‘Health-Promoting Properties Encoded within Fermented Food Microbiota’

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Traditional, culture-based, approaches to microbiology only reveal the ‘tip of the iceberg’

The success of culture based approaches is dependent on having types of agar/culture media/growth conditions that allow all microbes to grow

Such an agar does not exist!!

Indeed only a small % of microbes are easily cultured in the laboratory.

DNA sequencing-based approaches can allow an analysis to the entire population (and the genes present therein) regardless of whether it can be grown or not
Sequencing based approaches to study food microbes

Walsh et al. Annu Rev Food Sci Technol 2017
DNA-based community analysis

**Amplicon sequencing**
- PCR amplification
- 16S rRNA sequencing
- Sequence comparison

**Whole metagenome Shotgun sequencing**

**Community DNA**

**Community Composition**

**Taxonomy**
- Kingdom
- Phylum
- Class
- Order
- Family
- Genus

**Genes or Pathways**
Structure of presentation

1. Health-promoting fermented foods – Introduction
2. Microbial composition of selected foods
3. Particular focus on kefir
4. Feeding studies
5. Conclusion
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Questions:
• What is meant be ‘health-promoting’ fermented foods?
• What is the evidence that they are health-promoting?
• What microbes are present in these foods and how might they be contributing to health benefits?

Long-term Goal:
• Harnessing the microbes in these foods to generate fermented foods with genuine health-promoting features.

Everyone knows that drinking tea is good for your health but wouldn’t it be great if there were a way to make it even healthier? Well actually, there is! (Spoiler alert, it’s a fungus!)
Fermented beverages

Amasi, Aryan, Garris, Kefir, Kivuguto, Koumiss/Arag, Kumis, Nyarmie, Rob, Suusac, Shubat, Amazake, Boza, Bushera, Koko, Sour water, Kvass, Mahewu, Pozol, Togwa, Hardaliye, Kombucha, Water Kefir

Fermented beverages with health-promoting potential: Past and future perspectives

Alan J. Marsh\textsuperscript{a,b,c}, Colin Hill\textsuperscript{b,c}, R. Paul Ross\textsuperscript{a,b} and Paul D. Cotter\textsuperscript{a,b,*}

Trends in Food Science & Technology 38 (2014) 113–124
Health benefits of fermented foods

Type 2 diabetes,
Coronary heart disease,
mortality,
Cardiovascular disease,
Obesity,
Impaired glucose metabolism,
Hyperlipidemia,
Hypertension,
Osteoporosis,
Muscle soreness,
Depression,
Brain activity,
Infection control,
Bowel movement,
Irritable bowel syndrome
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Sequence-based analysis of the microbial composition of water kefir from multiple sources

Alan J. Marsh, Orla O’Sullivan, Colin Hill, R. Paul Ross & Paul D. Cotter
Kombucha

Bacteria

- Enterococcus
- Allobaculum
- Leuconostoc
- Gluconacetobacter

Fungi

- Naumovozyma
- Kluyveromyces
- Leucosporidiella
- Dekkera

Marsh et al. Food Microbiol 2014
Nunu

Spontaneously fermented yoghurt-like milk product from Africa....short shelf-life

Trained vs Untrained producers......

Walsh, Scott et al. AEM 2017
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Grouped by Fermented Food type

p = 0.01
Species level assignment

Log Relative Abundance

Type
- Staphylococcus aureus
- Paenibacillus lentum
- Lactobacillus johnsonii
- Bifidobacterium longum
- Acetobacter malorum
- Mycobacterium fortuitum
- Pichia membranaefaciens
- Leuconostoc gelidum
- Pichia kudriavzevii
- Serratia sp.
- Leuconostoc parabrevis
- Lactobacillus parabrevis
- Leuconostoc pseudomesenteroides
- Leuconostoc citreum
- Rastronia angularis
- Lactobacillus xiangfangensis
- Lactobacillus mucosae
- Gluconobacter oxydans
- Brettanomyces bruxellensis
- Clostridiodes difficile
- Leuconostoc mesenteroides
- Lactobacillus brevis
- Pediococcus parvulus
- Lactobacillus plantarum
- Lactococcus lactis
- Lactobacillus plantarum
- Lactobacillus brevis
- Leuconostoc mesenteroides
- Gluconobacter oxydans
- Lactobacillus mucosae

Lactococcus lactis
Lactobacillus plantarum
Lactobacillus brevis
Leuconostoc mesenteroides
Gluconobacter oxydans
Lactobacillus mucosae
Functionalities associated with fermented food types

Superfocus

- Soy
- Coconut kefir
- Brine
- Dairy
- Sugar
Bacteriocin production – putative probiotic trait

Cotter et al. Nature Reviews Microbiol 2005
Distribution of bacteriocin genes in cheeses

Abundance of bacteriocin gene classes across all cheeses

Class
- Type I
- Type II
- Type III

Sample

CPM

10^0  10^1  10^2

Groups:
- Lactococcus A (LCN-A)
- Acidocin
- Acidocin 8912
- Klebicin C activity
- Plantaricin F
- Plantaricin A
- Colcin E1
- Colcin E3
- Colcin E3 protein
- Colcin protein
- Linocin M18
- Helveticin
- garviseacin019 (garQ)
- Helveticin J
- Enterolysin A
- Linocin M18Bacteriocin
- Linocin M18
- Linocin M18Bacteriocin
- Linocin CPP29 homolog
- Bacteriocin helveticin J
- Bipu
- Bipx
- Lactococcus B (LCN-B)
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Health Benefits of Kefir

