

Harnessing Milk- and Water-Kefirs as Representative Fermented Beverage-Associated Communities

Paul Cotter
Head of Food Bioscience, Teagasc
Head of Microbiology, SeqBiome Ltd.



SEQBIOME 

Fermented Food Microbiomes



Water Kefir

- Marsh, et al. 2013
- Patel, et al. 2022
- Breselge, et al. 2025, 2026



Kombucha

- Marsh, et al. Food Microbiol. 2014
- Villarreal-Soto, et al. IJFM. 2020



Kefir

- Walsh, et al. mSystems. 2016
- Walsh et al iScience 2023
- And many, others



Bread Kvass, Carrot Kimchi, Boza, Turnip, Orange, Krauthehi (sauerkraut), Tepache, Ginger Beer, Tempeh, Cucumber, Milk Kefir, Water Kefir, Tofu Chilli, Daikon, Pickled vegetables, Raw Sauerkraut & Juniper berries, Brown rice amazake, Beetroot Kvass, Kefir and fennel soup, Mead, Sauerkraut, Dill dearg (sauerkraut), Kimchi, Golden child (sauerkraut), Water Kefir Hibiscus, Water Kefir lemon, Water Kefir Ginger, Kombucha Vinegar, Sauerkraut, Kombucha, Apple Cider Vinegar, Water Kefir(Pear, Ginger & Honey, Water Kefir(Pear, Ginger & Sugar), Dilly Carrots, Brussel Sprout Kimchi , Kimchi, Garlic Kraut, Dukkah Kraut, Ginger Sliced in 2% Brine, Daikon Radish in 2% Brine, Okra in 2% Brine, Tomatoes & mustard seeds in 2% Brine, Kombucha, Cherry Water Kefir, Beet Kvass, Coconut Kefir, Carrot sticks, Lemon and Ginger Fizz, Scallion Kimchi



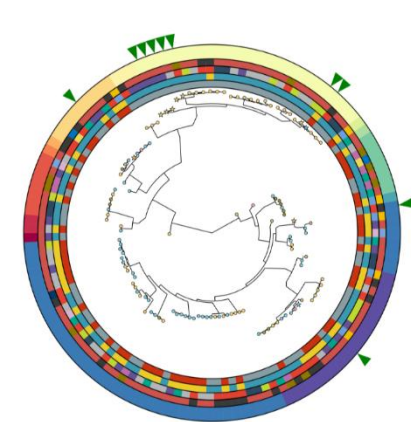
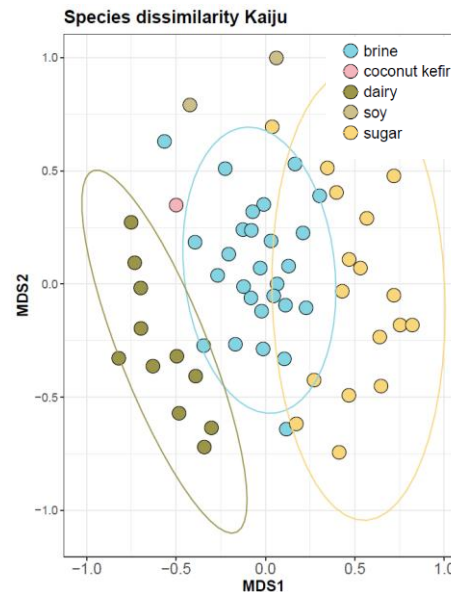
Nunu

- Walsh, et al. Appl Env Microbiol. 2017



Cheese

- Quigley, et al. mSystems. 2016
- Bertuzzi, et al. mSystems. 2018
- Walsh, et al. Nature Food. 2020
- And others



Multiple potentially novel species



Leech, et al. *mSystems*. 2020
Leech et al. *Microbiology* 2026

Food and Fermented Food Microbiomes

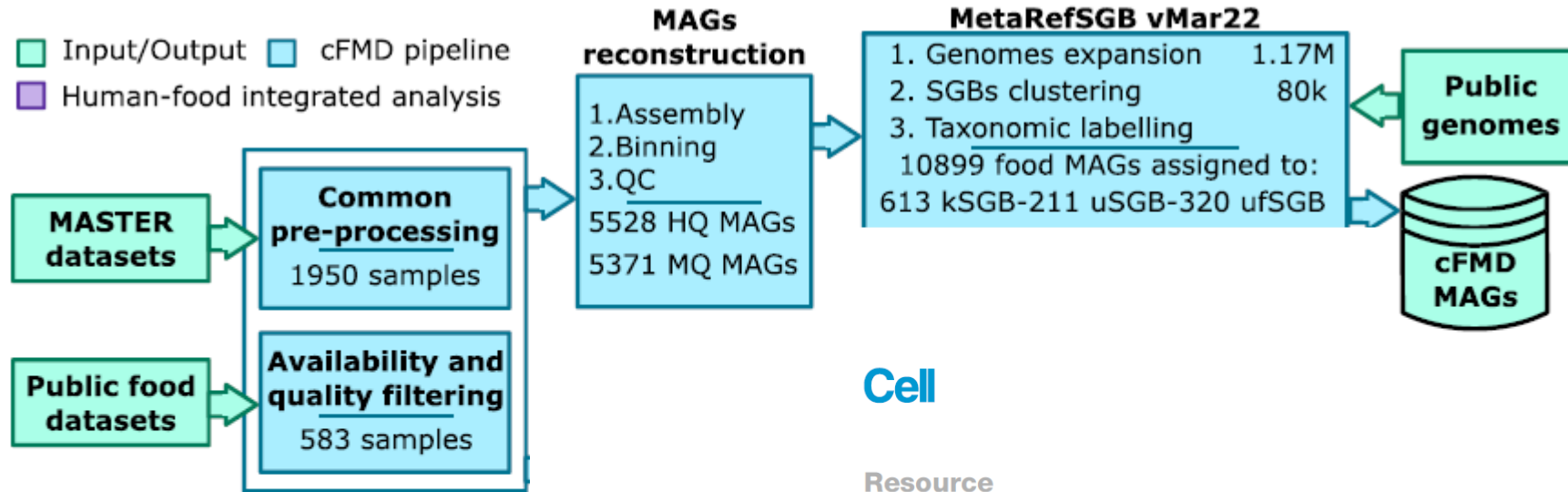
nature protocols

<https://doi.org/10.1038/s41596-023-00949-x>

Protocol

Check for updates

Improved sampling and DNA extraction procedures for microbiome analysis in food-processing environments



613 known SGBs, 211 unknown SGBs, 320 unknown food SGBs

Cell

CellPress
OPEN ACCESS

Resource

Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome

Niccolò Carlino,¹ Aitor Blanco-Míguez,¹ Michal Punčochár,¹ Claudia Mengoni,¹ Federica Pinto,¹ Alessia Tatti,^{2,3,4} Paolo Manghi,¹ Federica Armanini,¹ Michele Avagliano,⁵ Coral Barcenilla,⁶ Samuel Breselge,^{7,8} Raul Cabrera-Rubio,^{7,9} Inés Calvete-Torre,^{10,11} Mairéad Coakley,⁷ José F. Cobo-Díaz,⁶ Francesca De Filippis,^{5,12} Hrituraj Dey,¹ John Leech,⁷ Eline S. Klaassens,¹³ Stephen Knobloch,¹⁴ Dominic O'Neil,¹⁵ Narciso M. Quijada,^{16,17,18} Carlos Sabater,^{10,11} Sigurlaug Skirnisdóttir,¹⁴ Vincenzo Valentino,⁵ Liam Walsh,^{7,8,19} MASTER EU Consortium, Avelino Alvarez-Ordóñez,⁶ Francesco Asnicar,¹ Gloria Fackelmann,¹ Vitor Heidrich,¹ Abelardo Margolles,^{10,11} Viggó Thór Marteinsson,^{14,20} Omar Rota Stabelli,^{3,4} Martin Wagner,^{16,17} Danilo Ercolini,^{5,12,24} Paul D. Cotter,^{7,8,21,24} Nicola Segata,^{1,22,23,24,25,*} and Edoardo Pasolli^{5,12,24}

DOMINO

Cheese - De Filippis et al., NPJ Biofilms and Microbiome 2025

Resistome – Quijada et al. Nature Microbiol 2025

Acetic acid bacteria – Zhang et al iScience 2025

Phage – Walsh et al NPJ Biofilms and Microbiomes 2026

Hafnia – In prep

CAZymes – In prep

Gut brain modules – Balasubramanian et al NPJ Sci Foods Accepted

What is kefir?

Milk kefir = fermented **dairy** beverage traditionally made by inoculating **milk** with a **milk kefir grain** (containing a consortium of bacteria and yeasts bound by the exopolysaccharide, **kefiran**)

Water kefir = fermented **non-dairy** beverage made by inoculating a **sugar (sucrose)-rich solution** supplemented with fruits such as figs with a **water kefir grain** (containing a consortium of bacteria and yeasts bound by the exopolysaccharide, **dextran**).



Milk kefir

LAB - *Lactococcus lactis*, *Lactobacillus* species, *Leuconostoc*

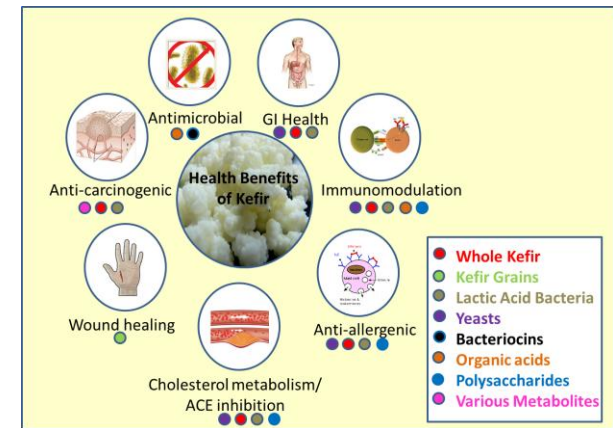
AAB e.g. *Acetobacter*

Yeasts e.g. *Saccharomyces* and *Kluyveromyces*



Walsh et al. mSystems 2016

<https://www.youtube.com/watch?v=uyibFCgXexA>



Bourrie et al. Front. Microbiol 2016.

van de Wouw et al. *Microbiome* (2020) 8:67
<https://doi.org/10.1186/s40168-020-00846-5>

Microbiome

RESEARCH

Open Access

Distinct actions of the fermented beverage kefir on host behaviour, immunity and microbiome gut-brain modules in the mouse



