

# Water Kefir - A Multi-Omics Study

**KombuchaKon**

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Samuel Mortensen, PhD

PI: Prof. Paul Cotter



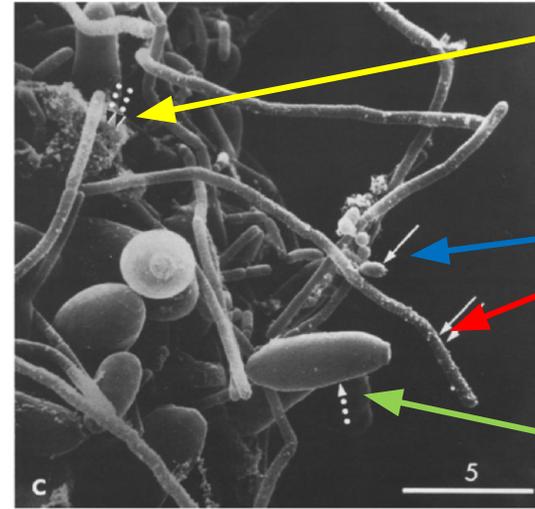
BSc & MSc  
Plant Biotechnology

PhD  
Biology

Postdoctoral Researcher  
Microbiology and Bioinformatics  
(Fermented Foods)

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

# Water Kefir



Dextran

Streptococci

Lactobacilli

Yeast

Gulitz, 2013

Moinas *et al.*, 1980

## Input:

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



## Dominant microorganisms:

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

## Products: fermented beverage

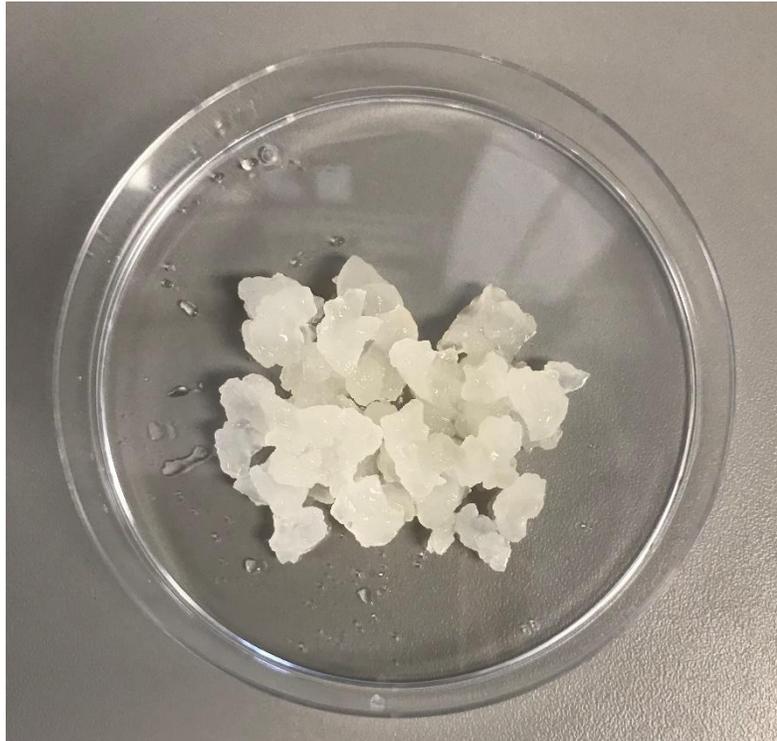
- Lactic acid
- Acetic acid
- Ethanol
- Carbon dioxide
- Aroma compounds

First Fermentation



Second Fermentation

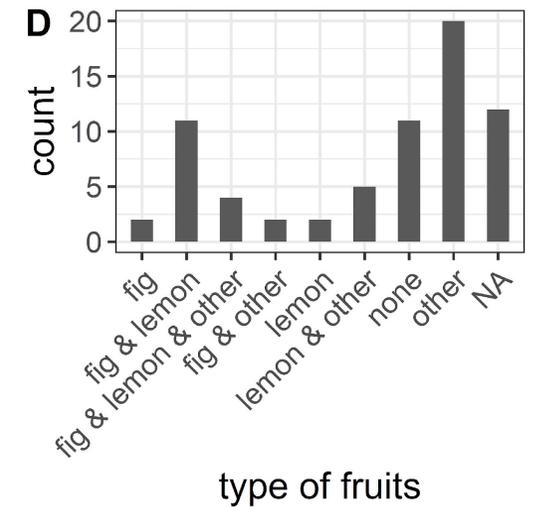
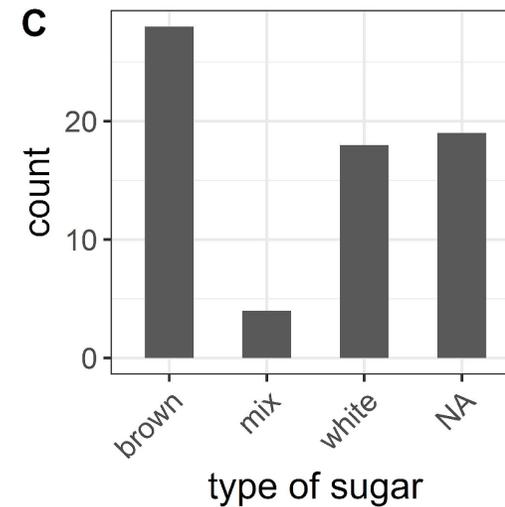
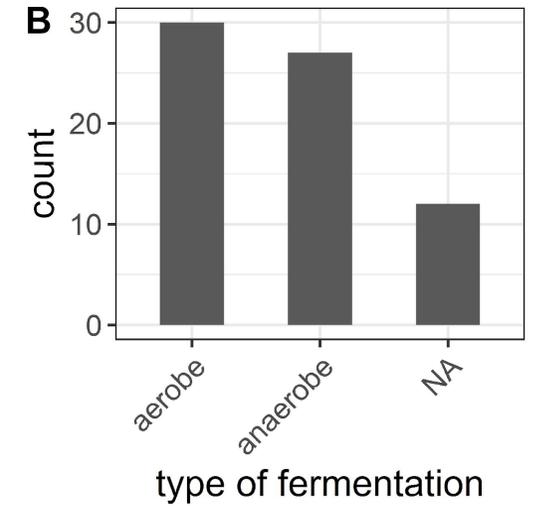
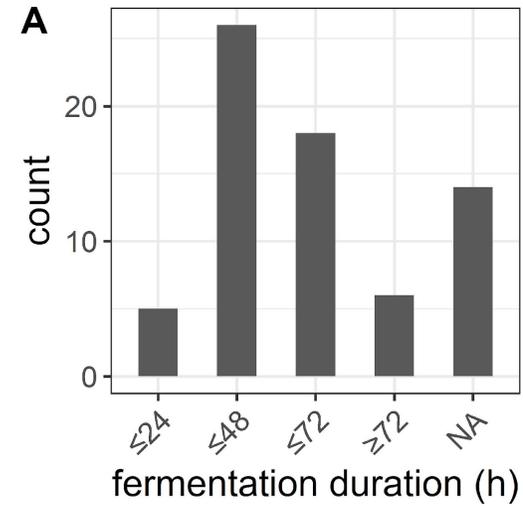
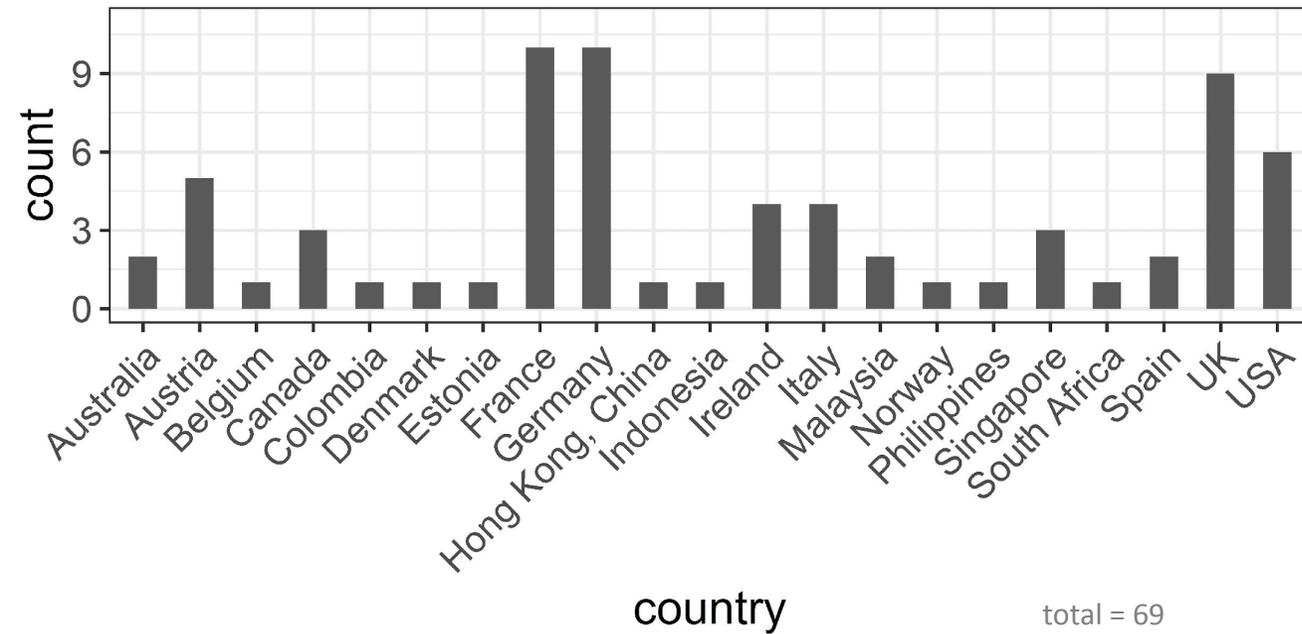


