Water Kefir - A Multi-Omics Study

KombuchaKon

20.04.2023

Samuel Mortensen, PhD

PI: Prof. Paul Cotter

Microbiome Applications for Sustainable food systems through Technologies and EnteRprise

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818368

My Journey

Leibniz Universität Hannover

Northeastern University

Microbiome Ireland

BSc & MSc
Plant Biotechnology

PhD
Biology

Postdoctoral Researcher
Microbiology and Bioinformatics
(Fermented Foods)

images: hannover.de, isbos.org, teagasc.ie

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Key Points

1. Introduction to water kefir
2. Global water kefir study
3. Microbial composition of water kefir
4. Using multi-omics for improved water kefir production

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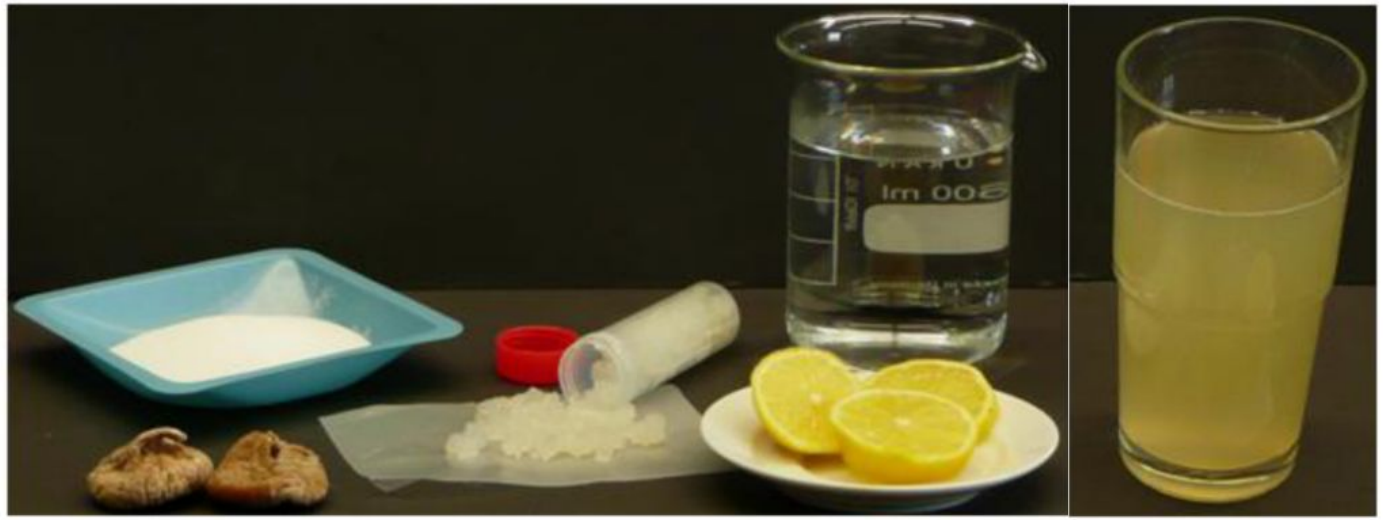
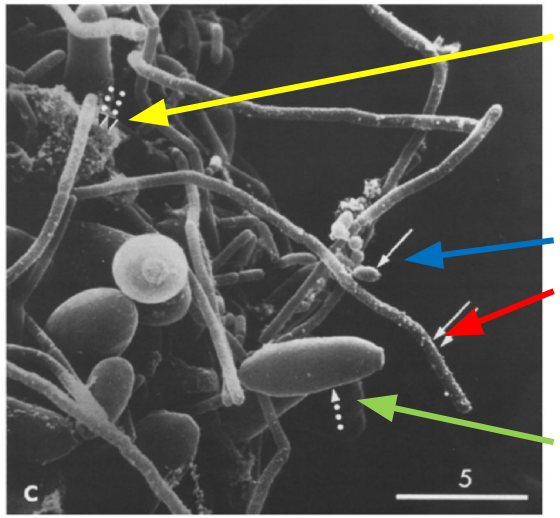
Water Kefir



Gulitz, 2013



Moinas *et al.*, 1980

Input:

- Kefir grains
- Water
- Sugar
- Dried & fresh fruits (e.g. fig & lemon)

~2 days at 21°C

Dominant microorganisms:

- Lactic acid bacteria (LAB)
- Acetic acid bacteria (AAB)
- Yeast

Products: fermented beverage

- Lactic acid
- Acetic acid
- Ethanol
- Carbon dioxide
- Aroma compounds

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Water Kefir Fermentation

First Fermentation

Second Fermentation

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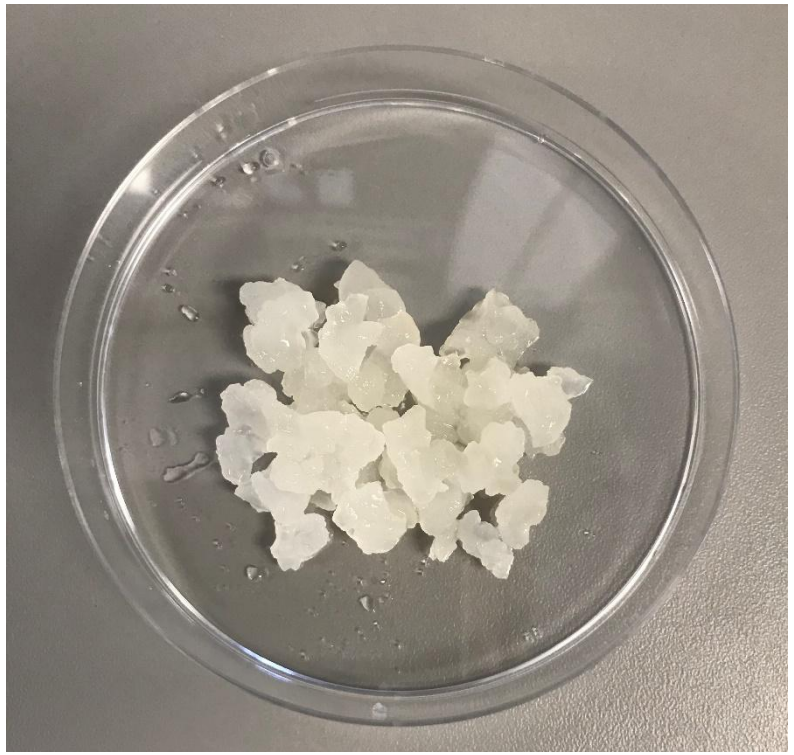
Water Kefir Fermentation in the Lab



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Global Water Kefir Study

What is the microbial composition of water kefir?

Which microbes should be present in a commercial water kefir?

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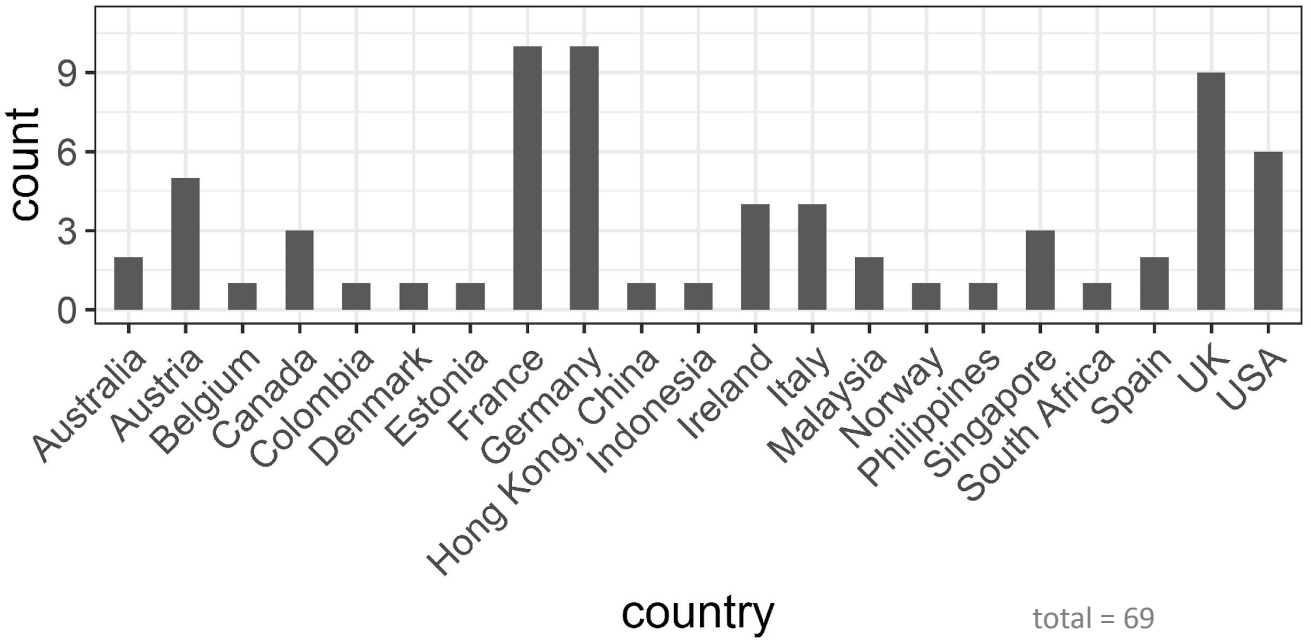
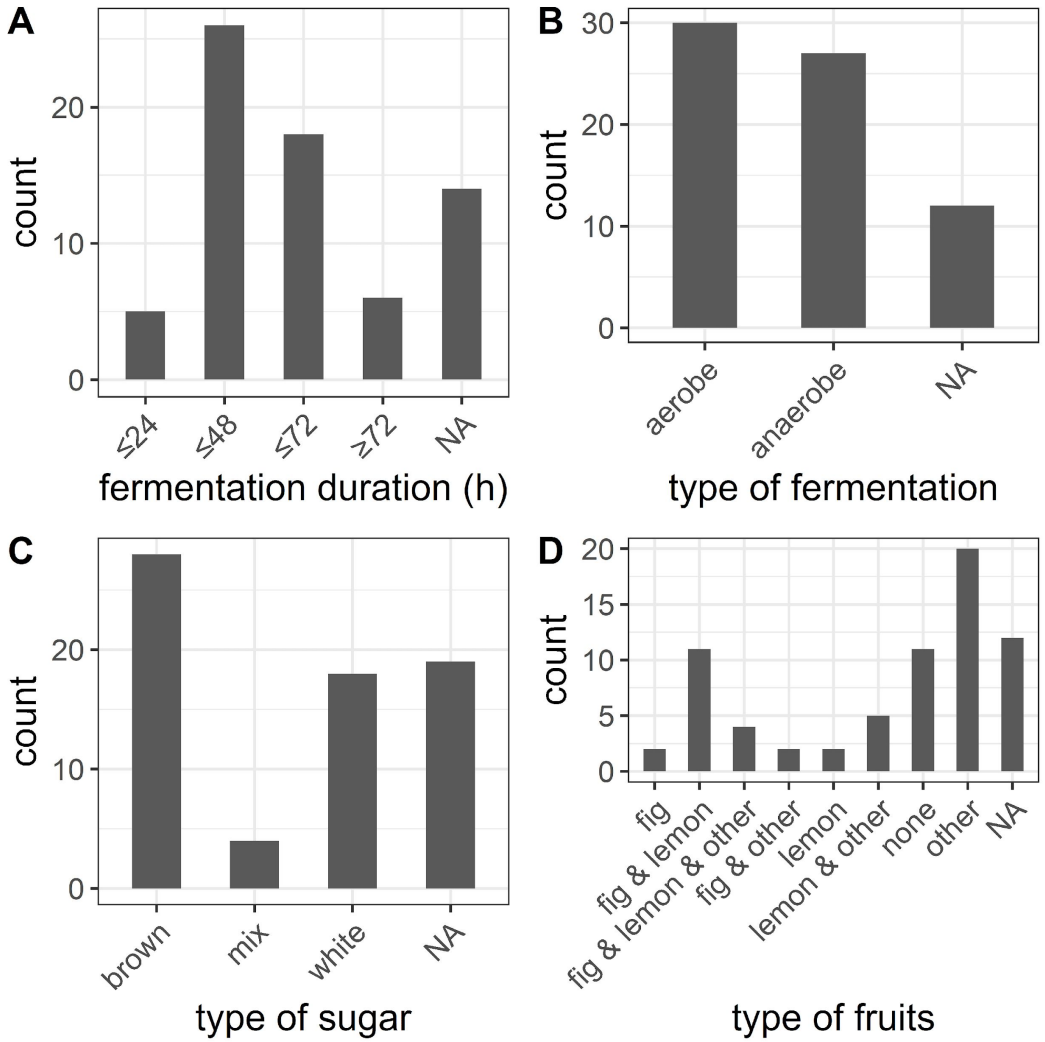
Water Kefir Collection



