

RESEARCH & EDUCATION FUND

**THE EFFECTS OF
LIVE-CULTURE BEER
ON ONE MAN'S
MICROBIOME.**



American Homebrewers Association®

I know my shit: The effects of live-culture beer on one man's microbiome.

By: Matthew J. Farber, Ph.D. and Derek Dellinger

Statement of Purpose:

The purpose of this research was to examine the influence of live-culture homebrew, including a *Lactobacillus* sour ale, on the bacterial populations of one man's gut.

Summary:

Because live-culture, sour homebrew contains a common probiotic genus, *Lactobacillus*, we were curious to see if moderate consumption of homebrew could influence Matt's microbiome and to start a discussion about whether live culture homebrew might be considered a probiotic beverage. ***It is important to note that the data and observations presented in this report were derived from only one experiment from one person and therefore should not be considered as conclusive.***

Introduction:

There are 10 times as many bacteria in the human body as there are human cells (Savage, 1977). Many are found in the colon; however the skin, mouth, nose, genitals, mammary glands, and even eyeballs contain a distinct population of bacteria termed the microbiome. The presence of beneficial microbes is thought to contribute to a healthy lifestyle by limiting growth of pathogens, producing antioxidants and beneficial neurotransmitters, reducing inflammation, and limiting nutrient malabsorption (Selhub, Logan, & Bested, 2014). With large scale, modern DNA sequencing, scientists can now investigate the influence of environmental or genetic differences on the microbiome, providing connections to various diseases. In general, the adult gut microbiome is resistant to large changes in population dynamics, however recent studies have shown that dietary changes can induce temporary microbiome fluctuations in as little as 24 hours (David et al., 2014). Thus diet is an important consideration in maintaining a healthy microbiome. Probiotics are defined as live-culture products which confer benefits to the consumer by positively influencing the intestinal microbiome.

One common probiotic found in yogurt, sauerkraut, kefir, and sausage is bacteria of the genus *Lactobacillus*. Historically, lactic acid bacteria (LAB), generally *Lactobacillus* or *Pediococcus*, unknowingly contaminated most styles of aged beer, often in collaboration with "funk-ifying" wild yeasts like *Brettanomyces*. A mild case of souring was, at least in many instances, an anticipated stage in the lifespan of a beer. For many beers, the slow attrition of fermentable sugar into tart lactic acid would have been part of the expected flavor profile. The further increase in acidity created by lactic acid bacteria even further stabilized the beverage from harmful bacterial contamination, as most microbes cannot survive in a low pH environment. In historic saisons, the succulent qualities of the lactic

sourness allowed a refreshing beer to better serve its intended function: a nutritious drink for farmhands. And in their beer, these thirsty farm workers consumed similar species of bacteria also found in their preserved, probiotic sauerkrauts and fermented milk products.

In the 19th century, fermentation was only understood in broad terms. Before Louis Pasteur and Emil Christian Hansen revolutionized sanitization and yeast culturing respectively, *Lactobacillus* bacteria were typically present throughout the brew house, settling into its niche in the microbial ecosystem alongside yeasts. Many modern brewers, however, draw their inspiration from the historic styles of Germany such as Berliner Weisse. Sour mashing (and the related method of kettle souring) relies on elevated fermentation temperatures which favor *Lactobacillus* to induce a quick, frenzied souring of the wört. Such a procedure is typically performed prior to the boil; so that, the microbes are killed and fermentation can continue as a 'clean' fermentation, without risk of infecting equipment and subsequent batches. As one can imagine, any probiotic potential of this particular fermentation product is then lost.

Direct and intentional pitching of LAB during primary or secondary fermentation permits the microbes to thrive long after fermentation and bottling. But many brewers find such mixed-culture fermentations to be more difficult to manage, with different microbes favoring different wört conditions and temperature ranges. Brewers may feel there is a risk to their equipment and future batches by letting 'infection' microbes remain alive in the beer. In addition, some (but not all) mixed-culture fermentations with living bacteria may require more time before packaging and distribution, thus adding budgetary drawbacks.

But with the rise of mixed-culture beers in modern craft brewing and homebrewing, the focus is predominantly on the flavor profiles and acid produced by the LAB, with little thought on additional benefits they may provide. One such benefit is as a probiotic. With the rise in popularity of citizen science projects such as The American Gut Project and UBiome, there arose an interesting opportunity to study the effects of live-culture homebrew on the microbial population of one man's gut.