Marcel van de Wouw^{1,2†}, Aaron M. Walsh^{1,3,5†}, Fiona Crispie^{1,3}, Lucas van Leuven¹, Joshua M. Lyte¹, Marcus Boehme¹, Gerard Clarke^{1,4}, Timothy G. Dinan^{1,4}, Paul D. Cotter^{1,3†} and John F. Cryan^{1,2,4†*}



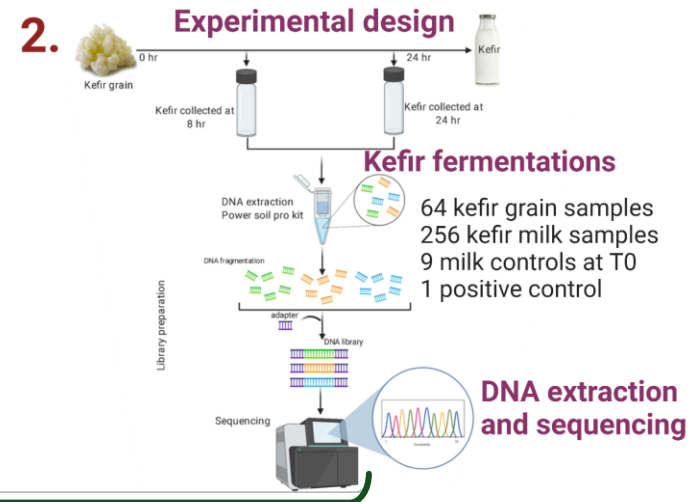
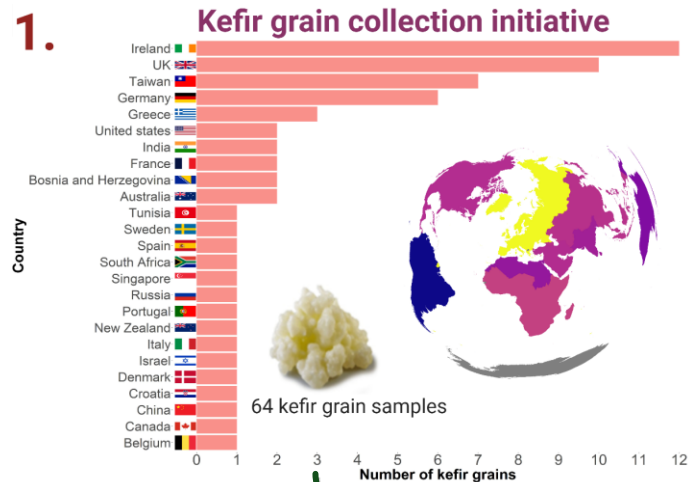
British Journal of Nutrition (2021), 125, 129–138
© The Author(s), 2020. Published by Cambridge University Press on behalf of The Nutrition Society

doi:10.1017/S0007114520002743

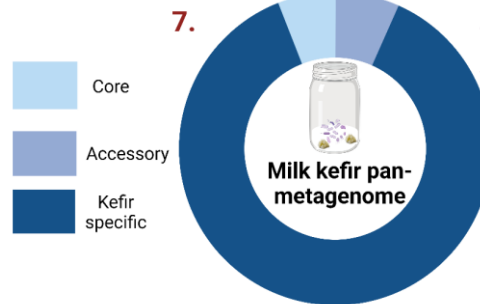
Kefir microbial composition is a deciding factor in the physiological impact of kefir in a mouse model of obesity

Benjamin C. T. Bourrie^{1,2}, Tingting Ju¹, Janelle M. Fohhse¹, Andrew J. Forgie¹, Consolato Sergi³, Paul D. Cotter^{2,4} and Benjamin P. Willing^{1*}

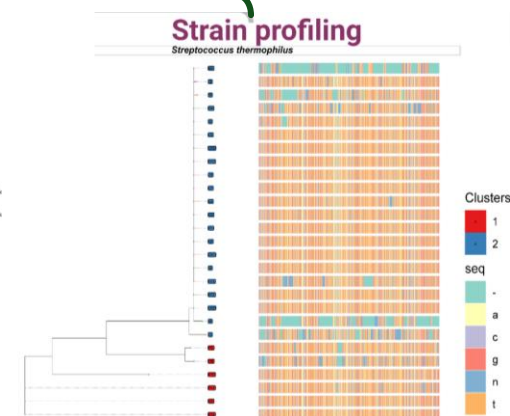
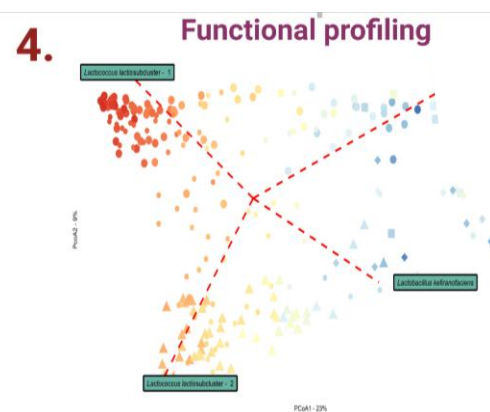
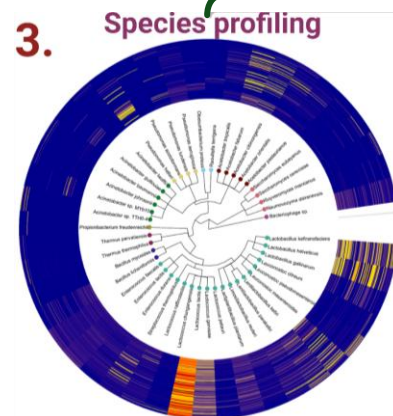
Milk Kefir (meta)pangenome



Walsh et al iScience 2023



The **pan-metagenome** is the collective study of all or several metagenomes from all possible units belonging to a particular type of ecosystem or host



Water kefir

LAB - *Lactobacillus* species (*L. hilgardii*, *L. nagelii*, *L. casei*, *L. paracasei*, *L. brevis*, and *L. hordei*) and *Leuconostoc*
 AAB e.g. *Gluconobacter*
 Yeasts e.g. *Saccharomyces*, *Brettanomyces*
 +/- *Zymomonas*



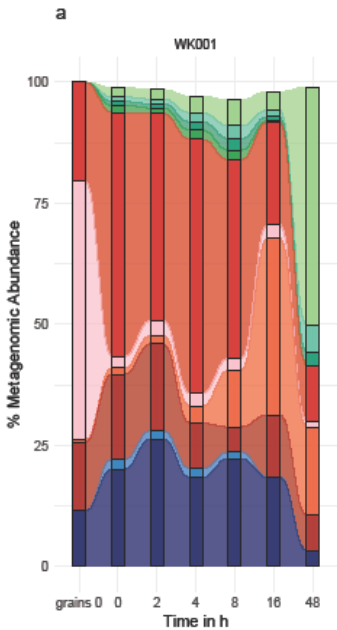
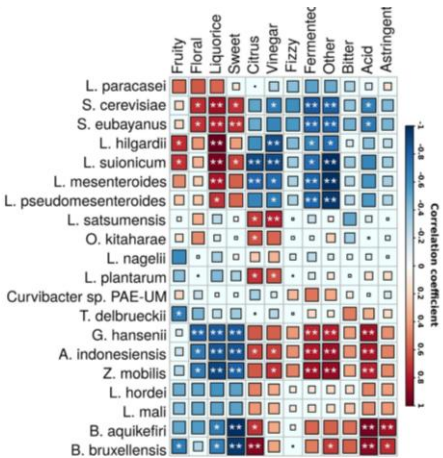
A temporal view of the water kefir microbiota and flavour attributes

S.H. Patel^{a,b}, J.P. Tan^c, R.A. Börner^c, S.J. Zhang^c, S. Priour^c, A. Lima^c, C. Ngom-Bru^c, P.D. Cotter^{a,b,d}, S. Duboux^{c,*}

^a SegBiom Ltd., Cork, Ireland
^b APC Microbiome Ireland, University College Cork, Cork, Ireland
^c Société des Produits Nestlé SA, Nestlé Research, Lausanne, Switzerland
^d Teagasc Food Research Centre, Moorepark, Ireland

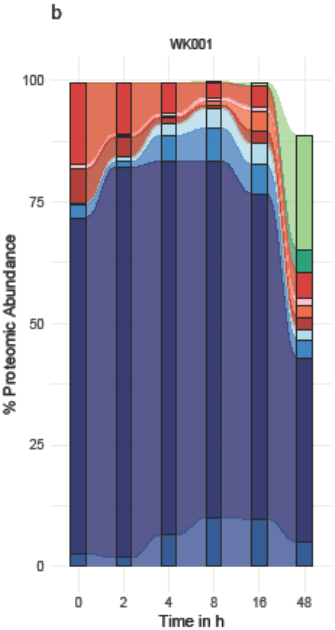


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Species

- Acetobacter_fabrum
- Acetobacter_orientalis
- Acetobacter_papayae
- Gluconobacter_cerinus
- Lactobacillus_paracasei
- Lentilactobacillus_hilgardii
- Leuconostoc_pseudomesenteroides
- Liquorilactobacillus_satsumensis
- Pichia_membranifaciens
- Saccharomyces_cerevisiae

