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Changes in human gut microbiota influenced by probiotic fermented milk ingestion

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ABSTRACT

We investigated the effect of consuming probiotic fermented milk (PFM) on the microbial community structure in the human intestinal tract by using high-throughput barcoded pyrosequencing. Six healthy adults ingested 2 servings of PFM daily for 3 wk, and their fecal microbiota were analyzed before and after 3 wk of PFM ingestion period and for another 3 wk following the termination of PFM ingestion (the noningestion period). Fecal microbial communities were characterized by sequencing of the V1–V3 hypervariable regions of the 16S rRNA gene. All subjects showed a similar pattern of microbiota at the phylum level, where the relative abundance of *Bacteriodetes* species increased during the PFM ingestion period and decreased during the noningestion period. The increase in *Bacteroidetes* was found to be due to an increase in members of the families *Bacteroidaceae* or *Prevotellaceae.* In contrast to PFM-induced adaptation at the phylum level, the taxonomic composition at the genus level showed a considerable alteration in fecal microbiota induced by PFM ingestion. As revealed by analysis of operational taxonomic units (OTU), the numbers of shared OTU were low among the 3 different treatments (before, during, and after PFM ingestion), but the abundance of the shared OTU was relatively high, indicating that the majority (>77.8%) of total microbiota was maintained by shared OTU during PFM ingestion and after its termination. Our results suggest that PFM consumption could alter microbial community structure in the gastrointestinal tract of adult humans while maintaining the stability of microbiota.
Key words: probiotic fermented milk, gut microbiota, pyrosequencing

INTRODUCTION

Fermented milk products have been consumed by many civilizations over centuries based on belief in their beneficial effects on human health. Yogurt is a coagulated dairy product from the fermentation of lactose in milk by the traditional yogurt starter cultures, such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Adolfsson et al., 2004). These bacteria are called probiotics, traditionally defined as viable microorganisms that offer beneficial effects when ingested by individuals (Rolfe, 2000) and have been used to enhance gut health for centuries. Some lactic acid bacteria, such as bifidobacteria and lactobacilli, have been combined with yogurt starters because of their probiotic properties. Probiotics are frequently included in yogurts, often together with some prebiotic compounds. Nondigestible food ingredients such as oligosaccharides, dietary fiber, and lactulose are generally used as prebiotics. Together, this combination of dietary supplements is referred to as a synbiotic yogurt (Palaria et al., 2012). Although these products have been used by many people, the mechanism(s) by which they act have yet to be identified (Bisanz and Reid, 2011).

The human gut microbiota contains at least 100 times as many genes as the human genome (Gill et al., 2006) and provides important human physiological functions such as glycan biosynthesis and metabolism (Gill et al., 2006; Sanz et al., 2008). Although a core human gut microbiota has been reported (Caporaso et al., 2011; Hunt et al., 2011), dietary habits can alter the com-

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position of the gut microbiota to change the metabolic potential (Hildebrandt et al., 2009). Consumption of yogurt has been proposed as a means to prevent obesity (Sun and Zemel, 2004; Diepvens et al., 2007) and to improve intestinal function in the gastrointestinal (**GI**) tract of some humans (Anukam et al., 2008; Schiffrin et al., 2009; Nikooyeh et al., 2011).

Although colonization in the gut by beneficial microorganisms may have significant effects on human health (Larsen et al., 2010) and may change the metabolic capacity of the gut (Claus et al., 2011), many probiotic bacteria are unable to colonize the human gut (Kullen et al., 1997) and pass through the human GI tract (Elli et al., 2006). Previously, McNulty et al. (2011) observed no changes in the abundance of a probiotic strain, *Bifidobacterium animalis*, in either humans or mice after yogurt ingestion, yet they did report definitive changes in the microbiota's metabolic pathways, such as increases in carbohydrate and nucleotide metabolism and decreases in amino acid and lipid metabolism. In addition, the effects of yogurt ingestion were observed with heat-sterilized yogurt (García-Albiach et al., 2008) as well as dry heat-killed probiotic powder (Shin et al., 2010; Vintiñi and Medina, 2011). These studies suggest that probiotics may indirectly influence gut microbiota without colonizing the human intestinal tract. Although there has been no direct evidence of the mechanisms for how probiotic bacteria can alter gut microbiota, secretion of antimicrobial compounds such as bacteriocins by some probiotic strains is known to prevent bacterial adhesion and evasion of epithelial cells (Gerritsen et al., 2011). Metabolic modeling of species interaction in the human microbiome predicts potential competition and syntrophy (Levy and Borenstein, 2013).

