
The nutritional power of fermentation and its management

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Fermentation is a biotechnology process for converting carbohydrates into alcohol or organic acids using microorganisms - yeasts or bacteria - under almost anaerobic conditions. Fermentation usually implies the action of desirable microorganisms naturally present or added in form of selected starters

Fermentation is an oxidative-reducing process where an organic compound acts both as donor and acceptor of electrons within a chain of reactions, which promotes the synthesis of ATP through the mechanism of phosphorylation at the substrate level.

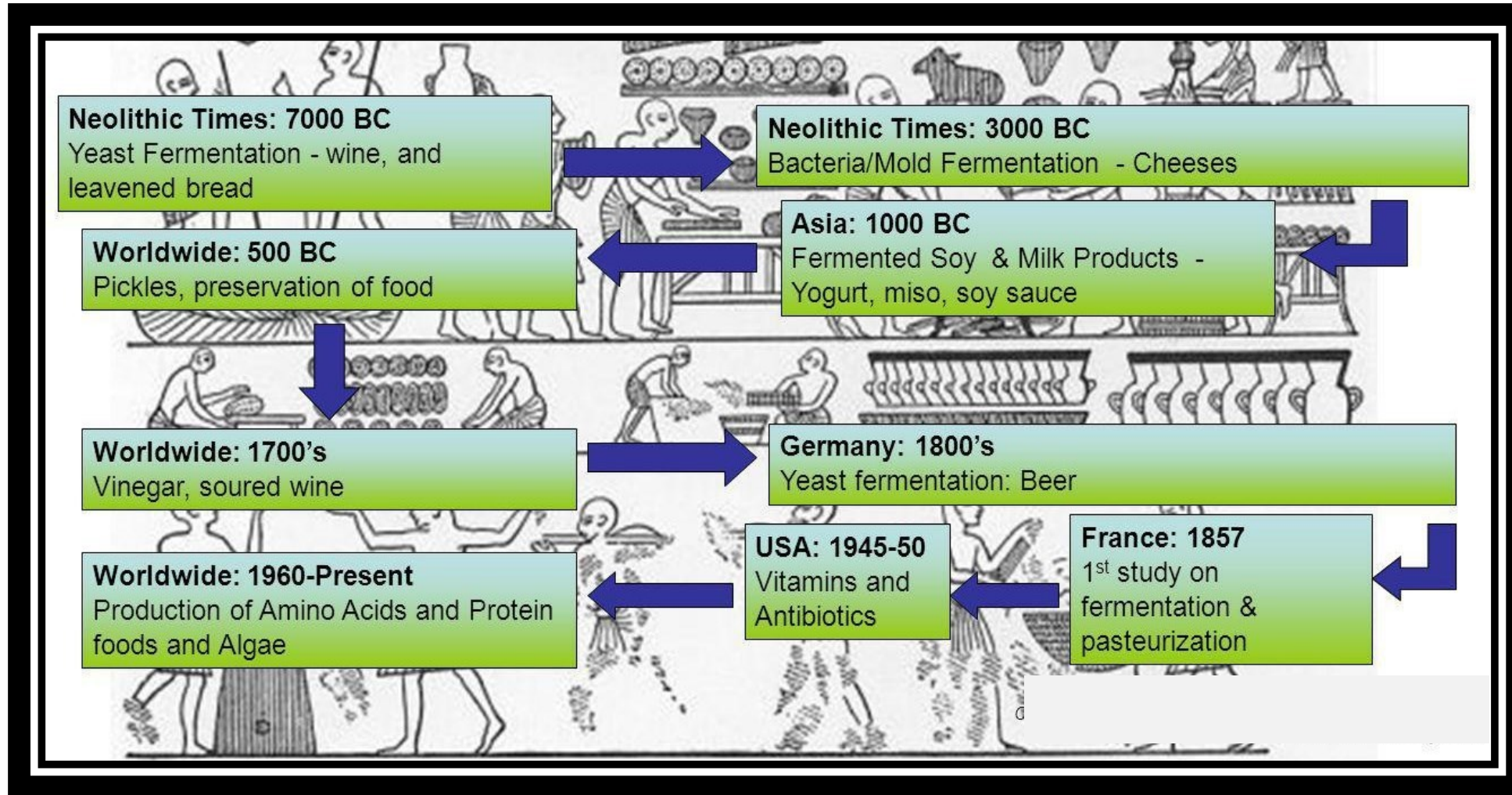
Fermentation is a very ancient process, which is inspiring the new biotechnologies (e.g., **precision fermentation**)

Fermentation may have been a greater discovery than fire.

- David Rains Wallace



Historical background of fermentation



(Claesson *et al.*, 2009. PLoS ONE, 4:e6669)

Fermented foods around the world: an issue of numbers

More than 3500 milk-, cereals-, legumes-, vegetables-, the-, meat- and fish-based fermented products

«there is ferment in consumers' diet»

- ✓ Thousands of different types of fermented foods are consumed worldwide, constituting 30-70% of the human diet.
- ✓ Many of them are emblematic of gastronomic culture and diversity, and in Europe they account for 20-50% of the daily diet.

(Xiang et al., 2019. Food Science and Human Wellness <https://doi.org/10.1016/j.fshw.2019.08.003>)

«There is ferment in kitchen»



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Fermented foods

The New York Times

Aug. 13, 2021

How Fermented Foods May Alter Your Microbiome and Improve Your Health

Foods like yogurt, kimchi, sauerkraut and kombucha increased the diversity of gut microbes and led to lower levels of inflammation.

“The vast majority came from somewhere else, and we don’t know where,” said Justin Sonnenburg, an author of the new study and a professor of microbiology and immunology at Stanford. “I think there were either low level microbes below the level of detection that bloomed, or the fermented foods did something that allowed for the rapid recruitment of other microbes into the gut environment.”

(Wastyk et al., 2021. Cell 184:4137)



Fresh kimchi, or Korean fermented vegetables, are an increasingly popular item at many supermarkets. Lanna Apisukh for The New York Times

From cow's milk to cheese: the microbiome fil rouge

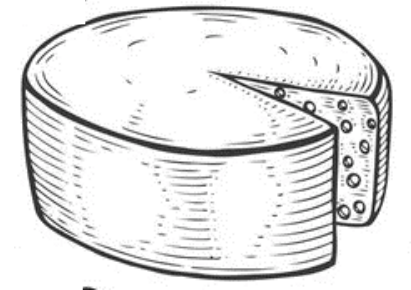
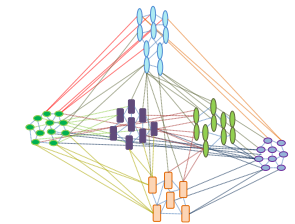
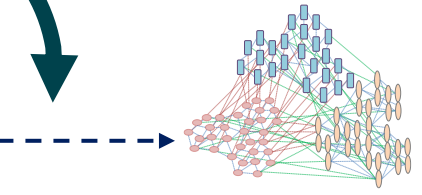
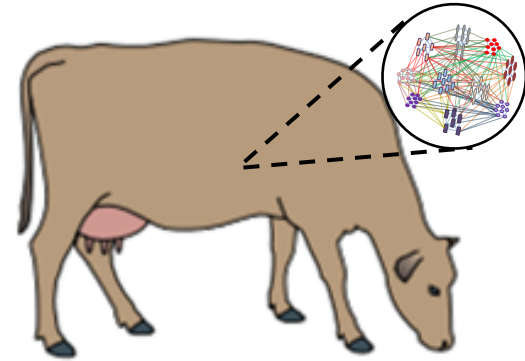
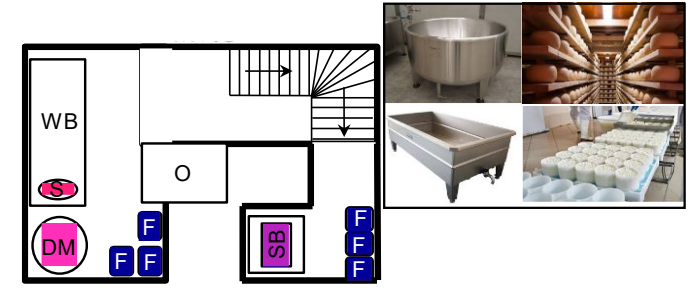
Management farm system – Feed



Raw vs pasteurized milk



House microbiota



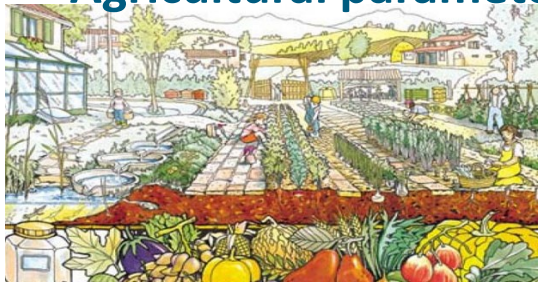
Farm or personnel hygiene

Cheese manufacture: biotic and abiotic factors

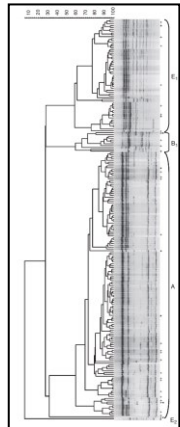
(Gobbetti et al., 2018. *Trends in Food Science & Technology*, 78:244)

From wheat plant to sourdough: the microbiome fil rouge

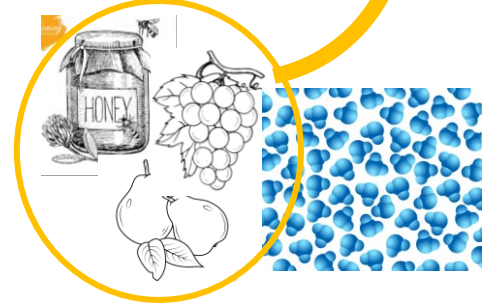
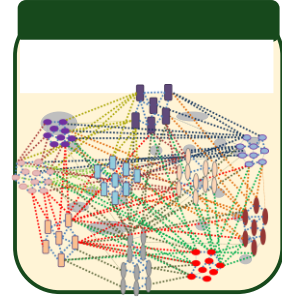
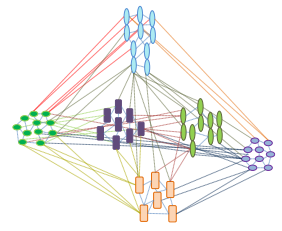
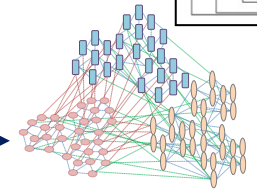
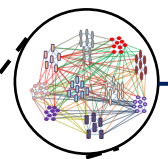
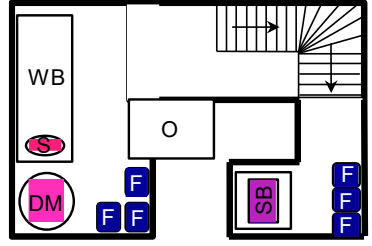
Agricultural parameters



Technological process parameters



House microbiota



Ingredients and tap water

(Gobbetti et al., 2019. *Crit. Reviews Food Sci Nutr.* In press)

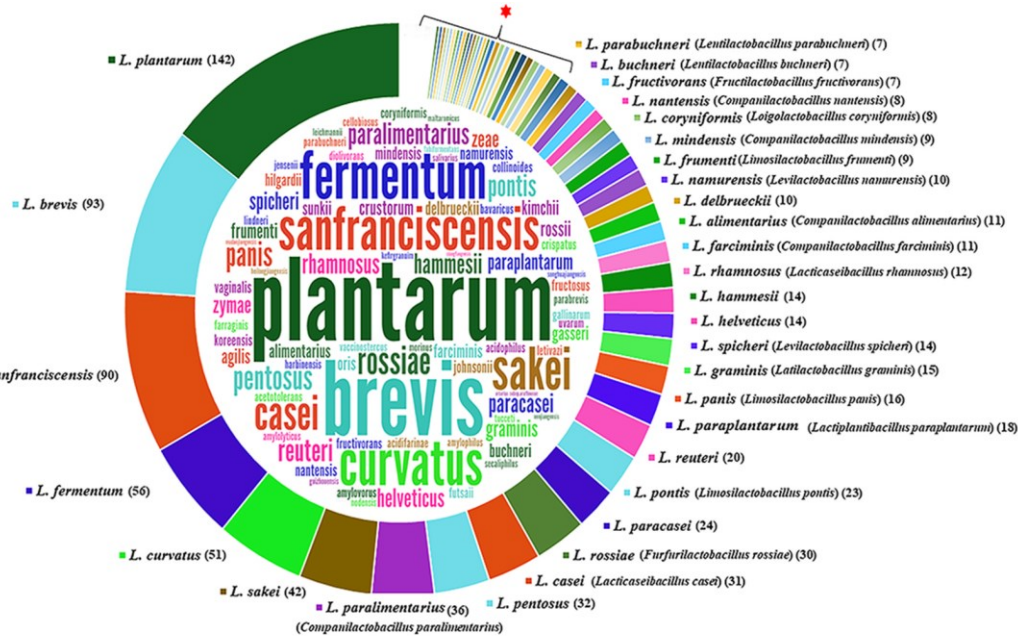
Sourdough microbial diversity

Bacteria (59 genera)

