

1 Fusicatenibacter Is Associated with Kefir Drinking *

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3 **ABSTRACT** Daily 16s rRNA-based microbiome sampling reveals that consumption of the fermented drink, kefir, is
4 associated with a previously-unexplored genus *Fusicatenibacter* of the *Firmicutes* phylum within family *Lachnospiraceae*.

5 Introduction

6 Kefir is a fermented milk drink produced by the action of bacteria and yeasts and believed to have medicinal uses. A
7 rigorous microbial analysis by Walsh et al. (2016)¹ recently showed precisely which microbes are present in kefir, at
8 various stages in the fermentation process. (See Figure ??). The grains themselves contain a combination of lactic
9 acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*), acetic acid bacteria (*Acetobacter*), and yeast, clumped together
10 with casein (milk proteins) and complex sugars in a matrix of a unique polysaccharide called Kefiran. The nutritional
11 content apparently varies depending on fermentation time and other factors.²

12 Numerous studies indicate that regular kefir drinking has positive effects on health, and it is reasonable to assume
13 that its known bacteriological properties would affect the gut microbiome, but we are unaware of previous research
14 that conclusively demonstrates that microbes in kefir make it successfully through the acidic environment of the
15 stomach.

16 Although similar doubts have been expressed about another fermented dairy product, yogurt, careful research has
17 shown several microbial strains that pass through the body successfully. (Uyeno, Sekiguchi, and Kamagata (2008))
18 Furthermore, several of the microbes apparently persist in the gut and can be observed a full 28 days after consumption.

19 We were interested to know if the same is true of kefir, and how it might alter the gut microbiome on a daily basis.
20 We sequenced the 16S rRNA gene in 500 near-daily samples of the microbiome of a single subject, a 50-year-old male
21 in excellent health. Replicating the experiment in David et al. (2014), we carefully tracked diet, sleep, location, activity,
22 and other variables. Most samples were from gut, but bimonthly samples were regularly taken of skin, nose, and mouth
23 as well.

24 Because we had several hundred days worth of daily microbiome sampling before the subject first encountered
25 kefir, we also wanted to find if any new microbes appeared (or disappeared) as a result. Finally, by continuing to test
26 long after the kefir consumption began, we were able to see how long any such microbes remain in the gut.

27 Results

28 We found that kefir consumption was associated with a clear change in the abundance of several organisms, including
29 one, *Lactococcus*, whose presence could be confirmed in the drink as well. To our surprise, we also found at least one
30 new organism, a novel one that had not been observed in hundreds of previous samples taken from the same subject.
31 Furthermore, the new organism, *Fusicatenibacter*, appears to remain in the gut after ending the kefir consumption,
32 indicating a persistent alteration of the gut microbiome.

33 The high levels of *Lactococcus* in the subject's gut was not unexpected. Previous studies have shown it to inhabit
34 kefir drinks³ and we also found it as the main genus of microbe found in our kefir samples as well. (see Table 1)
35 We found that, indeed, it survives passage through the stomach and presents in the subject's gut in high quantities.
36 Because we have the subject's near-daily gut microbiome records for more than one year prior to his first consuming
37 kefir (See Figure 1), we confidentially attribute the newly-found abundance to the drink.

38 We also spotted a new new microbe, *Fusicatenibacter* that appears to exactly trace the kefir consumption. (Figure 2).
39 A gram-stain-positive, obligately anaerobic, non-motile, non-spore-forming, spindle-shaped bacterium, our literature
40 search revealed nothing more of interest since its isolation in 2013⁴. Although it is seen regularly in the human gut, we

*Replication files are available on the author's Github account (<http://github.com/richardsprague>). **Current version:** November 15, 2017 ;
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¹also see a [2-minute Youtube presentation](#)

²Otles and Cagindi (2003) and <http://files.cienciapatodos.webnode.pt/20000022-79ffe7af9e/Kefir.pdf>

³Leite et al. (2013)

⁴Takada et al. (2013)

