnab093 Published: 26 July 2021 Article history •

Taking a microbial-centric approach to plant-based foods

Review

Hybrid Cheeses-Supplementation of Cheese with Plant-Based Ingredients for a Tasty, Nutritious and Sustainable Food Transition

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## Isolation, adaptation and screening for riboflavin-producing lactic acid bacteria in plant based fermentation using droplet microfluidics



Identify, select, and improve riboflavin producing lactic acid bacteria strains suitable for plant-based food fermentation.

### 

## Need for novel plant-based starter cultures

- LAB in dairy:
  - Adapted to dairy environment (lactose)
    - Loss of genes for utilizing plant carbohydrates
    - Unable to ferment plant bases
- Plant-based LAB:
  - Adapted to plant environment
    - better at fermenting complex plant-based carbohydrates (e.g. xylose and arabinose)
    - improve nutritional properties, sensory profile, texture
  - Avoid adding artificials clean product



Christensen, L.F. et al., 2022. Extracellular microbial proteases with specificity on plant proteins. International Journal of Food Microbiology 381(109889–109889. https://doi.org/10.1016/j.ijfoodmicro.2022.109889





### **Fermentation matrices**



## **The Best of Both Worlds?**



#### •Riboflavin (Vitamin B2):

• Water-soluble B-vitamin essential for energy metabolism.

#### •Function:

• Crucial for the formation of coenzymes (FMN and FAD) in energy production.

#### •Sources:

• Found in **dairy, meats**, green leafy vegetables, and enriched cereals.



soy powder













#### Traditional strain level microbial isolation and screening pipeline



#### Innovative community level microbial isolation and screening pipeline





Onyx







## **Onyx: Droplet Generation Device**

This device is used for generating emulsions.

Biological experiment perspective: ALE for plant-based foods High-throughput: Single cell level microculture







## Preparation of transparent soy milk



5% w/v soy flour (Sigma)

Decrease pH/Centrifuge

Buffering and increasing pH



Filtering with 0.2um pole filters



### B2 vitamin what is novel ?





## **Styx: Sorting Device**

Α

• Lasers: Yellow, Blue, Green, and Red



B

0: 0ms, E: 200us

Histogram: Green Linear

Green



## 16 fold increase in riboflavin production in TSM





## Comparasion of two different methods for detection of B2 vitamin





## 5 rounds of adaptation and sequencing of NFICC2835

#### Lactococcus lactis (NFICC2835)

Genome	CDS	Change	Amino Acid	Codon Change	Gene	
Location	Position		Change			
1051375	-	G -> C	-	-	ribG#	
1051399	-	T -> C	-			
1051552	-	A -> T	-	-	ribG#	
1051563	-	+A	-	-	ribG#	
1051783	145	G -> A	E -> K	GAA -> AAA	ribG	
1051915	277	A -> G	R -> G	AGA -> GGA	ribG	
1051988	350	A -> G	K -> R	AAA -> AGA	ribG	
1052011	373	A -> C	I -> L	ATC -> CTC	ribG	
1052348	710	G -> C	S -> T	AGT -> ACT	ribG	
1052356	718	T -> C	S -> P	TCA -> CCA	ribG	
1052374	736	G -> A	A -> T	GCT -> ACT	ribG	
1052414	776	T -> C	V -> A	GTG -> GCG	ribG	
1052560	922	A -> G	I -> V	ATC -> GTC	ribG	
1052584	946	G -> T	A -> S	GCC -> TCC	ribG	
1052588	950	C -> T	P -> L	CCT -> CTT	ribG	
1052988	262	T -> C	S -> P	TCA -> CCA	ribB	
1053793	408	A -> C	l -> L	ATT -> CTT	ribA	
1053799	414	A -> T	L -> F	TTA -> TTT	ribA	
1053827	442	A -> G	I -> V	ATT -> GTT	ribA	
1054614	5	A -> C	K -> T	AAA -> ACA	ribH	
1701211	949	GNC	T > P		purH	
1701313	746	A -> T	F -> Y	TTT -> TAT	purH	
1701818	241	T -> C	K -> E	AAA -> GAA	purH	
1701932	127	C -> T	E -> K	GAA -> AAA	purH	
1702010	49	C -> T	E -> K	GAA -> AAA	purH	
1702100	-	+T	-	-	purH#	
1702135	-	A -> G	-	-	purH#	
1702668	109	T -> C	I -> V	ATT -> GTT	hprT	



Sugar Utiliza	tion Sugar Utilization
Control (negative)	Esculine
Glycerol	Salicine
Erythritol	D-cellubiose
D-Arabinose	Maltose
L-Arabinose	Lactose
Ribose	D-Melibiose
D-Xylose	Sucrose
L-Xylose	D-Trehalose
Adonitol	Inulin
Beta-Methyl xyloside	Melezitose
Galactose	D-Raffinose
D-Glucose	Starch
D-Fructose	Glycogene
D-Mannose	Xylitol
L-Sorbose	Beta-Gentiobiose
Rhamnose	D-Turanose
Dulcitol	D-Lyxose
meso-Inositol	D-Tagatose
Mannitol	D-Fucose
Sorbitol	L-Fucose
alfa-Methyl-p-mannoside	D-Arabitol
alfa-Methyl-D-mannoside	L-Arabitol
N-acetyl-glucosamine	Gluconate
Amygdalin	2-ceto-gluconate
Arbutine	5-ceto-gluconate

## 28 mutations in common between 3 unique isolates



## Can we use this strain more globally for plant-based foods in general?

										Vitar	min
Products	Fat	Carbohydrate	Sugar	Protein	Calcium	Vitamir	D	Vitamin B <sub>2</sub>		B₁	2
Soy, Allos	2.3 g	1.1 g	0.5 g	3.6 g							
Soy, Spir	1.8 g	3.5 g	3.5 g	3.2 g							
Soy, Naturli	2.1 g	0.6 g	0.6 g	3.7 g							
*DCM, Naturli	2.0 g	3.4 g	2.3 g	3.4 g	120 mg	1.5 ug	0.2	21 mg 0.3		ug	
Rice, Allos	1.0g	12 g	5.5 g	<0. 5 g							
Rice, Spir	1.3 g	10 g	7 g	0 g							
Rice, Naturli	1.1 g	11.0 g	6.5 g	0.1 g							
Oat, Spir	1.9 g	8 g	3.2 g	0 g							
Oat, Naturli	1.7g	8 g	3.2 g	0.4 g							
Oat Bartista,											
Naturli	2.9 g	9.6 g	4.1 g	<u>1.0 g</u>	120 mg	1.5 ug	0.2	21 mg	0.38	ug	
Almond, Spir	2.5 g	4.1 g	3 g	1.1 g							
Almond, Naturli	0.9 g	2.7 g	2.4 g	0.3 g							





## HPLC results after soy milk fermentation





Fermented soymilk after centrifugation and filtering

1.2 mg/L riboflavin for overnight culture

#### **Recommended Amounts**

RDA: The Recommended Dietary Allowance (RDA) for men and women ages 19+ years is 1.3 mg and 1.1 mg daily, respectively. For pregnancy and lactation, the amount increases to 1.4 mg and 1.6 mg daily, respectively.

Harvard T.H. Chan School of Public Health https://www.hsph.harvard.edu > The Nutrition Source : Riboflavin – Vitamin B2 | The Nutrition Source



## **Concept of starter cultures is like LEGO**



Strain collection



Removal of off-flavors

# Acknowledgment





Data was generated by:

DTU

**Hang Xiao**: insights in microbial selection for more vitamin B<sub>2</sub> in plant based foods