total = 69



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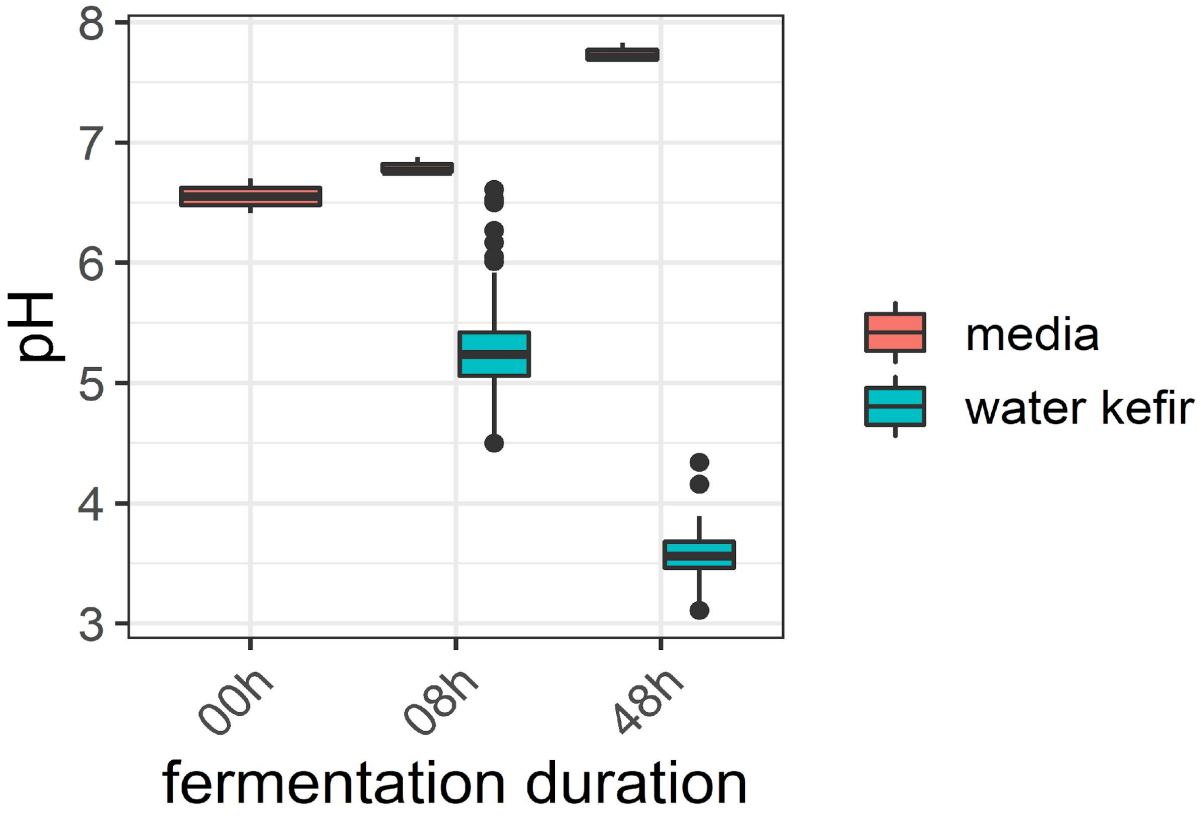
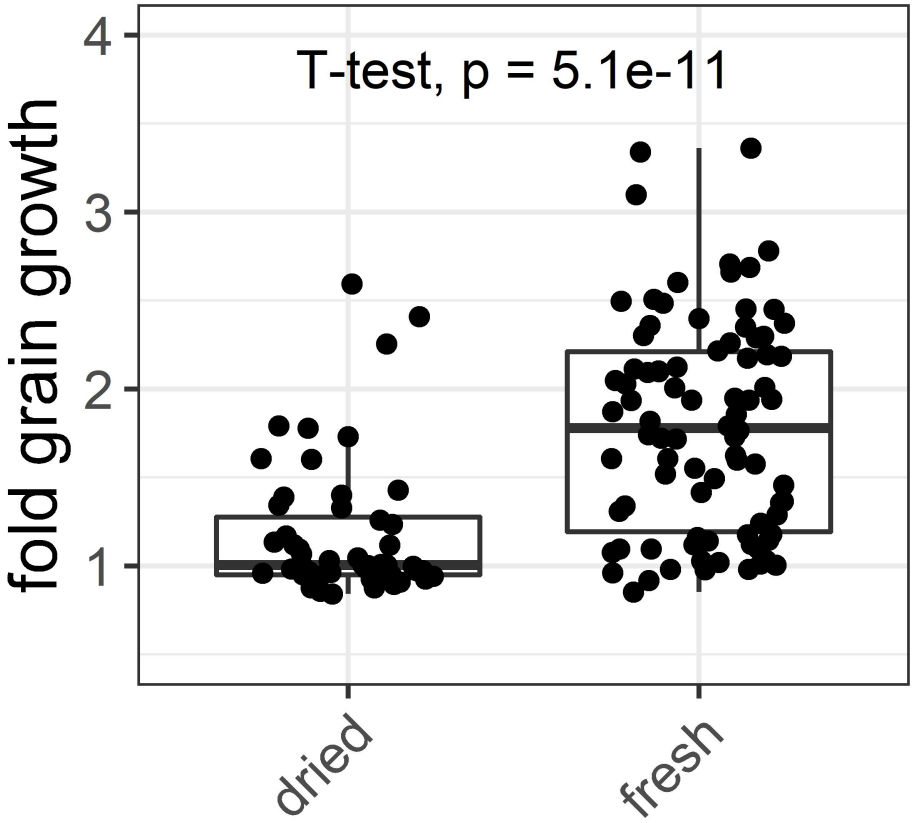
Water Kefir Fermentation