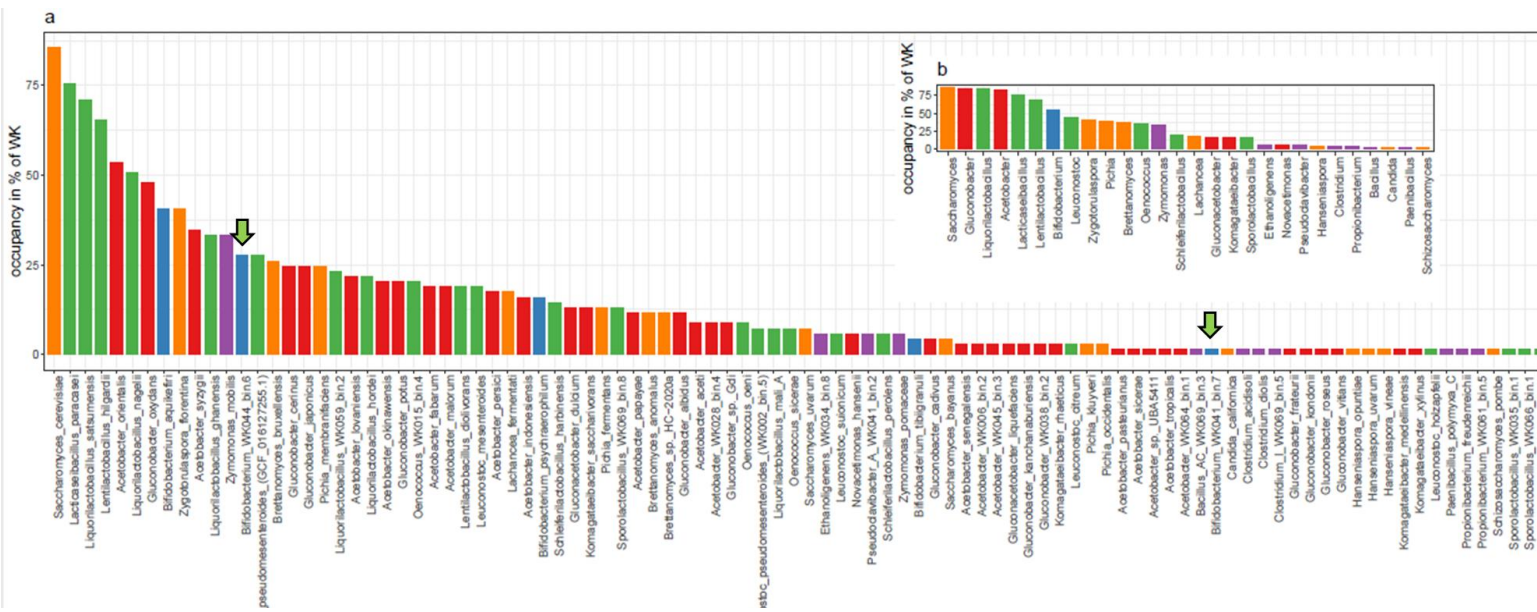


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- Lentilactobacillus_hilgardii
- Leuconostoc_pseudomesenteroides
- Liquorilactobacillus_satsumensis
- Hanseniaspora_mollemarum
- Pichia_membranifaciens
- Saccharomyces_cerevisiae
- Zygorhizula_florentina



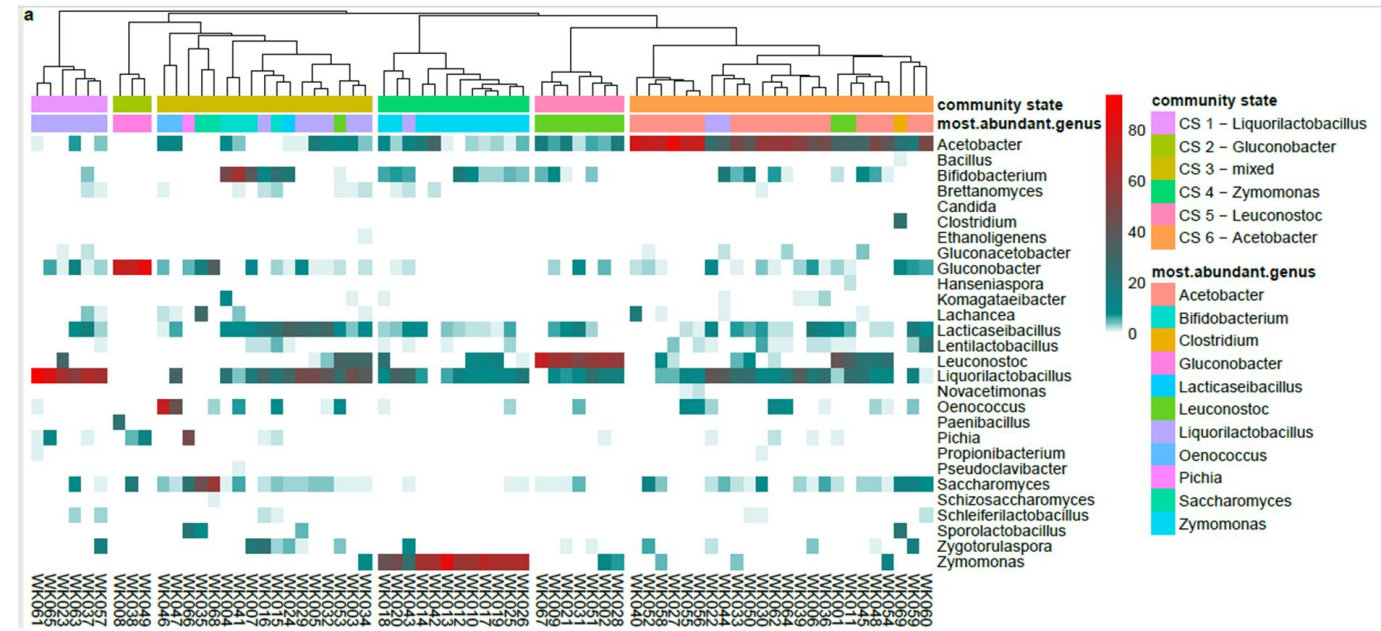
Breselge et al.
 NPJ Sci Food 2025



Breselge et al. Comms Biol 2025

Water Kefir (meta)pangenome

- 69 water kefir samples from 21 countries
- Shotgun metagenomics (2x 8h liquid, 2x 48 liquid, 1x grains)
- NMR metabolomics
- GC-MS volatilomics
- Additional metadata
- Detection of 39 different AAB species in water kefir (inStrain)
- 6 Community types dominated by *Liquorilactobacillus* (CS 1), *Gluconobacter* (CS 2), *Zymomonas* (CS 4), *Leuconostoc* (CS 5), and *Acetobacter* (CS 6), or were relatively balanced communities without a clearly dominant genus (CS 3)

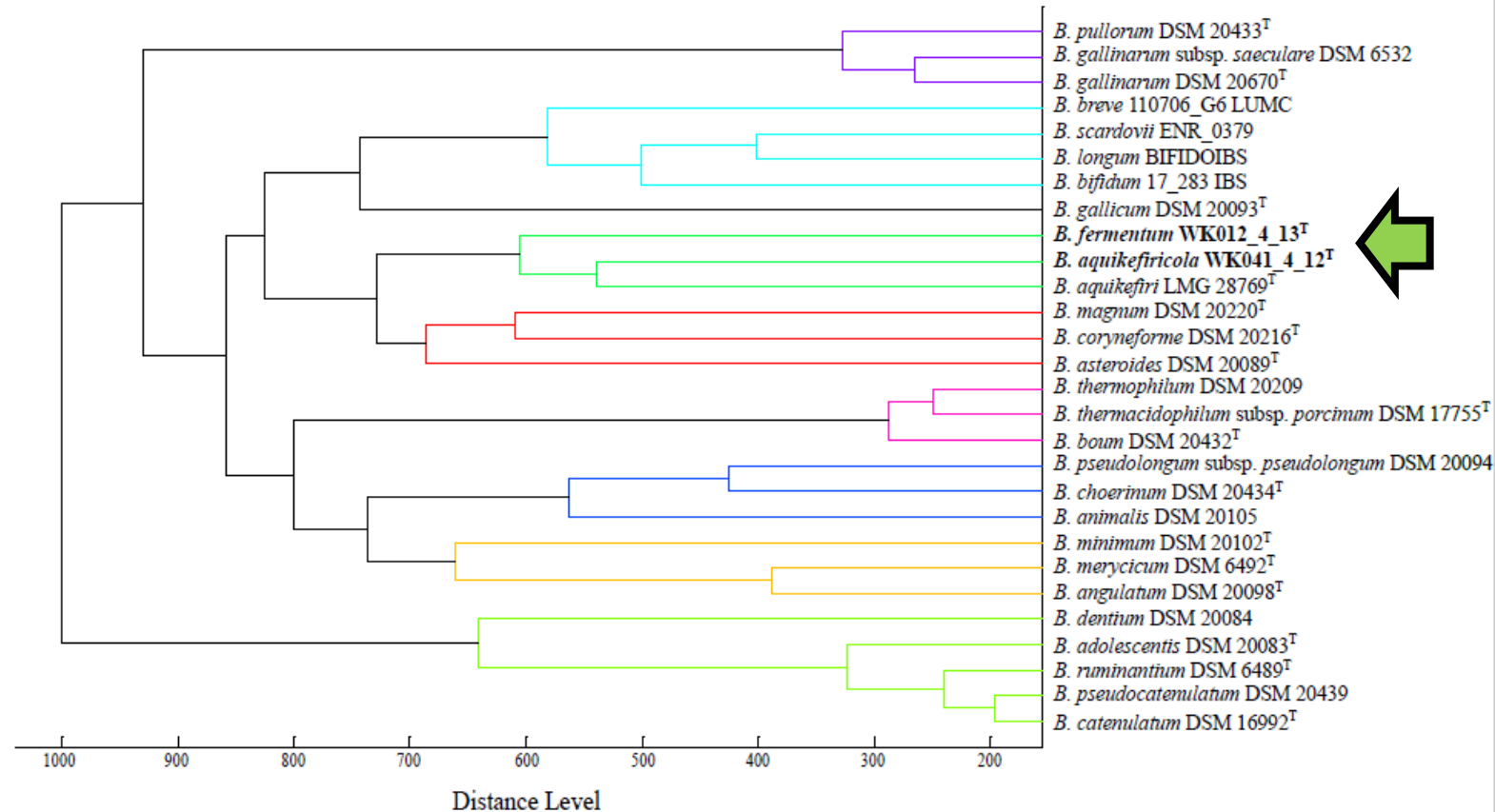
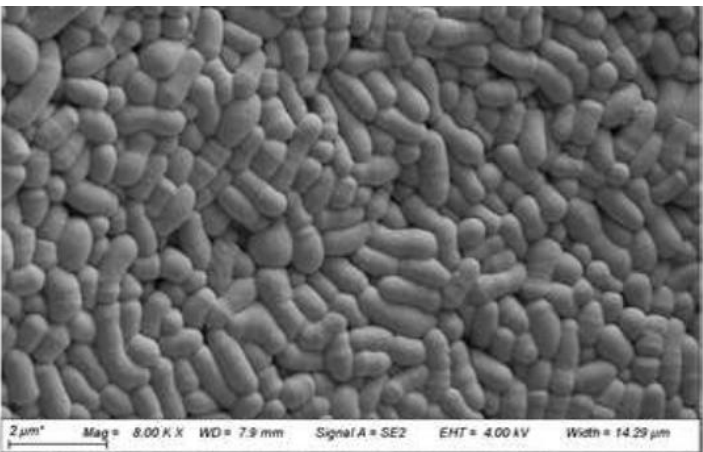
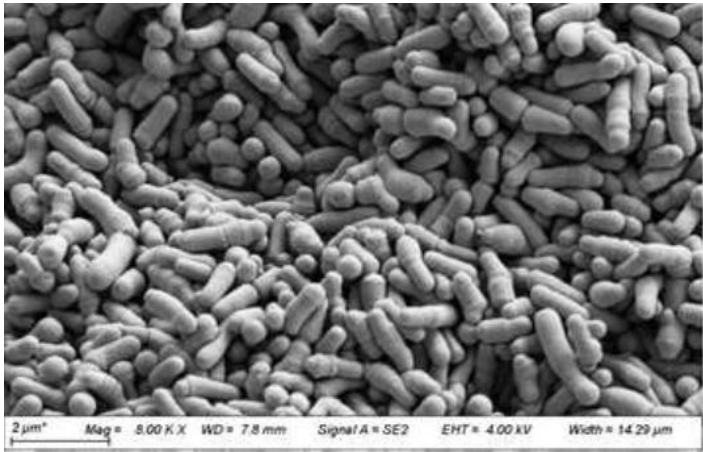


Can we culture the predicted novel species?