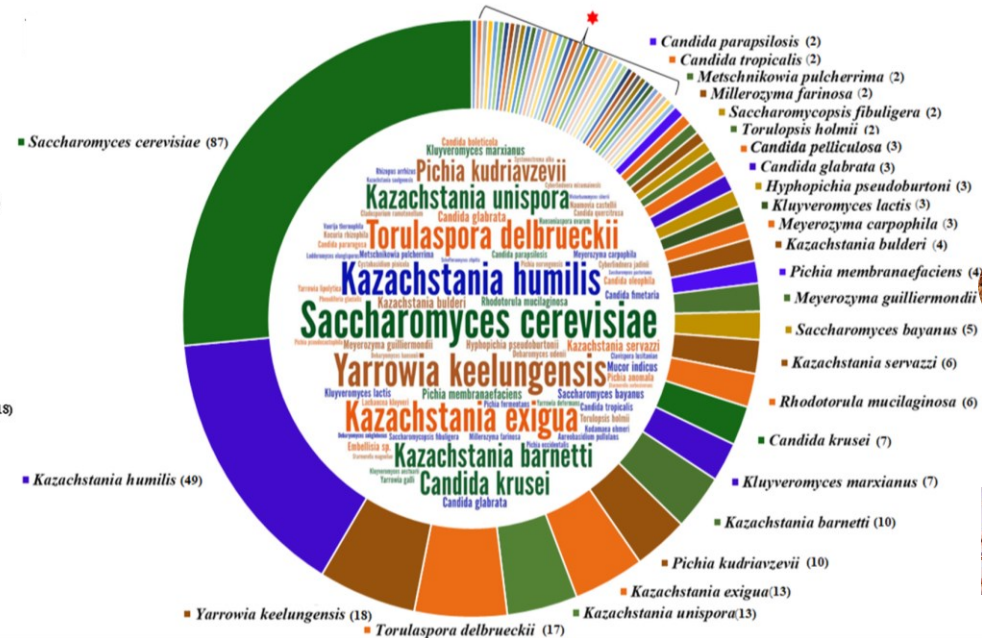
49 genera non lactic acid bacteria

10 genera lactic acid bacteria

Former *Lactobacillus* (82 species)



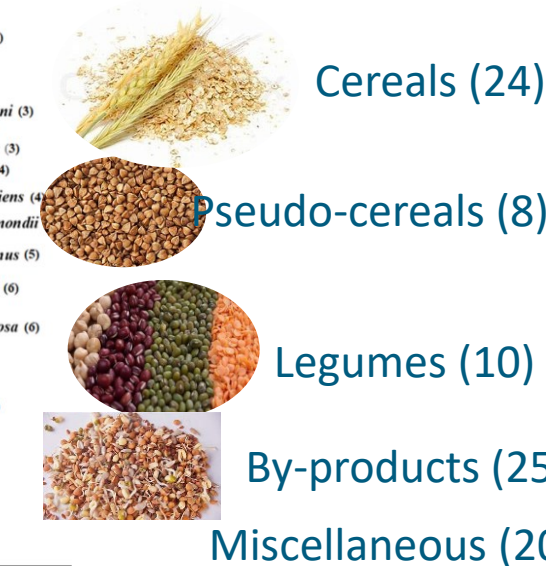
Yeasts (80 species)



357 Research articles e.g.,

- Bartkiene et al., 2017
- Stanzer et al., 2017
- Korcari et al., 2020
- Montemurro et al., 2020

Diversity of fermented matrices



Trends in Food Science & Technology

Volume 108, February 2021, Pages 71-83



Thirty years of knowledge on sourdough fermentation: A systematic review

Kashika Arora ^{a,1}, Hana Ameer ^{a,1}, Andrea Polo ^a, Raffaella Di Cagno ^a, Carlo Giuseppe Rizzello ^b, Marco Gobbetti ^{a,✉}

Nutritional attributes of sourdough

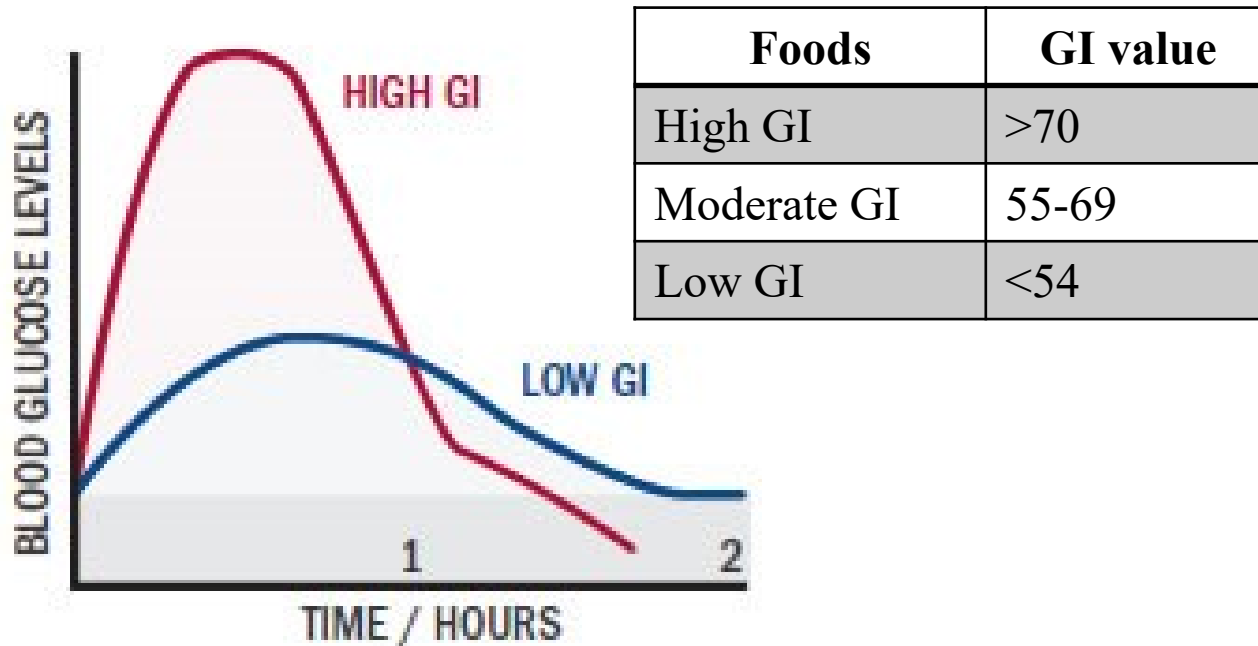
Word cloud representing the nutritional attributes focused during sourdough fermentation



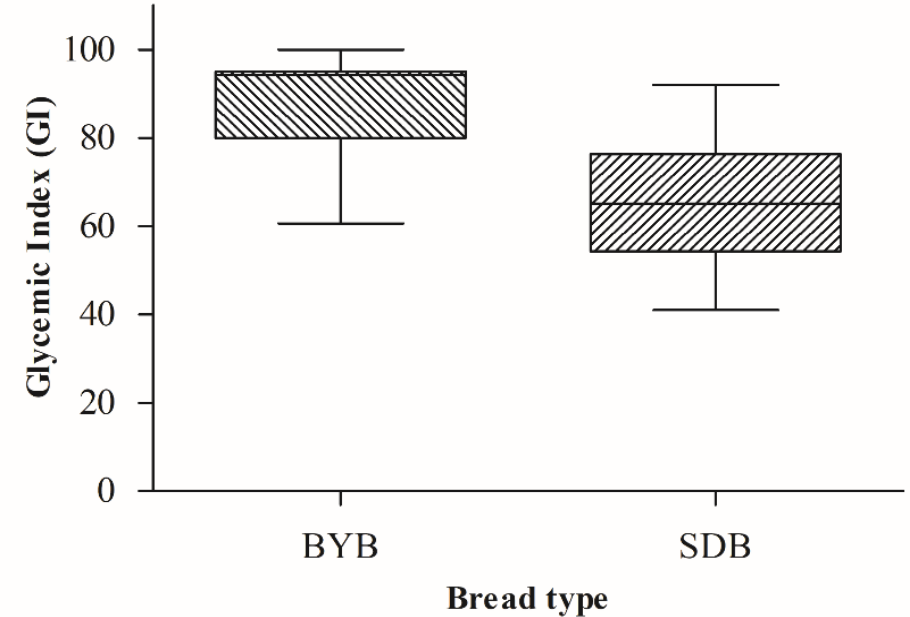
Arora et al., 2021. *Trends in Food Science & Technology* 108: 71-83

Glycemic Index (GI) of sourdough vs. baker's yeast breads

Glycemic Index (GI): A numerical value assigned to foods based on their capability to increase the blood glucose levels after consumption.



Curve for blood glucose levels vs. time
(Harvard Medical School)



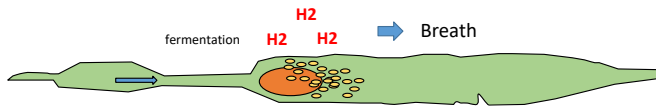
Median GI
Baker's yeast bread (BYB)=94.2
Sourdough bread (SDB)=65.1

Digestibility – *In vivo* clinical challenge

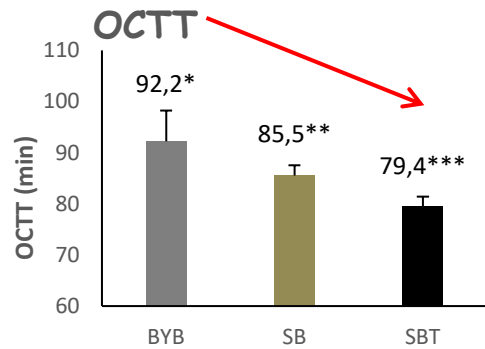
- 1) Baker's yeast bread (BYB) (1.5 h, baker's yeast 1.5%)
- 2) Sourdough bread (SB) (1.5 h, baker's yeast 1.5% + 20% type I sourdough previously incubated 4h)
- 3) Sourdough bread (SBT) (4 h, 20% type I sourdough previously incubated 24h)

Oro-cecal transit time (breath test)

faster transit for SB and SBT breads



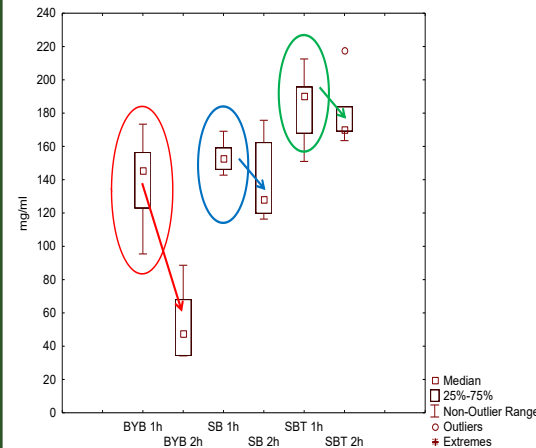
(more appetite for SB, higher satiety and fullness perceptions for SBT)



(Gobbetti *et al.*, 1994. *J. Food Sci.* 59:881)