The idea for this experiment was born out of a separate project undertaken by one of the authors. During the entirety of 2014, Derek's diet consisted of 100% fermented food. He will discuss the experience in an upcoming book, entitled *The Fermented Man*. Both authors of this report have long had a fascination with homebrewing, sour beers, and the microbes that produce them. While much attention is given to the probiotic benefits of microbes in many other fermented foods, little mention of their existence or possible effects is ascribed to sour beers which often contain the very same genus of LAB as yogurt or sauerkraut.

Materials and Methods:

Matthew Farber was the experimental subject and an author of this research. The data presented in this report only represents information from one test subject and thus should not be considered valid in any other context.

The Diet: A balanced and fixed diet was consumed each week (three consecutive weeks) of the experiment, Monday through Sunday. The meals varied each day, but a strict diet log was followed so that each week, the diet remained identical. Week 1 was no beer; week 2 included 2 Saison beers a day; and week 3 included 2 Lacto-Saison beers a day. Food brands were kept consistent across the entirety of the experiment. As an example, Table 1 describes the Monday diet. Foods with probiotics were excluded. Filtered or bottled water was imbibed without controlling for daily volume. Beers were consumed with dinner. Consistent steps were taken to ensure transfer of yeast / LAB sediment from bottle to glass.

Breakfast	Lunch	Dinner	Other
Multivitamin	Turkey, Cheese, Spicy brown mustard on wheat	Two breaded chicken fillets	Banana
Wheat toast, Peanut butter	Apple	BBQ sauce	afternoon cup of coffee
Cup of OJ	Nature's Bakery Fig Bar	1/2 Cup Frozen Corn	
Large coffee	Handful of almonds	Mixed greens salad with onion	
		Oil and Balsamic vinaigrette	
		Fruit popsicle	

Table 1: An example of the fixed diet consumed by Matt during the course of the experiment.

The Homebrew: Wört for the beer was prepared using a standard all-grain brewing procedure, utilizing a low mash temp for a more attenuable beer and thus a final product with as little residual sugar as possible. No hops were added to the wört at any point prior to fermentation, as they can inhibit LAB growth. Once cooled to room temperature and transferred into a carboy, the unhopped wört was pitched with White Labs Saison II and Mangrove Jack Belgian Saison, both *Saccharomyces* strains. At the same time, a mixture of four lactobacillus strains from a variety of sources was pitched, as well as *Brettanomyces lambicus*, *Brettanomyces custersianus*, and the yeast strain marketed as 'Brett Trois' (at the time of writing there is running debate about the accurate classification of this strain as a *Brettanomyces* species). All pitched microbes were allowed to carry out fermentation simultaneously and at the same temperature. Fermentation was carried out at ambient room temperature, approximately 72 – 75° F. Visible primary condition was concluded within one week. The beer was given 7 weeks to reach terminal gravity. The Lacto-Saison beer was then dry-hopped with 4 oz. of Citra leaf hops for one week before bottling and is referred to as Lacto-Saison in this report. In the Saison version, the four lactobacillus strains were omitted.

Sample Collection and Analysis: Distal Microbiome kits were ordered from UBiome (Figure 1). Samples were collected at the conclusion of each week on Monday morning. In brief, distal gut samples were collected from toilet paper with a sterile swab which was swirled in a collection tube for 1 minute. The tube was tightly capped and shaken by hand for an additional minute. The samples were then sent in the enclosed envelope to UBiome where they were further processed and sequenced. DNA sequences corresponding to specific microbial populations were analyzed by UBiome, the results of which were displayed via their Beta software (Figure 2). UBiome enables users to see the relative abundance of the bacterial Phyla in the sample as it compares to the Average for all samples received (Figure 2A). The microbiome data can also be arranged as a tree, organized by scientific taxa. While some species level information can be obtained in the Raw Data, only genus level information was displayed on the tree.