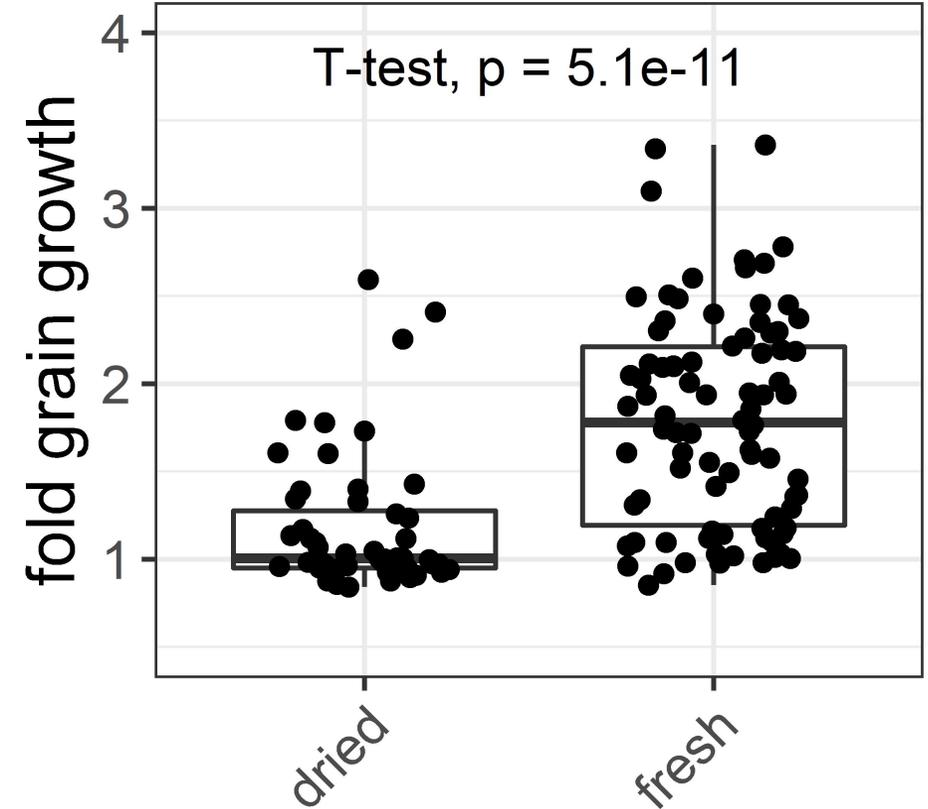
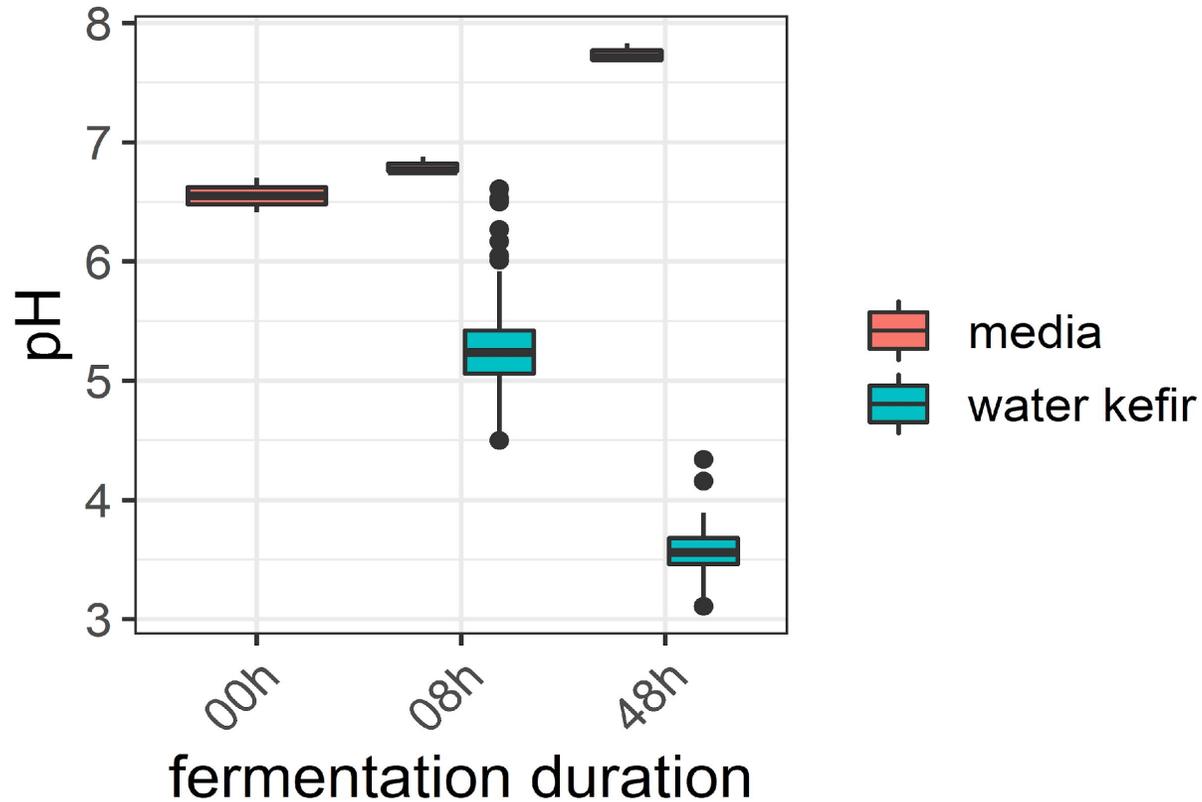


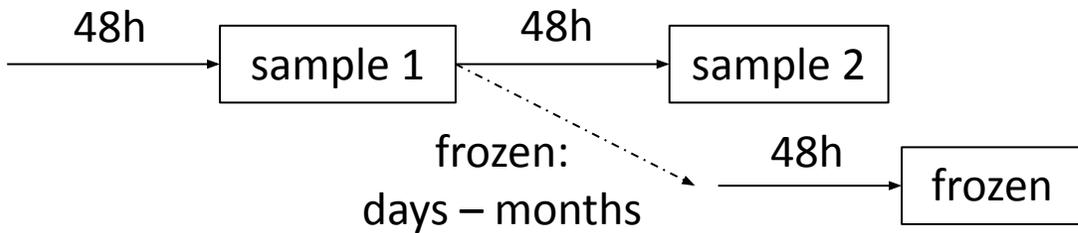
What is the microbial composition of water kefir?  
Which microbes should be present in a commercial water kefir?