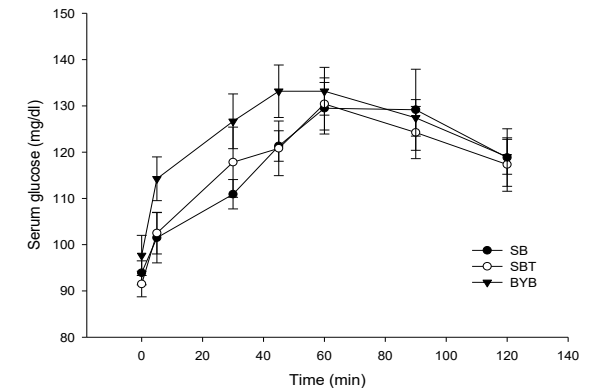
Amino acids in blood plasma

Higher amount at 1h and persistence at 2h after ingestion

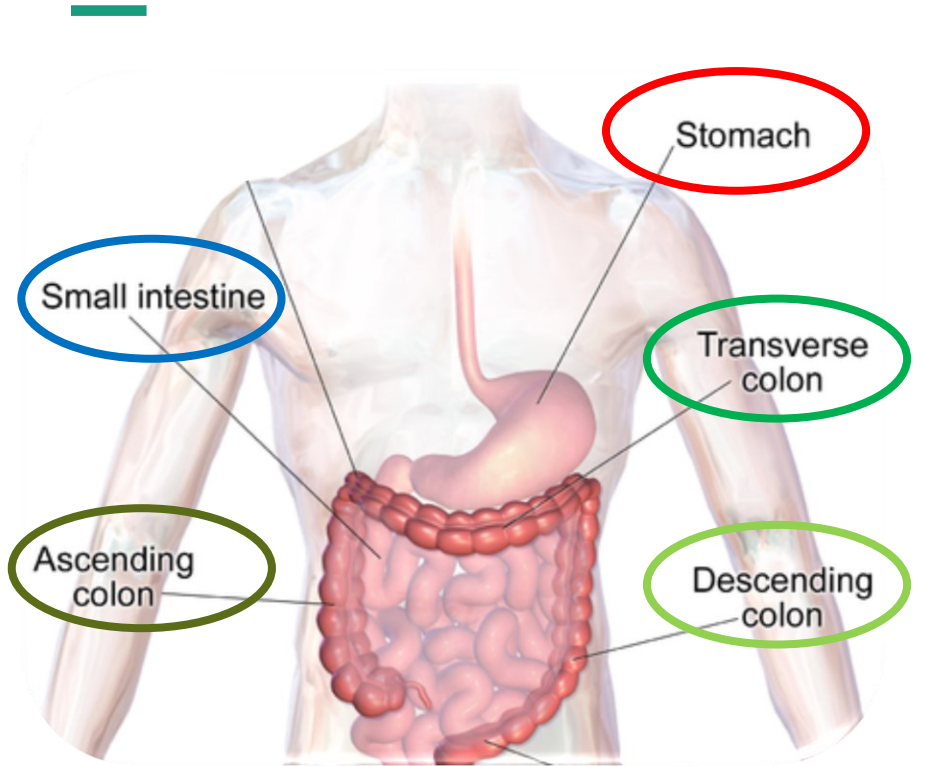


Post prandial glycemia

(glucose in blood after ingestion)
lower area under curve; lower glucose in the second part of the curve for SBT



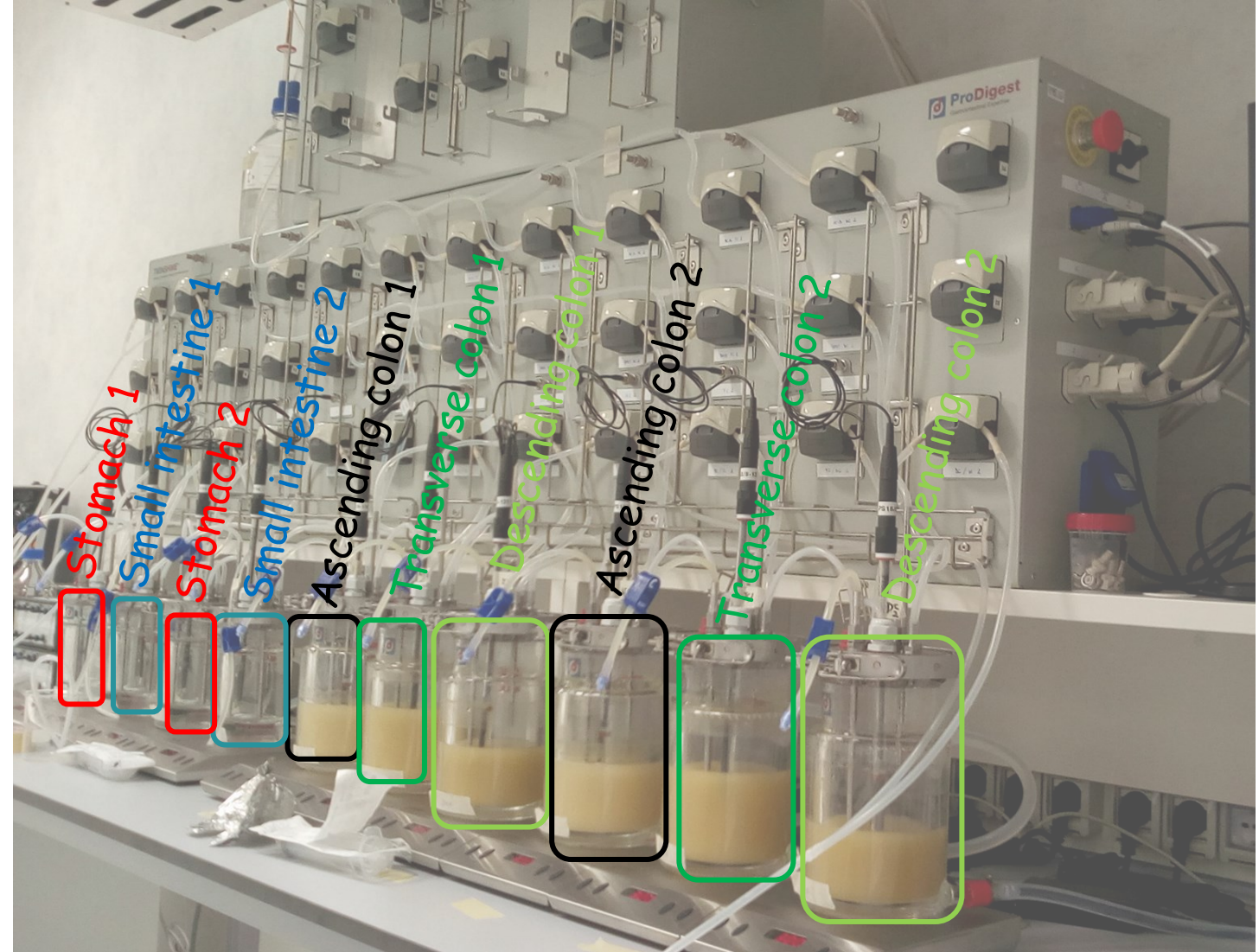
The Simulator of the Human Intestinal Microbial Ecosystem (SHIME)



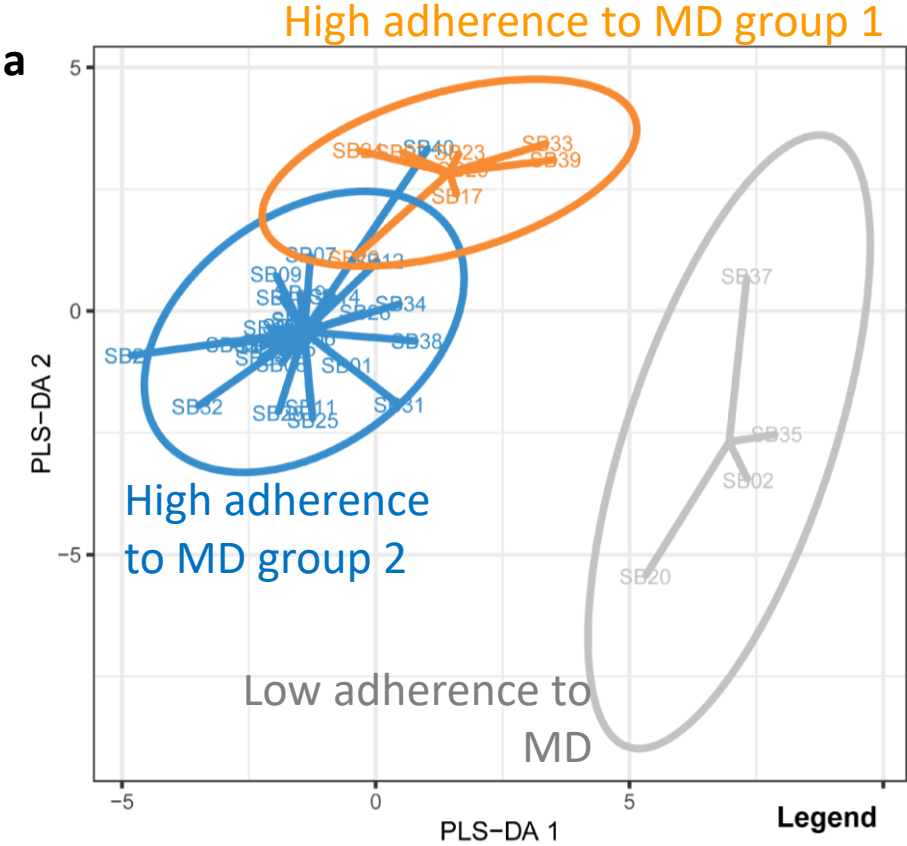
Gastrointestinal tract



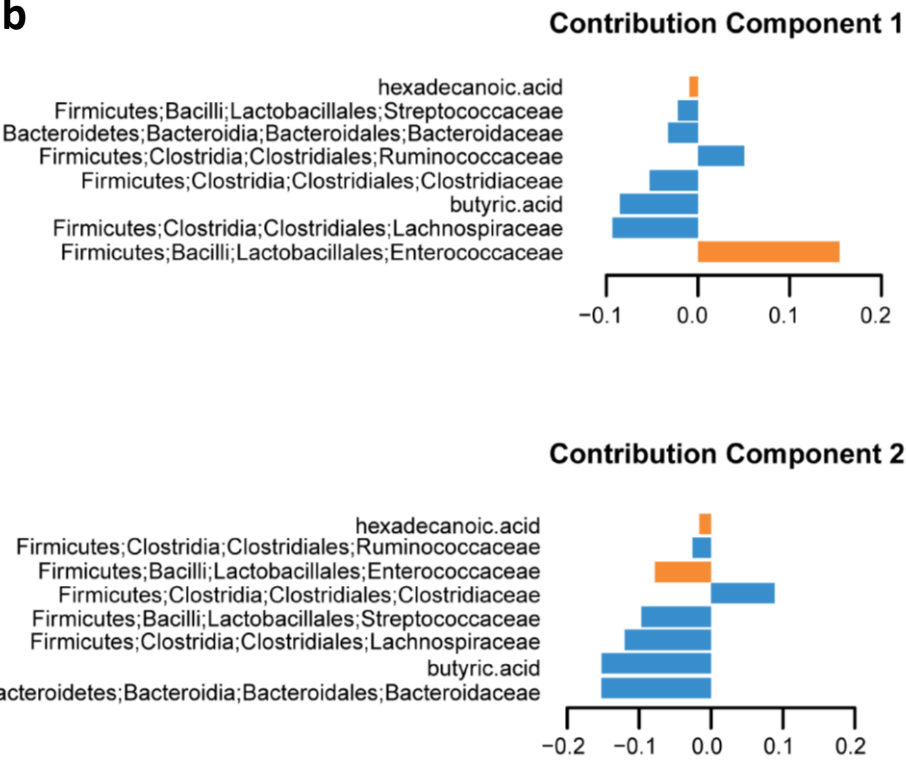
SHIME



Donor selection



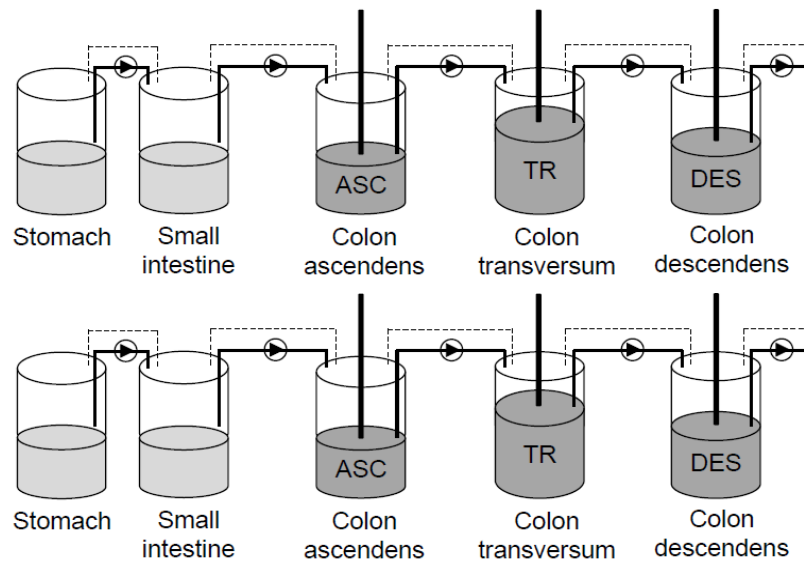
(a) Partial least square discriminant analysis (PLS-DA). Components 1 and 2 describe 77.87% and 0.05% of variance, respectively.