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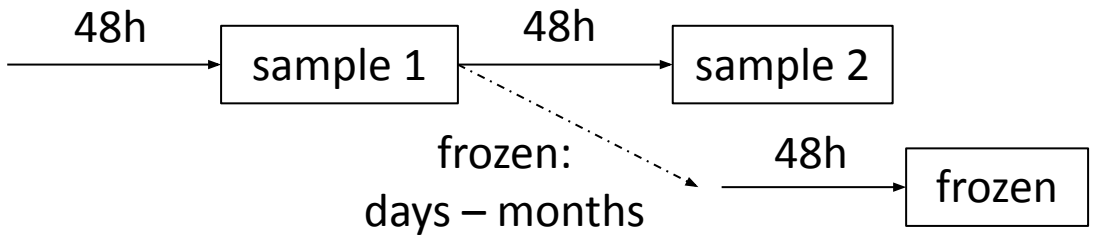
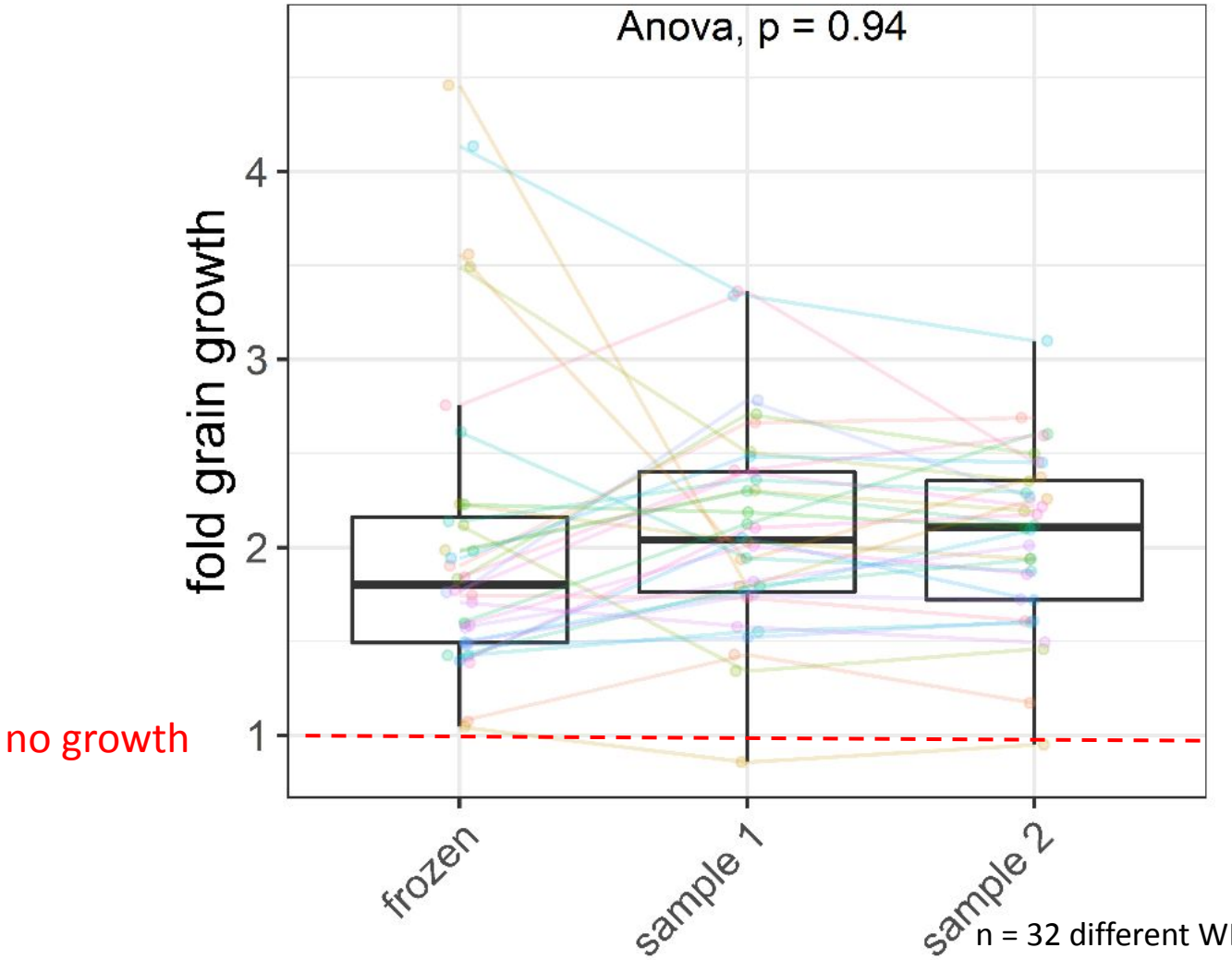
Freeze Tolerance of Water Kefir





n = 32 different WK

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Shotgun Metagenomics

Shotgun Metagenomics:

Water kefir (WK)

- 2x 08h liquid
- 2x 48h liquid
- 1x 48h grains

69 WK x 5 samples = 345 WK samples

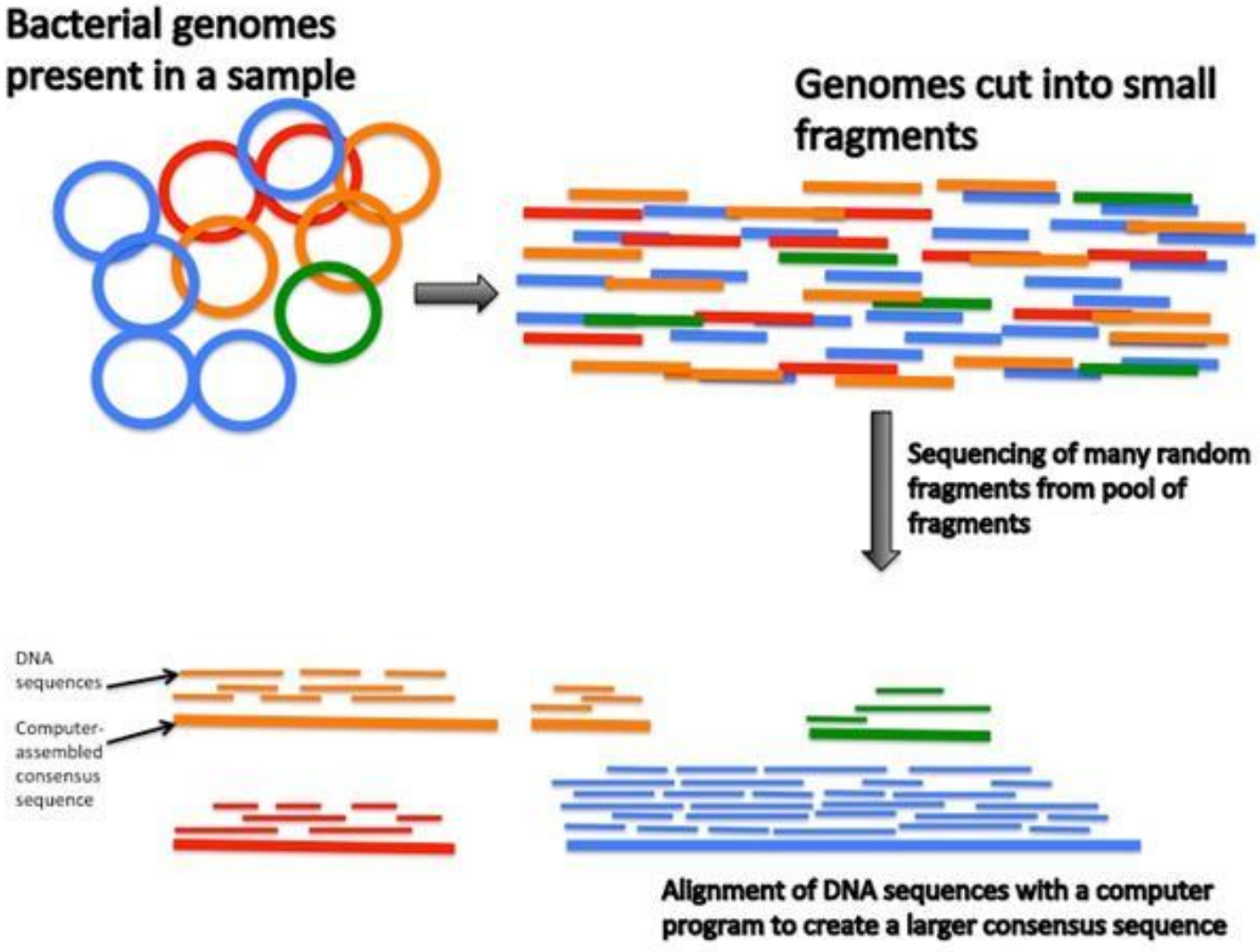

Fig: 1010genome.com

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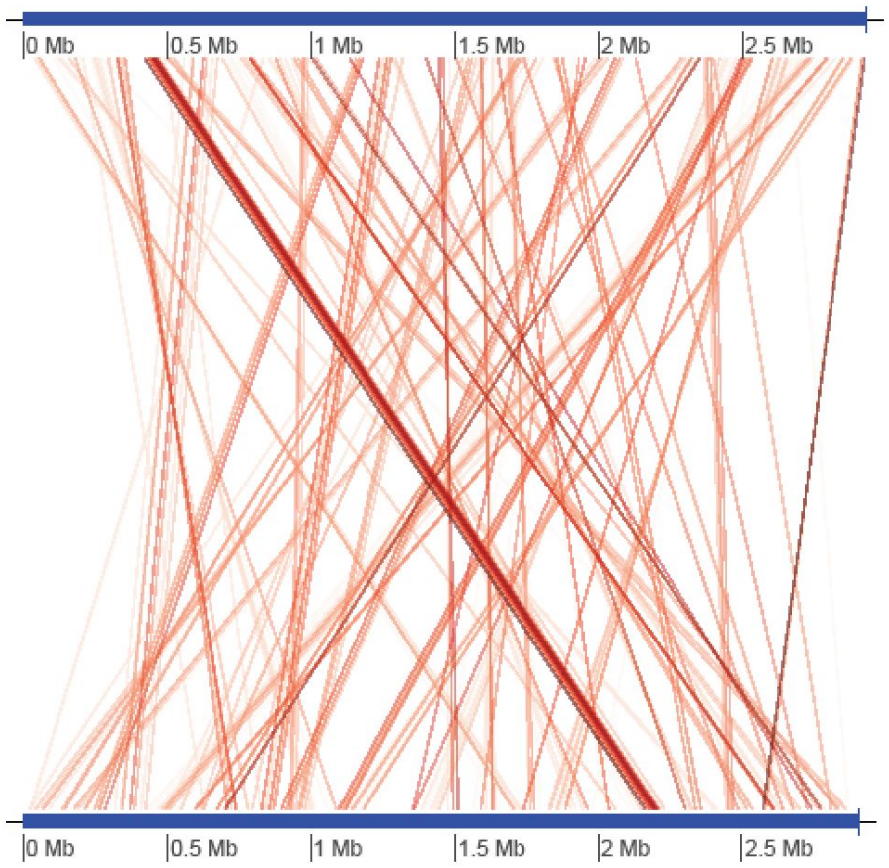
Metagenome-Assembled Genome (MAG) Analysis