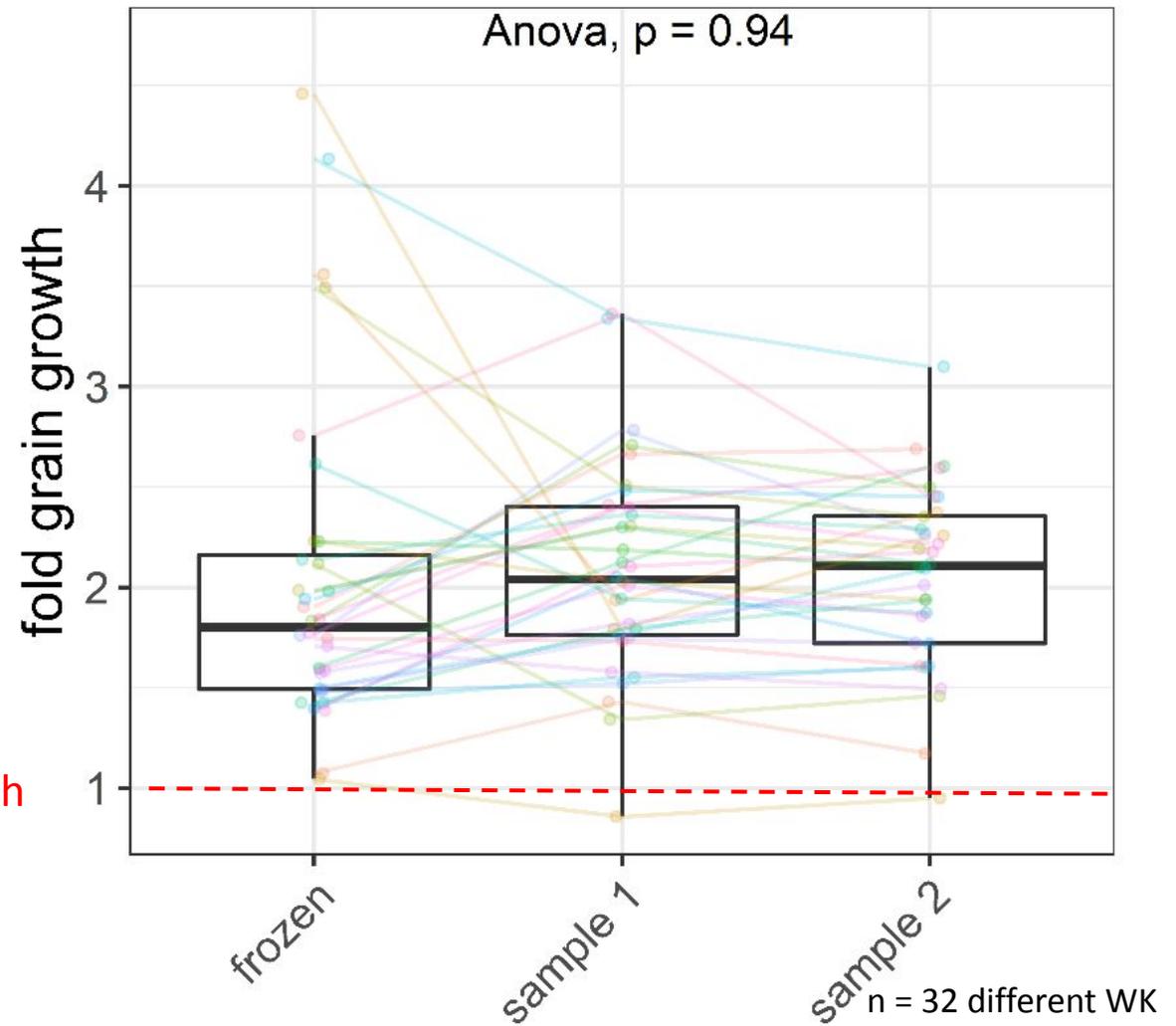
# Water Kefir Collection







no growth



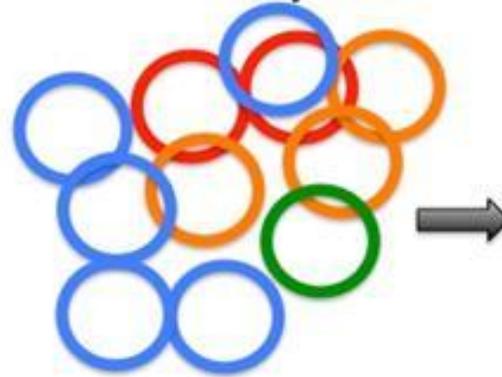
## Shotgun Metagenomics:

Water kefir (WK)

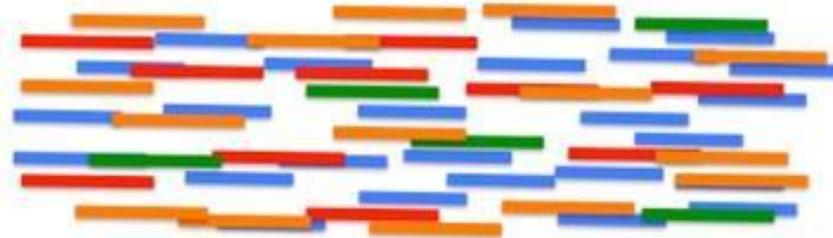
- 2x 08h liquid
- 2x 48h liquid
- 1x 48h grains