Kefir = milk fermented with kefir grain (containing a consortium of bacteria and yeasts)

Many putative health benefits but quality of many of the associated publications leaves a lot to be desired

Some of the products on the market that are called ‘kefir’ are not really kefir

Previous microbiota studies

1 kefir grain and milk (16S)
Dobson et al. 2011

23 kefir milk (16S and ITS)
Marsh et al. 2013
Microbiota – genotype screening

Collected samples at 0, 8 and 24 h of kefir fermentation (x 3 grains)
  – Extracted DNA at each time-point for sequencing
  – GCMS on milk samples to determine the flavour profile

Amplicon sequencing (16S and ITS) – Changes in microbial composition

Whole metagenome shotgun sequencing – Changes in gene content
High bacterial diversity at 0 hours…reflects the milk microbiota

Three genera dominated at 8 hours: *Lactobacillus, Leuconostoc* and *Acetobacter*

*Lactobacillus* decreased whereas *Leuconostoc* and *Acetobacter* increased between 8 and 24 hours
In contrast to the bacterial communities, there was considerable differences between the fungal communities (and low diversity)
Pathways involved in AA and FA metabolism increase as the fermentation proceeds.
Identified strong correlations between:

- *Acetobacter pasteurianus* and acetic acid - associated with vinegary flavours
- *Lactobacillus kefiranofaciens* and carboxylic acids associated with cheesy flavours
- *Leuconostoc mesenteroides* and 2,3-Butanedione (associated with buttery flavours)
- *Saccharomyces cerevisiae* and esters associated with fruity flavours

Adding *L. kefiranofaciens* NCFB 2797 to Fr1:

Increases in the levels of the esters ethenyl acetate (by 59.15%), ethyl acetate (100%), methyl-3-butyrate (26.83%), and 2-methylbutyl-acetate (11.44%) and the ketone 2-heptanone (65.86%).

Adding *L. mesenteroides* 213M0 to Ick:

Increases in the levels of acetic acid (168.28%) and 2,3 butanediol (14.91%), a precursor to 2,3-butanedione.
Health promoting features?

HUMANnN2 gene family table was inspected for genes associated with probiotic functionalities to better understand the basis of the health benefits of kefir.

We observed genes encoding

- EPS synthesis proteins
- Bile salt transporter proteins
- Adhesion proteins
- Mucus binding proteins
- Type III bacteriocins/bacteriolyisins
Structure of presentation

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Human feeding study

- Off the shelf probiotic drink - once daily
- Kefir - once daily
- Attempting to achieve 7g of inulin / day

28 day intervention

None of the treatments had large-scale effects on the overall bacterial diversity of the gut microbiota
(This isn’t a bad thing!)

Walsh et al Unpub
Family-level bacterial composition of the gut microbiota before/after each treatment

Inulin  Kefir  Probiotic

This graph indicates the average bacterial composition of the gut microbiota in each group/stage

Walsh et al Unpub
Harnessing the potential of kefir

Cultures from 16 kefir grains (and milk) from 6 countries

Phenotype - screening

- 2000 Bacterial and 2000 Fungal isolates
- pH tolerance (pH2) 75% of isolates resistant
- Bile tolerance (3% Oxgall) 75% of isolates resistant
- Antimicrobial production
- Cholesterol assimilation assays
- Adherence and anti-cytotoxicity

Bourrie, Willing et al Unpub
Cholesterol assimilation in milk

* = $P \leq 0.05$

** = $P \leq 0.01$

Bourrie, Willing et al Unpub
High fat fed mice

Groups:
• LFD control
• HFD control
• HFD + Commercial
• 4 X HFD + traditional kefir groups

* = Body weight taken
▲ = Fecal sample taken

Begin HFD/Kefir (Day 0)

Receive Mice

Terminate (Week 12)

HFD (40% calories + 1.25% cholesterol) or LFD
Daily gavage of 100 µl milk (controls) or kefir

Bourrie, Willing et al Unpub
Mouse (DIO) feeding studies

After 28 days intervention

Bourrie, Willing et al Unpub
Mouse feeding studies

Weight gain

Liver triglycerides

Plasma non-HDL Cholesterol

Some traditional, but not commercial kefir, reduced weight gain, levels of plasma LDL Cholesterol and liver TGs

Bourrie, Willing et al Unpub
Van de Wouw, Cryan et al Unpub
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Conclusion

• There are quite a number of putatively health-promoting fermented beverages (and foods) on the market but the science underlying the associated claims can often be poor

• We are trying to address this through a more rigorous examination of the microorganisms present and the health benefits they can confer

• These microorganisms can be used for the generation of fermented beverages that closely reflect existing artisanal products but for which health benefits have been clearly determined, and optimised

• The availability of strains to facilitate this will also ensure that the quality, flavour and shelf-life of such products can be ensured
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Food, Gut, Rumen, Exercise, Mycobiome, Probiotics, Bacteriocins