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Enterococcus faecium WEFA23 from infant lessens high-fat-diet-induced hyperlipidemia via cholesterol 7-alpha-hydroxylase gene by altering the composition of gut microbiota in rats

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ABSTRACT

Enterococcus faecium WEFA23 is a potential probiotic strain from Chinese infants with the ability to decrease cholesterol levels. Aiming to explore the mechanism of *E. faecium* WEFA23 in lowering cholesterol in vivo, we examined the gene transcriptions related to cholesterol metabolism, the composition of bile acids in feces, the synthesis of trimethylamine N-oxide (TMAO) in liver, and the composition of the gut microbiota of rats. We found that *E. faecium* WEFA23 enhanced the synthesis of bile acids by promoting cholesterol excretion, upregulating the genes transcript level relevant to cholesterol decomposition and transportation, and downregulating the genes involved in cholesterol synthesis. In addition, *E. faecium* WEFA23 not only downregulated the transcript levels of farnesoid X receptor and fibroblast growth factor 15 as well as flavin-containing monooxygenase 3, but also decreased the TMAO production followed by increasing the *CYP7A1* transcript level. Furthermore, when orally administered to rats for 35 d, *E. faecium* WEFA23 improved the gut microbiota diversity of rats fed a high-fat diet. Therein, the ratio of *Bacteroidetes* to *Firmicutes* and the abundance of *Rikenellaceae* increased, whereas the number of *Veillonellaceae* decreased. These results suggest that reduction of cholesterol level by *E. faecium* WEFA23 might be related to the changes in the gut microbiota. Our finding provides important information on lowering cholesterol by *E. faecium* and reveals that *Enterococcus* spp. might have the potential to decrease the TMAO level.

Key words: cholesterol 7-alpha-hydroxylase, trimethylamine N-oxide, intestinal microbiota, bile acid

INTRODUCTION

High cholesterol level is the leading cause of cardiovascular diseases (Prospective Studies Collaboration et al., 2007), which increase the number of human deaths worldwide (Mendis et al., 2011). Hypercholesterolemia leads to 45% of heart diseases in Western Europe and 35% of those in Central and Eastern Europe (Yusuf et al., 2004). The metabolism process of cholesterol includes synthesis, decomposition, and transportation in the host; among them, decomposition (i.e., bile acid synthesis) plays a crucial role in maintaining cholesterol homeostasis (Gupta et al., 2001). Cholesterol 7-α-hydroxylase (**CYP7A1**) is a critical enzyme that catalyzes the conversion of cholesterol into primary bile acids, which are then hydrolyzed into secondary bile acids. Parts of bile acids are reabsorbed in the ileum by active transport and return to the liver for secretion into the biliary system and gallbladder of the host (Hofmann, 1999). The other bile acids are eliminated by feces. Inhibiting the level of *CYP7A1* can reduce the biosynthesis of bile acids (Pullinger et al., 2002). *CYP7A1* is repressed by farnesoid X receptor (*FXR*), which functions as bile acid receptor (Chiang, 2009) and fibroblast growth factor 15 (*FGF15*; Inagaki et al., 2005). The *CYP7A1* is also inhibited by trimethylamine *N*-oxide (**TMAO**), which is converted from trimethylamine (**TMA**) by flavin-containing monooxygenase 3 (*FMO3*) in the liver (Tang et al., 2013).

Many publications have demonstrated that the synthesis of TMAO (Wang et al., 2011; Miller et al., 2014) and bile acids (Pavlović et al., 2012) is significantly affected by gut microbiota; however, few reports have stated that the regulation of *CYP7A1* affects the TMAO level by remodeling the microbiota. Moreover, no studies have reported that oral administration with probiotics (e.g., *Lactobacillus plantarum*, *Bifidobacterium* spp., and *Enterococcus* spp.) reshapes the gut microbiota structure and subsequently affects *CYP7A1* via TMAO.

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Cholesterol levels in patient with hypercholesterolemia are mainly reduced in humans by administering drugs. For instance, statins, specific inhibitors of hydroxymethylglutaryl-CoA reductase (*HMG-CoAR*), are the rate-limiting enzymes in cholesterol biosynthesis (Farmer, 1998). However, most drugs are expensive and may have side effects, such as muscle pain and increased risk of diabetes mellitus and abnormalities in liver enzyme tests (Naci et al., 2013). Development of novel alternative methods for lowering cholesterol is necessary.