69 WK x 5 samples = 345 WK samples

**Bacterial genomes present in a sample**



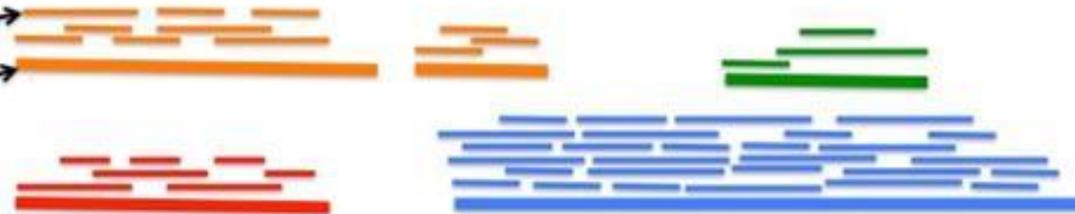
**Genomes cut into small fragments**



**Sequencing of many random fragments from pool of fragments**



DNA sequences  
Computer-assembled consensus sequence



**Alignment of DNA sequences with a computer program to create a larger consensus sequence**

## Analysis

MAG from  
water kefir



Known  
reference  
genome

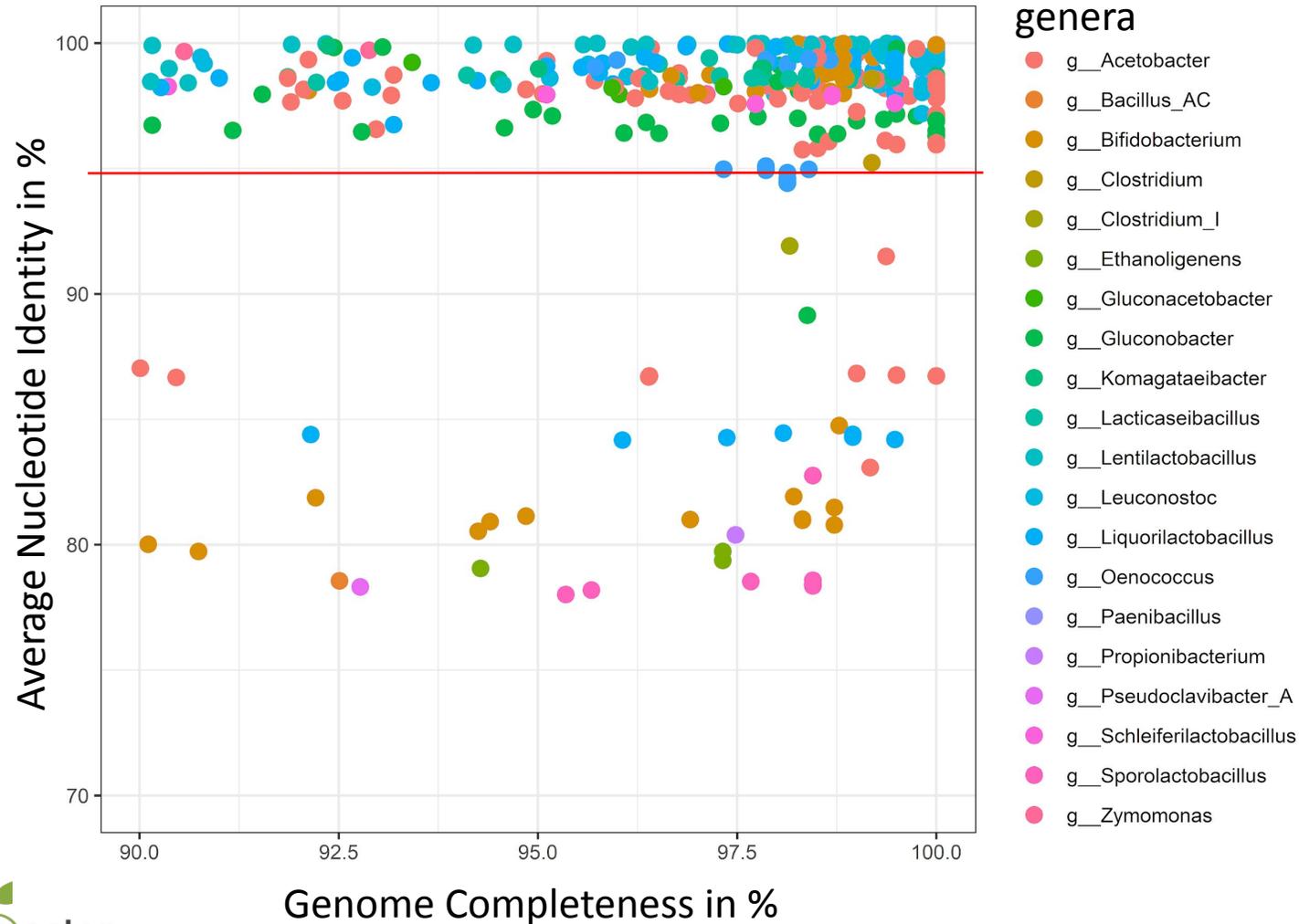


Genome Completeness: 98.8 %  
Average Nucleotide Identity (ANI): 98.8 %

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

## Analysis

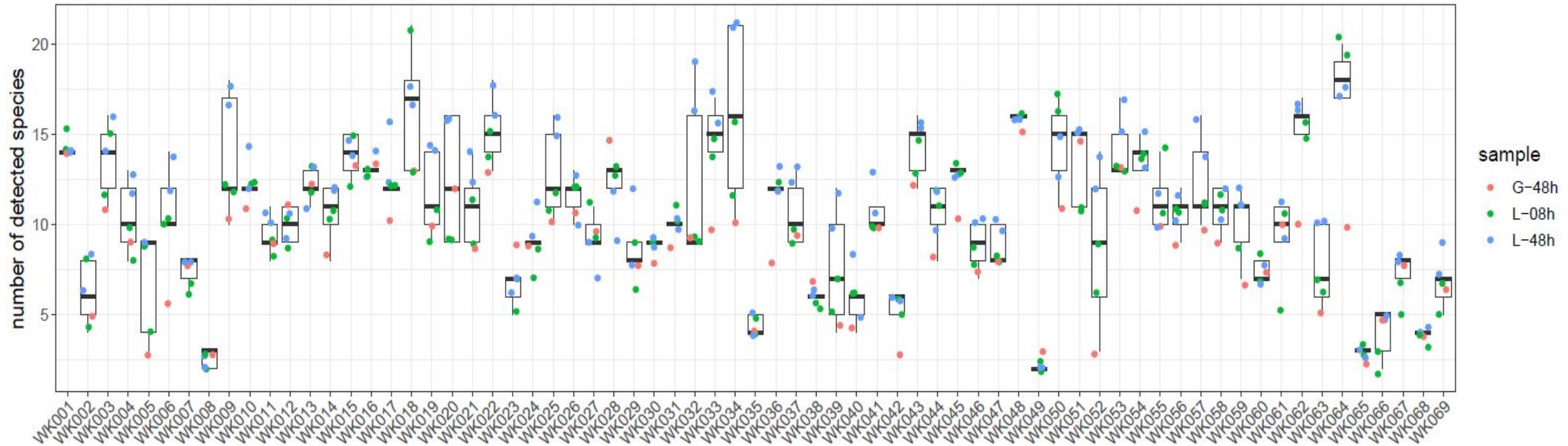
### Bacterial MAGs



54 MAGs not assigned at species level

□ 18 potential novel species

# Detected species



Acetobacter