In many studies, culture-based and molecular-based approaches that target specific groups of bacteria have been used to analyze changes in the gut microbiota upon various treatments (Elli et al., 2006; Schiffrin et al., 2009; Saxelin et al., 2010). Recently, high-throughput sequencing technologies such as pyrosequencing have been used to analyze microbial community composition and dynamics from diverse environments, and these approaches offer an opportunity to understand the whole microbial community much more comprehensively than traditional culture-based approaches (Claesson et al., 2009; Griffen et al., 2012; Osei-Poku et al., 2012). In this study, rather than studying the influence of specific probiotic strains on gut microbiota, we used high-throughput deep sequencing to investigate whether consumption of probiotic fermented milk (**PFM**) could result in alterations in the microbial community structure of human gut microbiota at different phylogenetic levels.

MATERIALS AND METHODS

In Vivo Experiment

Six healthy female volunteers, 20 to 24 yr of age, participated in this study (Supplemental Table S1; http://dx.doi.org/10.3168/jds.2014-8943). During the study period, the volunteers were asked to maintain their usual lifestyle and diet but to refrain from consuming any drugs and fermented milk products other than those provided by us. Written consent was obtained from each person, and the study was approved by the Research Ethics Committee of Chung-Ang University. The PFM was prepared using fresh milk, skim milk powder, sugar, and a stabilizer, and was fermented with the ABCT-BH starter culture (Culture System Inc., Mishawaka, IN) containing *Lactobacillus acidophilus* CSG (Han et al., 2005b), *Lactobacillus brevis* HY7401 (Lee et al., 2008), *Bifidobacterium longum* HY8001 (Han et al., 2005a), *Lactobacillus casei* HY2782, and *Streptococcus thermophilus*. The PFM was also supplemented with a dietary fiber mixture and lactulose as prebiotic components. The final preparation (140 mL serving size) contained dietary fiber (3 g), lactulose (1 g), nonfat milk solids (8.5%), protein (3.2 g), fat (3.0 g), and *Lactobacillus* (1.0×10^8 cfu/mL). The volunteers consumed 2 servings of PFM daily (one after breakfast and the other after dinner) for 3 wk, and then stopped consuming PFM for an additional 3 wk of the study period (Supplemental Figure S1; http://dx.doi.org/10.3168/jds.2014-8943). Fecal samples were collected from each subject at 3 time points: before PFM ingestion (d 0), at the end of PFM ingestion period (d 21), and another 3 wk following the end of PFM ingestion (d 42). All fecal samples were stored at −80°C until used.

DNA Extraction from Fecal Samples

Approximately 0.5-g aliquots of feces from each sample were washed twice with 5 mL of PBS (pH 7.4) and were subjected to DNA extraction using an UltraClean Fecal DNA Kit (Mo Bio Laboratories, Carlsbad, CA). Approximately 100 ng of DNA was used for PCR amplification of the 16S rRNA gene in samples.

Amplification of 16S rRNA Gene and Sequencing

The extracted DNA was amplified using primers targeting the V1 and V3 hypervariable regions of the bacterial 16S rRNA gene using primers 27F: 5′-X-AC-GAGTTTGATCMTGGCTCAG-3′ and 518R: 5′-X-AC-WTTACCGCGGCTGCTGG-3′, where X de-