***Enterococcus hirae* WEHI01 isolated from a healthy Chinese infant ameliorates the symptoms of type 2 diabetes by elevating the abundance of *Lactobacillales* in rats**

Min Wei,¹ Enyu Gu,¹ Jie Luo,¹ Zhihong Zhang,¹ Di Xu,¹ Xueying Tao,¹ Nagendra P. Shah,² and Hua Wei^{1*}

¹State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

²Food and Nutritional Science, School of Biological Science, University of Hong Kong, Pokfulam Road, Hong Kong, China

ABSTRACT

Enterococcus hirae WEHI01 is a potential probiotic strain isolated from a healthy Chinese infant. This strain has previously been characterized as having cholesterol-lowering potential and good dairy fermentation performance. In this study, we used rat models with obesity and type 2 diabetes mellitus (T2DM) induced by a high fat and sucrose diet and low-dose streptozotocin, respectively, and we evaluated the effect of *E. hirae* WEHI01 on glycolipid metabolism, glycolipid-related gene expression, organ histopathology, and intestinal flora changes in the 2 models. Our results showed that administration of 5.0×10^9 cfu of *E. hirae* WEHI01 for 4 wk decreased serum lipid levels and regulated glycolipid metabolism in the liver of obese rats. Following continuous administration of the same concentration of *E. hirae* WEHI01 to a T2DM rat model for another 5 wk, *E. hirae* WEHI01 improved glucose tolerance, recovered body weight loss, and led to significant decreases in tumor necrosis factor- α , IL-6, IL-10, and total bile acid in serum. We also found that *E. hirae* WEHI01 restored the morphology of the pancreas, kidney, and liver, and changed the composition of the gut microbiota (i.e., decreased the Shannon index, increased the Simpson index, and substantially increased the abundance of *Lactobacillales*). Combining the results for the obese model and the T2DM model, we speculated that beneficial effects of *E. hirae* WEHI01 on T2DM could be due to (1) a significant increase in *PPARA* expression and a tendency for increased *CYP7A1* expression in the liver of obese rats, promoting the conversion of cholesterol into bile acid and reducing serum total bile acid levels in T2DM model rats; or (2) a change in gut microbial diversity, especially elevated *Lactobacillales* abundance, which reduced the total bile acid in T2DM model rats.

These results demonstrated that *E. hirae* WEHI01 has the potential to ameliorate type 2 diabetes in rats and provide a promising rationale for further research into the prevention and treatment of T2DM.

Key words: type 2 diabetes, *Enterococcus hirae* WEHI01, gut microbiota, bile acid

INTRODUCTION

Diabetes is one of the leading causes of death around the world. The global population of people with diabetes is predicted to increase to 693 million by 2045 (Cho et al., 2018), and more than 90% of people with diabetes have type 2 diabetes mellitus (T2DM; Intagliata and Koch, 2007), which is characterized by hyperglycemia as a result of insulin resistance and relative insulin deficiency (Fujimoto, 2000). Chronic hyperglycemia can easily lead to other syndromes, such as retinopathy, nephropathy, neuropathy, and cardiovascular complications (Chen et al., 2014b). High blood glucose levels can be managed with several antidiabetic agents, including biguanides, sulfonylureas, meglitinides, thiazolidinediones, and α -glucosidase inhibitors. However, these treatments come with adverse effects such as flatulence, abdominal discomfort, or diarrhea (Phung et al., 2012). Natural substances with α -glucosidase inhibitory activity in plants (Ashwini et al., 2015), vegetables (Sarkar et al., 2015), traditional medicines (Ren et al., 2015), and probiotics (Li et al., 2016a) are often effectively used to treat T2DM.