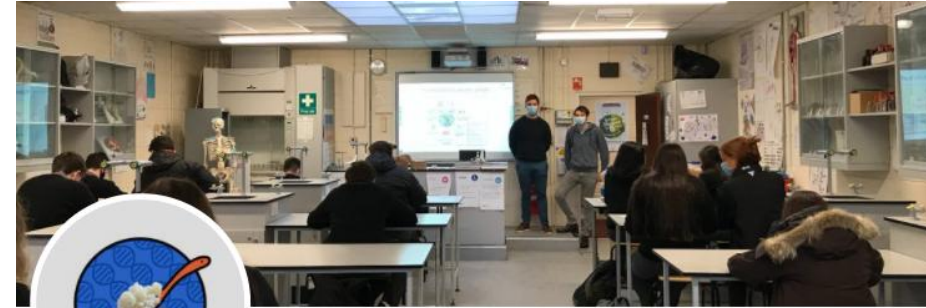
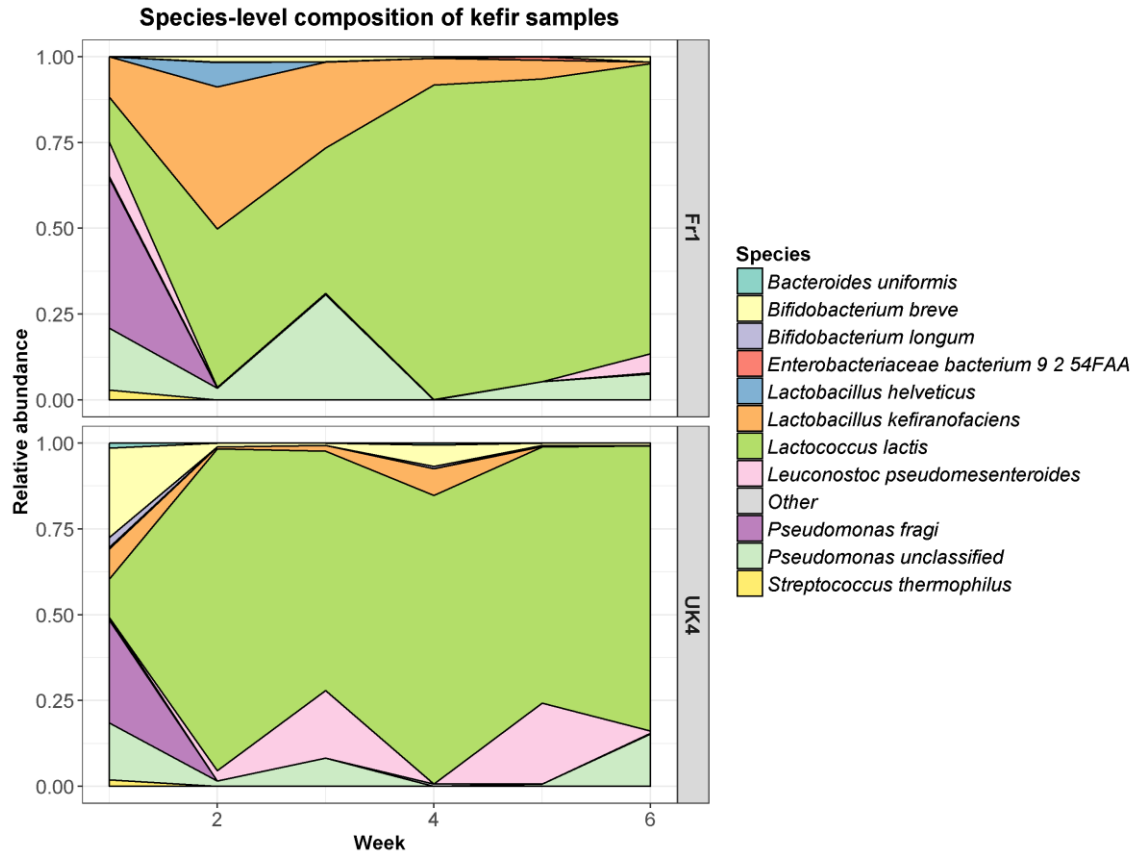
Two novel *Bifidobacterium* sp. from water kefir

Bifidobacterium fermentum sp. nov. (fermentum - that which causes fermentation) 12 of 69 kefir

Bifidobacterium aquikefircicola sp. nov. (aquikefircicola - water kefir inhabiting) 1 of 69 kefir



Kefir4All – Kefir evolution in the community



Kefir4All

@Kefir4All Follows you



Instructions for milk kefir fermentations

Step 1: Open Survey Monkey by opening the link below and fill in your ID and the type of kefir you are fermenting.

<https://www.surveymonkey.com/r/KefirFermentation>

Step 2: Study your fermentation jar, does the liquid look like any of the below pictures? Record your room temperature with the thermometer provided. Report your observations to Survey Monkey. Ignore this step if it is your first fermentation



Its Bubbling



It looks unchanged



The milk and whey have separated

Step 3: Unscrew and remove the top lid and strainer from the fermentation vessel.



Liam Walsh



Samuel Breslege



Guilherme Martin

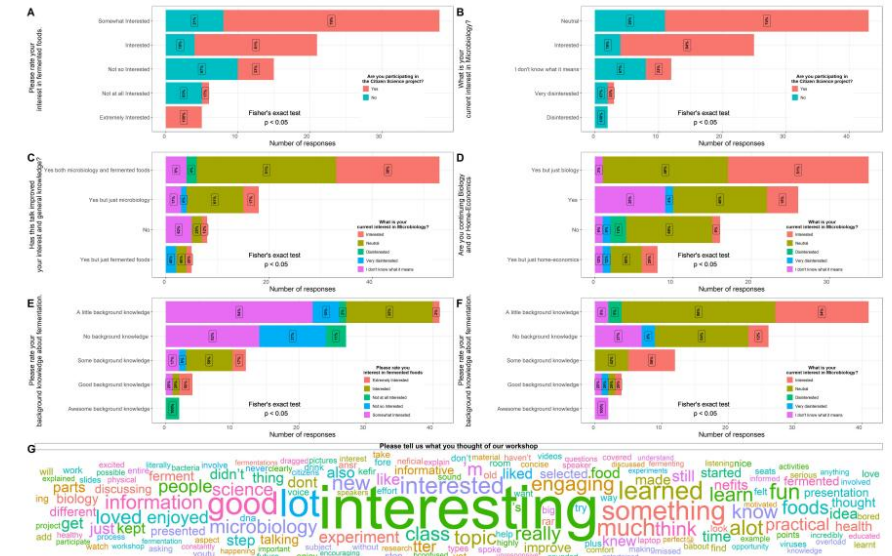
Kefir4All – insights into citizen science



Research Article

Kefir4All, a citizen science initiative to raise awareness of the roles that microbes play in food fermentation

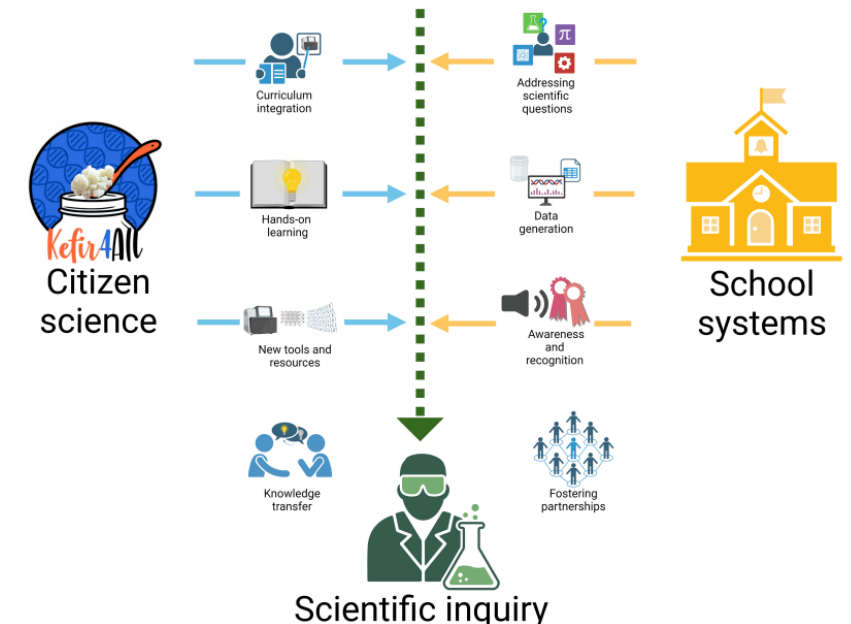
Liam H. Walsh,^{1,2} Samuel Breselge,^{1,3} José Guilherme Prado Martin,⁴ Mairéad Coakley,¹ Eimear Ferguson,^{1,5} Aimee Stapleton,³ Fiona Crispie,¹ Paul W. O'Toole,^{2,3} Paul D. Cotter^{1,3,5}



Perspective

The changing landscape with respect to scientific research and education for second-level students and how they can overlap: the Kefir4All example

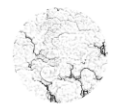
Liam H. Walsh,^{1,2} Cian O' Mahony,³ Paul D. Cotter^{1,4,5}





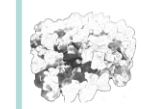
123 Citizen scientists

Milk kefir arm - 63 Citizen scientists



- A. Goat
- B. Cow- full fat
- C. Cow- low fat
- D. Cow- skimmed milk

Water kefir arm - 60 Citizen scientists

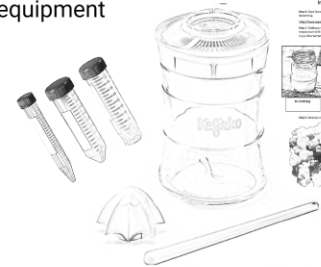


- A. 12 gram of white sugar and 1 dried fig
- B. 12 gram of white sugar, 1 dried fig, 1 slice of fresh lemon
- C. 12 gram of white sugar and 1 dried apricot
- D. 12 gram of brown sugar