MAG from water kefir

known species

novel species



Known reference genome

Genome Completeness: 98.8 %
Average Nucleotide Identity (ANI): 98.8 %

Genome Completeness: 98.5 %
Average Nucleotide Identity (ANI): 78.6 %

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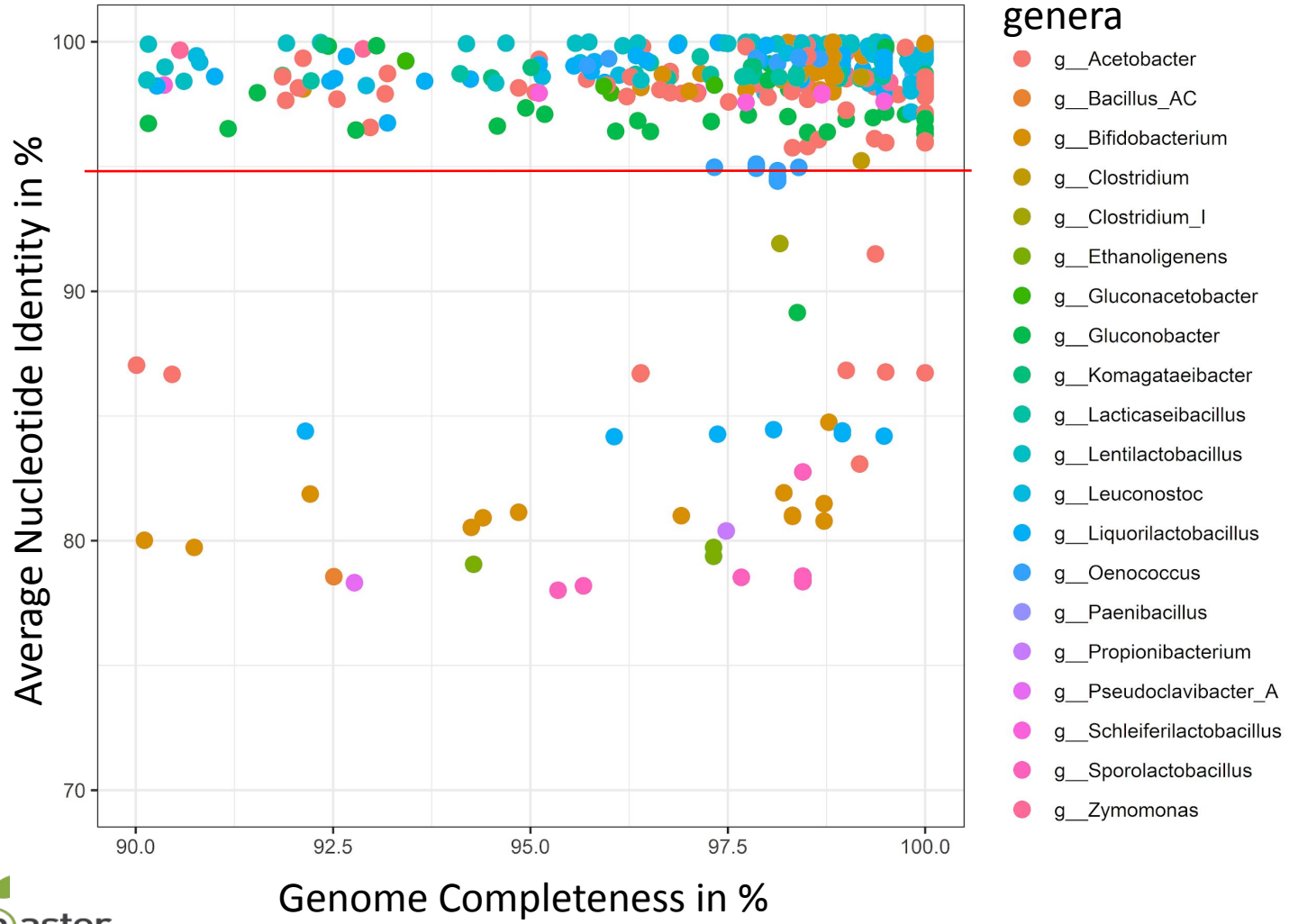
Metagenome-Assembled Genomes (MAGs) Analysis

Bacterial MAGs



54 MAGs not assigned at species level

- 18 potential novel species

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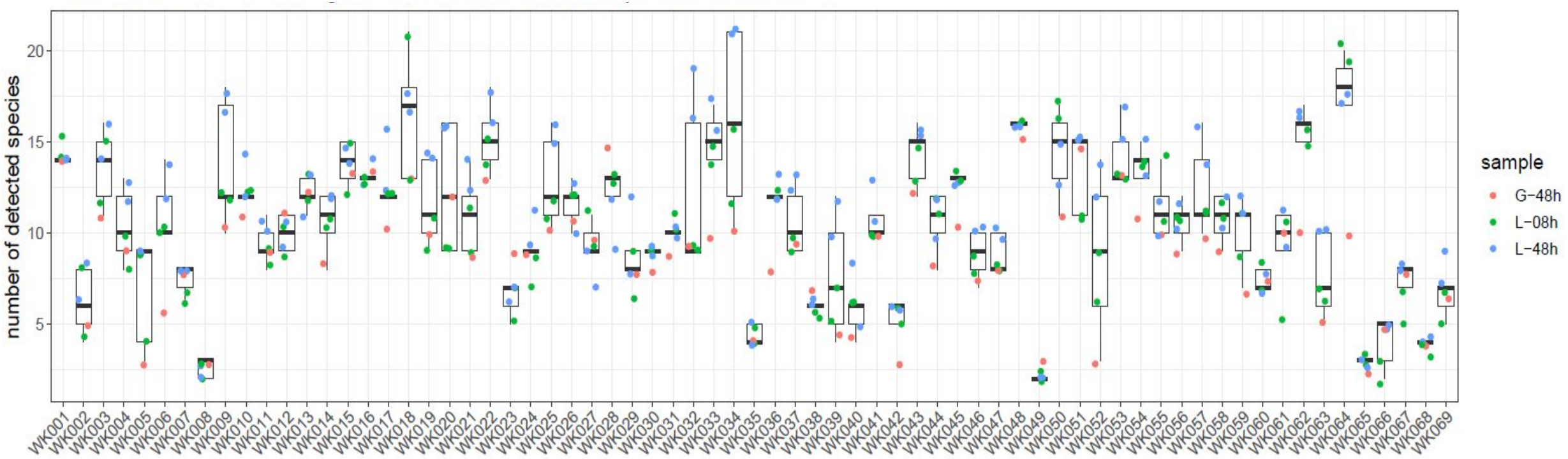
Detected species