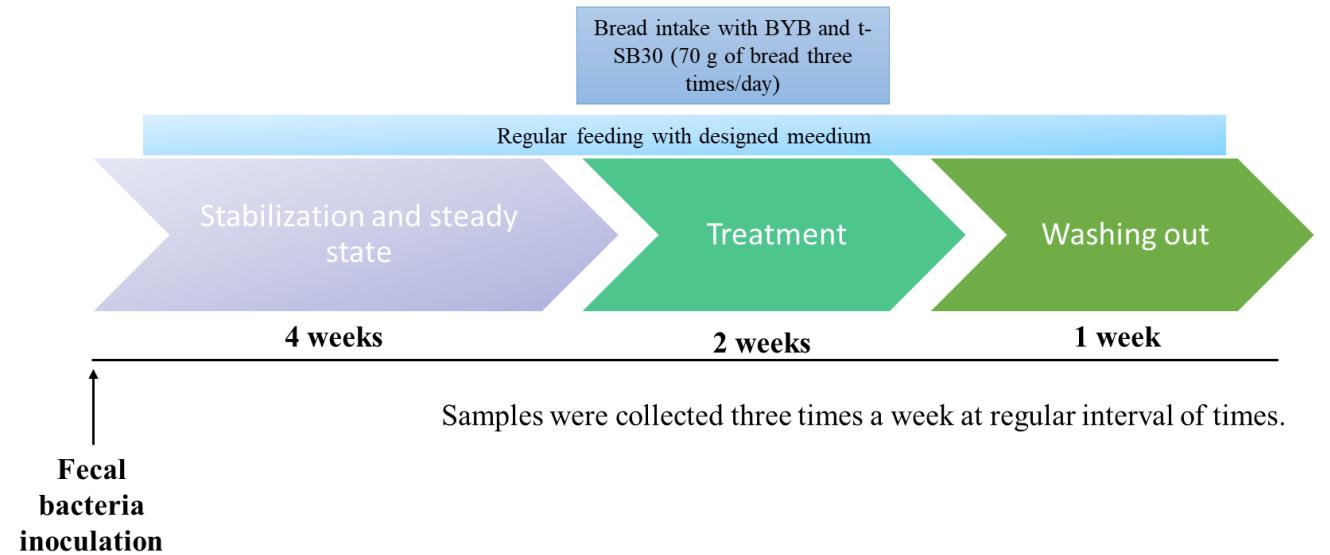
(b) Contribution of each variable to component loadings annotated by the groups with high adherence to

Experimental setup

- Baker's yeast bread (BYB)
- Sourdough bread (t-SB30)



Experimental setup of the Twin-Simulator of the Human Intestinal Microbial Ecosystem with mucosal environments (TWIN M-SHIME)



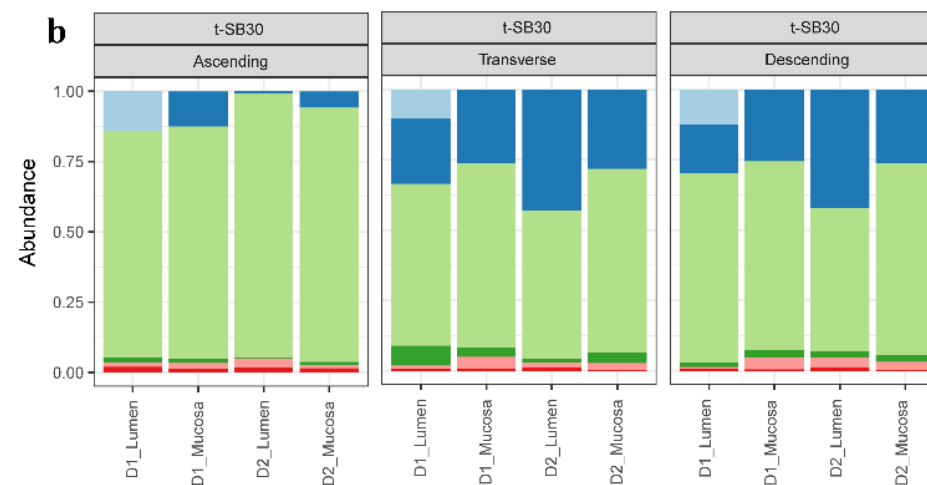
Experimental design of the in vitro experiment

Relative abundance at phylum level

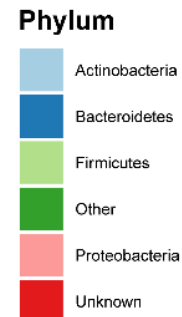
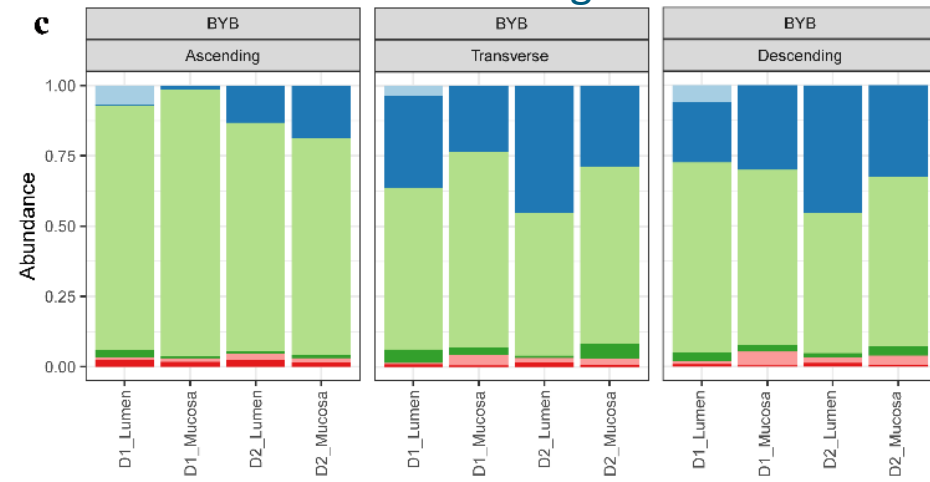
Before feeding



After feeding with t-SB30



After feeding with BYB

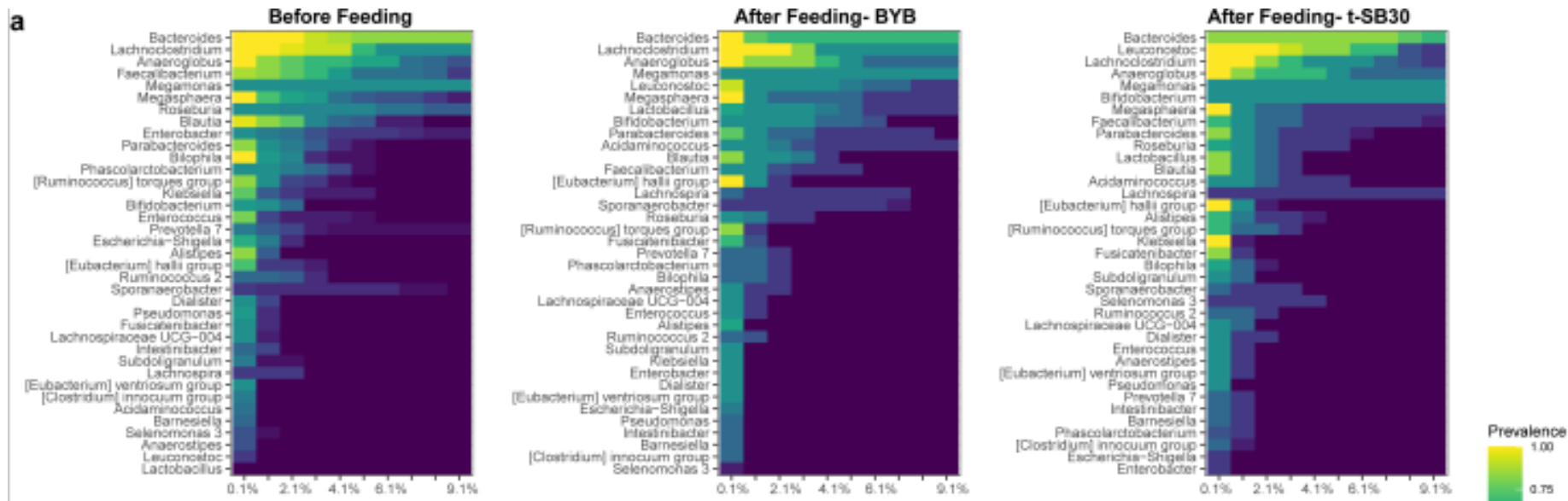


- Microbial phyla were consistent with faecal materials
- Similarities among colon tracts of both donors
- Firmicutes and Bacteroidetes were the taxa with the highest relative abundances
- The phylum distribution only slightly varied after 14 days of feeding with both breads
- Variations depend on bread feeding regardless of the type of bread

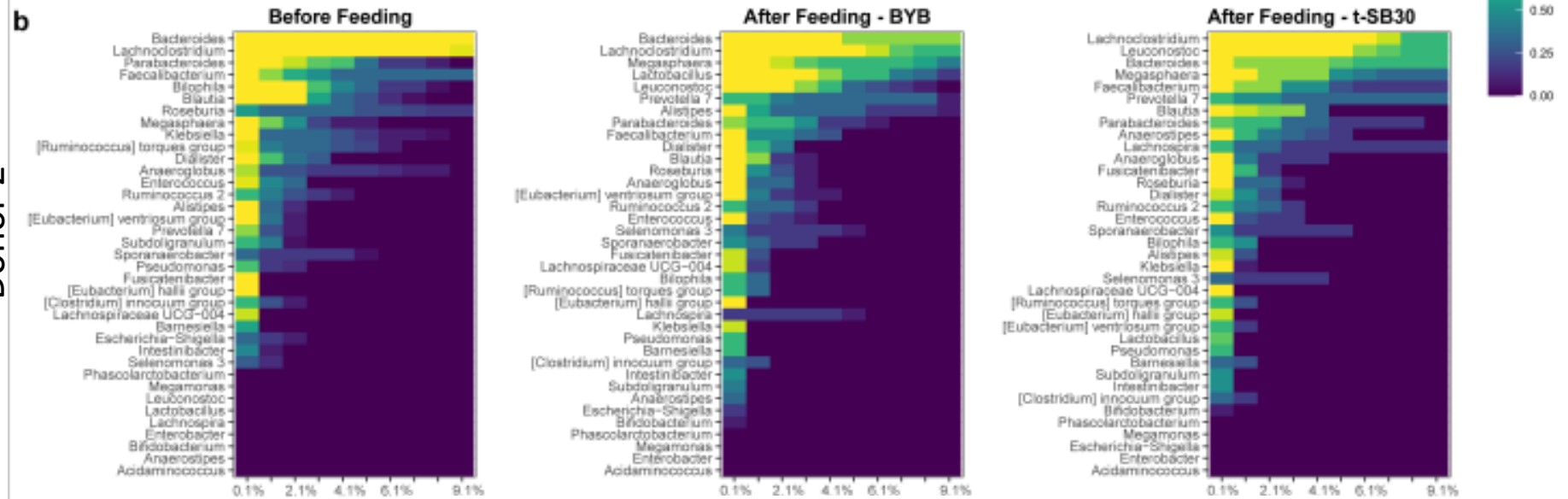
Core microbiome

- The genus core microbiota was investigated to search for diversity
- The composition only slightly changed after bread feeding
- When switching was observed, it was almost regardless of the type of bread and almost exclusively concerned a re-raking within the core microbiota

Donor 1



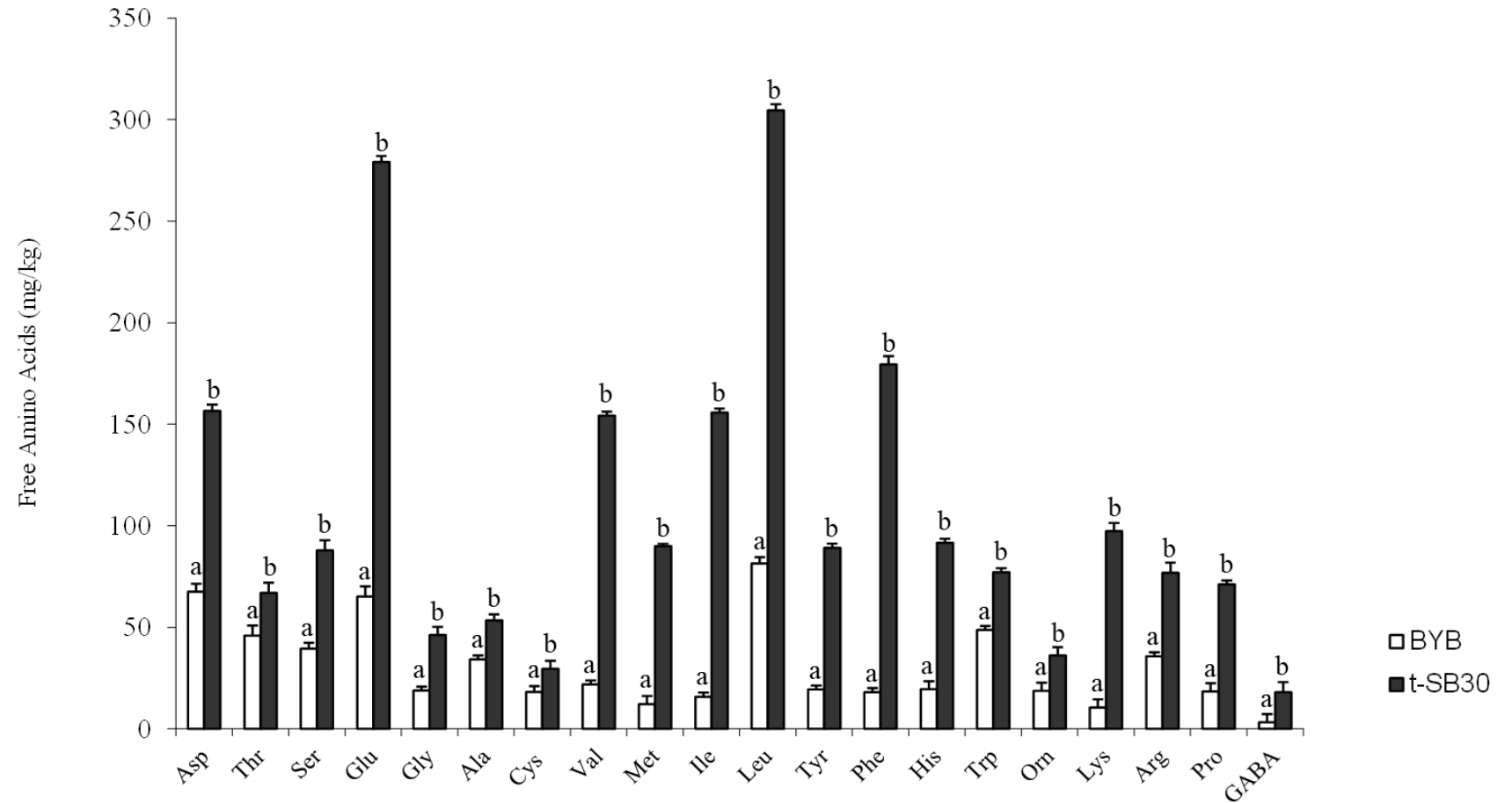
Donor 2



Detection Threshold (Relative Abundance (%))