Fermentation Categories

Citizen scientists selected one of four fermentation categories

Fermentation equipment

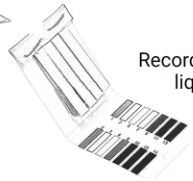


Fermentation instructions

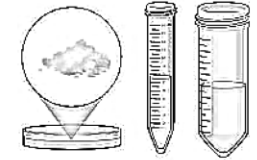
Record weight of grains



Record room temperature



Record pH of liquid



Samples collected at:	
Baselines*	at T0, T24 hr
Week 1	(day 7)
Week 5	(day 35)
Week 9	(day 63)
Week 13	(day 91)
Week 17	(day 119)
Week 21	(day 147)

1 Regular Fermentation

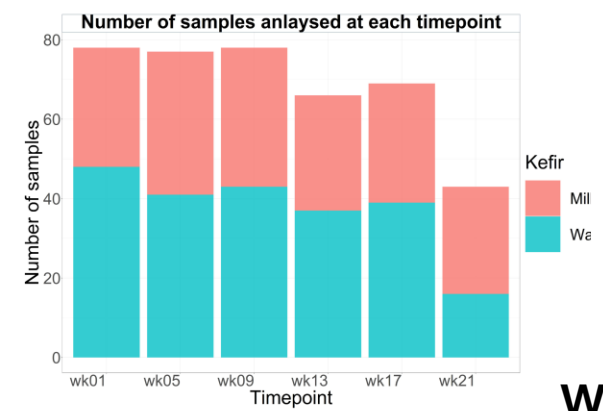
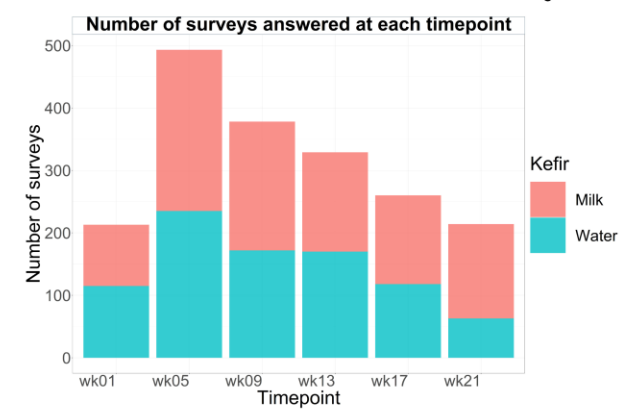
Fermentations were carried out by the citizen scientists in private households for up to 21 weeks.

2 Collect Metadata

Citizen scientists completed an online survey with every fermentation, to record information about the fermentation process e.g room temperature, pH of liquid.

3 Collect Samples

Citizen scientists provided liquid and grain samples after 1 week of continuous fermentation and 4 weeks thereafter for up to 21 weeks.



Walsh et al. Submitted

Early Fermentation Drives Rapid Change, e.g. beta diversity

Most compositional, functional, and volatile shifts occurred during weeks 1-9, with values stabilizing thereafter.

1

Weeks 1-5

Highest dissimilarity values. Rapid compositional transitions and community state shifts.

2

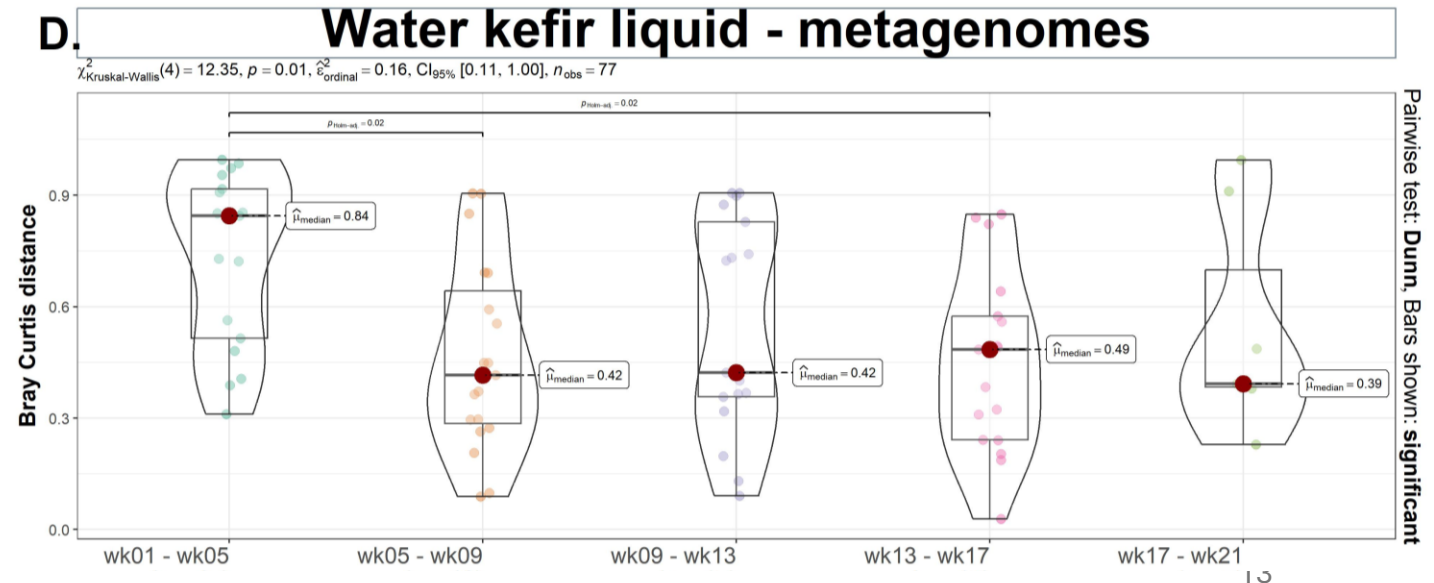
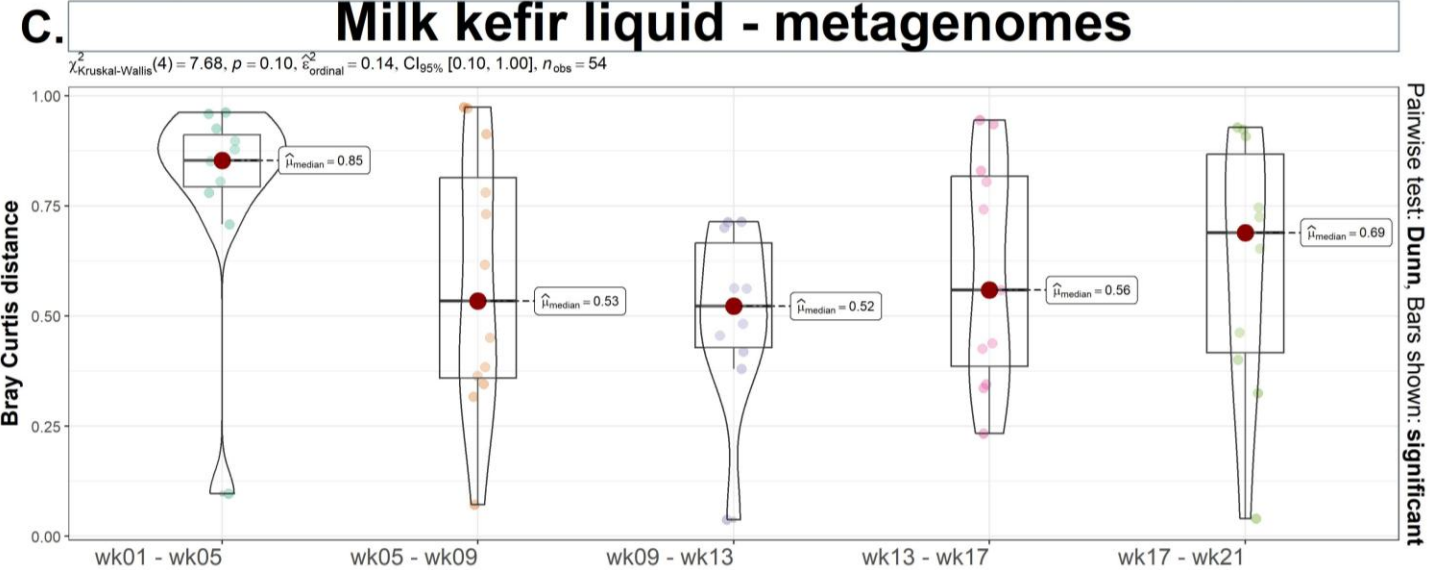
Weeks 5-9

Continued changes but decreasing rate. Functional profiles begin stabilizing.