Probiotics are defined as live microorganisms that can confer health benefits on the host when administered in adequate amounts (Hotel and Cordoba, 2001). Accumulating evidence suggests that oral administration of probiotics (e.g., *Bifidobacterium* spp. and *Lactobacillum* spp.) effectively alleviated the level of serum cholesterol and should be a promising option for treatment of cardiovascular diseases. A previous study reported that *Bifidobacterium bifidum* PRL2010 can decrease cholesterol levels by modulating the gut microbiota (Zanotti et al., 2015). Meanwhile, *L. plantarum* can reduce the absorption of cholesterol and accelerate the elimination of biliary cholesterol by downregulating Niemann–Pick C1-like 1 (*NPC1L1*) via upregulating the liver X receptor (*LXR*; Ishimwe et al., 2015).

Enterococcus spp. are common lactic acid bacterial species in humans, animals, and fermented foods (Franz et al., 2011), and exhibit cholesterol-lowering effect in vitro (De Rodas et al., 1996; Agerholm-Larsen et al., 2000). However, few studies have demonstrated the cholesterol-lowering effect of *Enterococcus* spp. in vivo (Hlivak et al., 2005; Zhang et al., 2017). Moreover, the mechanism underlying the cholesterol lowering property of the strain remains unclear.

In our previous study, *E. faecium* WEFA23 isolated from infants (Zhang et al., 2016) presented high activity for synthesis of bile salt hydrolase (**BSH**; Zhang et al., 2017), which can catalyze conjugated bile acids into nonconjugated bile acids (Pavlović et al., 2012). Another study showed the effect of lowering serum cholesterol in rats fed a high-fat diet by *E. faecium* WEFA23 (Zhang et al., 2017). However, the molecular mechanisms of lowering serum cholesterol in vivo are unknown yet.

In this study, critical factors (e.g., relevant genes and gut microbiota) and their relevance in the metabolism of cholesterol in vivo were systematically analyzed. The decrease in cholesterol level from the aspects of synthesis, decomposition, and transportation in rats fed a high-fat diet treated with *E. faecium* WEFA23 was also investigated. The whole study was performed as follows: establishment of a model of hyperlipidemia, analyzing the TMAO level by ultra HPLC/MS/MS and cholesterol metabolism-related gene transcript level by real-time quantitative PCR as well as the bioinformation of the gut microbiota composition by 16S rRNA gene amplicon sequencing.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Enterococcus faecium WEFA23 was grown on brain heart infusion agar (Oxoid, Basingstoke, Hampshire, UK) and incubated anaerobically at 37°C for 16 h.

Animals and Experimental Design

The protocol for the animal experiment was approved by Nanchang University animal ethical committee, all the ethical requirements for conducting the experiment were met (approval number 0064257). Fifteen 5-wk-old male Sprague Dawley rats were acclimatized for 1 wk and randomly divided into 3 groups: (1) **ND** group, fed with a standard chow diet and PBS (1.0 mL/d); (2) **HFD** group, which received a high-fat diet and PBS (1.0 mL/d); and (3) **WEFA23** group, which received a high-fat diet and *E. faecium* WEFA23 (5.0×10^9 cfu/mL in PBS, 1 mL/d). The composition of the high-fat diet was a normal diet (66.5%, wt/wt), lard (10.0%), sucrose (20.0%), cholesterol (2.5%), and sodium cholate (1.0%). The weight, food intake, and water consumption of the rats were measured once a week. After intervention for 35 d, the rats were fasted overnight and anesthetized using diethyl ether. Blood was collected from the orbit. Serum was separated from blood samples by centrifugation at 4°C and 4,000 × *g* for 20 min then stored at −80°C for use. All the rats were killed by cervical dislocation and autopsied in a sterile environment. The liver and feces in the colon and cecum were collected and stored at −80°C for analysis.

Cholesterol Content of Serum and Feces

The concentrations of serum lipids, including total cholesterol (**TC**), triacylglycerols (**TG**), high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**), were measured by corresponding kits (Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Total RNA Isolation and Quantitative Reverse-Transcription PCR Analyses

Primers (Supplemental Table S1; https://doi.org/10.3168/jds.2017-13713) for quantitative reverse-transcription PCR were designed then synthesized by