Figure 1: UBiome Distal Gut Microbiome Kit

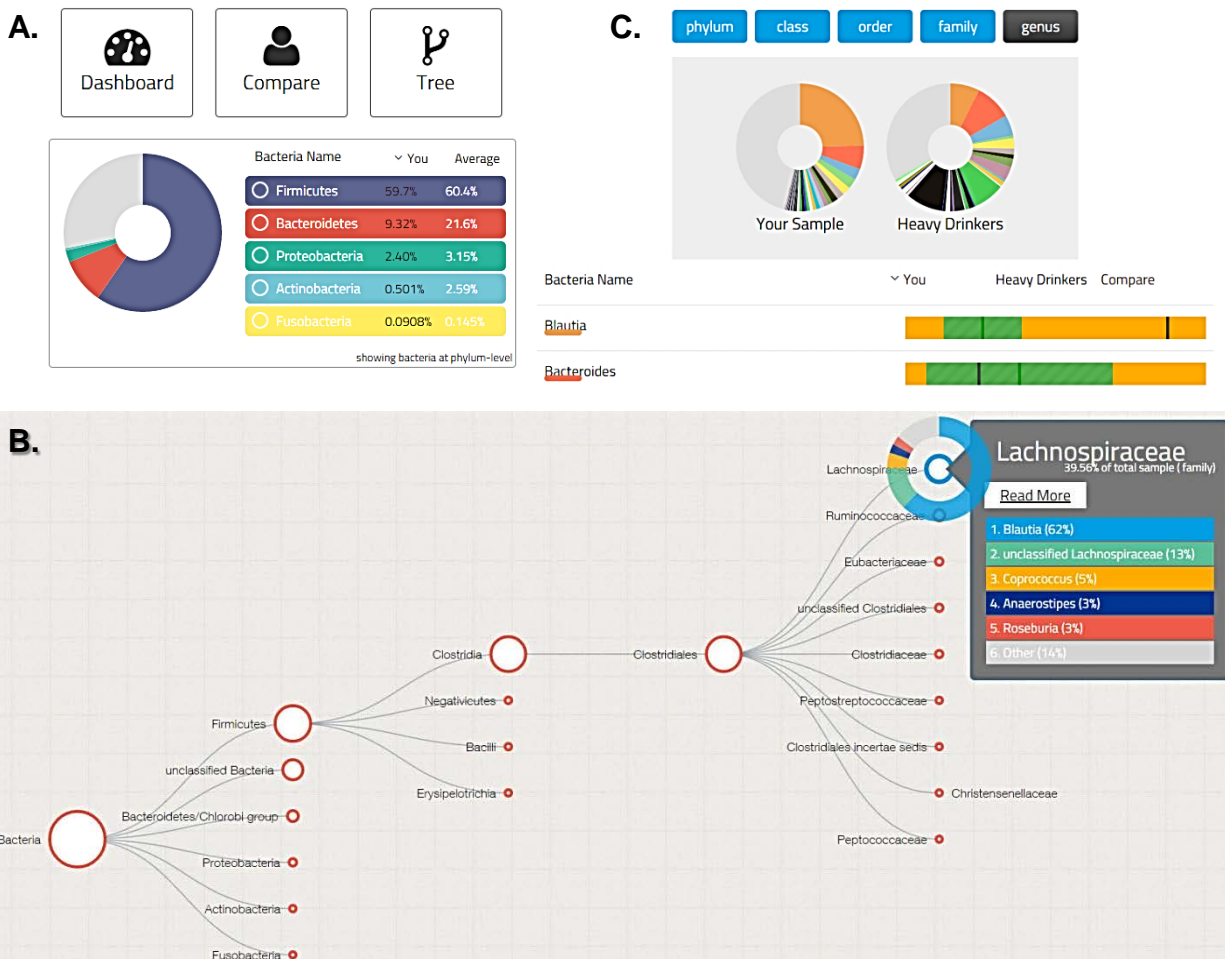


Figure 2: UBiome Microbiome Analysis Examples

UBiome enables users to select a bacterial taxon and read about microbe descriptions, trends, and known population differences. Based on a short survey all users complete prior to sample collection, the user data can be compared to the average data assigned to groups such as Heavy Drinkers, Vegetarians, or Healthy Omnivores (Figure 2C). Heavy Drinkers were defined by UBiome as people who reported consumption of “five drinks in a single sitting multiple times a month”. ***It is important to note, as stressed by UBiome, that this service should not be used to diagnose or identify potential diseases or problems. It is only intended as a citizen science service for inspiration and exploration.***

Results and Discussion:

The adult human, intestinal microbiome typically consists of six or seven bacterial Phyla which, in total, amounts to over 10,000 different species (NIH National Microbiome Project). Firmicutes and Bacteroidetes represent the majority of microbial Phyla in most Western (high protein, high fat) diets (Eckburg et al., 2005). The relative abundances of the major bacterial Phyla present in Matt's gut microbiome during the course of the experiment are presented in Figure 3a. There was a decrease in total Firmicutes with slight alterations in Bacteroidetes and Proteobacteria. Unfortunately, the number of unclassified bacteria increased which may be a result of UBiome's methods.