3

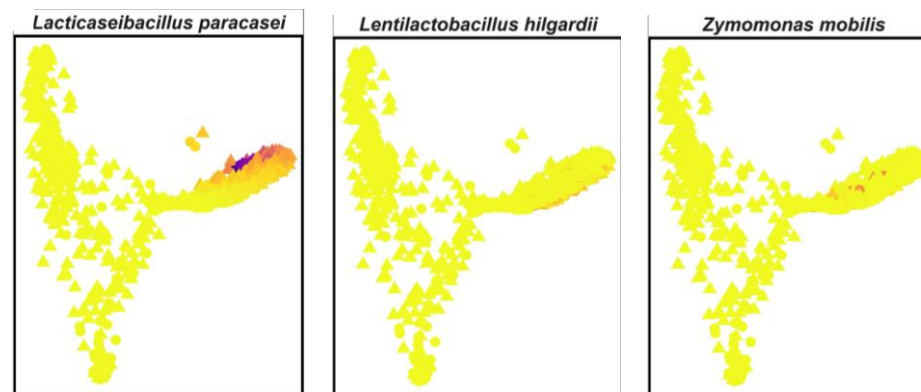
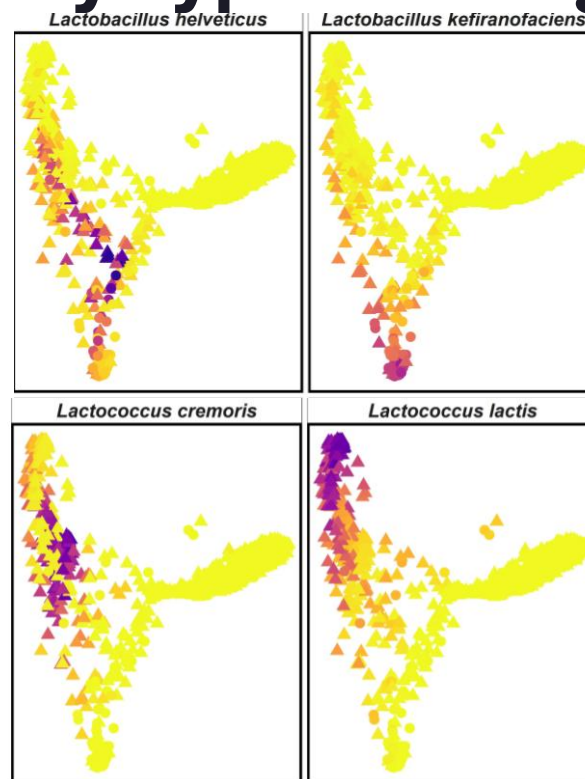
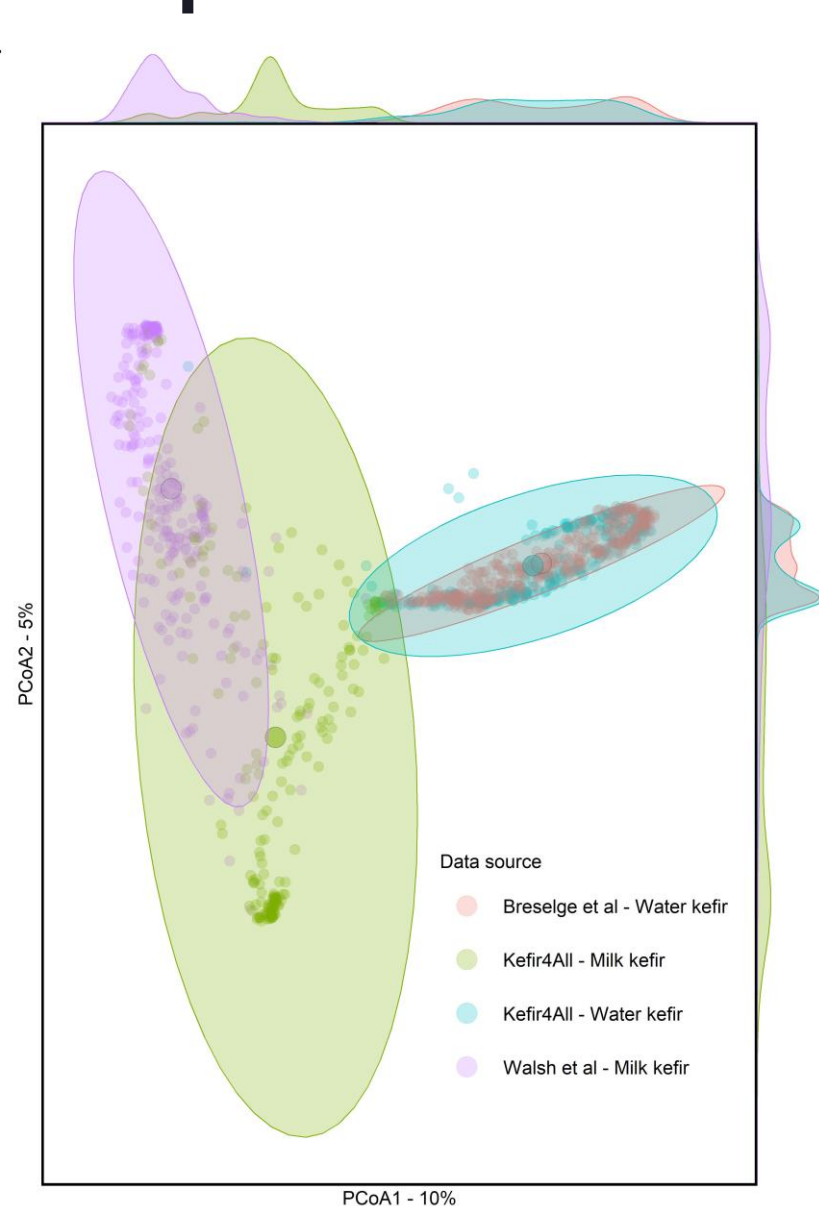
Weeks 9-21

Stable community states established. Minimal compositional changes observed.



Reproducible Community Types Emerge

A.



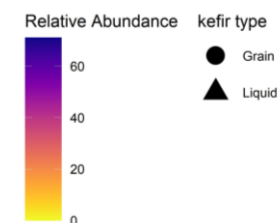
Despite starting from single grain sources, diverse community states emerged matching those from global kefir samples.

Milk Kefir States

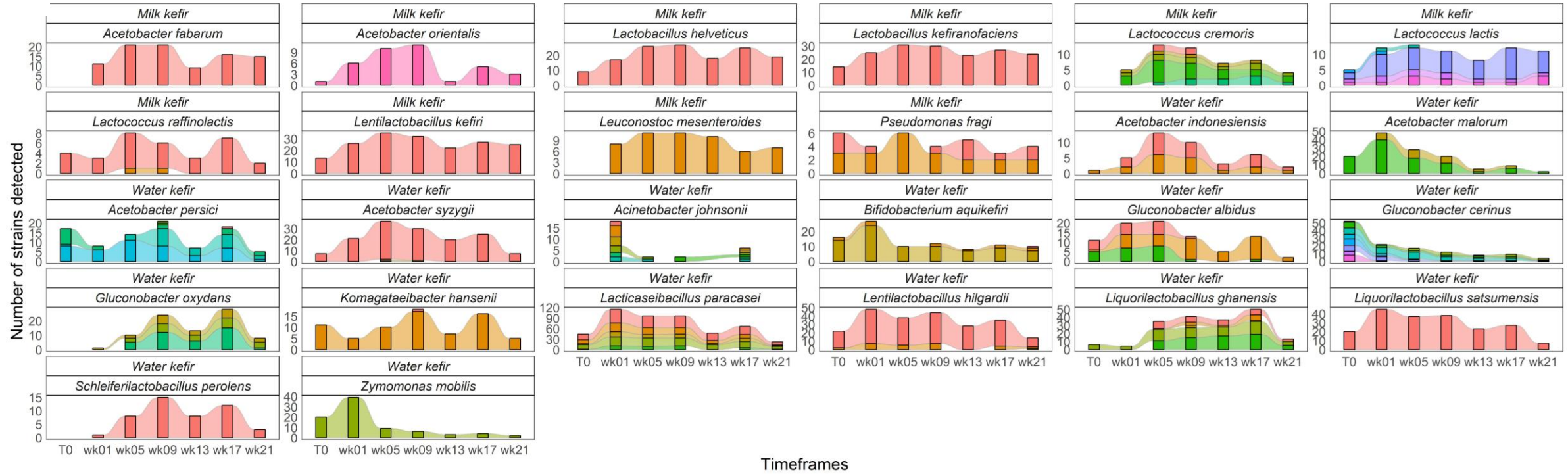
Communities dominated by *L. helveticus*, *L. kefiranofaciens*, *Lla. lactis*, or *Lla. cremoris* (4/4).

Water Kefir States

Communities dominated by *Lc. paracasei*, *Len. hilgardii*, or *Z. mobilis* (3/6).

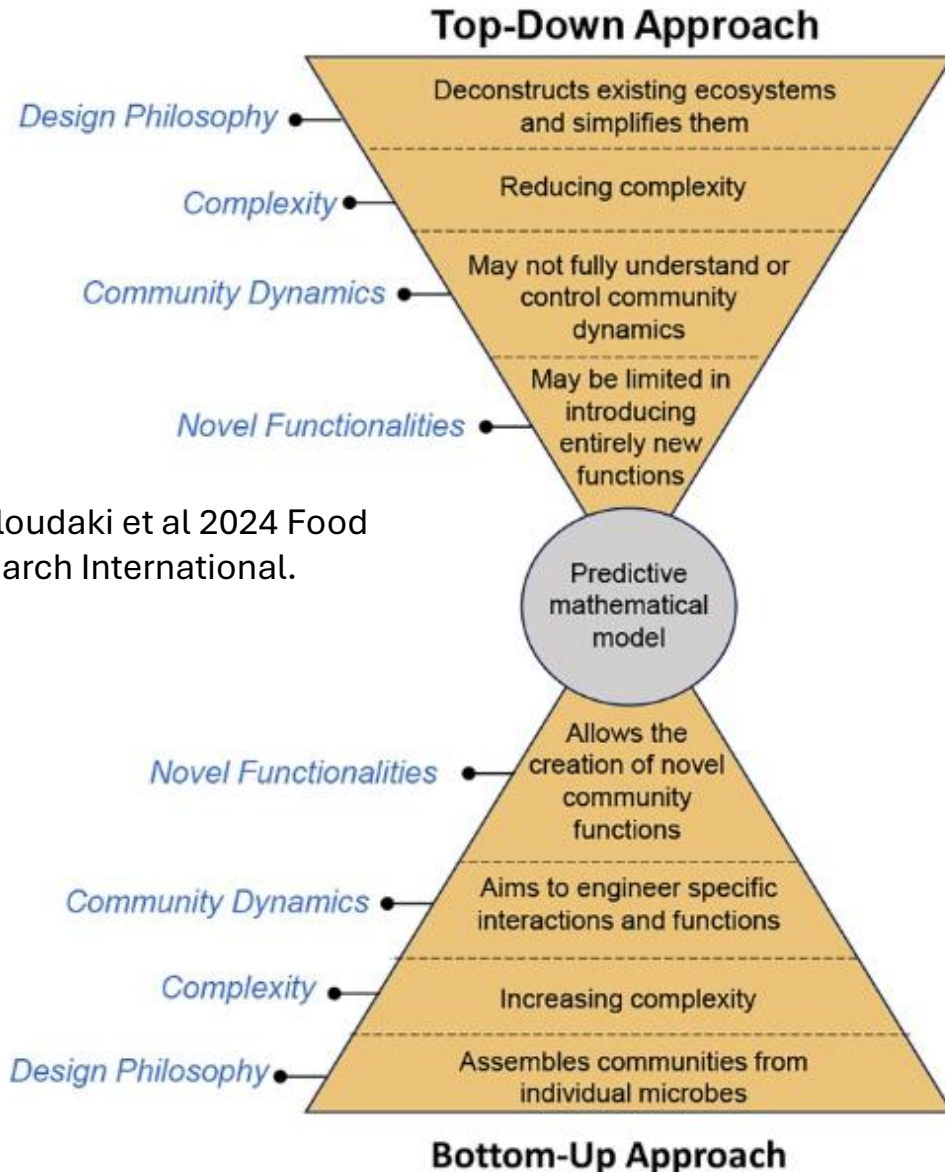


Strain-type analysis – emergence of strain types over time



Most species with multiple clusters showed temporal stability, with strains predominantly grouping under a single cluster. Exceptions included *Glu. albidus*, where strain clusters shifted over time. In *Lla. lactis*, *Lc. paracasei*, and *Len. hilgardii*, less predominant clusters also displayed temporal stability alongside the primary cluster.