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Acetobacter

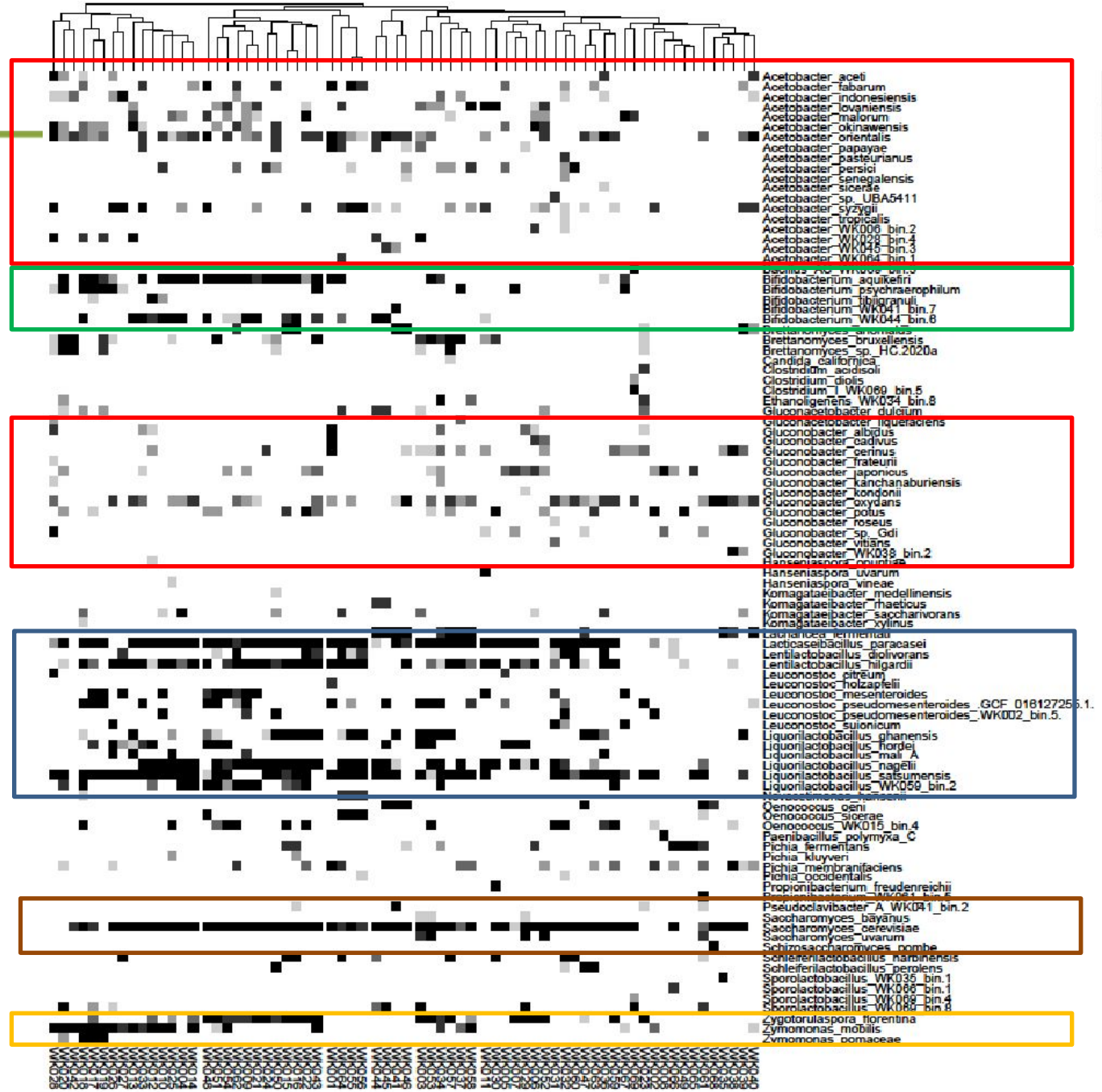
Bifidobacterium

Gluconobacter

LAB

Saccharomyces

Zymomonas



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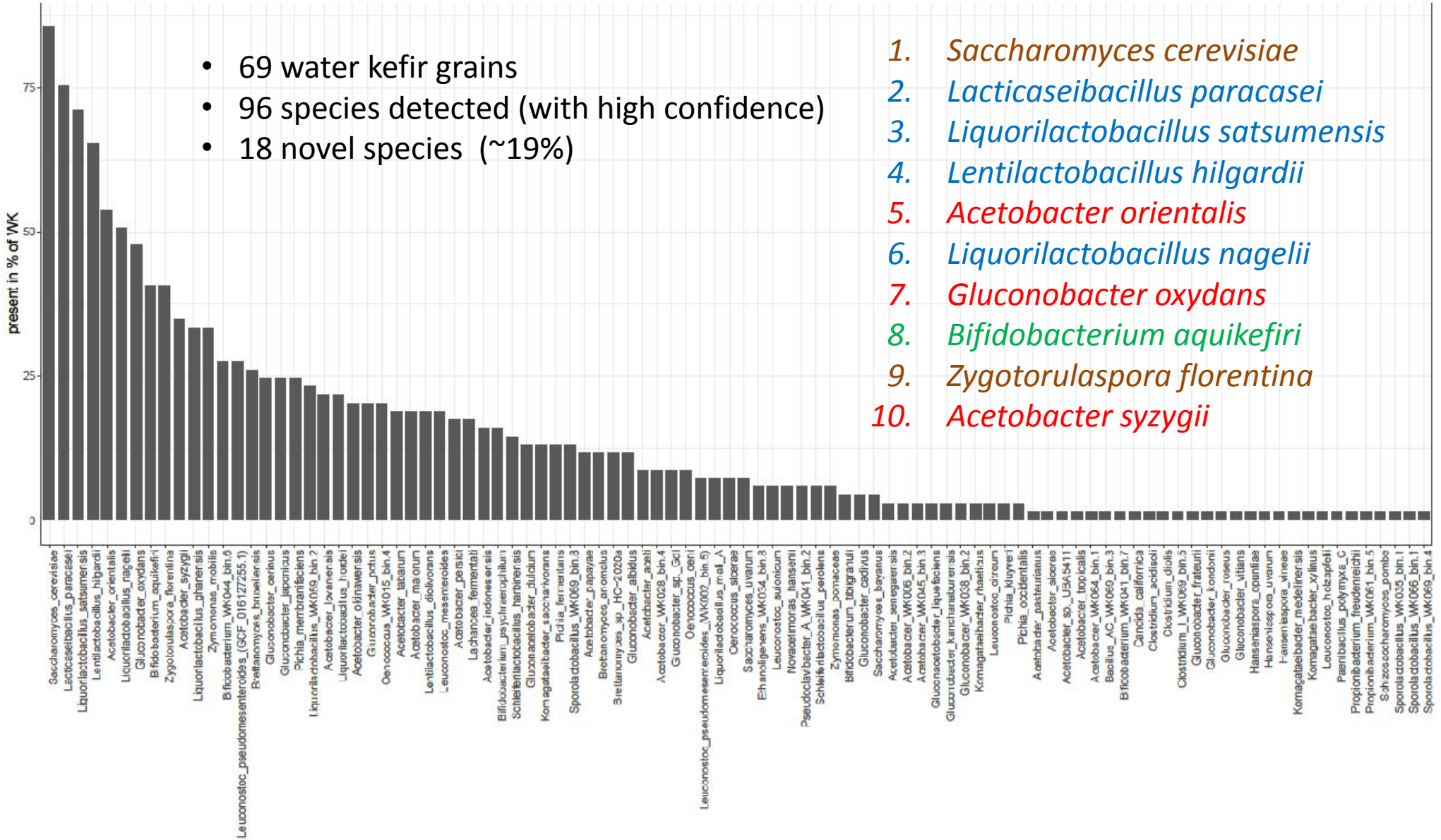
Detected species

- 69 water kefir grains
- 96 species detected (with high confidence)
- 18 novel species (~19%)



1. *Saccharomyces cerevisiae*
2. *Lacticaseibacillus paracasei*
3. *Liquorilactobacillus satsumensis*
4. *Lentilactobacillus hilgardii*
5. *Acetobacter orientalis*
6. *Liquorilactobacillus nagelii*
7. *Gluconobacter oxydans*
8. *Bifidobacterium aquikefiri*
9. *Zygotorulaspora florentina*
10. *Acetobacter syzygii*

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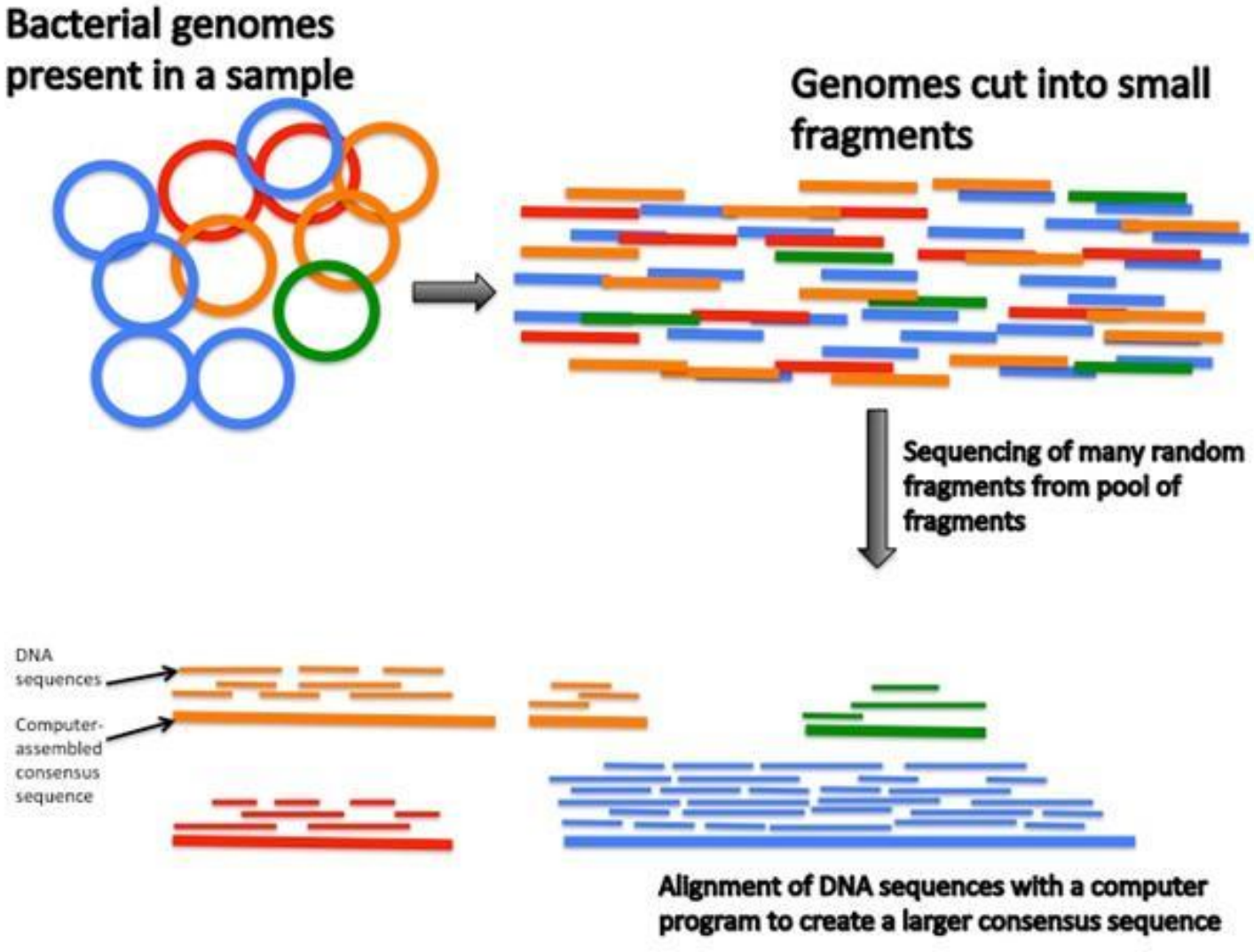