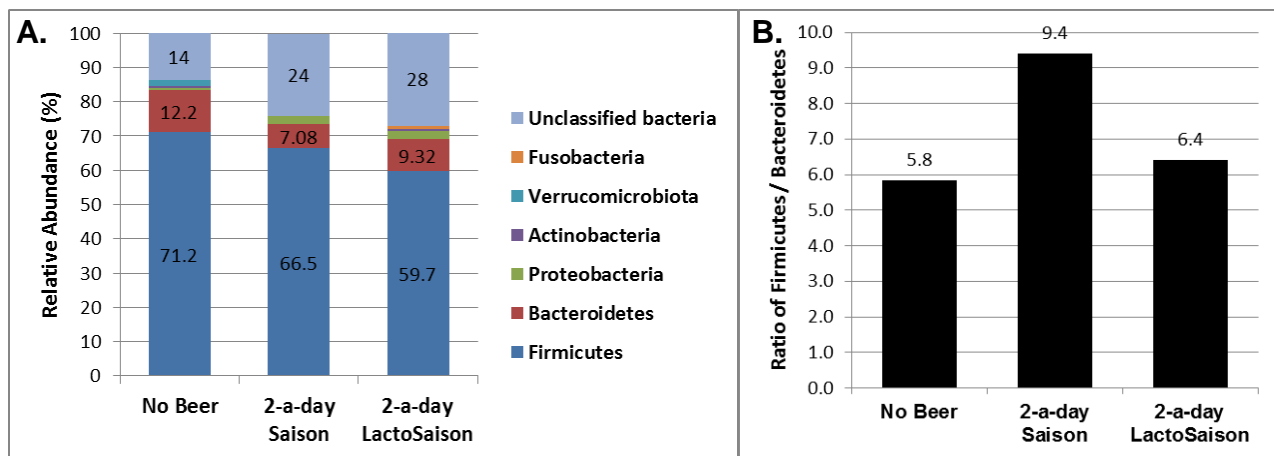


Figure 3: Microbiome analysis data (A) Relative Abundance of microbial Phyla from the distal gut at the conclusion of each diet represented as a percentage. (B) The Ratio between the relative abundance of Firmicutes / Bacteroidetes in the distal gut microbiome after each diet.

It has been demonstrated that the ratio of Firmicutes to Bacteroidetes correlates with body weight, whereby a higher ratio (less Bacteroidetes) is found in those who are obese (Armougom, Henry, Vialettes, Raccach, & Raoult, 2009). Bacteroidetes are thought to protect against obesity because they cannot digest fats, allowing them to pass through the digestive system (UBiome). Thus there is an interest in identifying conditions which decrease this ratio. In Matt's microbiome samples, there appeared to be an increase in the ratio of Firmicutes to Bacteroidetes between the No Beer diet and the 2-a-day Saison diet (Figure 3b). The ratio returned closer to baseline after consumption of the LactoSaison.

The relative abundance of Bacteroidetes can be reduced with excessive alcohol intake. Chronic alcohol consumption and alcoholic liver disease has been linked to lower abundances of Bacteroidetes and higher Proteobacteria (Mutlu et al., 2012). In addition, another experiment evaluated the microbiome of mice whose diet was supplemented with 5% (v/v) ethanol for 6 weeks. A decline of both Bacteroidetes and Firmicutes phyla was observed, with a proportional increase in Proteobacteria and Actinobacteria (Bull-Otterson et al., 2013). One interesting speculation from Matt's microbiome data might be that even moderate consumption of beer reduces Bacteroidetes levels. However, in the study of chronic alcohol consumption from Bull-Otterson *et al.*, the bacterial genera which increased the most were *Alcaligenes* and *Corynebacterium* (Bull-Otterson et al., 2013). There were no *Alcaligenes* present in Matt's

microbiome data and *Corynebacterium* was extremely minimal with hardly any change (Table 2) which suggests a difference in the effects of chronic versus moderate alcohol consumption on the microbiome.

Interestingly, Matt’s Firmicutes / Bacteroidetes ratio returned closer to baseline after consumption of the Lacto-Saison (Figure 3b). Might the presence of LAB in the beer further influence the microbiome? It has previously been reported that the addition of probiotics can actually improve some of the negative microbiome effects of chronic alcohol, as administration of lactobacillus attenuated ethanol-induced liver damage in mice (Nanji, Khettry, & Sadrzadeh, 1994). This remains an interesting possibility for future experiments.