Concentration of free amino acids

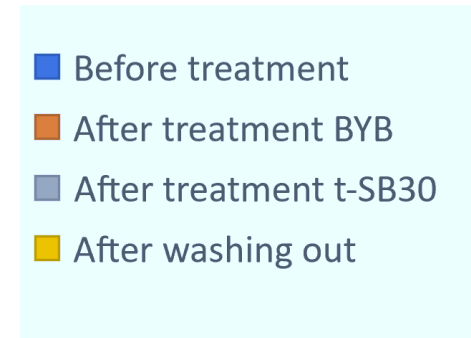
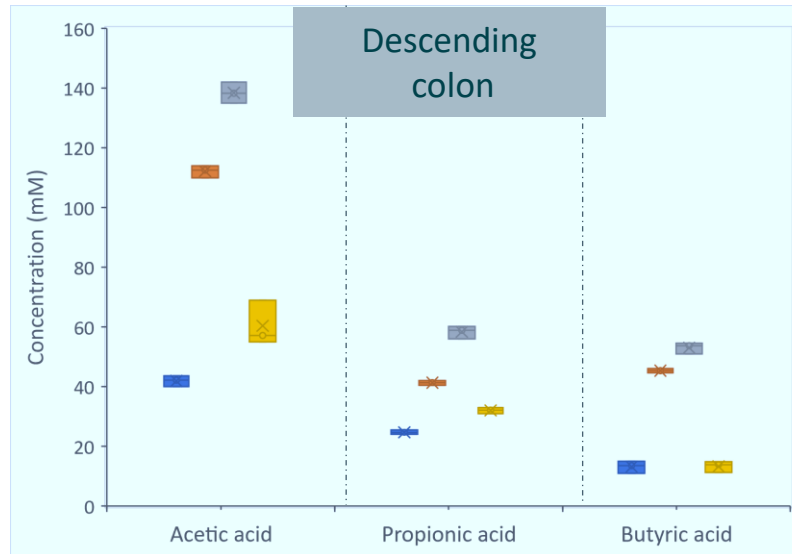
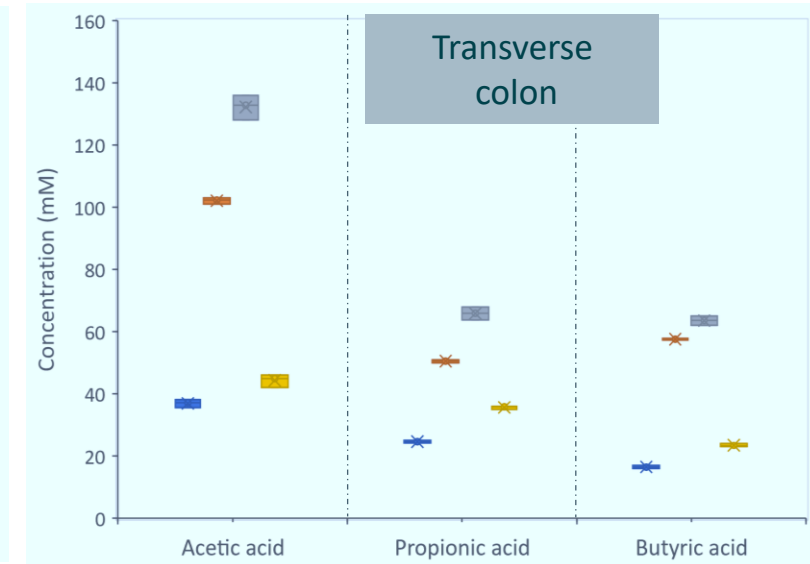
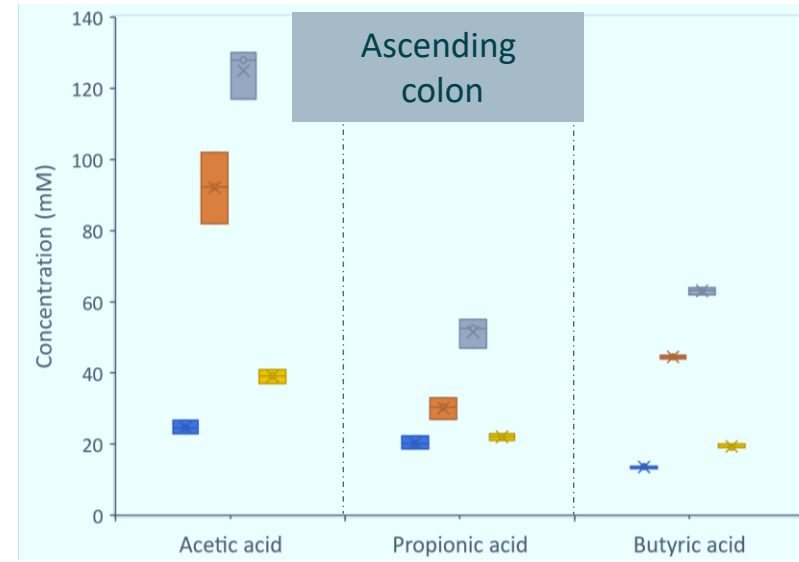
- Most of the individual FAA were at the highest levels in t-SB30
- Mainly: Leu and Glu, followed by Ile, Val and Asp



^{a-b} FAA with different superscript letters differ significantly ($P < 0.05$) based on one-way ANOVA (Tuckey-Kramer). The statistical analysis was performed separately for each amino acid

Short chain fatty acids

- A significant ($P < 0.05$) increase of SCFA was evident after 14 days of feeding with t-SB30 bread compared to BYB
- Following 1 week of wash out, the content SCFA trended to similar absolute values found before bread feeding



BYB vs t-SB30

Bread chemical, technological and nutritional characteristics

	BYB	t-SB30
pH	5.6 ± 0.1 ^a	4.2 ± 0.1 ^b
Volume increase (%)	245 ± 5 ^a	210 ± 4 ^b
Hardness (g)	3230 ± 22 ^b	3494 ± 18 ^a
Resilience	0.85 ± 0.02 ^a	0.69 ± 0.02 ^b
Fracturability (g)	3080 ± 5 ^a	2168 ± 10 ^b
Black pixel area (%)	48.0 ± 1.8 ^b	41.1 ± 1.3 ^b
In vitro protein digestibility (%)	64.2 ± 0.7 ^b	82.6 ± 1.5 ^a
Total FAA (g/kg)	0.61 ± 0.06 ^b	2.16 ± 0.06 ^a
Total peptides (g/kg)	4.4 ± 0.1 ^b	9.3 ± 0.1 ^a
Protein score (%)	18.5 ± 0.3 ^b	62.0 ± 0.5 ^a
Biological Value (BV)	36.5 ± 0.5 ^b	68.4 ± 1.2 ^a
Predicted Glycemic Index (pGI)	95 ^a	72 ^b
Resistant starch (% d.m.)	1.1 ± 0.1 ^b	2.5 ± 0.1 ^a

^{a-b}Values in the same row with different superscript letters differ significantly ($p < 0.05$) based on one-way ANOVA (Tuckey-Kramer). The data are the means of three independent analysis ± standard deviations (n =3).

Sourdough and its challenge

Is sourdough a supra-entity (e.g., fermentome) comprising not just the dominant species?

Metabolic framework of spontaneous and synthetic sourdough metacommunities to reveal microbial players responsible for resilience and performance

Francesco Maria Calabrese^{#1}, Hana Ameer^{#2}, Olga Nikoloudaki², Giuseppe Celano¹, Mirco Vacca¹, Wilson J F Lemos Junior², Caterina Manzari³, Fabienne Vertè⁴, Raffaella Di Cagno^{2*}, Graziano Pesole³, Maria De Angelis¹, Marco Gobbetti².

BMC Part of Springer Nature

Microbiome

✓ Investigation of the complex metabolic interactions among dominant lactic acid bacteria and yeasts and the subdominant ones, and the less abundant satellite members



- Sourdough resistance, resilience (gene redundancy)
- Longer stability
- Promote widespread use at artisanal and industrial levels

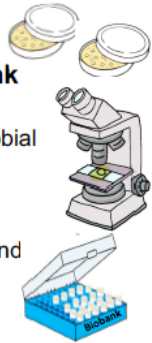
“FERMENTOME”

Methods Traditional sourdoughs collection and omics analysis

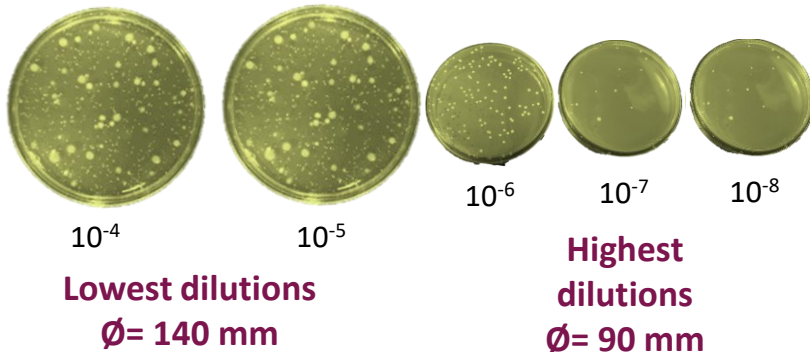
1. Culturomics – Biobank creation

Enumeration of diverse microbial populations

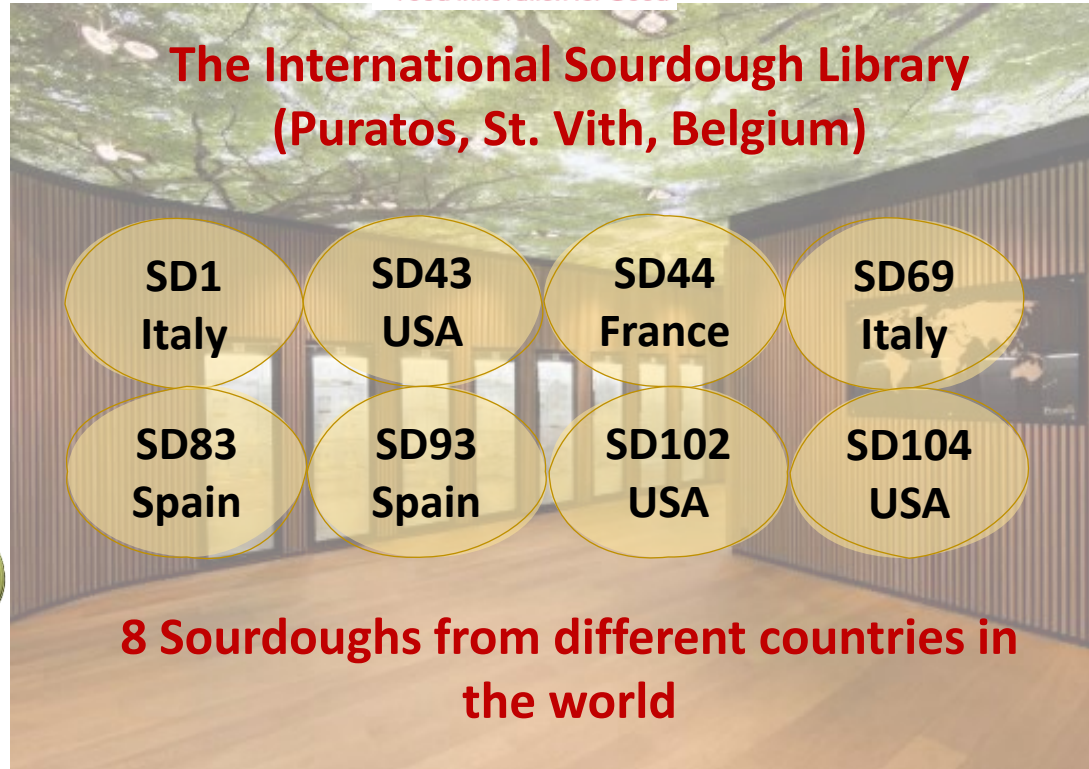
Isolation, purification and characterization of bacteria and yeasts



To investigate the subdominant culturable community of lactic acid bacteria, isolation was carried out by using 140 mm diameter plates and considering lowest dilutions.