Probiotics from humans, animals, dairy products, and fermented foods are regarded as potential biotherapeutics for T2DM (Panwar et al., 2013), especially isolates from healthy people and those from natural dairy products. Oral administration of *Lactobacillus paracasei* TD062 in mice ameliorated the metabolism of lipids and glucose and reduced oxidative stress because of its α -glucosidase inhibitory activity (Dang et al., 2018). Treatment with IL-10-inducing lactic acid bacteria alleviated metabolic disorders by suppressing chronic inflammation (Toshimitsu et al., 2016). *Lacto-*

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*Corresponding author: weihua@ncu.edu.cn

bacillus plantarum strain Ln4 showed inhibitory effects on obesity and insulin resistance by adipogenesis and glucose uptake in 3T3-L1 mice fed a high-fat diet (Lee et al., 2018). Several strains of lactic acid bacteria have been shown to attenuate the adverse effects of T2DM and obesity by preventing metabolic disorders and ameliorating insulin resistance by stimulating the immune system (Takemura et al., 2010; Xiao et al., 2014).

Enterococcus has shown a wide range of beneficial properties in vitro and in vivo (Hlivak et al., 2005; Cavallini et al., 2009; Guo et al., 2016). As an important species of nonstarter lactic acid bacteria in dairy products, it produces aromatic volatile compounds and bacteriocins against food spoilage or pathogenic bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium* spp.) in a variety of cheeses during ripening (Giraffa, 2003). To date, most studies have focused on non-toxigenic *E. faecium* (Cavallini et al., 2009; Bhat and Bajaj, 2018; Hu et al., 2019) and *E. faecalis* (Mishra et al., 2019). Few studies have focused on *E. hirae* (Daillère et al., 2016), which has been isolated from fermented food (Sheoran and Tiwari, 2019), Brazilian artisanal cheese (Cavicchioli et al., 2019), and raw cow milk (Bouymajane et al., 2018), but seldom from humans. Daillère et al. (2016) observed that *E. hirae* and *Barnesiella intestinihominis* facilitated a cyclophosphamide-induced therapeutic immunomodulatory effect. Sheoran and Tiwari (2019) reported that bacteriocins from *E. hirae* LD3 and *L. plantarum* LD4 inhibited the growth of *S. aureus* in milk. However, to the best of our knowledge, the effect of *E. hirae* in chronic diseases, particularly T2DM, has not yet been reported.

In a previous study, we isolated *E. hirae* WEHI01 from a healthy Chinese infant (Zhang et al., 2017a). This strain is used in the fermentation of yogurt and has a wide range of beneficial properties, including cholesterol-lowering effects, high glycodeoxycholic acid-hydrolase activity, and strong adhesion capacity to Caco-2 cells (Zhang et al., 2017a). In this study, we further investigated the biological effects of *E. hirae* WEHI01, particularly its protective effect against T2DM in rat models of pre-insulin resistance induced by a high fat and sucrose (HFS) diet and T2DM induced by low-dose-streptozotocin and an HFS diet.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures involving animals were approved by the Animal Care Review Committee (approval number 0064257) of Nanchang University, Jiangxi, China, and

adhered to the institutional animal care committee guidelines.

Male Sprague-Dawley rats (5 wk old, 140–160 g) were acclimated for 1 wk using a standard diet and fresh water under a 12 h light/dark cycle at a temperature of $23 \pm 1^\circ\text{C}$ and a humidity of $54 \pm 2\%$. The experimental model is shown in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-17185>). All rats were randomly assigned to 1 of 3 groups (9 rats per group): fed a normal chow diet (**NDm**); fed a HFS diet consisting of 10% lard, 10% sucrose, 55% corn starch, 20% casein, 1% vitamin mix, and 4% bone powder (**Dm**); WEHI01, fed the same diet as the Dm group plus *E. hirae* WEHI01 (5.0×10^9 cfu/rat per day). After 4 wk, 3 rats from each group (NDm, Dm, and WEHI01) were assigned as obesity models (normal chow, HFS, and HFS-WEHI01 groups, respectively). The remaining 6 rats from the NDm group were given PBS, and the remaining rats from the Dm and WEHI01 groups were given a tail vein injection of streptozotocin (dissolved in citrate buffer to pH 4.5, 30 mg/kg of BW; Sigma, St. Louis, MO) to establish a T2DM model. We measured BW and 2-h postprandial blood glucose in all rats once per week.