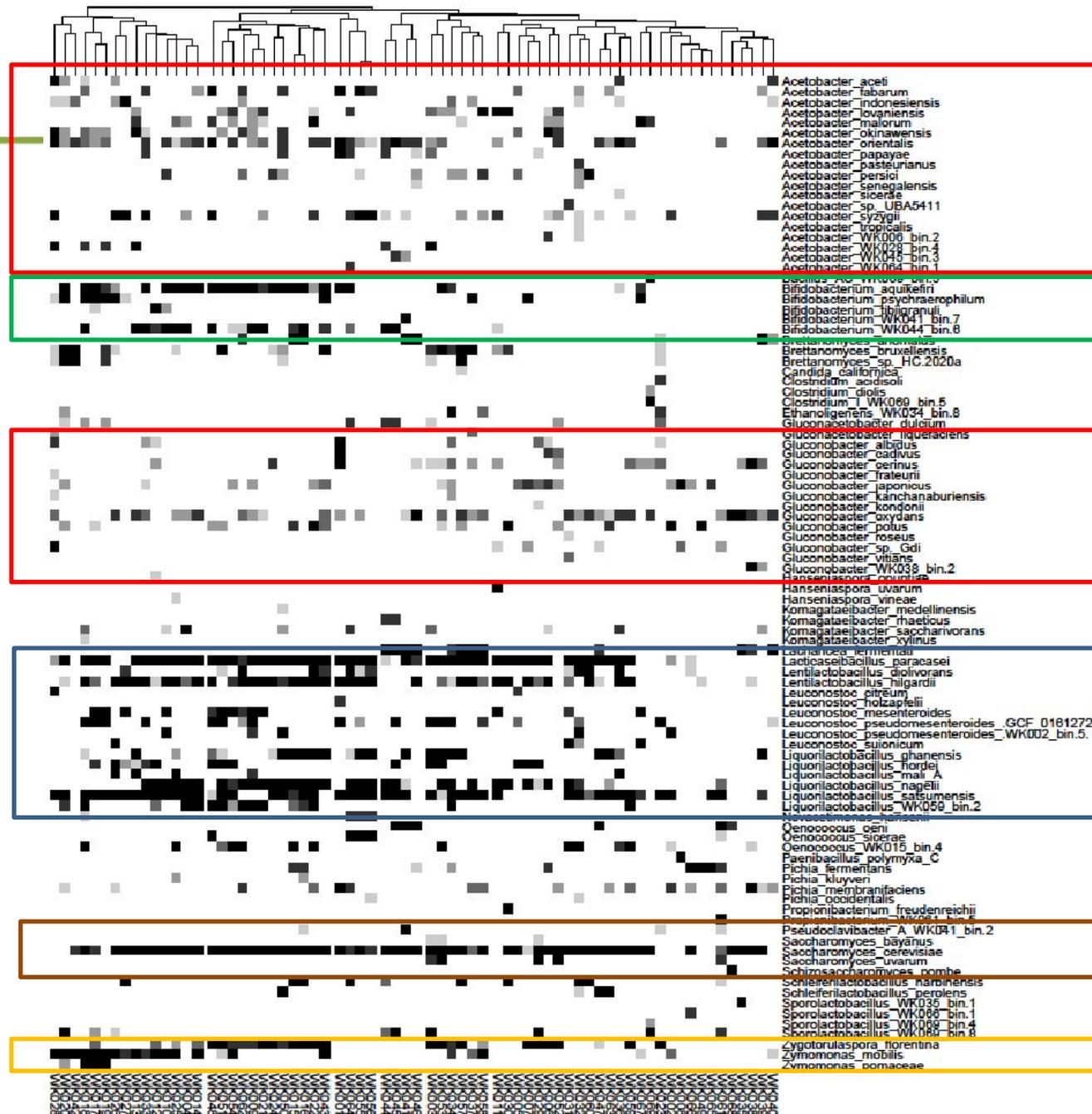
Bifidobacterium

Gluconobacter

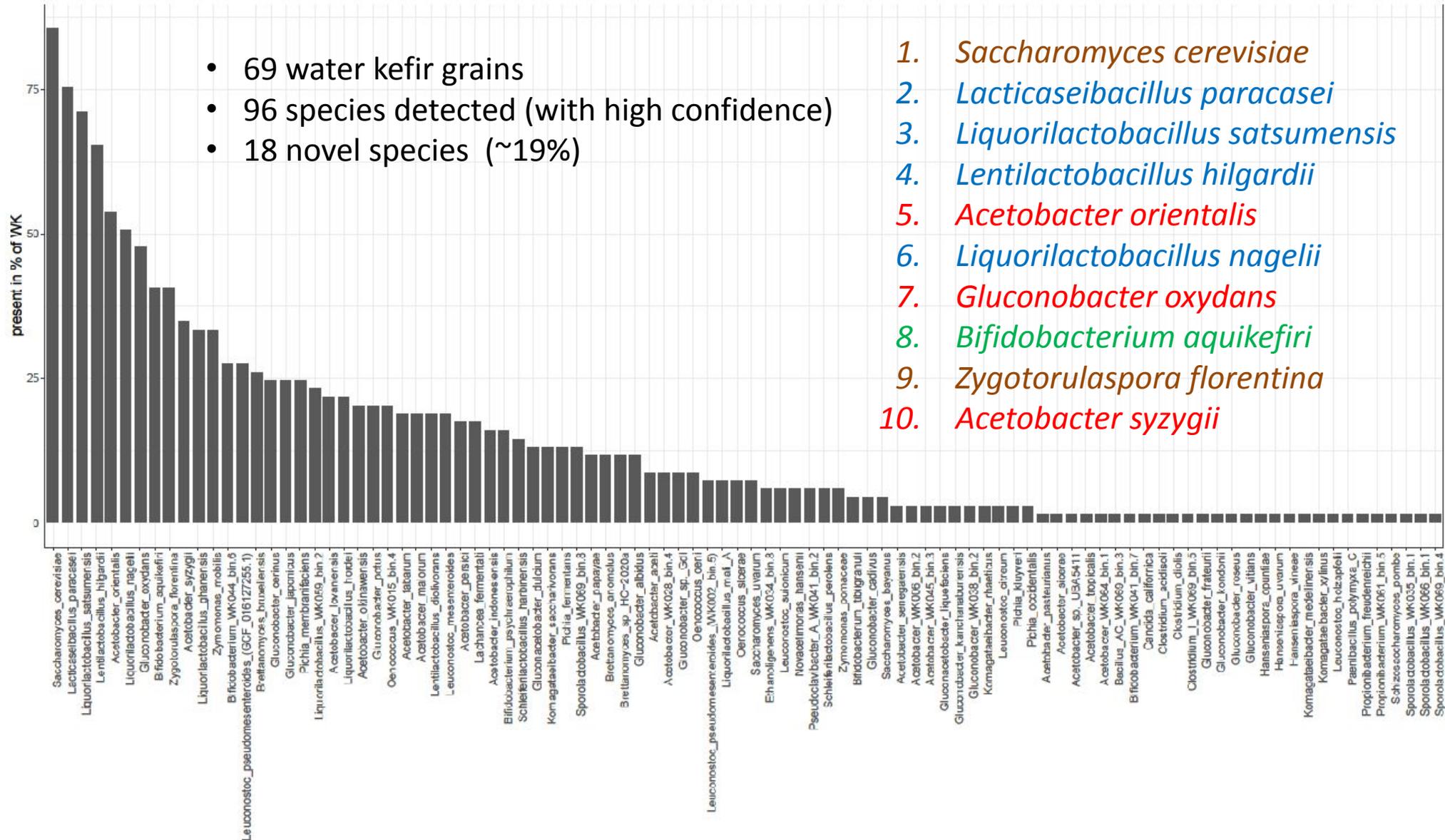
LAB

Saccharomyces

Zymomonas



# Detected species



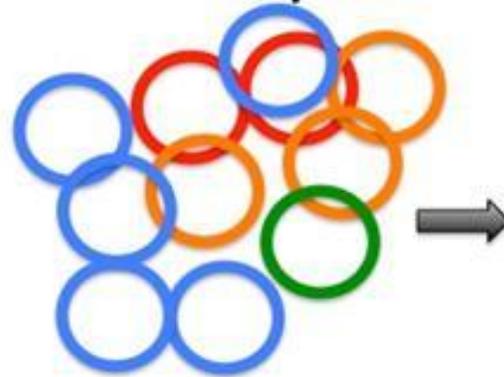
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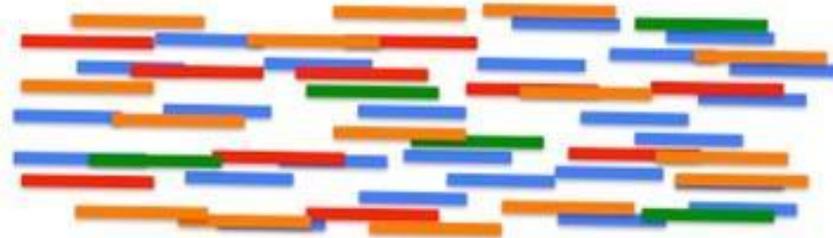
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Bacterial genomes present in a sample



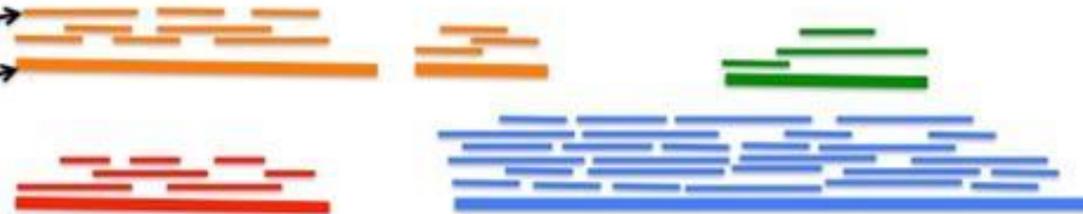
Genomes cut into small fragments



Sequencing of many random fragments from pool of fragments

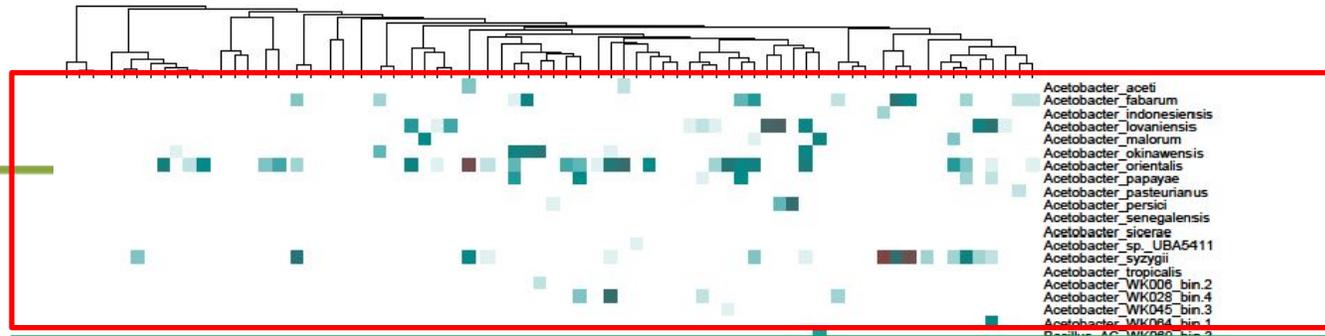


DNA sequences  
Computer-assembled consensus sequence

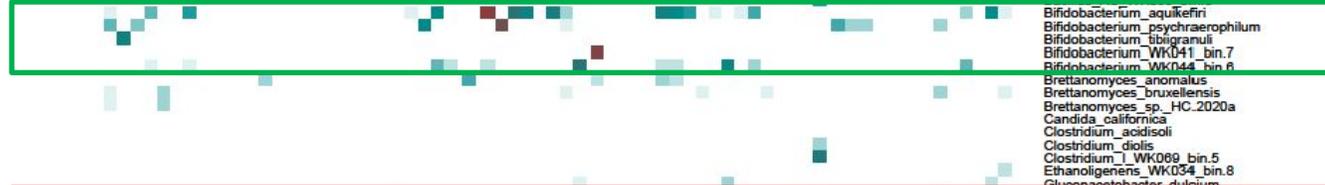


Alignment of DNA sequences with a computer program to create a larger consensus sequence

