The Mysteries Of Kefir Grain

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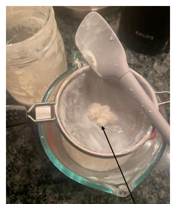
Abstract

Kefir grains are a symbiotic culture of both bacteria and yeast, known for their ability to ferment milk. Traditionally, kefir is made with whole milk, therefore, studies on the effects of varying milk fat percentages on the microbial composition of kefir grains is limited. As a result, this project strives to observe and investigate the impact milk fat percentage has on microbial activity, and characteristics of fermentation on kefir grains utilizing DNA barcoding methods. Furthermore, fermentation behavior of the grains (separation into curd and whey) will be documented as well. Samples will be plated to sufficiently discern any differences. Results will provide clarity to comprehending how the availability of fat may alter the stability and growth of kefir grains.

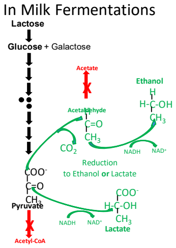
Introduction

Kefir is a fermented milk beverage that is made from the process of inoculating milk with kefir grains, which are symbiotic communities of lactic acid bacteria, acetic acid bacteria, yeasts, and fungi (Prado et al., 2015). Kefir itself originates in Caucasus and Tibet, where nomadic herders had accidentally discovered them as they transported milk in animal-skin bags. It was used to preserve milk, and provide nutrition. It is believed that kefir and substances like that of kefir, could have been used up to as many as 5,000 years ago. Kefir also has ties to Islam, as legends suggest the Prophet Muhammad gifted the grains to the Karachai people. Its rich history is now widely shared due to the fact that kefir has gained global popularity because of its probiotic potential, and ability to break down the lactose sugar in milk, making it much easier to digest (Rosa et al., 2017). During kefir fermentation, microorganisms break down the lactose sugars within milk under anaerobic conditions. The process of glycolysis allows for glucose to be converted into pyruvate, thus yielding ATP and NADH. Due to the absence of oxygen, pyruvate experiences fermentation pathways that will regenerate NAD+, which will typically produce lactic acid, and metabolites that make up the characteristic acidity and texture to kefir. Kefir also rivals yogurt, as yogurt contains only bacteria and no fungi, leading to less nutritional value than that of kefir grains. The healthiest of these grains usually develop over multiple cycles of fermentation, instead of just one. This leads to the formation of the curd. However, sometimes this can also result in the spontaneous production of both curd and whey. This lends itself to the mysterious nature of kefir grains, as predicting its presence can be quite difficult, and the factors that cause spontaneous products to form are not fully understood. It may be due to fat content within milk, which has the potential to alter how stable a grain is, as well as its microbial balance. Vieira et al., 2015 demonstrated that the fat content within milk affects how fermentation by kefir grains changes milk's fatty acid profile. Only high-lipid matrices showed an increase in polyunsaturated fatty acids after the event of fermentation.

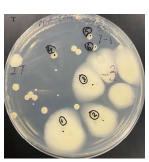


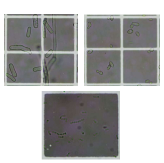
Kefir Grains

Anaerobic Fates of Pyruvate In Milk Fermentations



Additionally, milk fat also plays a crucial role in ensuring the viscosity of kefir. The higher the percent of fat, the more viscous Kefir is (Magra et al., 2012). Therefore, there is a likely possibility that kefir grains react differently within lower fat percentage milks. Despite the likelihood of such, controlled studies that analyze how fat content within milk may affect kefir grains microbial composition, and fermentation patterns has remained scarce. Comprehending this relationship is highly important to bettering kefir production in household settings and enhancing understanding on what the true ecological needs of kefir grains are to ensure the best possible quality. Fermentative microbiomes like kefir are made up of both yeast, and bacteria. Lactic acid is known as the dominant group that is responsible for causing the acidity and sourness within kefir. Since the grains depend on stability among the fungal and bacterial communities, changes in the chemistry of the milk, like its content of fat, can influence their composition as well as activity. In order to measure such, we will evaluate the amount of wet grains (grams) in every type of milk, and the presence of different bacteria and fungi within our samples. DNA barcoding applications will be applied and especially helpful for comparing microbial communities. This project will analyze how and if different milk types (whole milk, skim, low-fat, 1%, and 2%) change the health of the grains, the formation of the grains, diversity in microbial colonies, and the fermentation outcomes. By utilizing both observations of morphological characteristics with microbial plating and DNA barcoding, our goal is to characterize how the content of fat within milk influences the overall function of kefir grains, and thus serving to uncover a part of the mystery behind kefir.





Methods

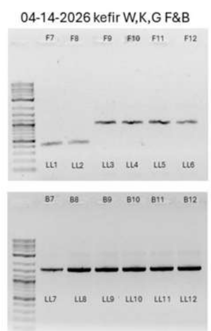
•The Kefir was made in a home environment, naturally it separates into 3 groups; Kurd, Whey, and Kefir Grains. In order to get an extensive overview of fungi and bacterial cultures that grew in each category we plated, extracted, purified, and sequenced each group separately.

•**Isolating Cultures:** The kefir was made in a home environment and naturally separated into three groups: curds, whey, and kefir grains. To get an extensive overview of the fungi and bacteria in each category, we plated, extracted, and sequenced each group separately. We started with 100mg of each sample and performed a 10-fold dilution series. To isolate fungal cultures, we used PDA–CAM (Potato Dextrose Agar with Chloramphenicol) to promote fungal growth while inhibiting bacteria. To isolate bacterial cultures, we used MRS Agar plates to specifically cultivate lactic acid bacteria.