Measurement of Blood Glucose Levels

To measure blood glucose levels, we conducted oral glucose tolerance tests (OGTT). Rats were fasted for 12 h and then administered glucose 2 g/kg BW orally. Blood glucose levels were measured at 30, 60, 90, and 120 min using a portable Contour blood glucose monitor (Ascensia Diabetes Care Shanghai Co. Ltd., Shanghai, China).

Measurement of Blood Cytokines and Lipids

On the final days of wk 4 and 9, animals were fasted for 12 h and anesthetized, and we collected blood samples from the orbital venous plexus and centrifuged them at $3,000 \times g$ at 4°C for 15 min to obtain the serum. We measured serum levels of tumor necrosis factor α (**TNF- α**), IL-6, and IL-10 using ELISA kits (YuanYe, Shanghai, China). Serum lipid concentrations, including total cholesterol (**TC**), triglycerides, high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**), were evaluated at the Nanchang Qianmai Medical Laboratory Co., Ltd. (Jiangxi, China).

Histopathological Evaluation

At the end of the experiment, rat kidney, liver, and pancreas tissues were dissected, fixed with 4% para-

formaldehyde solution, extracted, dehydrated, and embedded in paraffin. Then, 4-mm-thick samples were prepared, stained with hematoxylin and eosin, observed (Eclipse Ci, NIS_F_Ver46000_64bit_E; Nikon, Tokyo, Japan), and photographed under an optical microscope (Digital sight DS-FI2; Nikon).

Total RNA Isolation and Quantitative RT-PCR Analysis

Total RNA was extracted from the liver using the Takara MiniBEST Universal RNA Extraction Kit (Takara, Shiga, Japan), and cDNA was obtained by reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Vilnius, Lithuania). The qPCR reactions were performed using a 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq (Takara, Kusatsu, Japan). We used the housekeeping gene *ACTB* for normalization. The sequences of primers (Invitrogen China, Ltd., Beijing, China) used for real-time quantitative PCR are shown in Table 1. Data analysis was carried out by using the $2^{-\Delta\Delta C_t}$ method (Norris et al., 2017), and all gene transcript levels were standardized using the basic value of the HFS group.

High-Throughput Sequencing and Bioinformatics Analysis

The contents of the rats' ceca were collected and stored at -80°C for use. Total DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and used for 16S ribosomal (r)RNA gene amplicon sequencing on the Illumina MiSeq platform (BGI TechSolutions Co. Ltd., Shenzhen, China; Caporaso et al., 2012). The primers targeting the hypervariable V4 region of 16S rRNA gene were designed as described by Pan et al., (2016).

The Quantitative Insights into Microbial Ecology pipeline was used for bioinformatics analysis of the sequencing data. We obtained clean reads from the raw data after removing contamination and low-quality data. Briefly, raw reads were blasted with the National Center for Biotechnology Information Sequence Read Archive database (<https://www.ncbi.nlm.nih.gov/>). High-quality clean reads were stitched into tags 252 bp long using Fast Length Adjustment of Short reads (FLASH, version 1.2.11; <http://www.cbcb.umd.edu/software/flash>). Clean reads were filtered from raw reads to eliminate low-quality reads, and then high-quality paired-end reads were combined with tags using FLASH. Sequences with 97% similarity were clustered to operational taxonomic units (OTU) at 97% sequence similarity using UPARSE ([**Table 1.** Gene primers used in the study](http://www</p>
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Primer ¹	Oligonucleotide sequence (5'-3')
Actin F	CACCATGTACCCAGGCATTG
Actin R	ACAGTCCGCCTAGAAGCATT
<i>FMO3</i> F	CTCAGGTTGTGACATCGCTG
<i>FMO3</i> R	TGATCACCACCATGTCCCAA
<i>TNFA</i> F	GGGCAGGTCTACTTTGGAGTCATTG
<i>TNFA</i> R	GGGCTCTGAGGAGTAGACGATAAAG
<i>ChREBP</i> F	CAGATGCCGGGACATGTTTGA
<i>ChREBP</i> R	AATAAAGTCCGGATGAGGATGCT
<i>CYP7A1</i> F	GATCCTGATGCTCTCCTGCT
<i>CYP7A1</i> R	CGCCAAGTGACATCATCCAG
<i>CRTC2</i> F	TGCCTACTGACCAGCGATTG
<i>CRTC2</i> R	AATTGCTCCGATGAGGATGCT
<i>GKRP</i> F	AAACGATATCAGCATGTGATCGA
<i>GKRP</i> R	GCCGCTTCATACCCTGACA
<i>LDLR</i> F	TACTCTCTGGTGACCGAGGA
<i>LDLR</i> R	GCTGGAGATGGAGTGGAGTT
<i>PPARA</i> F	TCAAACCTGGGTTCCATGAT
<i>PPARA</i> R	TGAACAAAGACGGGATG