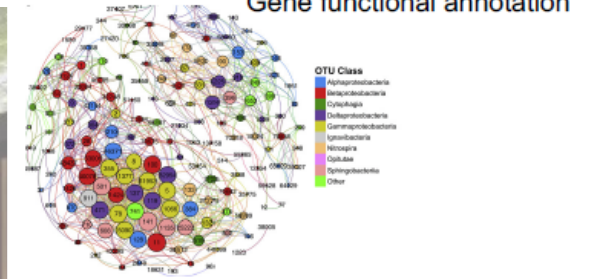


Sourdough Biobank: 1,661 isolates (1,488 bacteria and 173 yeasts)



2. Metagenomics Taxonomic annotation

Gene functional annotation

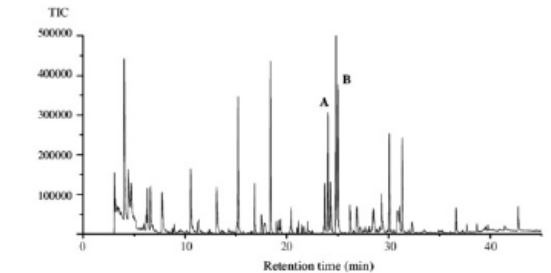


3. Metatranscriptomics

Transcript profile annotation and comparison

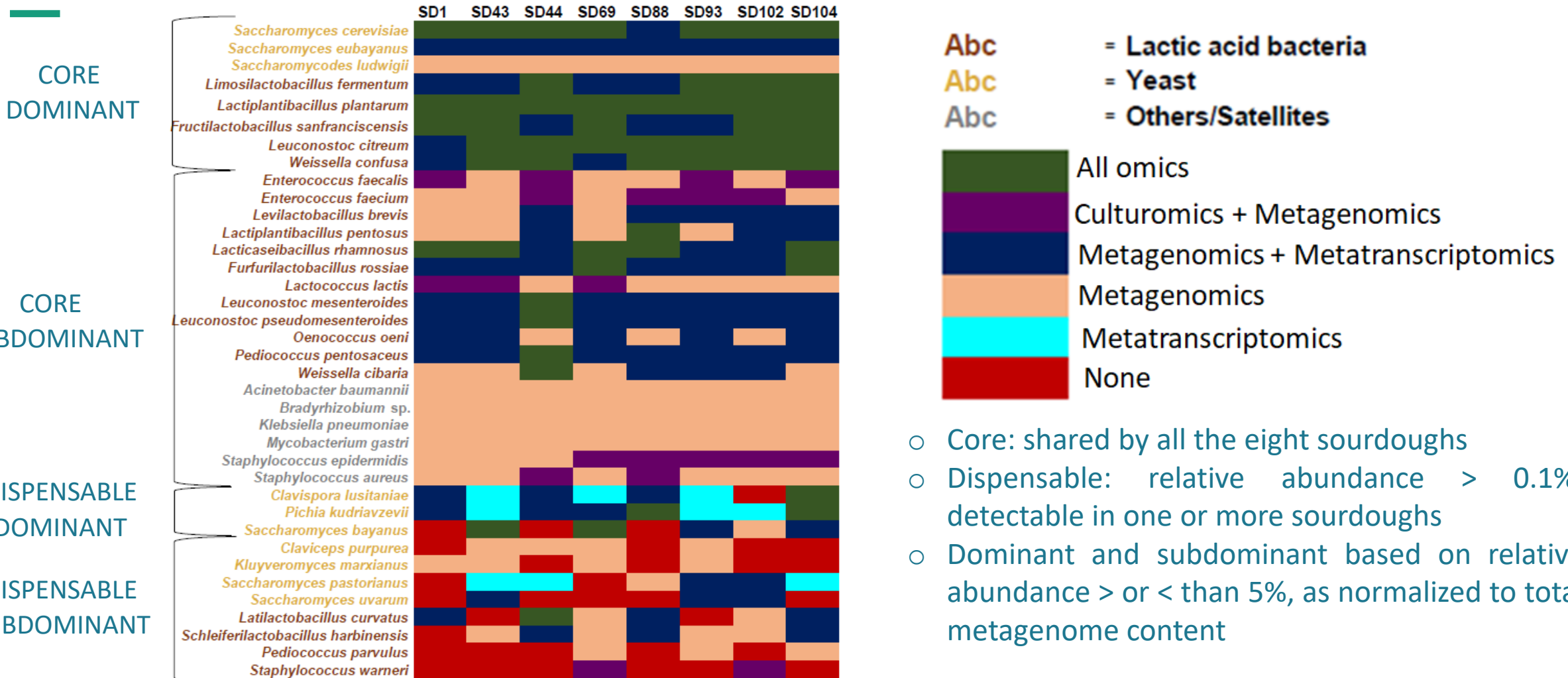
4. Metabolomics

Volatile compound identification



Taxonomic annotation by culturing-shotgun metagenomics-metatranscriptomics

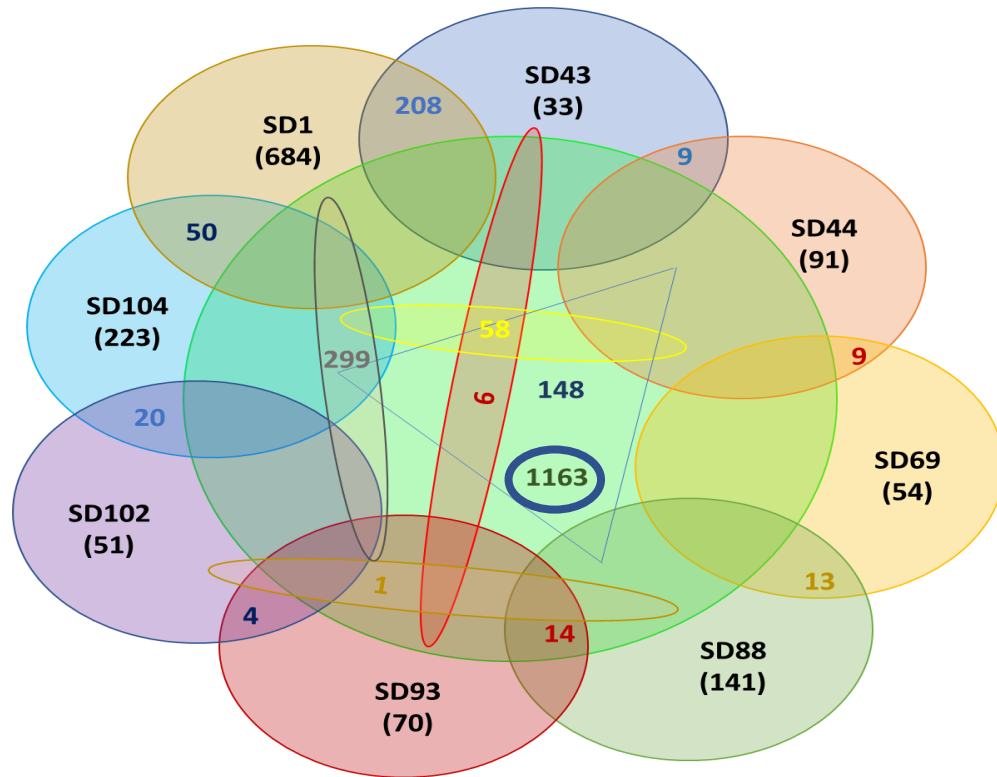
Core and dispensable species in the eight spontaneous sourdoughs



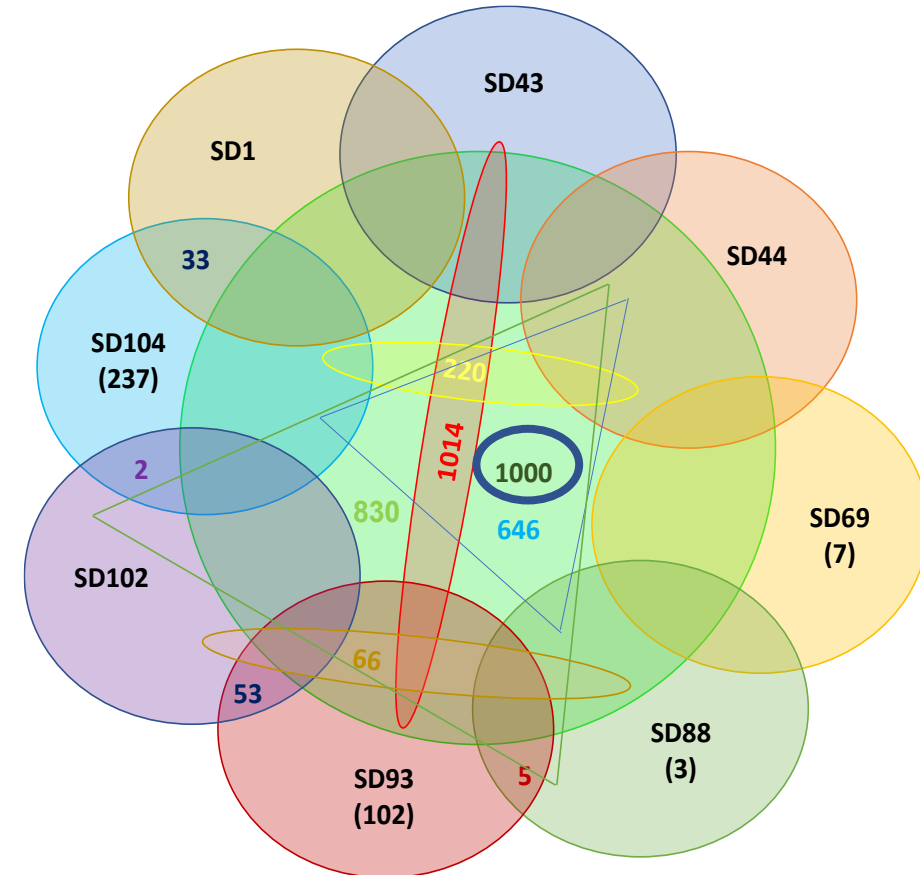
- Core: shared by all the eight sourdoughs
- Dispensable: relative abundance > 0.1%, detectable in one or more sourdoughs
- Dominant and subdominant based on relative abundance > or < than 5%, as normalized to total metagenome content

Deciphering the functional redundancy of sourdough genomes

Shotgun metagenomics



Metatranscriptomics



Gene abundances in each sample was mapped as a single set of reads (from each individual sample) against the total assembly obtained by pooling reads from all samples

Metabolic pathways investigated in 8 traditional sourdoughs

Carbohydrate, pyruvate and energy metabolism related pathways

- ❖ Starch, non-starch polysaccharides and sucrose pathways
- ❖ Pentoses and pyruvate metabolism

Nitrogen metabolism

- ❖ Peptide utilization encoding genes
- ❖ Catabolism of branched chain amino acids and aromatic amino acids (BCAA and ArAA)
- ❖ Catabolism of free amino acids (FAA)

