Metagenomic study links microbes to flavors in kefir

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Credit: American Society for Microbiology

A team of food scientists and microbiologists in Ireland have used high-

throughput sequencing to analyze how microbial populations change as kefir ferments. It's a new frontier in food analysis: Using the data, collected over a 24-hour fermentation period, the researchers were able to connect the presence of individual microbial species and their associated pathways to flavor compounds in the fermented milk beverage. They reported their findings in *mSystems*, an open access journal of the ASM.

Such a deep understanding of microbial behavior during fermentation could point to new ways to improve kefir. Food scientists might be able to optimize production, customize the flavor, or improve the health benefits of the food by tweaking the microbial mix. They might even create synthetic versions of kefir, using only select microbes.

kefirdrinkInterest is growing among food researchers in combining high-throughput sequencing with metabolomics to better understand how individual microbial species influence the characteristics of food. However, previous studies on fermented foods have been conducted using lower resolution sequencing techniques, like 16S rRNA gene sequencing.

"We didn't know until now what the individual species are doing," says lead author Aaron Walsh, who is working on his PhD at Teagasc Food Research Centre in Cork, as part of the APC Microbiome Institute.

The new analysis also shows how kefir can serve as a robust model for studying communities where multiple bacterial species interact in complex ways, like the gut or soil. Kefir has fewer microbial species than other fermented foods and only requires 24 hours to ferment at room temperature. (Cheese, for comparison, requires three months or more to ferment.) The new study has already led the researchers to consider similar, high-throughput analyses of microbial populations in other foods.

"We're looking at how the concept can be extended to the entire fermented foods industry," says Paul Cotter, senior author on the study and a microbiologist at Teagasc and the APC Microbiome Institute. "That includes cheese, yogurts, even fermented meats and sourdough breads. We might be able to study alcoholic beverages, as well."

To create the kefir for their analysis, the researchers began with grains - a starter mix of bacteria and yeast that resembles cauliflower - from three different geographic regions. These grains were used to ferment pasteurized milk into kefir. The researchers found that the bacterial populations of the kefir milks changed in similar characteristic ways during fermentation.

To obtain species-level data, the microbiologists used whole metagenome shotgun sequencing - a technique used to study microbial populations too complex or diverse for individual species to be cultured individually. They reported that bacterial diversity decreased rapidly during the 24-hour fermentation period. Sequencing showed that during the early stages of fermentation, Lactobacillus kefiranofaciens dominated the bacterial population. Later in the process, however, Leuconostoc mesenteroides became more prevalent.

They identified links between individual bacterial species and certain flavors. Acetobacter pasteurianus, for example, was correlated with an acidic, vinegar-type flavor. Lb. kefiranofaciens was correlated with cheesy flavors. Other species were connected to the metabolites responsible for buttery and fruity flavors.

Previous studies have suggested that consuming kefir can improve intestinal health and reduce inflammation and cholesterol. Microorganisms in the kefir grains may be responsible, but scientists don't know for sure - and they don't know the underlying mechanism. Cotter says metagenomics may help answer such outstanding questions

about the fermented beverage.

"In the past, kefir has been associated with many health benefits," he says. "In my mind, they haven't really been pinned down or studied in a scientifically rigorous manner."

More information: Microbial Succession and Flavor Production in the Fermented Dairy Beverage Kefir, DOI: 10.1128/mSystems.00052-16 , msystems.asm.org/content/1/5/e00052-16

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