¹F = forward; R = reverse.

[.drive5.com/uparse/](https://drive5.com/uparse/)). We used Ribosomal Database Project Classifier version 2.2 (Wang et al., 2007) to analyze the phylogenetic affiliation of each 16S rRNA gene sequence. Finally, we analyzed differences using α -diversity and β -diversity. The α -diversity analysis included indexes of Shannon diversity and Simpson diversity. The β -diversity analysis mainly explored the difference in microbial flora structure between samples, generally using principal component analysis (PCA). Principal components were developing using OTU abundance information, the relative abundance of each OTU in each sample was calculated, and then the PCA of OTU was conducted using the relative abundance values and ade4 (<https://pbil.univ-lyon1.fr/CRAN/web/packages/ade4/index.html>) of software R (v. 3.1.1; <https://cran.rstudio.com/>).

Statistical Analysis

All experimental data were shown as mean $\pm$ standard error of the mean (SEM). Multiple groups were tested using 1-way ANOVA and analyzed using 2-tailed unpaired Student's *t* test. We considered $P < 0.05$ as statistically significant. All statistical analyses were carried out using GraphPad Prism v5.01 (Graph Pad Software Inc., La Jolla, CA).

RESULTS AND DISCUSSION

Effects of *E. hirae* WEHI01 on BW and Blood Glucose in Rats

The intervention protocol investigating *E. hirae* WEHI01 in obesity and T2DM is summarized in Supplemental Figure S2 (<https://doi.org/10.3168/jds.2019>

Figure 1. Effects of *Enterococcus hirae* WEHI01 on (A) BW; (B) oral glucose tolerance test (OGTT); (C) area under the curve of glucose (AUC_{glucose}); and (D) 2-h postprandial blood glucose. Data are presented as mean $\pm$ SEM. ## $P < 0.01$; ### $P < 0.001$ vs. NDm group; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. Dm group. Dm = rats with diabetes mellitus; NDm = rats without diabetes mellitus; WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01.

-17185), including indexes of BW and blood glucose levels. Streptozotocin has been widely used to induce a model of diabetes that is prone to weight loss due to abnormal carbohydrate and lipid metabolism, and increased catabolism of protein. As shown in Figure 1, before injection of streptozotocin, the Dm and WEHI01 groups showed a similar tendency toward low BW compared to the NDm group; after injection of streptozotocin, the WEHI01 group demonstrated a significant increase in BW at 8 and 9 wk compared with the Dm group ($P < 0.05$). In contrast, the Dm group maintained a significantly low BW compared to the NDm group. Administration of *E. hirae* WEHI01 led to

recovered BW, indicating that *E. hirae* WEHI01 might have a protective effect in terms of BW in T2DM. Still, it is important to note that in rats who received *E. hirae* WEHI01, BW was lower than in rats in the NDm group. This finding was consistent with a previous study (Chen et al., 2014a) showing that administration of *L. casei* CCFM0412 prevented excessive decreases in BW in mice with T2DM, but their BW was still lower than that of the control mice.