Stress response

Vitamin and cofactor metabolism

Fatty acid metabolism

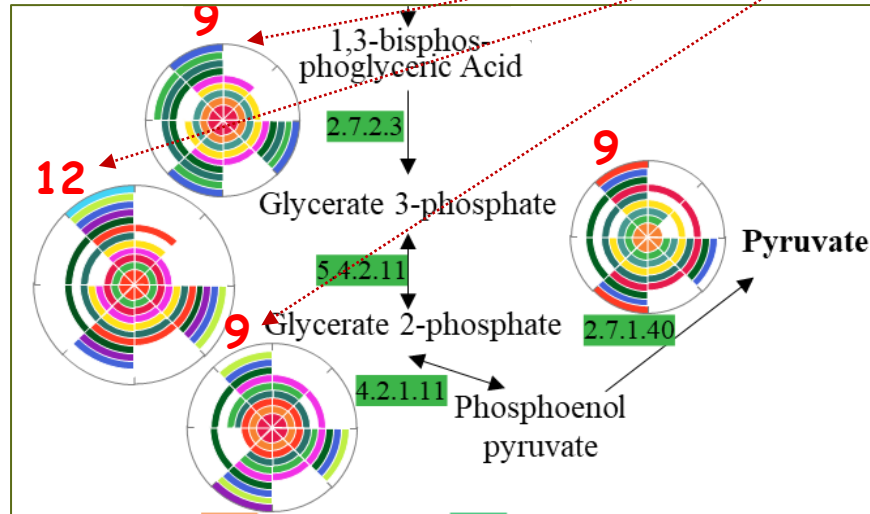
PTS transport

Biosynthesis of secondary metabolites

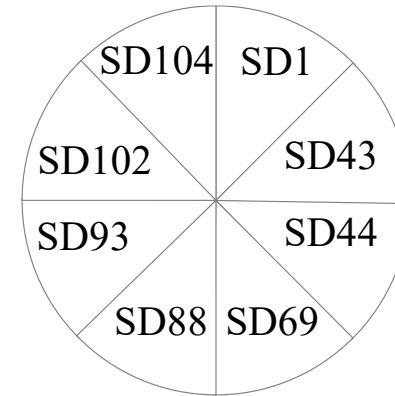
... will follow in a further publications

Reconstruction of main metabolic pathways - pentoses and pyruvate metabolism

9, 12, or 9 species have expressed the same gene within one sourdough. High diversity leads to functional redundancy



Phosphoglycerate kinase [EC:2.7.2.3]; Phosphoglycerate mutase [EC:5.4.2.11]; Phosphopyruvate hydratase [EC:4.2.1.11];



- | | | | |
|--------------------------------------|---|--|--------------------------------|
| <i>Saccharomyces cerevisiae</i> | <i>Levilactobacillus brevis</i> | <i>Lacticaseibacillus paracasei</i> | <i>Erwinia persicina</i> |
| <i>Lactiplantibacillus plantarum</i> | <i>Fructilactobacillus sanfranciscensis</i> | <i>Pediococcus parvulus</i> | <i>Naumovozyma castellii</i> |
| <i>Leuconostoc citreum</i> | <i>Leuconostoc mesenteroides</i> | <i>Leuconostoc pseudomesenteroides</i> | <i>Latilactobacillus sakei</i> |
| <i>Weissella confusa</i> | <i>Furfurilactobacillus rossiae</i> | <i>Lactococcus lactis</i> | <i>Bacillus cereus</i> |
| <i>Pediococcus pentosaceus</i> | <i>Pichia kudriavzevii</i> | <i>Lacticaseibacillus rhamnosus</i> | <i>Leuconostoc sp</i> |
| <i>Limosilactobacillus fermentum</i> | <i>Latilactobacillus curvatus</i> | <i>Limosilactobacillus reuteri</i> | |

Reconstruction of Synthetic Microbial Communities (SMCs)



SDG

Taxonomic, genomic potential and expression profiles from spontaneous sourdoughs

Methods

Culturomics

Metagenomics

Metatranscriptomics

Construction of SDG

Selection of species based on the setting up of specific thresholds

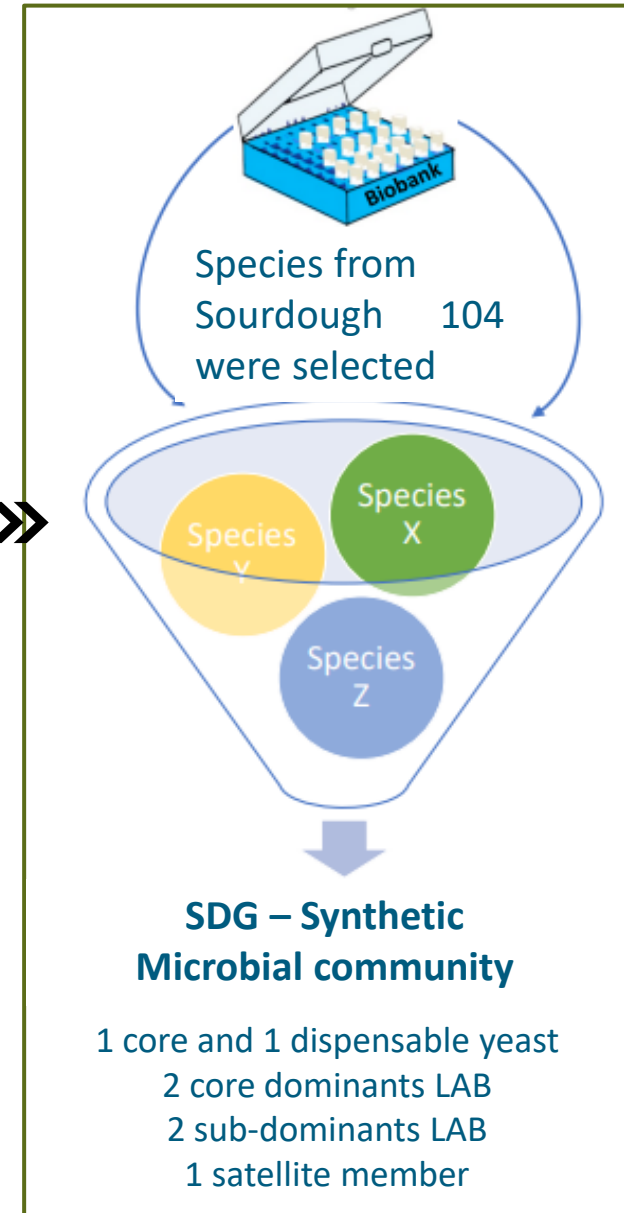
1. Species present at least in 50% of sourdoughs
2. Harbour and express at least 20 key genes representative for carbon and nitrogen metabolisms
3. Species have been found by culturomics

Thirteen species out of 49 dominant and subdominant lactic acid bacteria and yeasts met these criteria.

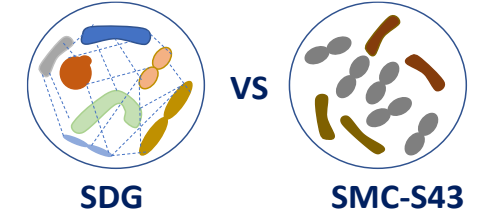
Construction of SMC-43 was based only on culturomics results of SD43 traditional sourdough



SMC-S43



Final species composition of SMCs



SDG				SMC-SD43			
Taxa	Cell density (cfu mL ⁻¹)	Group	n. of species	Taxa	Cell density (cfu mL ⁻¹)	Group	n. of species
Lactic acid bacteria				Lactic acid bacteria			
<i>Lactiplantibacillus plantarum</i>	9.96	Core dominant	4	<i>Lacticaseibacillus paracasei</i>	2.11	Core sub-dominant	7
<i>Limosilactobacillus fermentum</i>	8.45	Core dominant		<i>Lactiplantibacillus plantarum</i>	6.48	Core dominant	
<i>Furfurilactobacillus rossiae</i>	5	Core sub-dominant		<i>Lacticaseibacillus rhamnosus</i>	2.48	Core sub-dominant	
<i>Pediococcus pentosaceus</i>	3.23	Core sub-dominant		<i>Fructilactobacillus sanfranciscensis</i>	9.96	Core dominant	
			<i>Lactococcus lactis</i>	2.7	Core sub-dominant		
			<i>Leuconostoc citreum</i>	3.18	Core sub-dominant		
			<i>Weissella confusa</i>	8.48	Core dominant		
Other bacteria				Other bacteria			
<i>Staphylococcus epidermidis</i>	2.48	Satellite	1	<i>Staphylococcus sp.</i>	2	Satellite	1
Yeasts				Yeasts			
<i>Saccharomyces cerevisiae</i>	6.23	Core dominant	2	<i>Saccharomyces cerevisiae</i>	6.26	Core dominant	1
<i>Pichia kudriavzevii</i>	5.85	Dispensable dominant					

The cell densities of all species corresponded to those found in traditional sourdoughs as estimated by culturomics

Investigation of resilience, stability and performance in sourdough-like environment (WFH) and *in situ*

7 depleted SMCs at exponential (T1) and stationary (T2) growth phases in sourdough-like environment simulation (WFH) (12 h at 30°C)

Species interaction within SMC – ONLY SDG

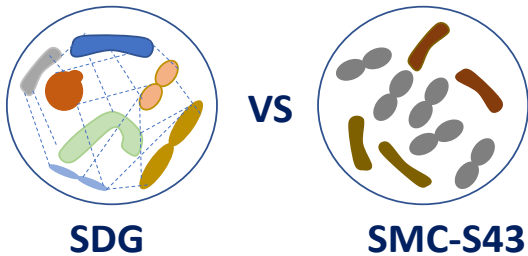
Methods
Metatranscriptomics

Assessment of transcriptomics profiles by depleting one species at a time from the consortium



Shared and unique species contribution

2 SMCs at exponential (T1) and stationary (T2) phases in sourdough-like environment simulation (WFH) and *in situ*



Metabolic reconstruction of active pathways - simulation in WFH

Methods Culturomics, Metatranscriptomics, Metabolomics

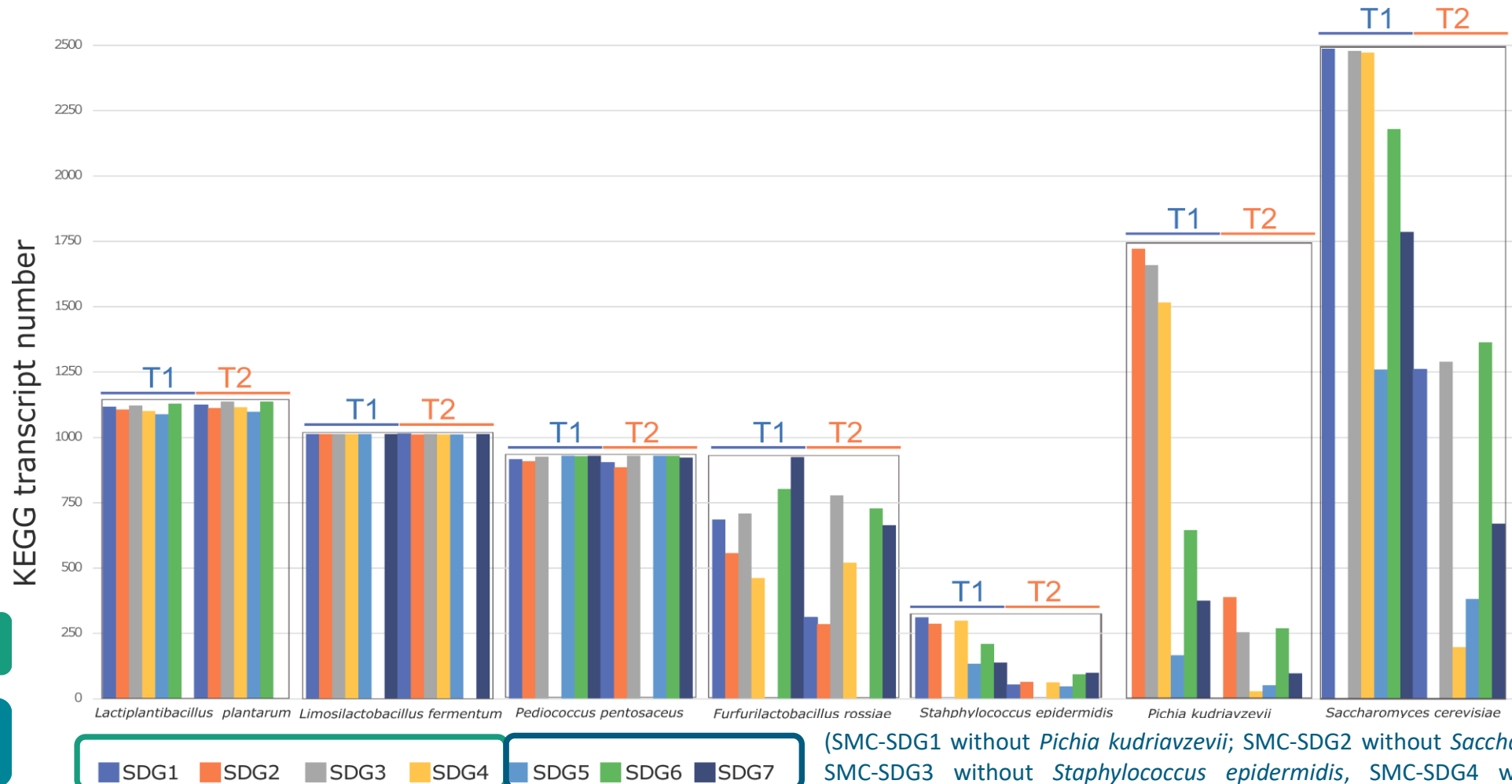
Shared and unique species contributions - simulation in WFH