Acetobacter



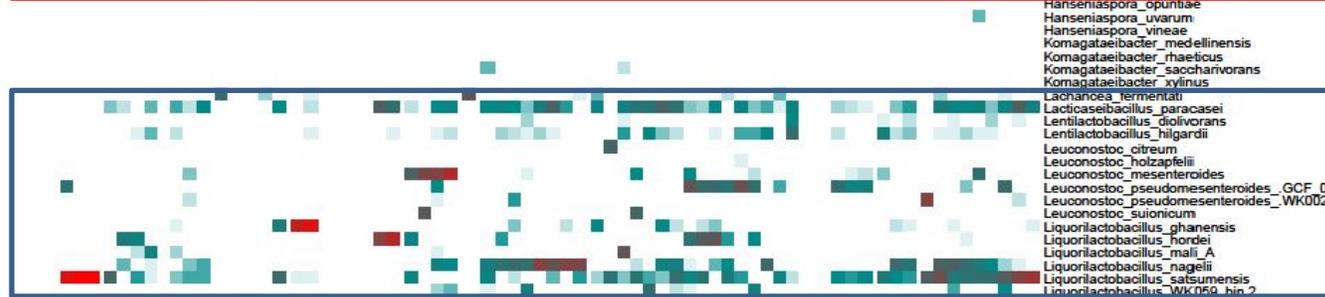
Bifidobacterium



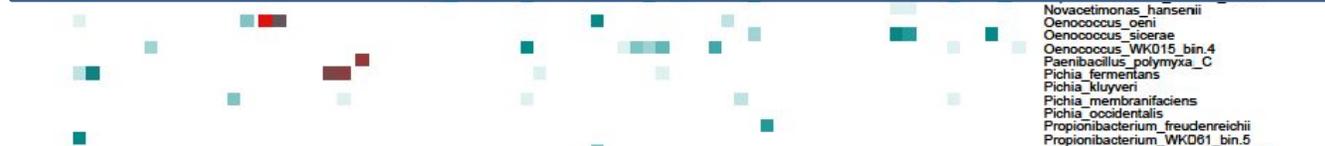
Gluconobacter



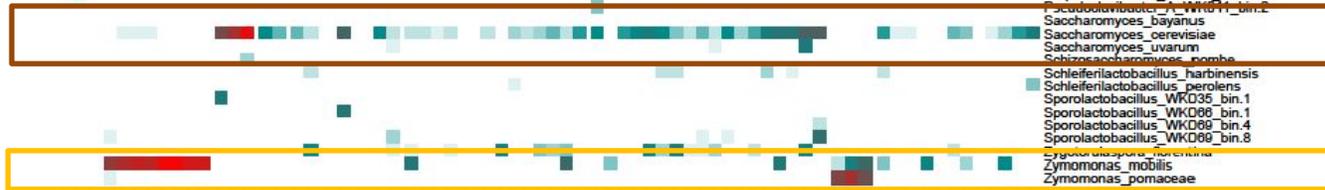
LAB



Saccharomyces



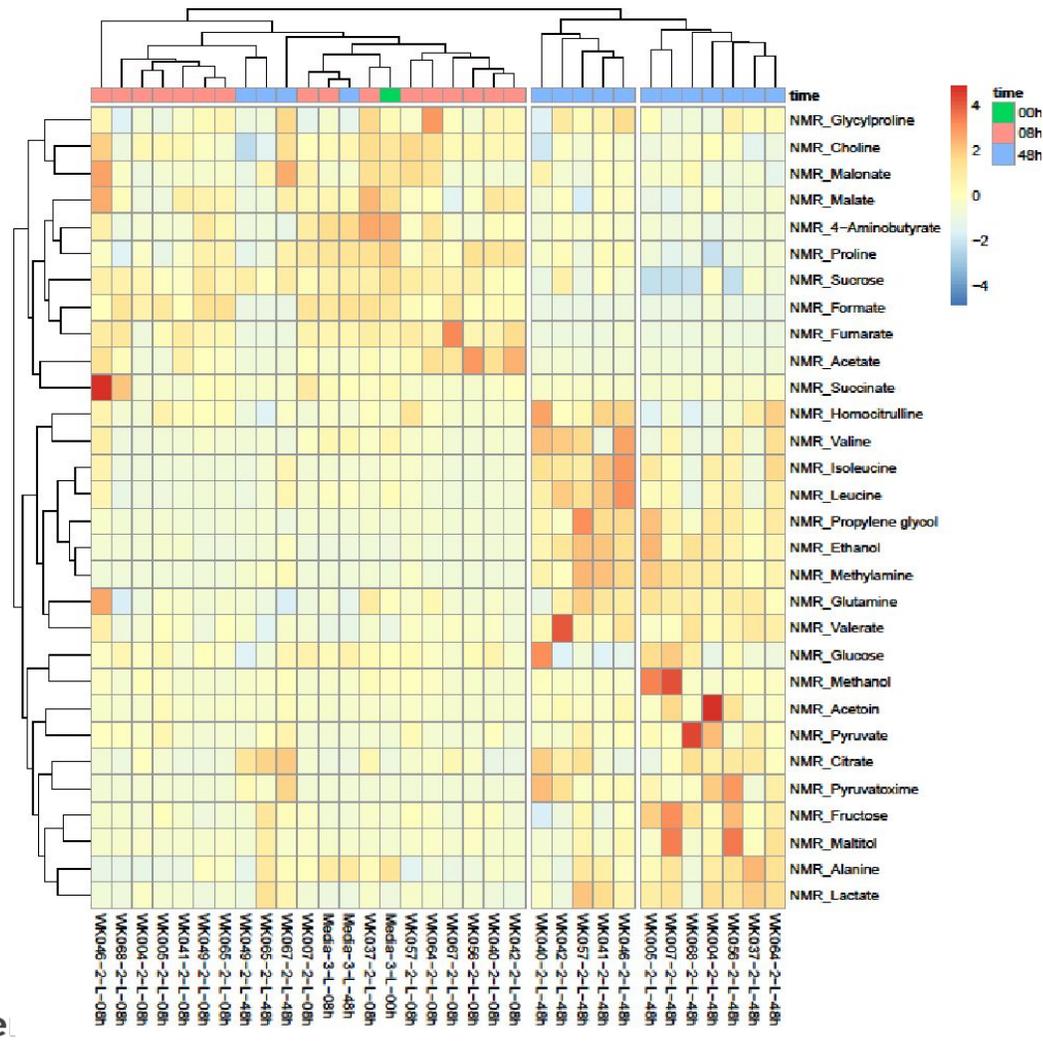
Zymomonas



- Acetobacter\_aeeti
- Acetobacter\_fabarium
- Acetobacter\_indonesiensis
- Acetobacter\_lovianensis
- Acetobacter\_malorum
- Acetobacter\_okinawensis
- Acetobacter\_orientalis
- Acetobacter\_papayae
- Acetobacter\_pasteurianus
- Acetobacter\_persici
- Acetobacter\_senegalensis
- Acetobacter\_sicerae
- Acetobacter\_sp\_UBA5411
- Acetobacter\_szygii
- Acetobacter\_tropicalis
- Acetobacter\_WK008\_bin.2
- Acetobacter\_WK028\_bin.4
- Acetobacter\_WK045\_bin.3
- Acetobacter\_WK045\_bin.4
- Acetobacter\_WK050\_bin.3
- Acetobacter\_WK050\_bin.4
- Bifidobacterium\_aquikefiri
- Bifidobacterium\_psychraerophilum
- Bifidobacterium\_tibigranuli
- Bifidobacterium\_WK041\_bin.7
- Bifidobacterium\_WK044\_bin.6
- Brettanomyces\_anomalus
- Brettanomyces\_bruvelliensis
- Brettanomyces\_sp\_HC.2020a
- Candida\_californica
- Clostridium\_acidisoli
- Clostridium\_diolis
- Clostridium\_1\_WK009\_bin.5
- Ethanoligenens\_WK034\_bin.8
- Glucoacetobacter\_dulcium
- Glucoacetobacter\_liquefaciens
- Gluconobacter\_albidus
- Gluconobacter\_cadivus
- Gluconobacter\_cerinus
- Gluconobacter\_frateuri
- Gluconobacter\_japonicus
- Gluconobacter\_kanchanaburiensis
- Gluconobacter\_kondonii
- Gluconobacter\_oxycans
- Gluconobacter\_potus
- Gluconobacter\_roseus
- Gluconobacter\_sp\_Gdi
- Gluconobacter\_vitians
- Gluconobacter\_WK038\_bin.2
- Hanseniaspora\_opuntiae
- Hanseniaspora\_uvarum
- Hanseniaspora\_vineae
- Komagataeibacter\_medellinensis
- Komagataeibacter\_rhaeticus
- Komagataeibacter\_saccharivorans
- Komagataeibacter\_xylinus
- Laanocaea\_fermentati
- Lactocaseibacillus\_paracasei
- Lentilactobacillus\_diolivorans
- Lentilactobacillus\_hilgardii
- Leuconostoc\_citreum
- Leuconostoc\_holzapfelii
- Leuconostoc\_mesenteroides
- Leuconostoc\_pseudomesenteroides\_GCF\_016127255.1
- Leuconostoc\_pseudomesenteroides\_WK002\_bin.5
- Leuconostoc\_suionicum
- Liquorilactobacillus\_ghanensis
- Liquorilactobacillus\_hordei
- Liquorilactobacillus\_mali\_A
- Liquorilactobacillus\_nagelii
- Liquorilactobacillus\_satsumensis
- Liquorilactobacillus\_WK069\_bin.2
- Novacetimonas\_hansenii
- Oenococcus\_oeni
- Oenococcus\_sicerae
- Oenococcus\_WK015\_bin.4
- Paenibacillus\_polymyxa\_C
- Pichia\_fermentans
- Pichia\_kluyveri
- Pichia\_membranifaciens
- Pichia\_occidentalis
- Propionibacterium\_freudenreichii
- Propionibacterium\_WK061\_bin.5
- Propionibacterium\_WK071\_bin.2
- Saccharomyces\_bayanus
- Saccharomyces\_cerevisiae
- Saccharomyces\_uvarum
- Schizosaccharomyces\_pombe
- Schleiferilactobacillus\_harbinensis
- Schleiferilactobacillus\_perolens
- Sporolactobacillus\_WK036\_bin.1
- Sporolactobacillus\_WK066\_bin.1
- Sporolactobacillus\_WK069\_bin.4
- Sporolactobacillus\_WK069\_bin.8
- Zygodontia\_sporobolus
- Zymomonas\_mobilis
- Zymomonas\_pomaceae