Fig: 1010genome.com

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Acetobacter

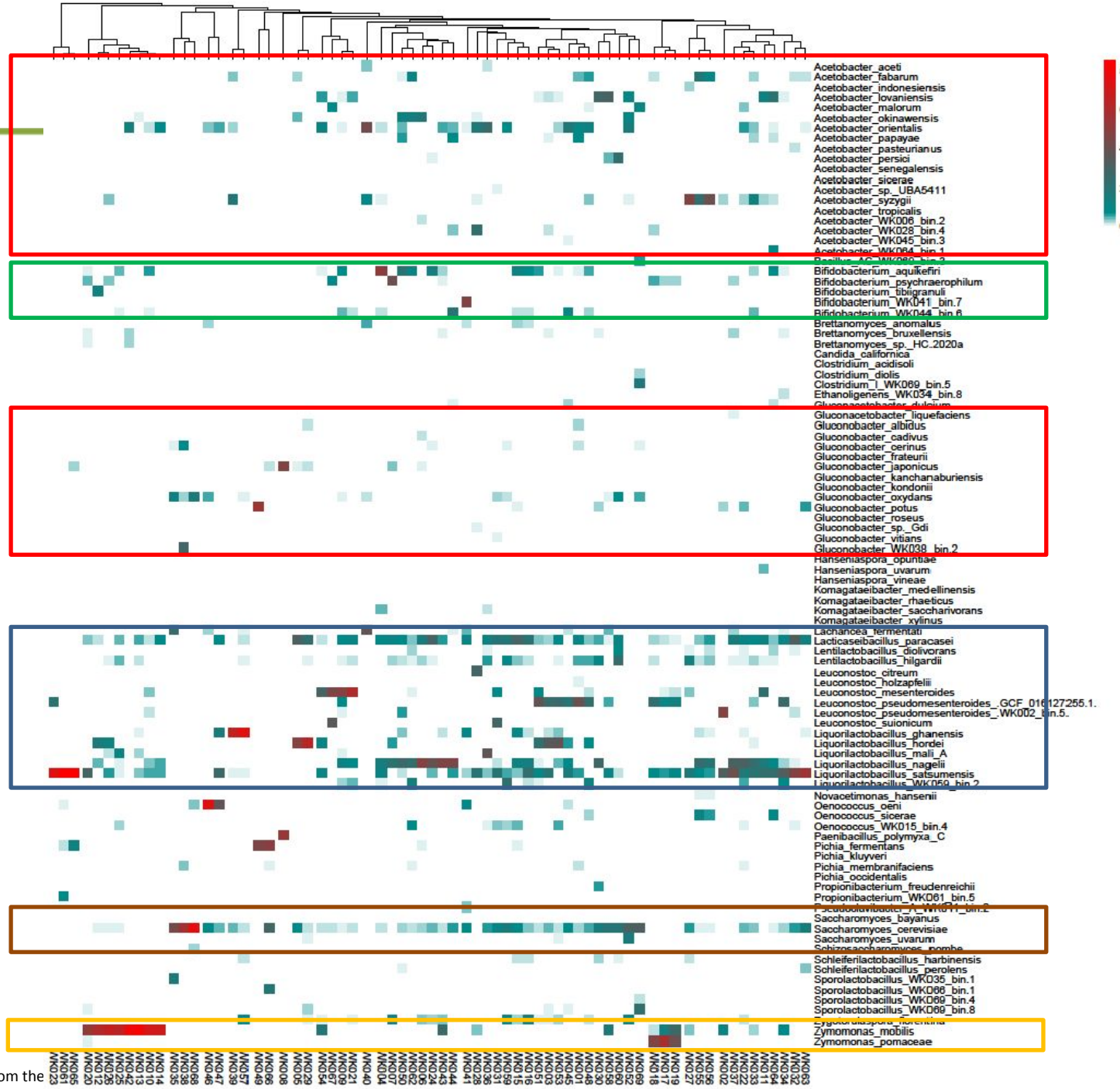
Bifidobacterium

Gluconobacter

LAB

Saccharomyces

Zymomonas



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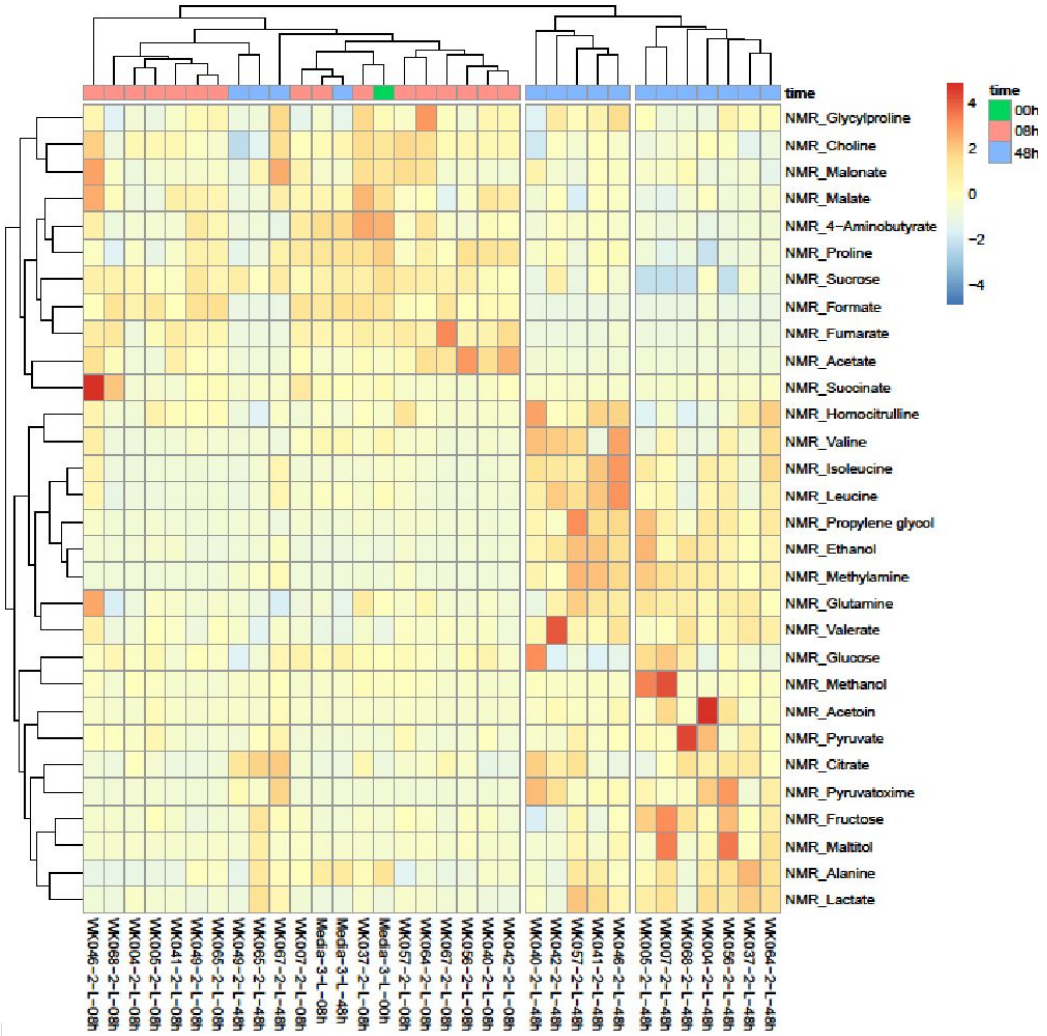
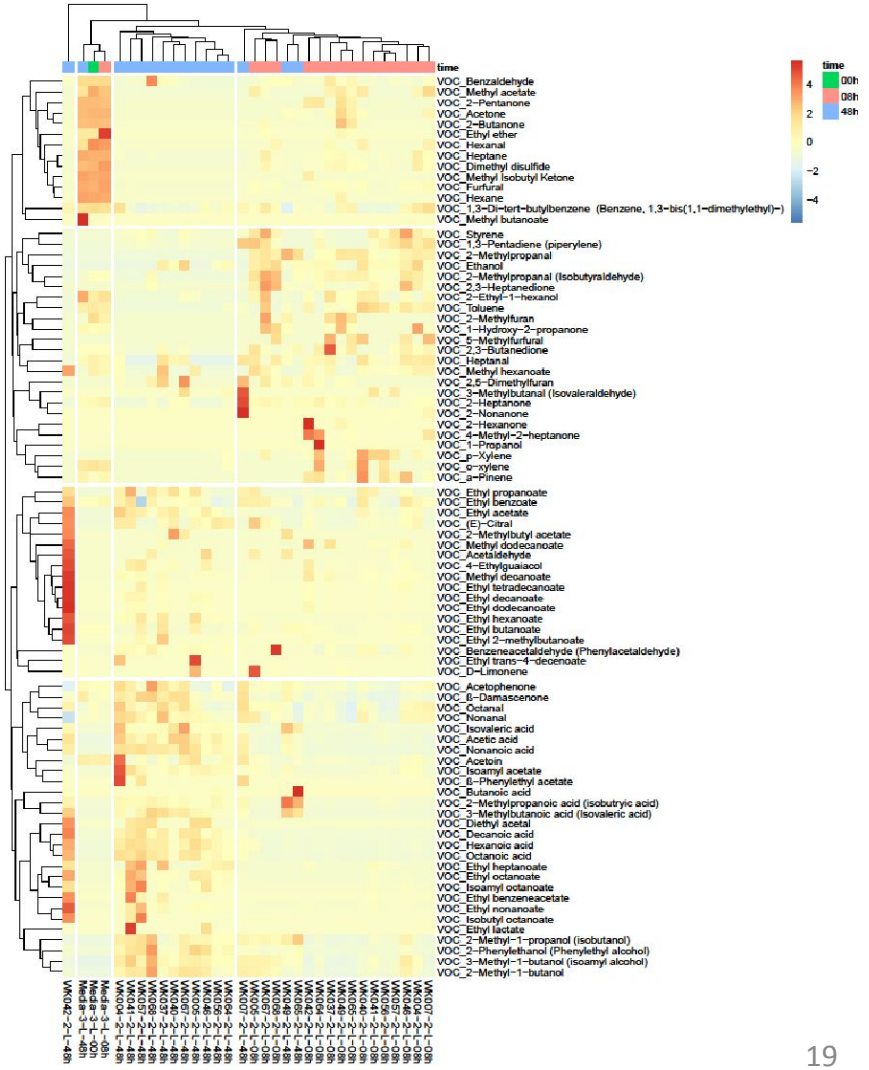
Metabolites & Flavour Results