Persistence RAPD-PCR profiling
30 days of daily back slopping *in situ*

Day 0 > Day 1 > Day 10 > Day 30

Volatilome *in situ*

Transcription level (KEGG function number) of SDG species



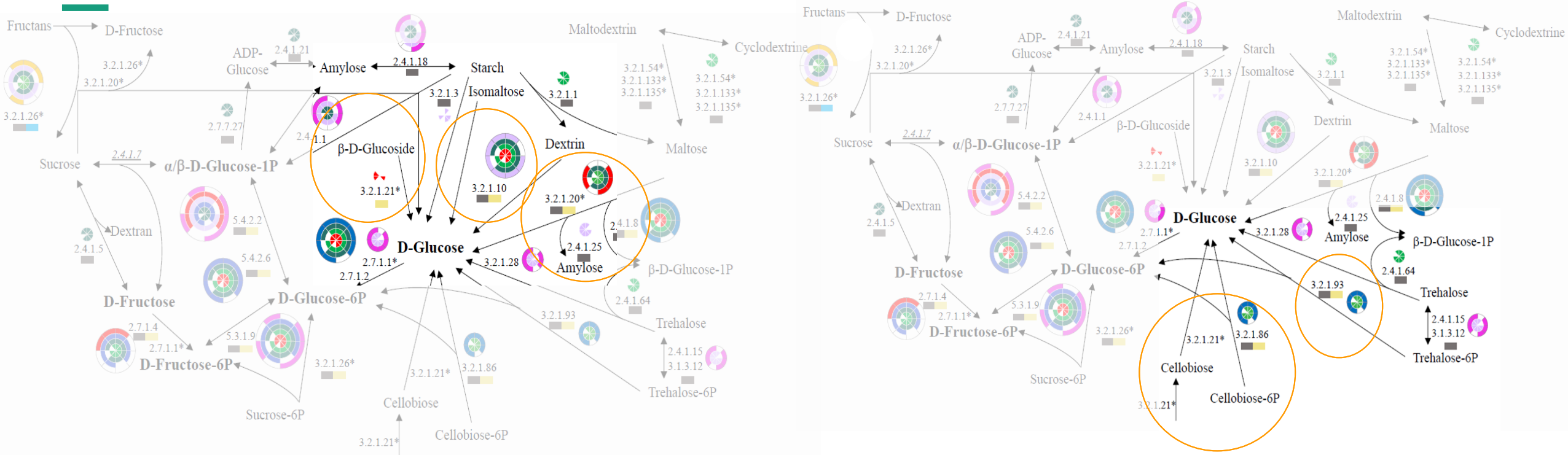
Core dominant

Core subdominant

(SMC-SDG1 without *Pichia kudriavzevii*; SMC-SDG2 without *Saccharomyces cerevisiae*, SMC-SDG3 without *Staphylococcus epidermidis*, SMC-SDG4 without *Pediococcus pentosaceus*, SMC-SDG5 without *Furfurilactobacillus rossiae*, SMC-SDG6 without *Limosilactobacillus fermentum* and SMC-SDG7 without *Lactiplantibacillus plantarum*)

The irreplaceability of sub-dominants members in sourdough functionality

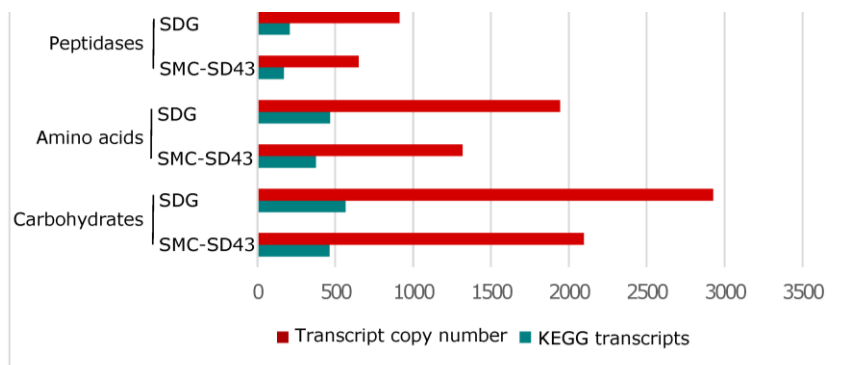
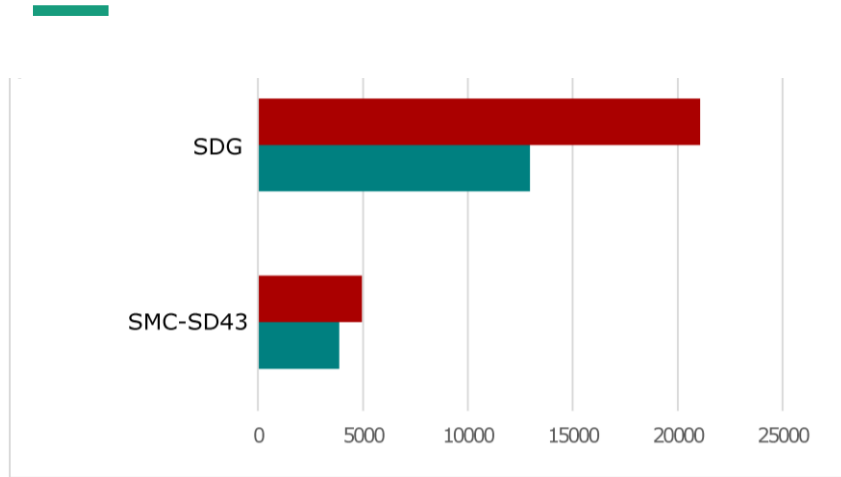
Sub-dominants: *Furfurilactobacillus rossiae*; *Pediococcus pentosaceus*



- ✓ *Fur. rossiae* showed the capability to ferment dextrin/isomaltose and maltose through **oligo-1,6-glucosidase (EC:3.2.1.10)** and **alpha-glucosidase (EC:3.2.1.20)**. β -D-Glucosidase (EC:3.2.1.21) gene transcripts were unique to *Fur. rossiae*.
- ✓ Arabinoxylan metabolism leading to **D-xylose** and **arabinose** was exclusively found in *Fur. rossiae*.

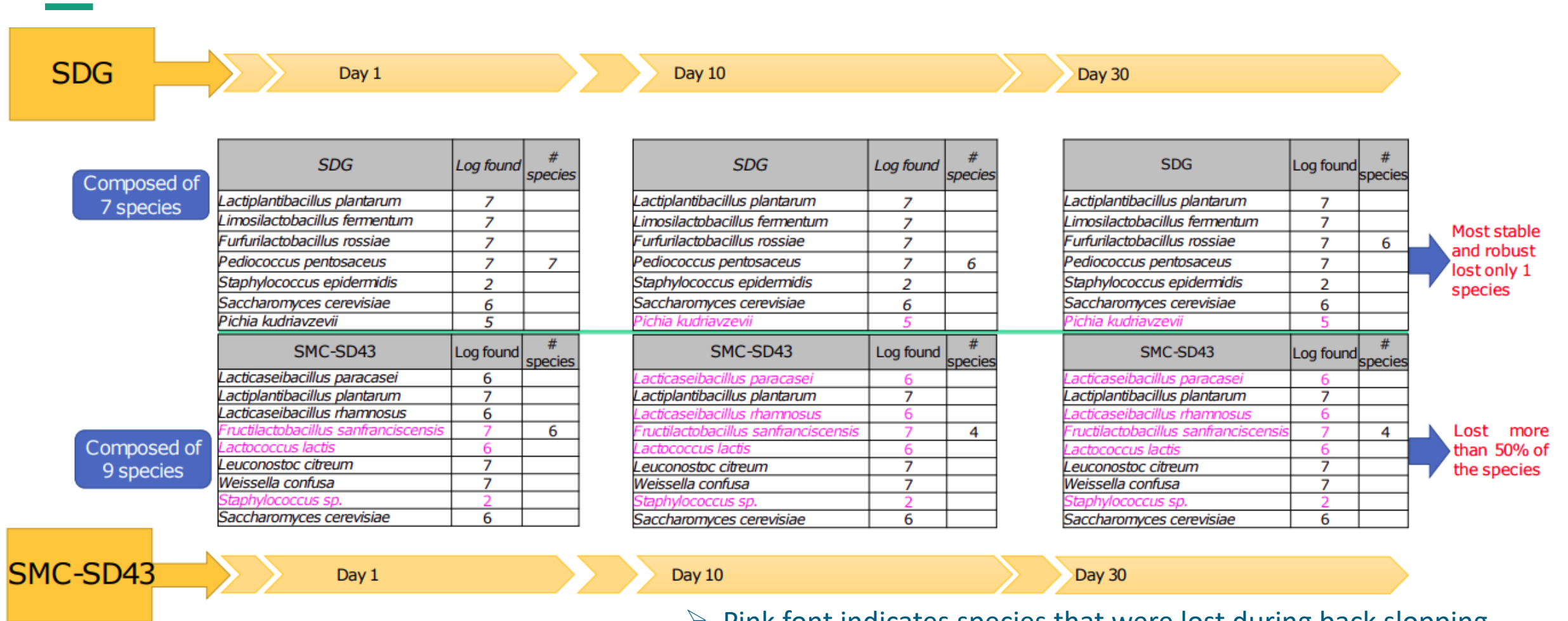
- ✓ *Ped. pentosaceus* showed the unique capability to use alternative energy sources such as trehalose and cellobiose via 6-phospho-beta-glucosidase (**EC:3.2.1.86**) and trehalose-6-phosphate hydrolase (**EC:3.2.1.93**).

Sourdough-like environment simulation (WFH): the *de novo* synthetic community (SDG) shows transcriptome redundancies



- The number of total KEGG transcripts in SDG was 4 times higher than that in SMC-SD43.
- This overall trend was also confirmed for each of the main reconstructed pathways.
- The highest number of gene transcripts in SDG belonged to carbohydrate metabolism, which was represented by more than 2500 gene copies.
- Dominant species maintain their expression levels in both exponential (T1) and stationary phase (T2). Subdominant species and satellite members despite their reduced performance play a key role in the metabolisms with unique genes.
- Species interactions modified the KEGG profiles when any single species was removed from the pools.

The *de novo* synthetic community (SDG) shows steady state under *in situ* conditions

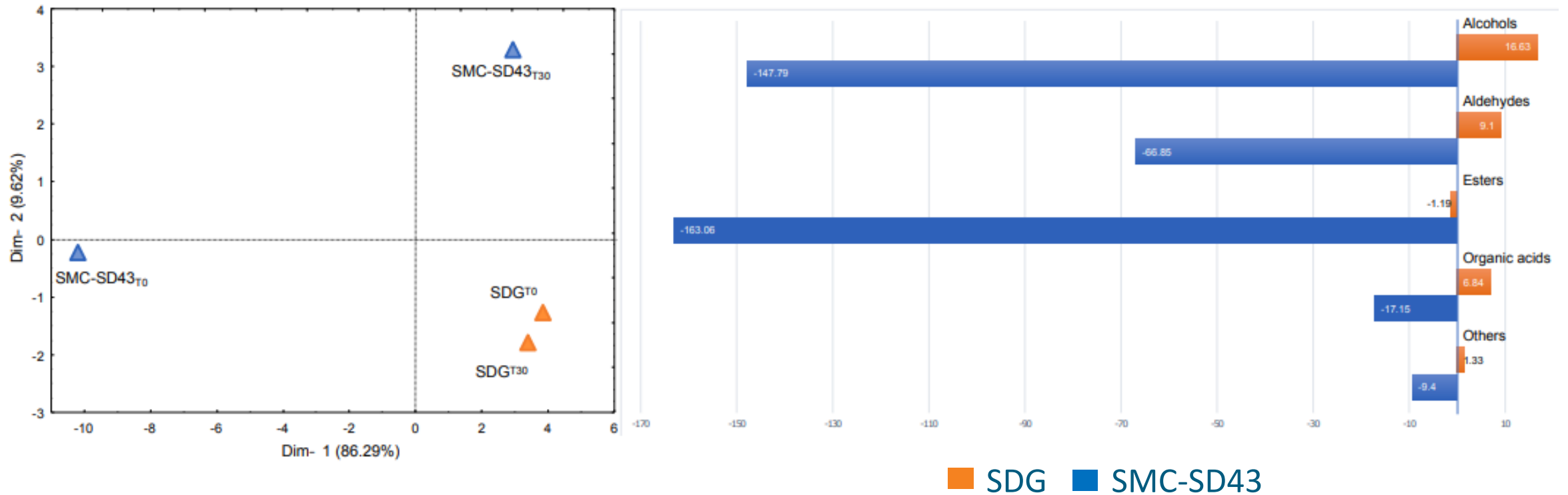


➤ Pink font indicates species that were lost during back slopping

Volatilome profile of SDG after *in situ* long-term back slopping

- PCA based on volatile profile normalized data matrix for SDG and SMC-SD43 communities at one and thirty days of propagation

Resulting metabolic delta (T30 minus T0) of VOC abundances grouped according to chemical class



Fermentation: a metabolic labyrinth