More specific changes to Matt’s microbiome after addition of beer can be observed at the Family and Genus level. The most abundant taxa and relevant probiotic taxa (*Lactobacillus* and *Bifidobacteriales*) are displayed in Table 2 with their relative abundances displayed as a percentage. Unfortunately no increases in *Lactobacillus* or *Bifidobacteriales* (both probiotics) could be observed in Matt’s data (Table 2).

Phylum	Family or Genus	No Beer	Saison	Lacto-Saison	Average Healthy Omnivore	Descriptions (from UBiome)
Firmicutes	<i>Blautia</i>	14.14%	32.54%	24.54%	7.59%	-Indicates a healthy gut -Digests complex carbohydrates
Firmicutes	<i>Ruminococcus</i>	13.09%	3.93%	2.85%	6.36%	-Digests complex carbohydrates
Firmicutes	<i>Faecalibacteria</i>	6.38%	0.23%	0.15%	9.83%	-Digests complex carbohydrates -Reduced in Crohn’s Disease
Firmicutes	<i>Roseburia</i>	6.01%	2.45%	1.09%	3.66%	-Indicates a healthy gut -Digests complex carbohydrates -Releases anti-inflammatory butyrate
Firmicutes	<i>Lactobacillus</i>	0.02%	0.01%	0.02%	0.70%	-Probiotic
Bacteroidetes	<i>Bacteroidaceae</i>	11.49%	4.68%	6.20%	9.8%	-Drop out in high fat diets -Alcoholics have fewer than the general population -Diabetics have fewer as well
Bacteroidetes	<i>Rikenellaceae</i>	0.02%	0.85%	1.62%	1.14%	-Indicates healthy gut -Thrive on high fat diets / enriched in obesity
Actinobacteria	<i>Bifidobacteriales</i>	0.29%	0.04%	0.03%	1.07%	-Probiotic
Actinobacteria	<i>Corynebacterineae</i>	0.00%	0.01%	0.02%		-Babies born via C-section tend to have more

Table 2: Relevant bacteria in Matt’s microbiome and their relative abundances during the experiment.

One noticeable observation is the high percentage of bacteria capable of complex carbohydrate digestion in the No Beer control. This is likely a reflection on Matt’s diet which typically consisted of high fiber (Table 1) during and as part of his routine diet prior to the experiment. With the exception of *Blautia* many of these bacteria were reduced after addition of Saison or Lacto-Saison to the diet. Unfortunately there were few resident probiotic bacteria in Matt’s microbiome with no appreciable effect on the population after addition of Saison or Lacto-Saison.

While the results of this study cannot correlate moderate beer consumption with specific changes in the microbiome, they do highlight the complexity and importance of a balanced microbiome. Ultimately, changes in diet, including consumption of alcohol, can affect the microbial diversity in the gut. A few scientific studies of the microbiome have focused on chronic alcohol consumption, but greater focus is needed on the benefits of moderation consumption. As an example, red wine has been shown to increase Bifidobacterium levels (Clemente-Postigo et al., 2013). In another study, fermentation of polyphenols by gut microbes indirectly stimulated proliferation of Bifidobacteria and decreased the ratio of Firmicutes to Bacteroidetes (Parkar, Trower, & Stevenson, 2013). Although this research did not focus on beer, beer is rich in polyphenols, many of which are derived from hops. Interestingly, dry hop additions were included in the Lacto-Saison, so perhaps this sample contained more polyphenols which could explain the decreased Firmicutes to Bacteroidetes ratio in Figure 3b.

Ultimately, this research raises the question, can beer be considered a probiotic beverage if it contains live-culture LAB? The National Institutes of Health states that although promising research supporting probiotics and health have been reported, strong and significant evidence for the effects on probiotics on specific conditions is lacking, especially with long-term considerations. As such, the Food and Drug Administration has not approved any probiotics (NIH, 2013). Ultimately we cannot call live-culture beer a probiotic, but it remains a distinct possibility. In the future, careful research will be needed to examine not only the effect of LAB-containing beer on the microbiome, but how total LAB cell numbers, and thus health benefits, might be increased without affecting beer flavor.

In conclusion, we are still a long way from understanding the potential health benefits of LAB beer on the microbiome, but this research has provided an interesting introspective on the possibility. We are excited to start the conversation of live-culture beer as a probiotic beverage, but clearly more scientific studies will be needed to validate the claim. We will conclude with an interesting question and possibility: What if a beer gut is created not solely by an increase in calories but by alteration of the microbiome, such as an increase in gut microbes associated with fat adsorption?