NMR metabolomics



MS volatolomics



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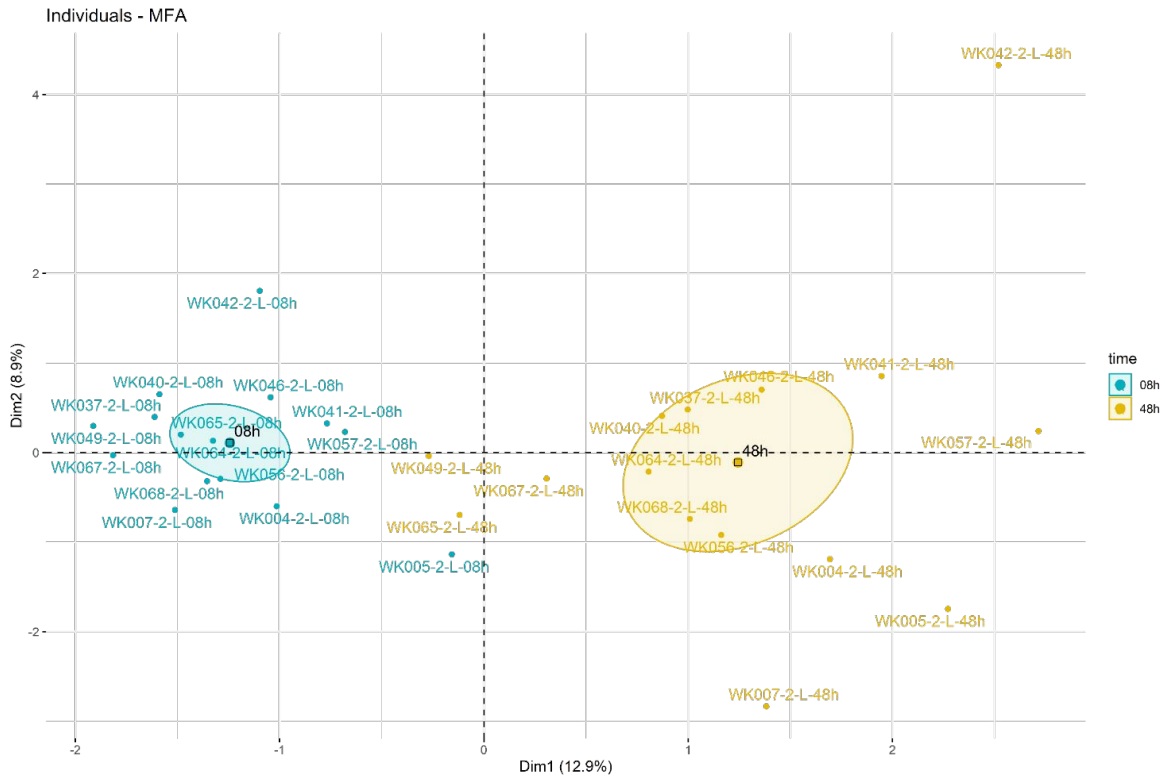
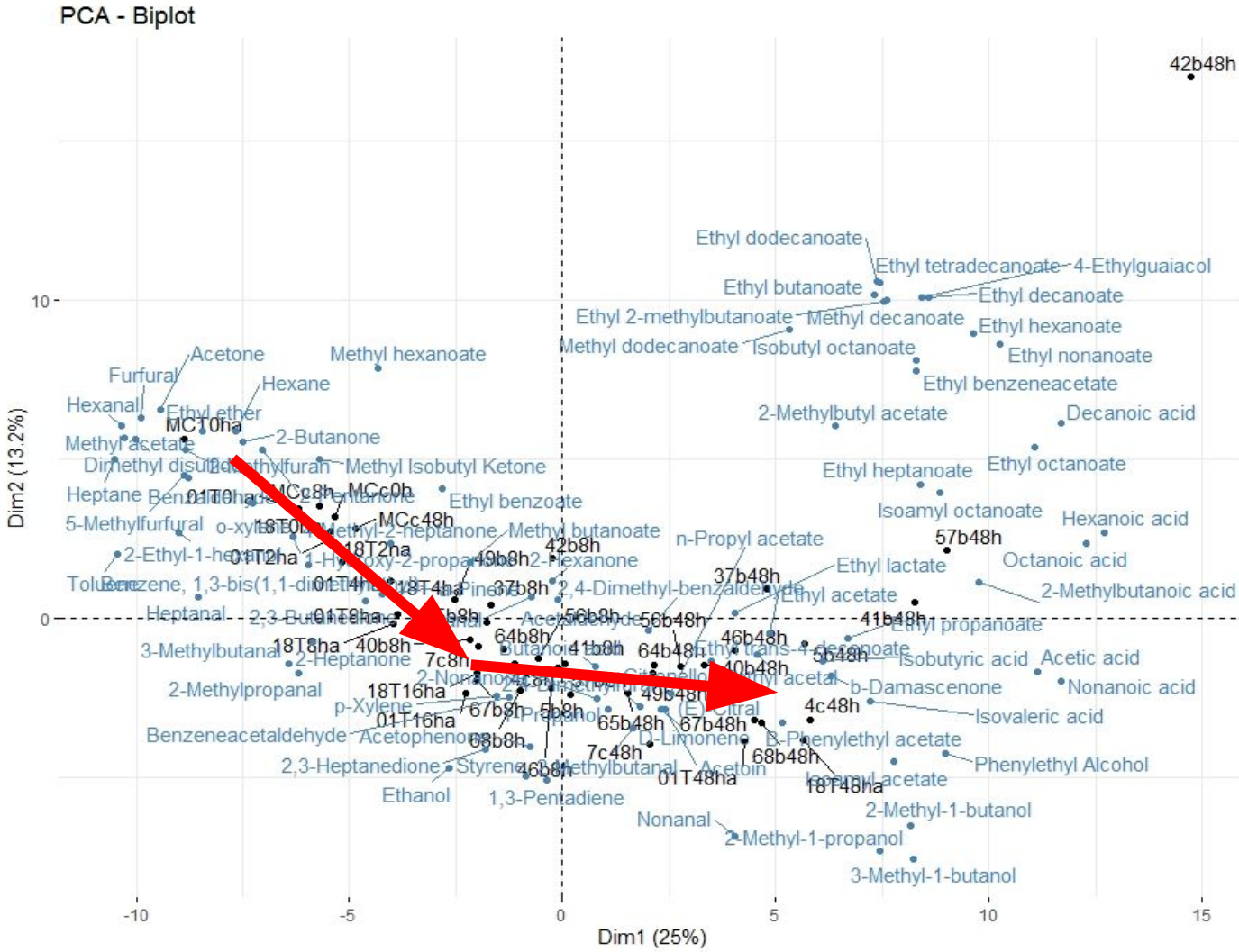
Development of Water Kefir Characteristics

Species & Flavour & Metabolites



Flavour Development



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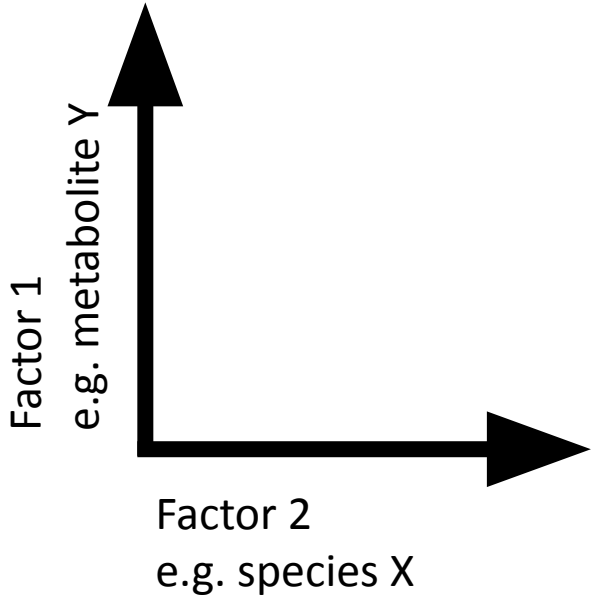
Correlation Analysis