Designing new defined communities – bottom up and top down



Nikoloudaki et al 2024 Food Research International.

DOMINO  Funded by the European Union  UK Research and Innovation

WP3 & WP4 training session:
Webinar on genome-scale modelling for consortia design

May 28th 2024. 9 to 11 a.m.

Elham KARIMI, Valentin LOUX and Stéphane CHAILLOU (INRAE)



<https://doi.org/10.1038/s41467-026-74251-1>

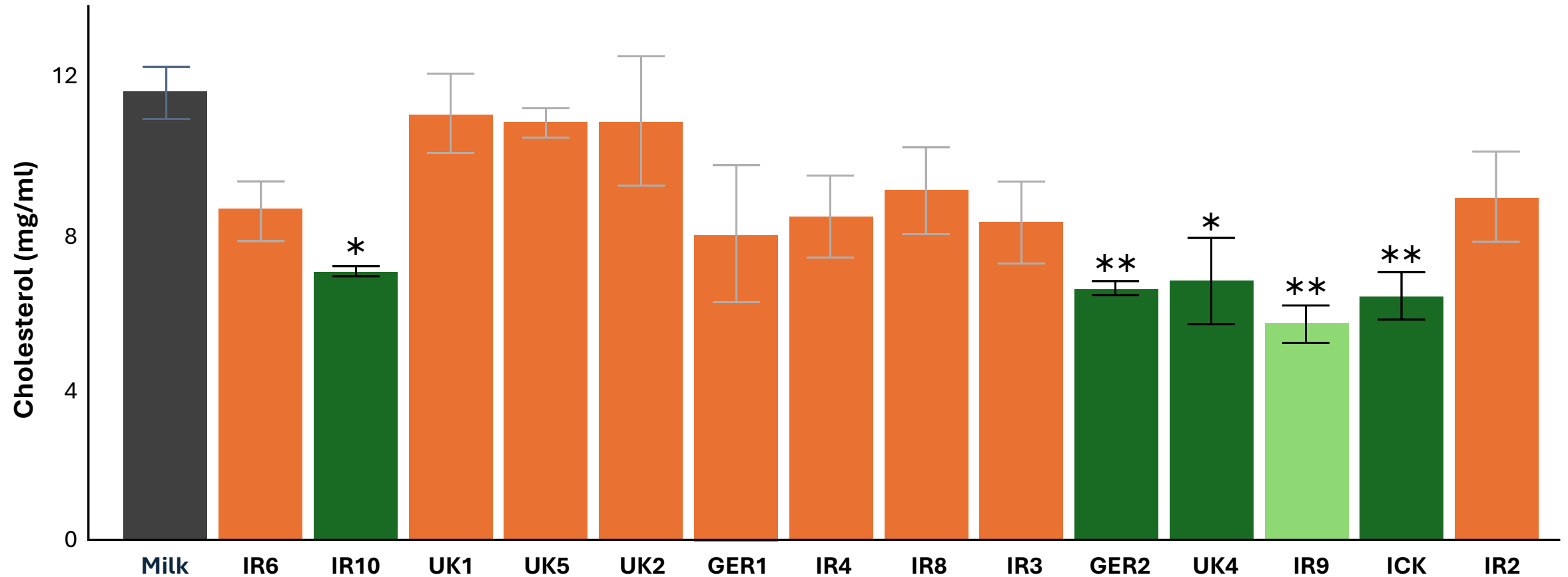
From “synthetic” to defined microbial communities for clearer terminology

Hanna Koch, Thomas Clavel, Cintia Mayr, Benjamin L. Coltman, Michael Schlöter, Julia A. Vorholt, Yolanda Sanz, Tomislav Cernava, Gwyn A. Beattie, Lene Lange, Stéphane Chaillou, Ákos T. Kovács, Hauke Smidt, Corné M. J. Pieterse, Tanja Kostic, Omri M. Finkel, Christopher E. Lawson, Luca Cocolin, Jesús Mercado-Blanco, Robert D. Finn, Kalliope K. Papadopoulou, Matthew Ryan, Marco Candela, Paul D. Cotter, Gabriele Berg, Orla O'Sullivan, Manuel Delgado-Baquerizo, Pankaj Trivedi, Trevor C. Charles, Brajesh K. Singh, Günter Brader, Malek Marian & Angela Sessitsch

 Check for updates

Koch et al 2026 Nature Comms

Cholesterol Assimilation in Milk



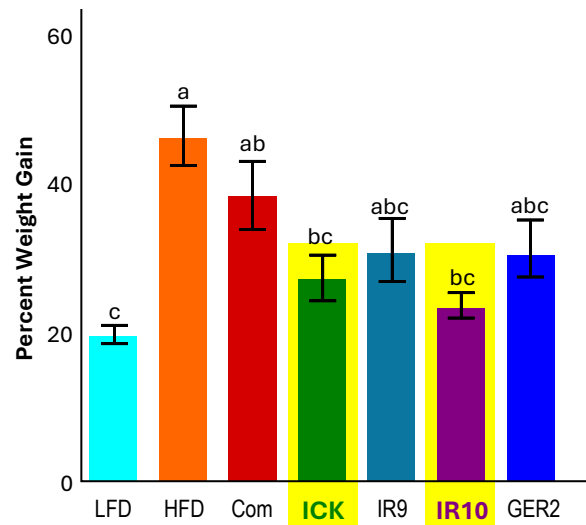
*=P≤0.05; **=P≤0.01

Bourrie BC, Cotter PD, Willing BP. *J Funct Foods*. 2018;46:29–37.

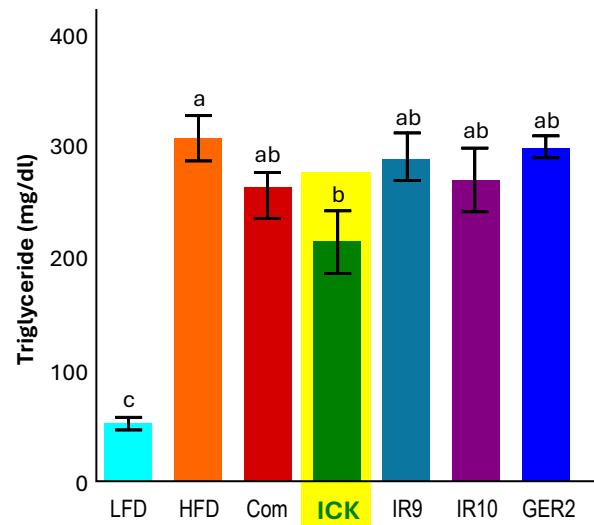
There Are Tremendous Opportunities to Optimise This Potential and Ensuring Quality Control, Including Through Combining Specific Strains With Particular Attributes

Major differences in health promoting attributes of kefir depending on the microbes present

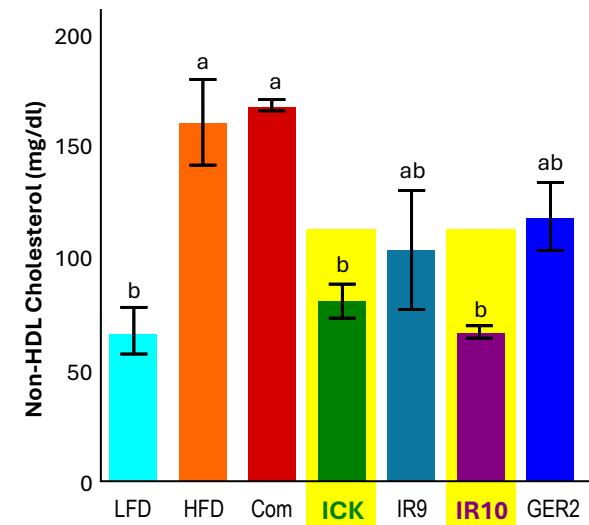
Weight Gain¹



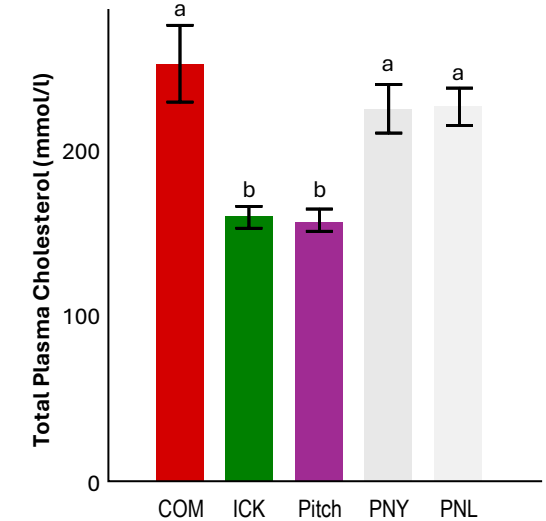
Liver Triglycerides¹



Plasma Non-HDL Cholesterol¹

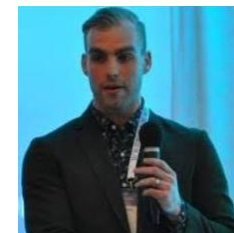


Plasma Cholesterol²



Benefits (cholesterol and TAG) of an artisanal kefir (ICK) recreated through use of a 'Pitched' kefir (5 bacteria & 4 yeast).

Benefits lost in absence of LAB or yeast



Significant differences are represented by different letters (a, b, c)

1. Bourrie BC, Cotter PD, Willing BP. *J Funct Foods*. 2018;46:29–37; 2. Bourrie BC, et al. *British Journal of Nutrition*. 2021;125(2):129–138.

Human study (males) – Randomised crossover pilot study

Cardiometabolic risk factors in males fed either a commercial (Com) or lab produced (Pitch) kefir for 4 weeks.

n = 21 days
2 x 4 weeks

When compared to baseline, pitched kefir consumption reduced LDL-Chol, ICAM-1, and VCAM-1, while commercial kefir consumption increased TNF- α .

Pitched kefir consumption resulted in greater reductions in IL-8, CRP, VCAM-1, and TNF- α when compared to commercial kefir consumption.