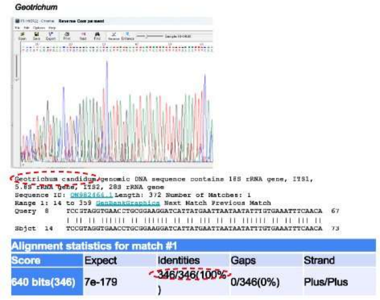
•**Identifying Colonies:** After incubation, we used visual characteristics to infer which species were growing. On the PDA–CAM plates, large white colonies were identified as *Geotrichum*, and small white colonies were identified as *Saccharomyces*. Micrographs validated these assumptions, showing that the large white colonies had a filamentous shape (*Geotrichum*) and the smaller ones were budding yeast (*Saccharomyces*). While we couldn't identify the bacteria visually because the medium-sized beige colonies look similar to many species, micrographs allowed us to identify them as *Lactococcus* due to their spherical, egg-like shape.

•**DNA Purification:** We initially used a Chelex boil procedure for PCR, but it gave us inconsistent results. To ensure better accuracy and standardization, we switched to a more reliable method. We isolated the cultures through streak-plating, then harvested the material using the Qiagen Pro-Soil DNA Extraction Kit. This method provided much better purification and higher DNA concentrations than the boiling method we tried first.

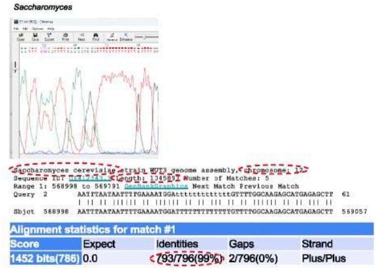
•**Sanger Sequencing and NCBI-Blast:** The purified DNA was sequenced, and we used electropherograms to check the quality of our tests. We then ran the sequence data through NCBI-BLASTN to compare our results with sequences in the GenBank database for final identification.



Geotrichum

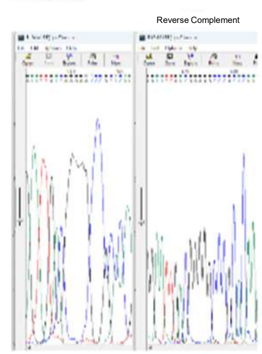


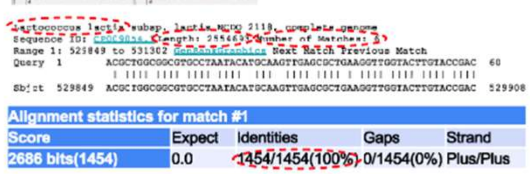
Saccharomyces



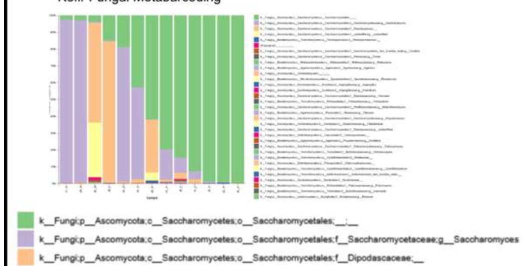
Lactococcus

Reverse Complement

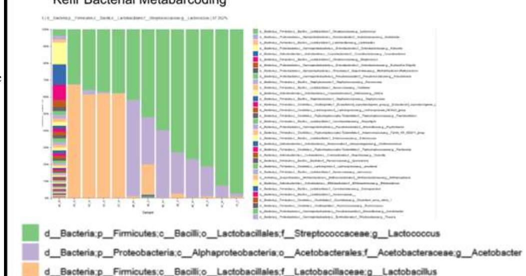




Kefir Fungal Metabarcoding



Kefir Bacterial Metabarcoding



Discussion

The purpose of the kefir grain is a central question in this study. Because kefir naturally separates into curds, whey, and grains, it is unclear if the system functions as a single unified fermentation or as three distinct fermentations that happen simultaneously. Our results suggest that separate fermentation processes occur simultaneously within the different phases. While Lactococcus is distributed throughout the kefir, acting as the primary fermenter of the liquid phases, the kefir grain serves as a specialized biological reservoir.

The grains provide a space that specifically concentrates Lactobacillus and Saccharomyces, species that were largely absent or less prevalent in the whey and curds. This suggests that the grains act as a permanent "seed", preserving the essential microbial diversity required to start the next batch, while the surrounding liquid undergoes a more rapid, localized fermentation. Rather than being a homogenous mixture, we suggest that kefir is a partitioned system where the physical separation of phases allows different microbial communities to thrive in their own specific roles.

Results

The results of the Sanger sequencing, utilizing overlapping forward and reverse reads, confirmed the identity of our isolates through both genetic and morphological data. Analysis of the fungal amplicons and electropherograms identified the large filamentous colonies as Geotrichum candidum (F7, F8) and the slower-growing, non-filamentous colonies as Saccharomyces cerevisiae (F9, F10). Bacterial sequencing (B7-B12) consistently identified Lactococcus lactis, matching the complete genome and chromosome in the NCBI database. Fungal metabarcoding further revealed that while Geotrichum (salmon) and general yeasts (green) were distributed throughout, Saccharomyces (purple) was heavily concentrated in the kefir grains, indicating that Kefir grains may be a primary reservoir for this yeast. Bacterial metabarcoding showed that Lactococcus (green) and Acetobacter (purple) dominated the liquid whey and kefir, but Lactobacillus (salmon) was found almost exclusively in the kefir grains. These findings indicate that while Lactococcus is present in the kefir as a whole, the kefir grain provides a specialized environment that concentrates Lactobacillus and Saccharomyces.

References and Acknowledgements

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Slide 1

RN1 In the introduction add background on the kefir grains specifically what is mysterious about them

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