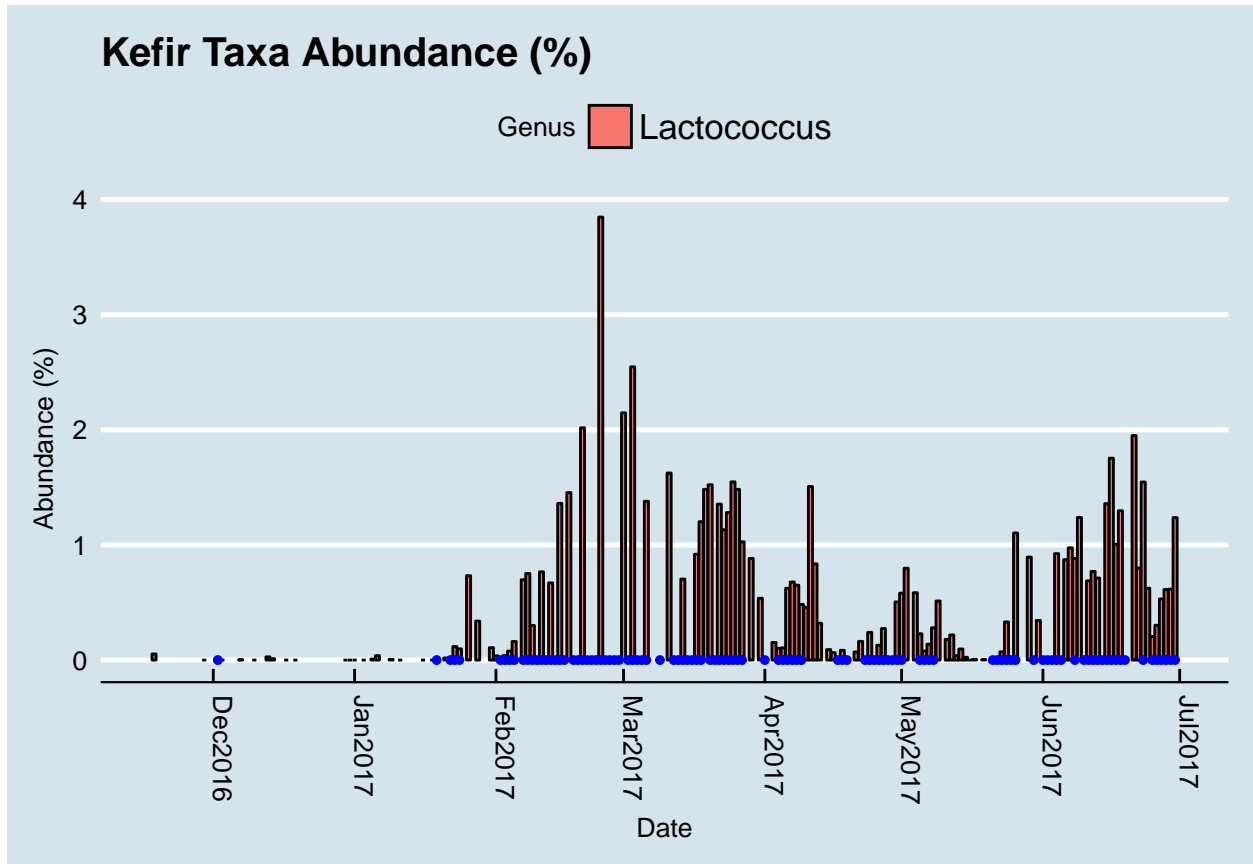


Figure 1: Abundance levels of Genus *Lactococcus* in a 55-year-old male subject's gut. Kefir was consumed only on the dates indicated in blue. Samples were taken near-daily throughout the period, so abundance levels are zero unless otherwise indicated. We note that levels seem to dip when on days when the kefir is not consumed, such as during trips out of town in mid-April and mid-May.

Table 1: Sequenced abundances found in the kefir drink before consumption.

	Kefir (%)
Lactococcus	96.06
Leuconostoc	3.02
Lactobacillus	0.22
Faecalibacterium	0.14
Roseburia	0.06

41 are unaware of any reports of its connection to diet. We believe this is the first reported instance of its association with
42 a specific type of food.