Measure	Com Baseline	Com Post-Treatment	Pitch Baseline	Pitch Post-Treatment	Com Delta	Pitch Delta	Bonferroni Adjusted <i>P</i> Value Pre vs Post-Treatment Com	Bonferroni Adjusted <i>P</i> Value Pre vs Post-Treatment Pitch	Bonferroni Adjusted <i>P</i> value Pitch vs Com Delta
Triglyceride (mmol/L)	1.68±0.90	1.56±0.81	1.56±0.94	1.63±0.91	-0.12±0.33	0.08±0.56	0.228	1.000	0.131
Cholesterol (mmol/L)	6.16±0.89	6.05±0.78	6.35±1.00	6.29±1.03	-0.11±0.47	-0.06±0.36	0.540	1.000	0.928
LDL Cholesterol (mmol/L)	4.20±0.87	4.10±0.78	4.37±0.81	4.20±0.81	-0.10±0.38	-0.13±0.20	0.514	↓ 0.042	0.516
Non-HDL Cholesterol (mmol/L)	4.97±0.88	4.84±0.72	5.01±0.90	4.93±0.87	-0.12±0.45	-0.04±0.31	0.488	0.406	0.912
Glucose (mmol/L)	5.26±0.76	5.36±0.80	5.08±0.56	5.02±0.55	0.10±0.34	-0.06±0.33	0.416	0.984	0.219
Insulin (pmol/L)	80.27±56.02	77.94±52.97	89.88±90.01	106.41±103.84	-2.33±19.63	16.53±45.48	1.000	0.330	0.063
CRP (mg/L)	1.87±1.73	1.83±1.50	1.75±1.91	1.32±1.66	0.01±0.65	-0.32±0.61	1.000	0.19	↓ 0.033
sICAM-1 (ng/mL)	351.11±47.95	344.68±45.89	358.03±56.60	329.43±42.78	-6.44±36.37	-28.61±28.63	0.950	↓ 0.002	0.126
sVCAM-1 (ng/mL)	335.75±57.68	338.38±45.63	342.75±52.48	318.56±33.53	2.63±37.21	-24.19±27.02	1.000	↓ 0.004	↓ 0.007
IL-6 (pg/mL)	0.49±0.24	0.51±0.35	0.47±0.25	0.41±0.20	0.02±0.18	-0.06±0.15	1.000	0.234	0.223
IL-8 (pg/mL)	3.45±0.83	3.60±0.94	3.90±1.34	3.43±1.32	0.05±0.50	-0.47±0.79	1.000	0.072	↓ 0.030
TNF α (pg/mL)	1.19±0.71	1.69±0.49	1.21±0.72	1.13±0.65	0.50±0.61	-0.08±0.21	↑ 0.012	0.266	↓ 0.007

LDL = low density lipoprotein; HDL = high density lipoprotein; CRP = C-Reactive Protein; sICAM-1 = secretory Intercellular Adhesion Molecule 1; sVCAM-1 = secretory Vascular Cell Adhesion Molecule 1; IL-6 = Interleukin 6; IL-8 = Interleukin 8; TNF α = Tumor Necrosis Factor alpha. Values are presented as means ± standard deviation. n=16-20.

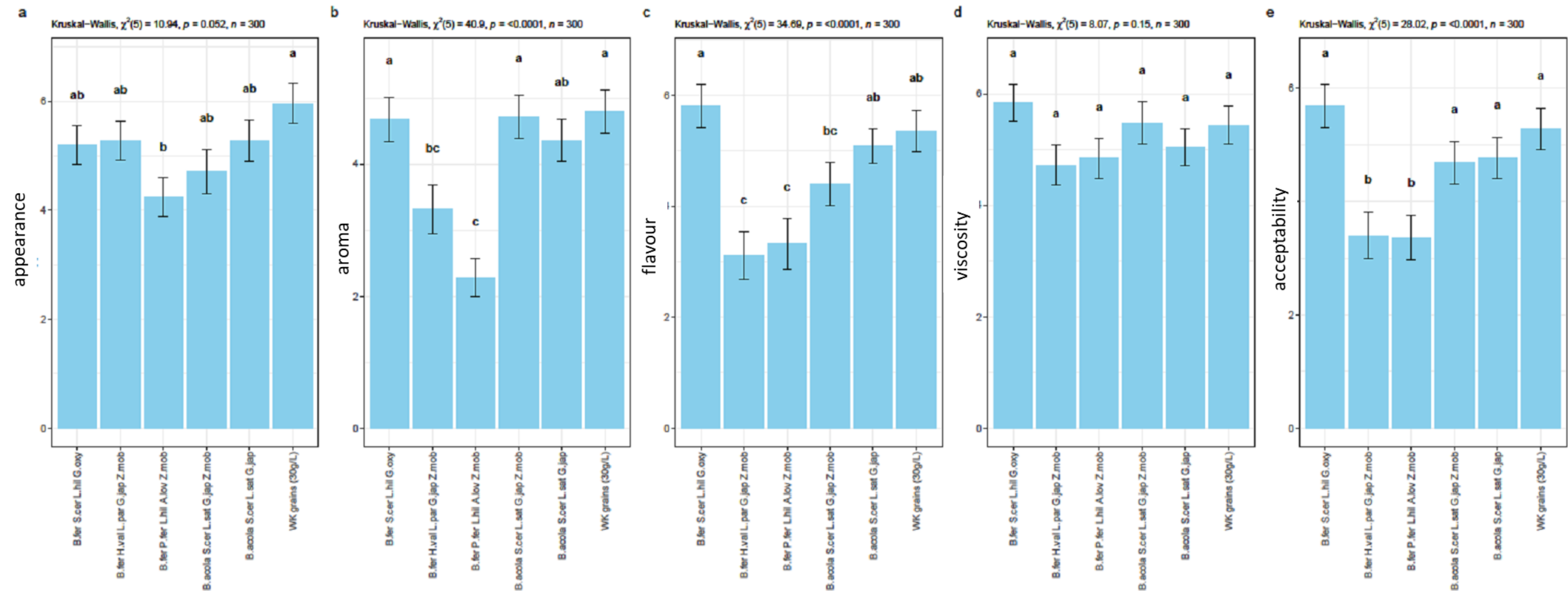
Designing new defined communities for water kefir production

Yeasts *Brettanomyces bruxellensis*, *Hanseniaspora valbyensis*, *Pichia fermentans*, and *Saccharomyces cerevisiae*

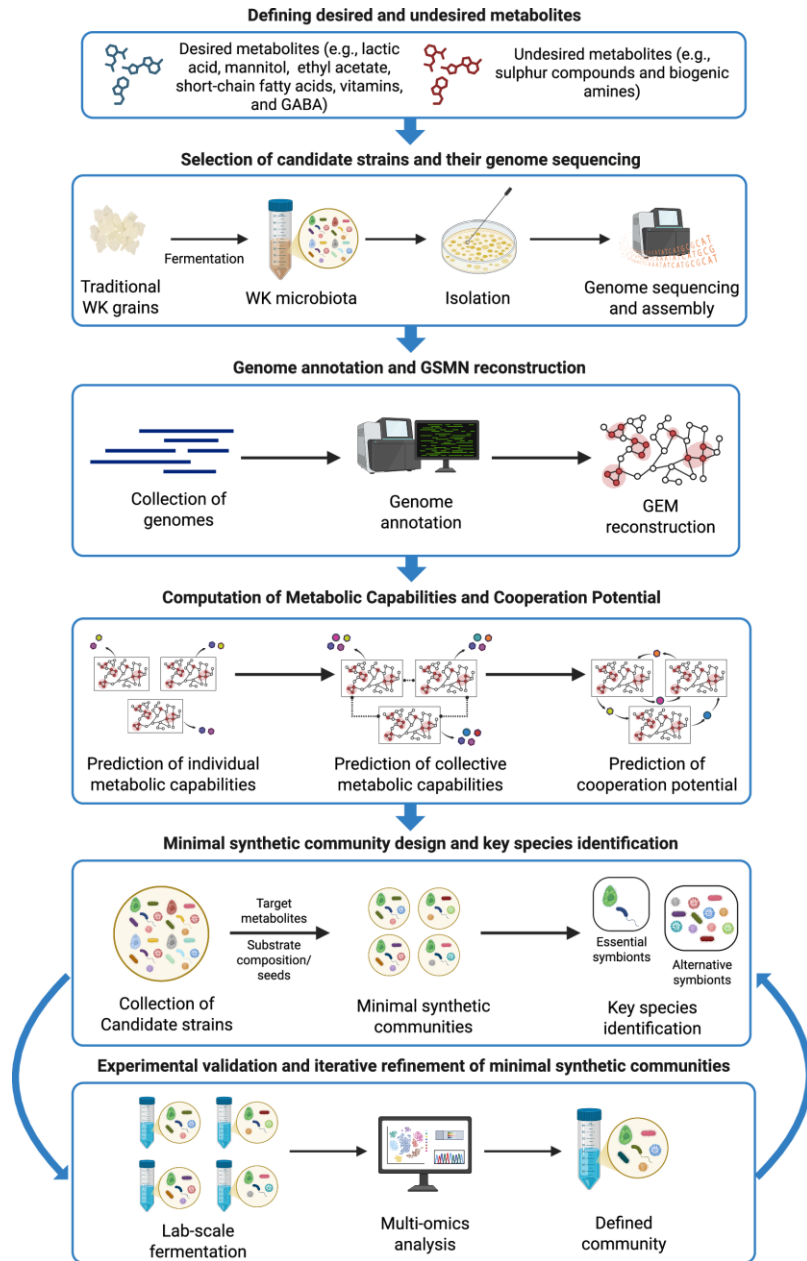
LAB *Lactocaseibacillus paracasei*, *Lentilactobacillus hilgardii*, *Leuconostoc pseudomesenteroides*, and *Liquorilactobacillus satsumensis*:

All possible combinations of communities consisting of:
1 yeast + 1 LAB + 1 AAB + 1 *Bif* +/- *Z. mobilis*

256 pitched fermentations



Bottom up based approaches to defined community development



Water Kefir – Anik Khan In prep
Kombucha – Erkang Zhang In prep
Pot ale valorisation – Ana Soriano In prep
Non-alcoholic beer – Ana Soriano In prep
Fruit valorisation – Ines Calvete In prep

Conclusion

- Milk and water kefir have served as excellent models to study fermented food associated microbiomes
- Study of these beverages has
 - Highlighted the global diversity of these microbiomes
 - Connections between microbiomes and other features
 - The extent through which artisanally produced beverages can change with time
 - Different ways in which defined communities can be developed for a variety of applications



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Conor Feehily

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