AN001 AN002 AN003 AN004 AN005 AN006 AN007 AN008 AN009 AN010 AN011 AN012 AN013 AN014 AN015 AN016 AN017 AN018 AN019 AN020 AN021 AN022 AN023 AN024 AN025 AN026 AN027 AN028 AN029 AN030 AN031 AN032 AN033 AN034 AN035 AN036 AN037 AN038 AN039 AN040 AN041 AN042 AN043 AN044 AN045 AN046 AN047 AN048 AN049 AN050 AN051 AN052 AN053 AN054 AN055 AN056 AN057 AN058 AN059 AN060 AN061 AN062 AN063

## NMR metabolomics

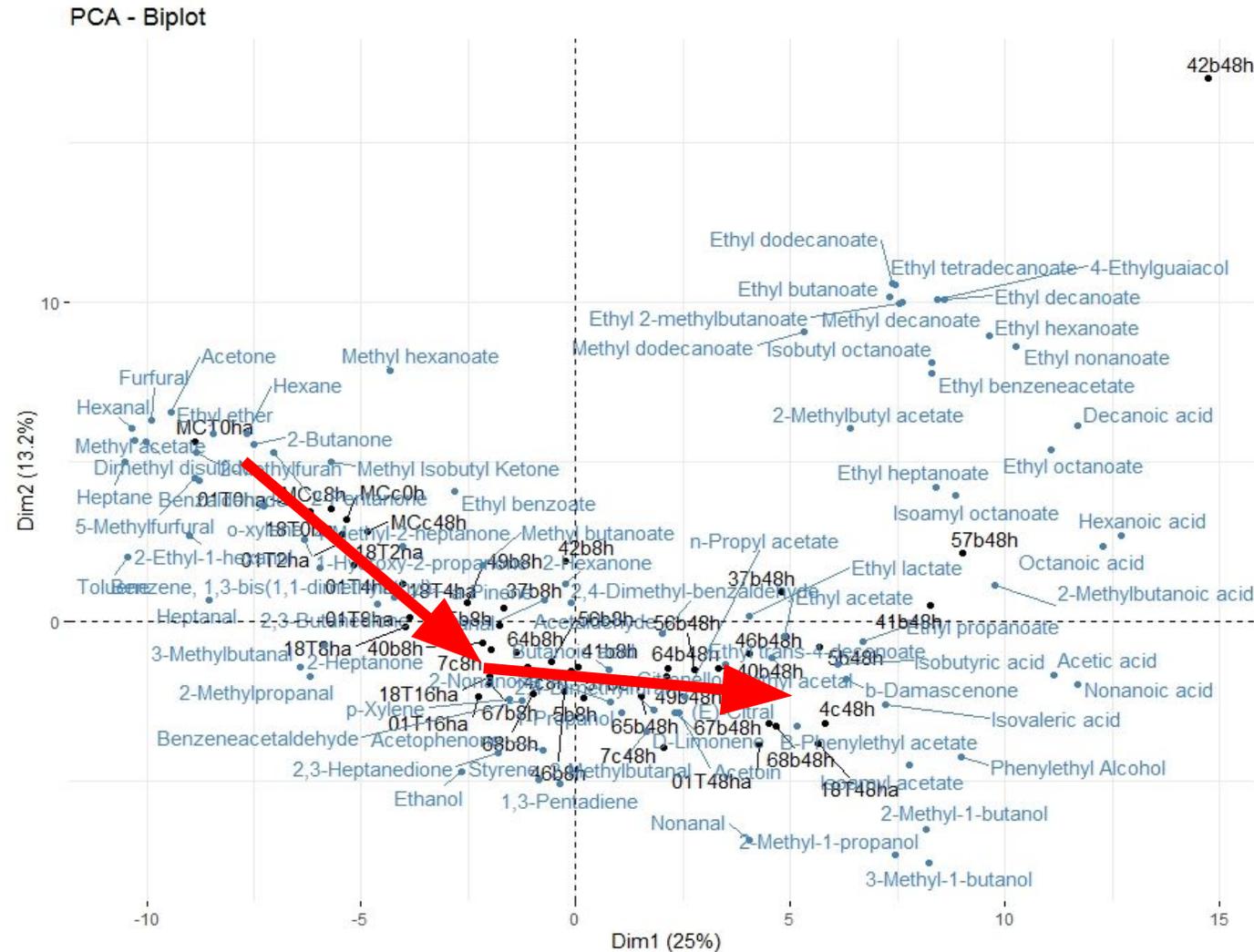
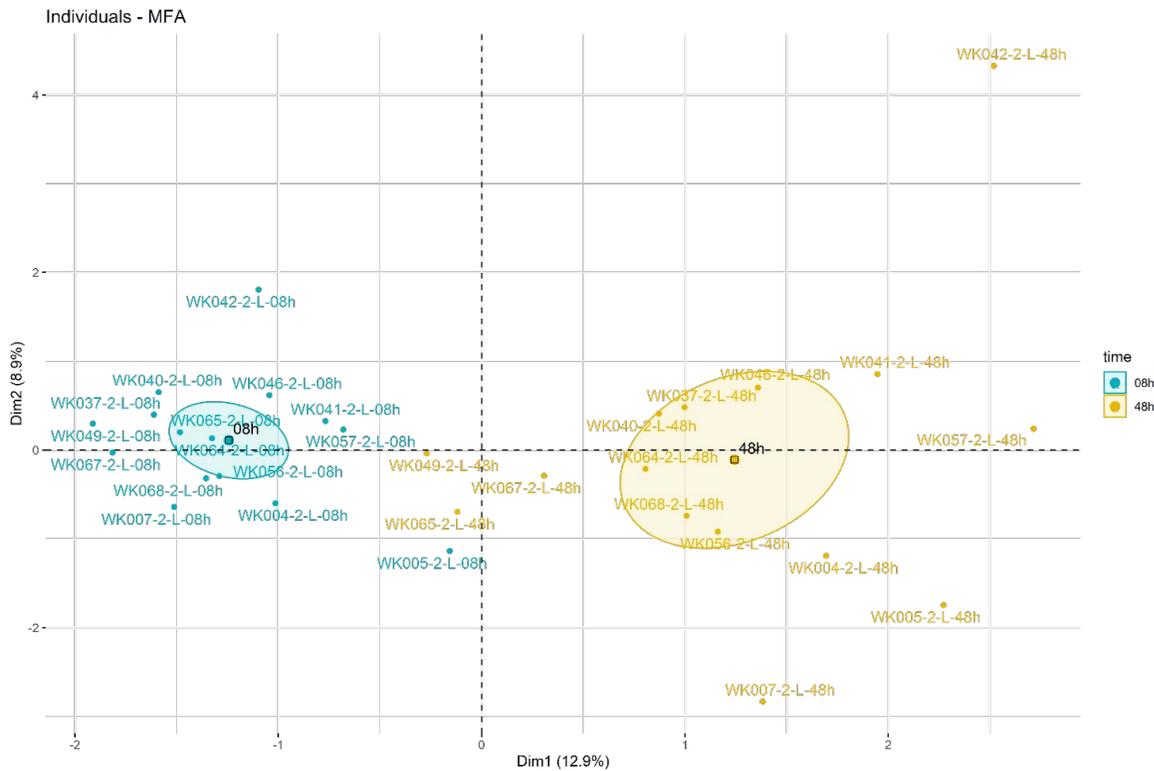