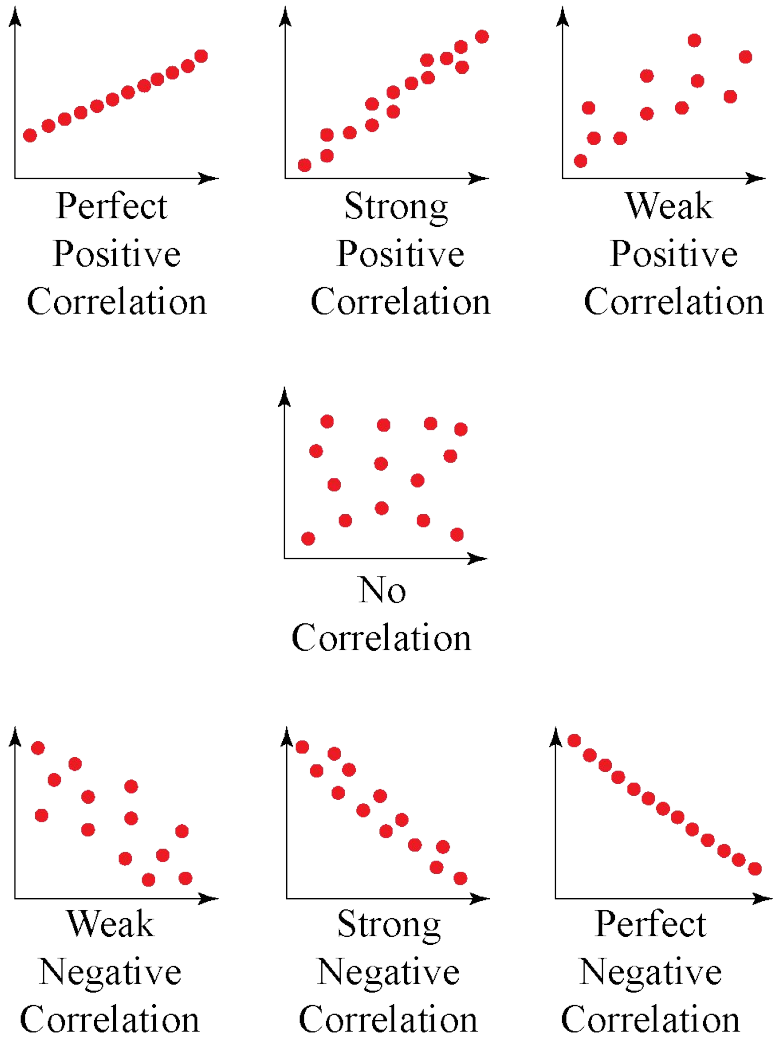


Fig: cuemath.com

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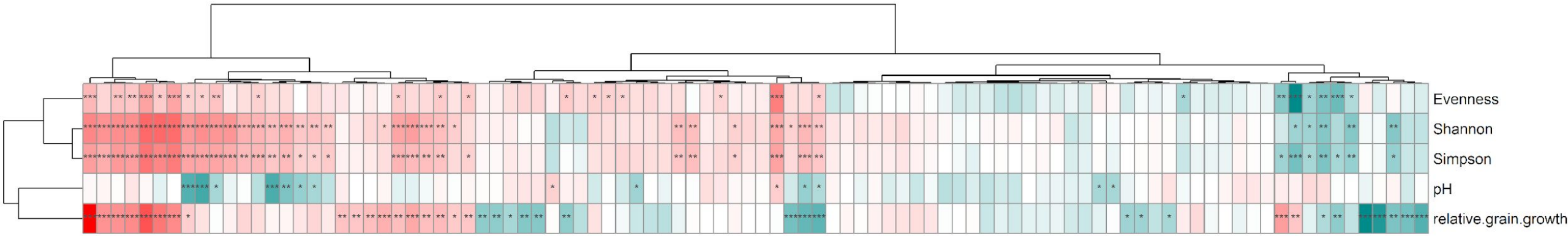
Correlation Analysis



Lentilactobacillus hilgardii

Acetobacter spp.

Saccharomyces cerevisiae

Zymomonas mobilis

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Correlation Analysis
Species vs Metabolites

- *Lentilactobacillus hilgardii:* *** + Maltitol
- *Aceto. orientalis*: ** + Leucine & Isoleucine
- *Gluconobacter oxydans*: ** + Pyruvate
- …

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Correlation Analysis Species vs Flavour

*** + Esters

- *Zymomonas mobilis*
- *Brettanomyces spp.*
- *Acetobacter spp.*
- *Gluconacetobacter spp.*

Most negative VOC correlations:

- *Pichia fermentans*

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Acknowledgements

- Prof. Paul Cotter
- Dr. Mairead Coakley
- Dr. Fiona Crispie
- Vision 1 members:
  - Dr. John Leech
  - Dr. Wiley Barton
  - Elaine Lawton
  - Liam Walsh
  - …

NMR analysis @UCD:

Dr. Lorraine Brennan

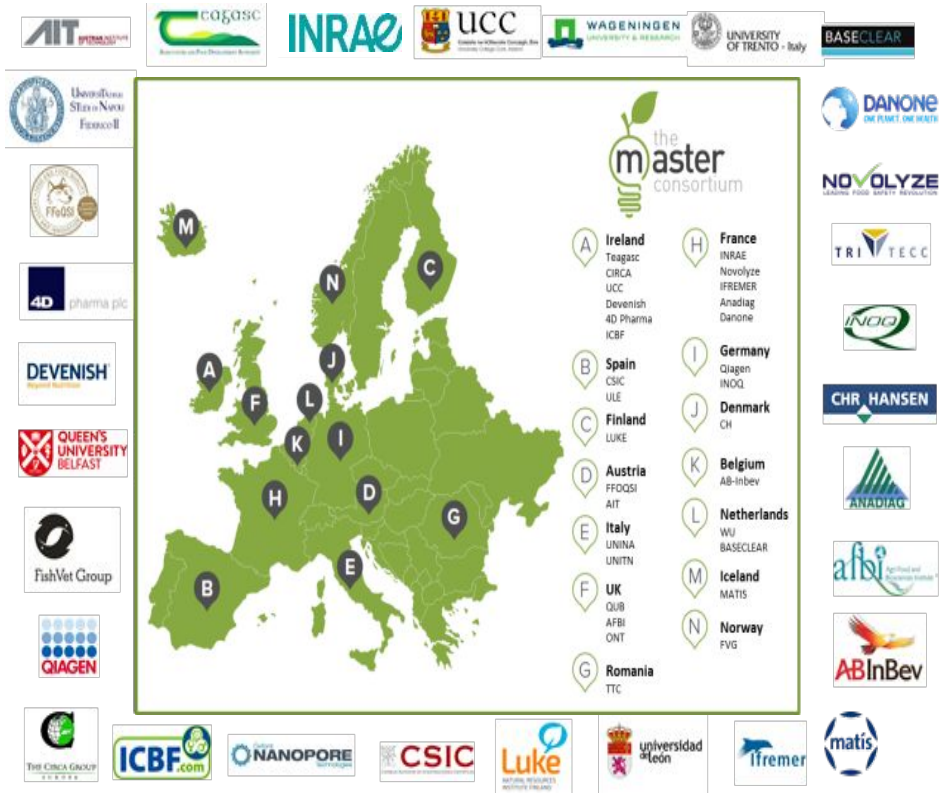
Dr. Xiaofei Yin

VOC analysis @Teagasc

Dr. Kieran Kilcawley

Iwona Skibinska

The MASTER Consortium



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Summary

1. Introduction to water kefir
2. Global water kefir study
3. Microbial composition of water kefir
   - Water kefir shows great microbial diversity
   - Discovery of several new species (MAGs).
   - Isolated two novel species (data not shown, more in process).
   - Lack of strict “core microbiome”, but some species are regularly present in water kefir.
4. Using multi-omics for improved water kefir production
   - Correlations between species, flavours, & metabolites
   - Roadmap for which species could/should be present in commercial & pitched water kefir.

Thank you for your attention :-)
Happy to take questions!

Email: samuel.mortensen@teagasc.ie
Twitter: @SamuelM_Biology
Instagram: @the_science_of_fermentation (w/ Dr. John Leech)

@THE_SCIENCE_OF_FERMENTATION

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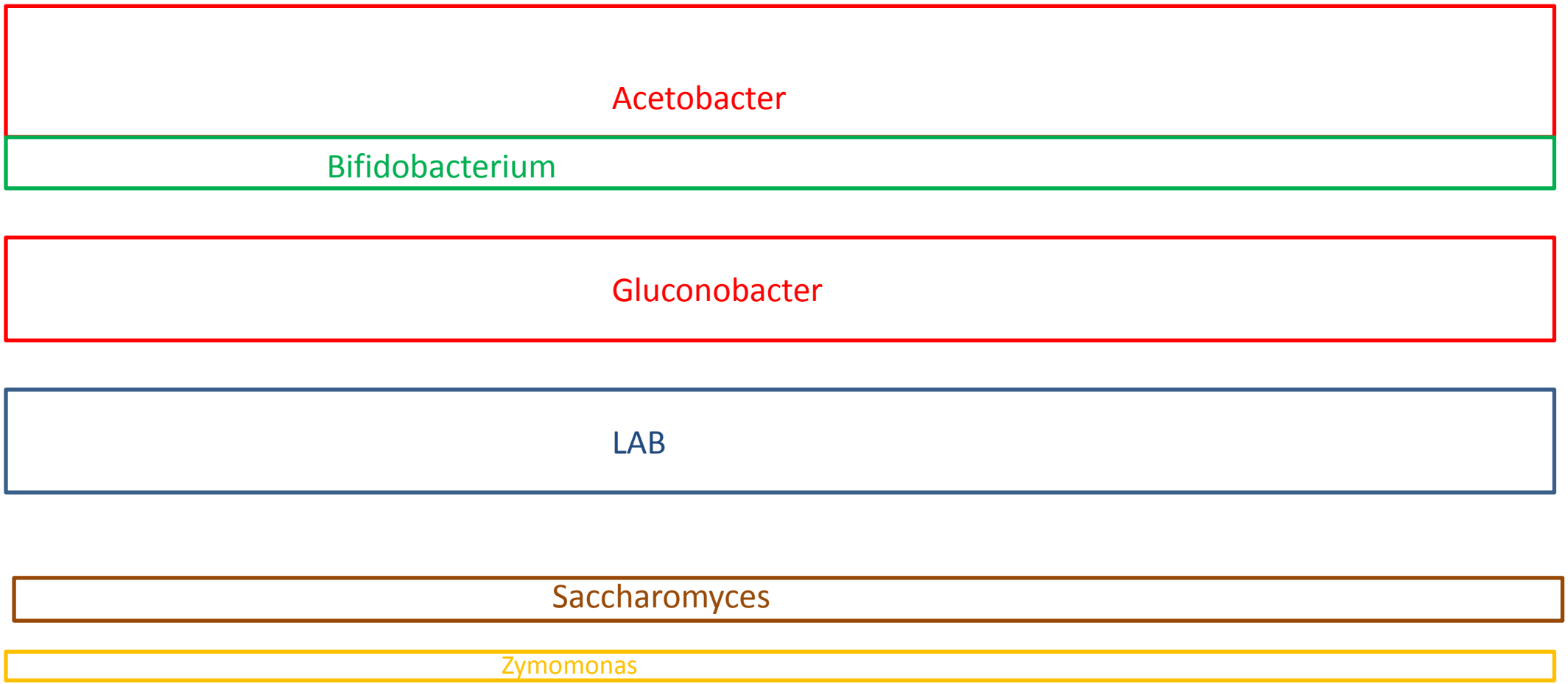
Species abundance (%)



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