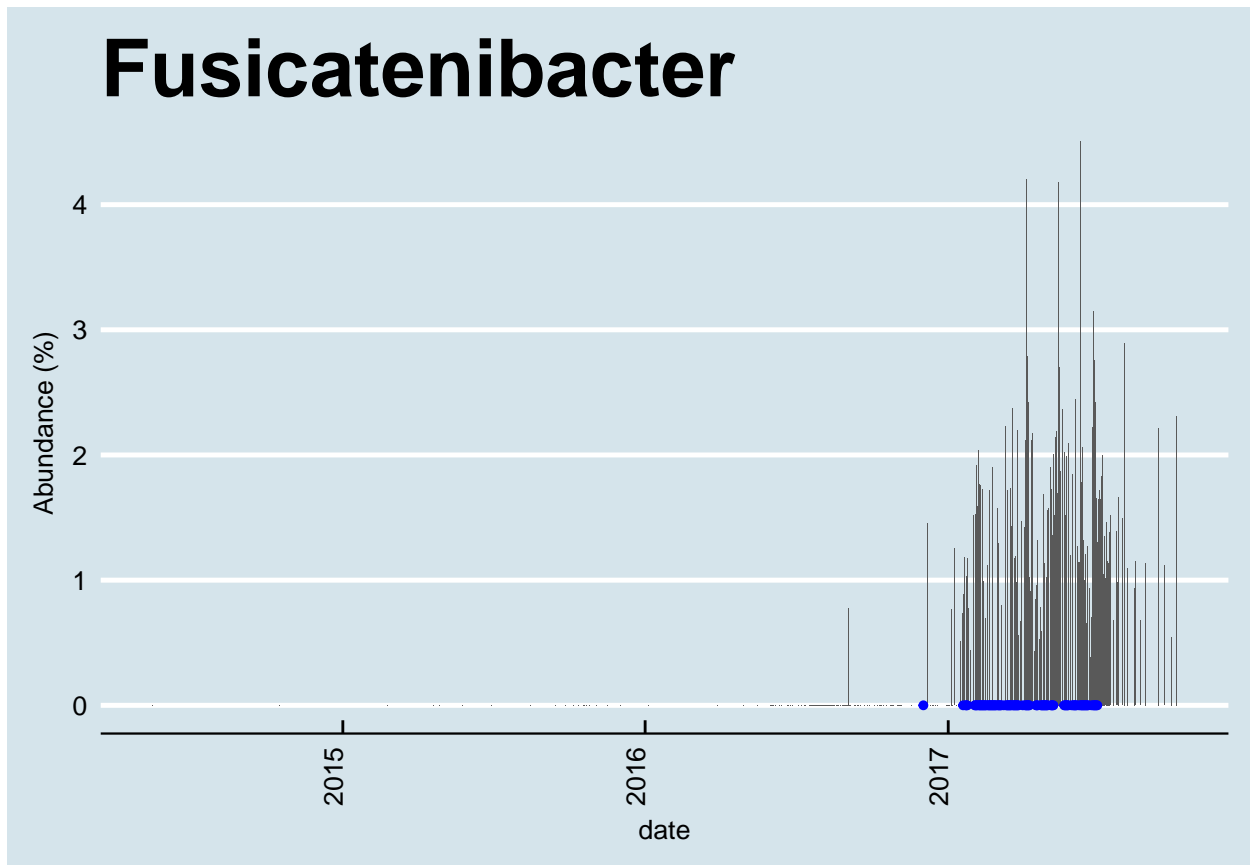


Figure 2: *Fusicatenibacter* is found at high abundance after drinking kefir. This chart shows abundance levels were zero since testing began more than two years previously.

43 Discussion

44 Although we were pleased to see that one of the microbes in the original drink made it through the body and could
45 be found in the gut, this result alone merely confirms what intuition would suggest: microbes that go in the mouth
46 can successfully navigate the entire gastrointestinal tract. Other researchers have reported similar persistence of the
47 same microbe *Lactococcus* after yogurt consumption, another fermented drink. Interestingly, our subject, an occasional
48 yogurt eater, showed virtually none of this microbe in the years of measurement before drinking kefir. We speculate
49 that there may be something uniquely robust about the particular species of *Lactococcus* found in this sample, one that
50 may not be found in the commercially-available yogurt previously consumed by the subject.