Glucose intolerance is a well-established diagnostic criterion for insulin resistance in T2DM. Glucose tolerance was impaired in the Dm group after streptozotocin injection. As shown in Figure 1B, administration

of *E. hirae* WEHI01 led to OGTT levels at 60 and 90 min close to those of the NDm group, a significant decrease compared to the Dm group. Furthermore, taking the total area under the curve of glucose over 120 min as the quantitative result of the OGTT, the WEHI01 group showed a significant improvement in oral glucose tolerance (Figure 1C, $P < 0.05$). Correspondingly, administration of *E. hirae* WEHI01 led lower postprandial blood glucose levels than the Dm group from 5 wk, levels similar to the NDm group. These results suggest that *E. hirae* WEHI01 can improve glucose tolerance in rats with T2DM to some extent.

Influence of *E. hirae* WEHI01 on Serum Lipid Levels in Rats

As shown in Figure 2, TC and LDL-C levels in the HFS group were significantly higher than those in the normal chow group. However, TC, LDL-C, and HDL-C levels in the HFS-WEHI01 group were significantly lower than those of the HFS group ($P < 0.05$). In addition to improving blood glucose status, *E. hirae* WEHI01 decreased serum TC, LDL-C, and HDL-C levels in obese rats, indicating the potential lipid-lowering activity of *E. hirae* WEHI01. Low-density lipoprotein

cholesterol is the most dangerous factor in blood lipids because oxidized LDL-C is transported to the arterial wall to form atherosclerosis; the decreases in LDL-C we found suggest that *E. hirae* WEHI01 could play a role in alleviating atherosclerosis.

Relative Expression of Genes Related to Inflammation, Blood Glucose, and Lipid Metabolism in Rat Liver

Serum lipid and inflammatory levels may be partially affected by the expression of relevant genes in liver, such as *CYP7A1*, *HMG-CoAS*, *SCD1*, *FAS*, *SCARB1*, *LDLR*, and *FMO3* (Huang et al., 2018). After lowering of serum lipid levels with *E. hirae* WEHI01, we also examined the expression of *TNFA*, *CYP7A1*, *GKRP*, *ChREBP*, *CRTC2*, *PPARA*, *LDLR*, and *FMO3*. As shown in Figure 3, transcription levels of *CYP7A1* tended to be increased in the liver ($P > 0.05$), but levels of *PPARA* and *LDLR* increased significantly ($P < 0.01$ and $P < 0.05$, respectively), and levels of *TNFA*, *GKRP*, *CRTC2*, and *FMO3* decreased significantly ($P < 0.05$). Based on our findings and the results of previous studies, we propose the mechanism glycolipid metabolism regulation with *E. hirae* WEHI01 in the

Figure 2. Effect of *Enterococcus hirae* WEHI01 on (A) serum total cholesterol (TC); (B) serum low-density lipoprotein cholesterol (LDL-C); and (C) serum high-density lipoprotein cholesterol (HDL-C). HFS = high fat and sucrose diet; HFS-WEHI01 = high fat and sucrose diet combined with *E. hirae* WEHI01; NC = normal chow diet. Values are expressed as mean $\pm$ SEM ($n = 3$ per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 3. Relative expression of genes related to inflammation, blood glucose, and lipid metabolism in the liver of rats by quantitative PCR analysis using *GAPDH* as the internal reference. Relative expression indicates the group fed a high fat and sucrose diet combined with *Enterococcus hirae* WEHI01 (HFS-WEHI01) compared with the group fed a high fat and sucrose diet only (HFS). Values are expressed as mean $\pm$ SEM (n = 3 per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

liver of obese mice as follows: *E. hirae* WEHI01 significantly upregulated transcription levels of *PPARA* ($P < 0.01$), accelerating the metabolism of total bile acids and significantly downregulating *ChREBP* and *CRTC2* ($P < 0.001$) to promote sugar metabolism and

reduce fat accumulation. For cholesterol metabolism, downregulation of *FMO3* ($P < 0.001$) coupled with an increase in *CYP7A1* might contribute to a reduction in yield of *TMAO*, alleviating the negative feedback regulation of cholesterol 7-hydroxylase to accelerate cholesterol decomposition, as suggested by our previous study (Huang et al., 2018).