The Guts of the Report:

- Live-culture beer with Lactic Acid Bacteria might be considered a probiotic as similar LABs are used in yogurt, sauerkraut, and other fermented foods.
- Minor fluctuations in Matt's microbiome after consumption of two beers a day could be observed between the Saison and the Lacto-Saison. The Firmicutes / Bacteroidetes ratio increased with Saison consumption, but decreased with the Lacto-Saison. High Firmicutes / Bacteroidetes ratios have been associated with obesity, thus from this data, the Lacto-Saison may be a "healthier" option.
- No changes in specific probiotic species could be detected in the microbiome, supporting the notion that probiotics need to be routinely consumed (in moderation) to receive health benefits.
- The data and observations presented in this report were derived from one experiment and from one person and therefore should not be considered as conclusive.

About the Authors:

Matthew Farber is a Teaching Postdoctoral Fellow at the University of the Sciences in Philadelphia, PA where he studies fermentation science and biotechnology, specifically the effects of various proteases on beer quality. He is a member of and has presented at the American Homebrewers Association, the American Society of Brewing Chemists, and the Master Brewers Association. He tweets nuggets of beer science @HighBeerQ as “Dr. Sarah Visiae” or can be reached via email at m.farber@usciences.edu

Derek Dellinger is a writer and brewer living in Beacon, NY. In 2014, Derek lived for a full year consuming nothing but fermented products and will write about the experience in his upcoming book *The Fermented Man*, out winter 2016. Derek blogs about fermentation and brewing at www.bear-flavored.com, and he is the Brewmaster at Kent Falls Brewing, a brewery and hop farm in Connecticut. Follow him on Twitter @bearflavored or email him at bearflavored@gmail.com.

Works Cited:

Armougom, F., Henry, M., Vialettes, B., Raccach, D., & Raoult, D (2009). Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and *Methanogens* in anorexic patients *Plos One*, 4(9), e7125. doi:10.1371/journal.pone.0007125

Bull-Otterson, L., Feng, W., Kirpich, I., Wang, Y., Qin, X., Liu, Y., et al (2013). Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment *Plos One*, 8(1), e53028. doi:10.1371/journal.pone.0053028

Clemente-Postigo, M., Queipo-Ortuño, M. I., Boto-Ordoñez, M., Coin-Aragüez, L., del Roca-Rodríguez, M. M., Delgado-Lista, J., et al (2013). Effect of acute and chronic red wine consumption on lipopolysaccharide concentrations *The American Journal Of Clinical Nutrition*, 97(5), 1053-61. doi:10.3945/ajcn.112.051128

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al (2014). Diet rapidly and reproducibly alters the human gut microbiome *Nature*, 505(7484), 559-63. doi:10.1038/nature12820

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., et al (2005). Diversity of the human intestinal microbial flora *Science (New York, N.Y.)*, 308(5728), 1635-8. doi:10.1126/science.1110591

Mutlu, E. A., Gillevet, P. M., Rangwala, H., Sikaroodi, M., Naqvi, A., Engen, P. A., et al (2012). Colonic microbiome is altered in alcoholism *American Journal Of Physiology. Gastrointestinal And Liver Physiology*, 302(9), G966-78. doi:10.1152/ajpgi.00380.2011

Nanji, A. A., Khettry, U., & Sadrzadeh, S. M (1994). *Lactobacillus* feeding reduces endotoxemia and severity of experimental alcoholic liver (disease) *Proceedings Of The Society For Experimental Biology And Medicine. (New York, N.Y.)*, 205(3), 243-7.

NIH – National Center for Complementary and Alternative Medicine (2012). Oral Probiotics: An Introduction. Web Access on 12/10/14. < <http://nccam.nih.gov/health/probiotics/introduction.htm>>.

Parkar, S. G., Trower, T. M., & Stevenson, D. E (2013). Fecal microbial metabolism of polyphenols and its effects on human gut microbiota *Anaerobe*, 23, 12-9. doi:10.1016/j.anaerobe.2013.07.009

Savage, D. C (1977). Microbial ecology of the gastrointestinal tract *Annual Review Of Microbiology*, 31, 107-33. doi:10.1146/annurev.mi.31.100177.000543

Selhub, E. M., Logan, A. C., & Bested, A. C (2014). Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry *Journal Of Physiological Anthropology*, 33, 2. doi:10.1186/1880-6805-33-2

Acknowledgements:

Thank you to the Research and Education Fund of the American Homebrewers Association for funding this research and to the REF committee for their support, especially Aimee Garlit our REF liason.