51 It is interesting to note that the subject was drinking homemade kefir, fermented overnight in his kitchen, and thus
52 exposed to the same environmental microbes that would have surrounded the subject himself. We hypothesize that
53 the known high variance in microbial environments may play a significant role in which microbes appear in the gut.
54 Commercially-purchased kefir is produced in sanitized industrial environments which, while enabling a consistent
55 product and protective against pathogens, may inevitably result in differences in microbial strains.

56 We do not understand why a novel microbe, *Fusicatenibacter* would appear in the gut in such large quantities
57 immediately after the first drink. We confirmed with the lab that this microbe was unlikely to result from contamination.
58 Although it had not been found in this subject previously, the lab reports that it is found regularly in samples from
59 other people. Analysis of the plates on which the subject's samples were processed indicated no irregularities; in fact,
60 the wells directly adjacent to the subject's sample did not show any of this microbe, though that was present in other
61 samples processed in the same run.

62 A literature search reveals nothing of clinical or other apparent interest about this microbe, a Clostridium that
63 appears within the family *Lachnospiraceae* of phylum *Firmicutes*. We can find no apparent link to health or other
64 conditions documented by other projects. Since it persists and makes up from 1-4% of the subject's post-kefir microbiome,
65 we think it must have found a role in the microbial ecosystem.

66 Note that the subject remained in excellent health before and after the kefir consumption. We could detect no
67 significant differences in blood chemistry or other quantitative health metrics. A review of his activity, sleep, and diet
68 reveals no other significant differences that might compound the microbiome changes that occurred after beginning
69 kefir.

70 Methods

71 Samples were collected on a daily basis, following instructions from commercially-available kits from uBiome, Inc.
72 Fecal samples were lightly mixed and swabbed throughout to lessen distribution anomalies within the sample. The
73 swabs were stirred into a lysis buffer and then transported at room temperatures to the uBiome lab. Published accounts
74 of the uBiome protocol indicate that genomic DNA was extracted by a liquid-handling robot, amplified up to 30 times
75 using PCR, with primers inserted at the V4 subunit of the rRNA gene ((515F: GTGCCAGCMGCCGCGTAA and 806R:
76 GGACTACHVGGGTWTCTAAT) using Illumina NextSeq platform rendering 2 x 150bp pair-end sequences.

77 Samples were barcoded with a unique combination of forward and reverse indexes allowing for simultaneous
78 processing of multiple samples. De-multiplexing of samples was performed using Illumina's BCL2FASTQ algorithm.
79 Acquired reads were filtered using an average Q-score > 30. Primers and any leading bases were subsequently trimmed
80 from the reads, and forward and reverse reads were appended together. To effectively cluster real biological sequences
81 and to identify reads that contain errors as a product of sequencing, reads were clustered using the Swarm algorithm
82 (Mahé et al. 2014) using a distance of 1 nucleotide. The most abundant sequence per cluster was considered the real
83 biological sequence and was assigned the count of all reads in the cluster.

84 The representative reads from all clusters were subjected to chimera removal using the VCHIME algorithm (Rognes
85 et al. 2016). Reads passing all above filters were aligned using an in-house uBiome database of 16S sequences derived
86 from the NCBI-nr database (Benson et al. 2013). Decreasing sequence identities were used to map reads to different
87 taxonomic rankings: > 97% sequence identity was used for the assignment to a species, > 95% sequence identity for the
88 assignment to a genus, > 90% for assignment to a family, > 85% for assignment to an order, > 80% for assignment to
89 a class, and > 77% for assignment to a phylum. The relative abundance of each taxonomic group was calculated by
90 dividing the abundance of the taxonomic group to all sequences that map to any sequence in the bacterial domain.

91 Bioinformatics was performed in R using Phyloseq (McMurdie and Holmes (2013)). Source code is available on our
92 Github page.

93 Conclusions

94 Interesting associations with microbes of unknown function are commonly found in all microbiome experiments, and
95 the volume of data collected ensures that some "statistically-significant" results will be found simply due to random
96 chance. Although the function of *Fusicatenibacter* is unknown, the association in this subject was so pronounced, and
97 so clearly associated with the start of kefir drinking that we felt other researchers may benefit from learning about our
98 experience. Despite the n=1 oddity of this experiment, we have seen the organism in other kefir-drinking subjects, and
99 we present this work in the hopes that it may be useful others working to understand the effect of kefir and other
100 fermented drinks on human health.

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