## MS volatolomics

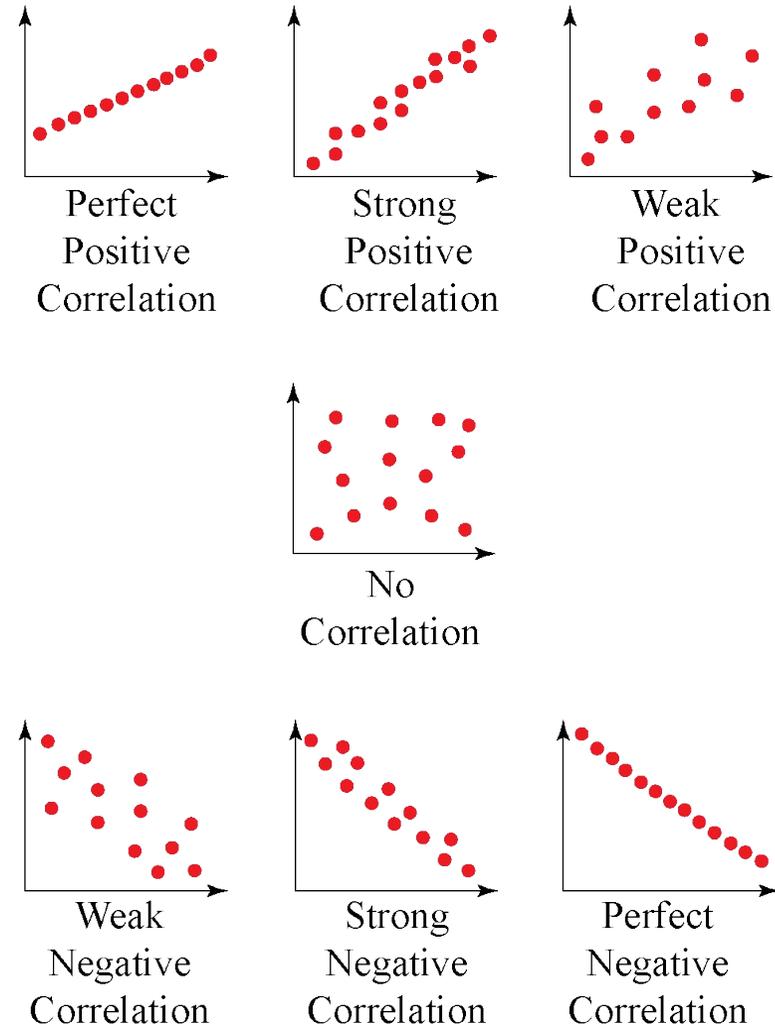
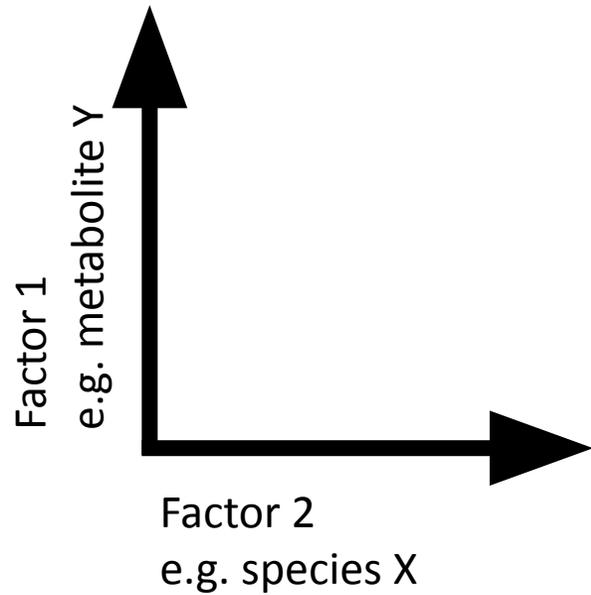


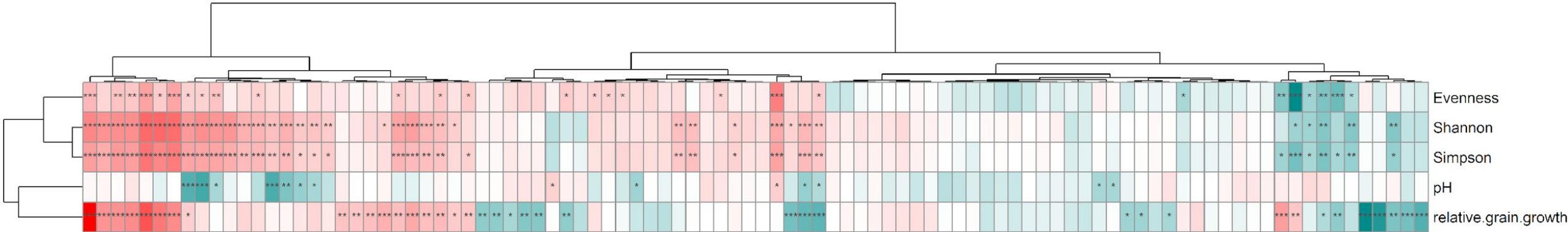
## Species & Flavour & Metabolites

## Flavour Development



# Correlation Analysis



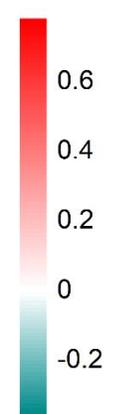


*Lentilactobacillus hilgardii*

*Acetobacter* spp.

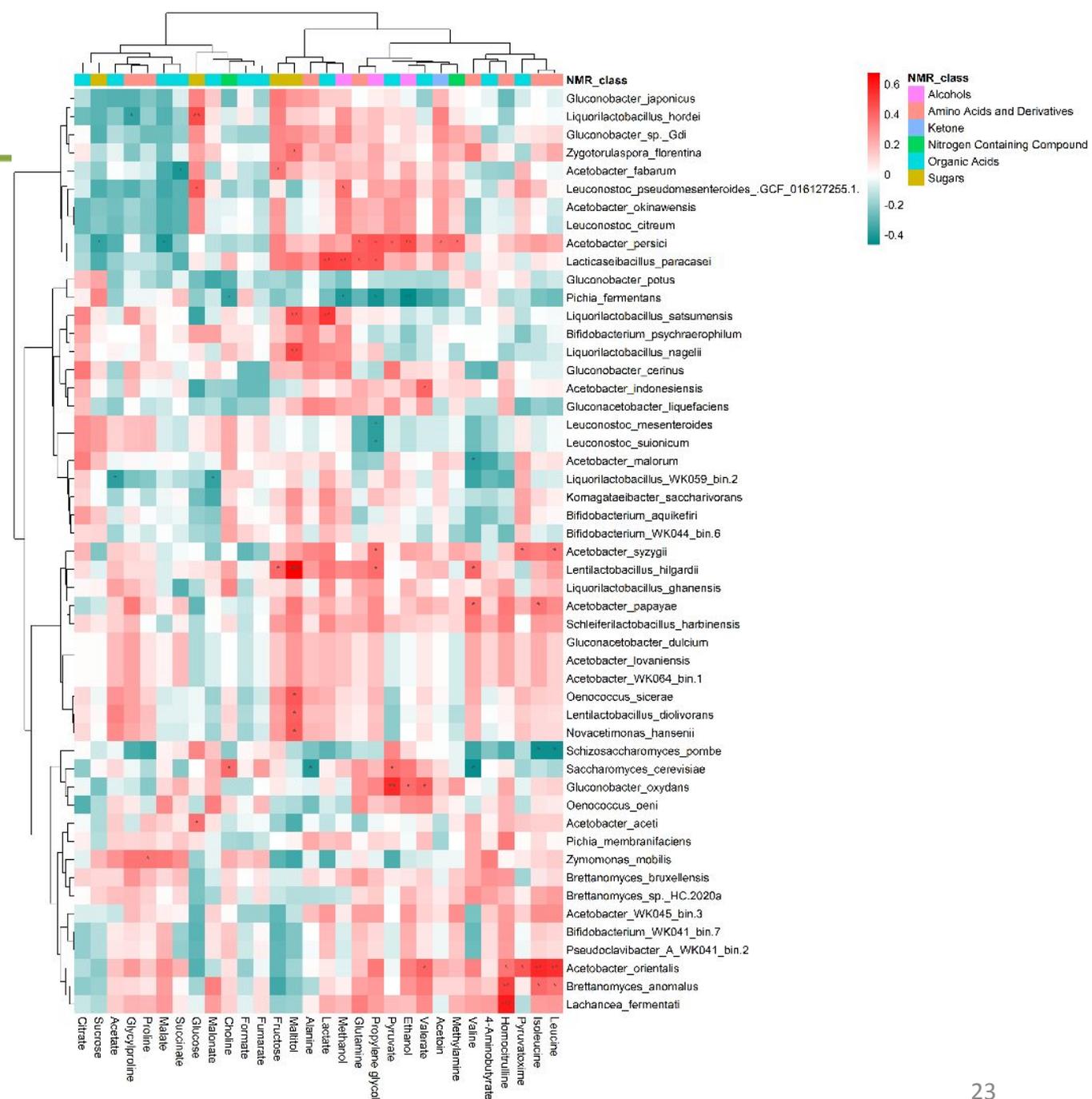
*Saccharomyces cerevisiae*

*Zymomonas mobilis*



# Correlation Analysis Species vs Metabolites

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



# Correlation Analysis Species vs Flavour

\*\*\* + Esters

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

Most negative VOC correlations:

- *Pichia fermentans*



- 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



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!*

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Twitter: @SamuelM\_Biology

Instagram: @the\_science\_of\_fermentation (w/ Dr. John Leech)



@THE\_SCIENCE\_OF\_FERMENTATION



# Species abundance (%)

Acetobacter

Bifidobacterium

Gluconobacter

LAB

Saccharomyces

Zymomonas