Influence of *E. hirae* WEHI01 on Serum Inflammatory Cytokines in Rats with T2DM

Type 2 diabetes mellitus is a common complication in obesity, characterized by low-grade inflammation (Burdick et al., 2011) and higher levels of proinflammatory cytokines in serum (Tian et al., 2016). Consistent with a previous report (Wang et al., 2017), we observed that *E. hirae* WEHI01 significantly reduced serum levels of IL-6 ($P < 0.05$), IL-10 ($P < 0.05$), and TNF- α ($P < 0.01$) in the WEHI01 group compared with the Dm group (Figure 4). Interleukin-6 acts on islets during onset of T2DM, and high concentrations of IL-6 inhibit insulin secretion (Rabinovitch, 1998). Tumor necrosis factor α interacts with other cytokines to affect insulin secretion (Liu et al., 2016), and plays an important role in the development of T2DM (Mishima et al., 2001). In contrast, IL-10 plays a vital role in the regulation of immune response and inflammatory response (Toshimitsu et al., 2016), and elevated levels in people with diabetes may be a precursor to hypertension (Zietz et al., 2000). Consistent with evidence that *L. casei* CCFM419 reduces inflammatory cytokines levels in mice with diabetes (Li et al., 2016b), administration

Figure 4. Effect of *Enterococcus hirae* WEHI01 on serum (A) tumor necrosis factor α (TNF- α); (B) IL-6; and (C) IL-10 in each group. Values are expressed as mean $\pm$ SEM (n = 5 per group). * $P < 0.05$; ** $P < 0.01$. Dm = rats with diabetes mellitus; NDM = rats without diabetes mellitus; WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01.

of *E. hirae* WEHI01 exhibited the potential to alleviate inflammation in rats with T2DM.

Effect of *E. hirae* WEHI01 on Histological Changes in Rats with T2DM

To confirm improvements in the syndromes of T2DM with *E. hirae* WEHI01, we performed histological analysis of the pancreas (Figure 5A), kidney (Figure 5B), and liver (Figure 5C) of the rats in the study. The NDm group showed normal proportions and normal structure of the islets of Langerhans. Acinar cells stained strongly and were arranged closely. We observed a remarkable difference between the Dm and NDm groups with respect to the shape of the pancreas and the number of islets of Langerhans, suggesting necrosis of some acinar cells around the pancreatic islet. Importantly,

oral administration of *E. hirae* WEHI01 led to partial restoration of islet cells and decreased β -cell necrosis and vacuolation (Figure 5A). This finding was consistent with the results of a previous study (Li et al., 2016b), which demonstrated that administration of *L. casei* CCFM419 and dead *L. casei* CCFM419 protected against pancreatic impairment, and the extent of cellular repair was greater with *L. casei* CCFM419 than with dead *L. casei* CCFM419.

Similarly, destruction of renal tissue (e.g., more renal tubular epithelial cell necrosis, deep staining or fragmentation of the nucleus pyknosis and glomerulus mild congestion) in the Dm group was reversed by *E. hirae* WEHI01 treatments in the WEHI01 group (Figure 5B); namely, some renal tubular epithelial cells were shed into the lumen, and this renal injury was alleviated.

Figure 5. Effect of *Enterococcus hirae* WEHI01 on histology (200 $\times$) of the (A) pancreas, (B) kidney, and (C) liver in rats in the NDm, Dm, and WEHI01 groups. Hematoxylin and eosin staining. Dm = rats with diabetes mellitus; NDm = rats without diabetes mellitus; WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01.

In the liver (Figure 5C), hepatocyte edema and hepatic sinus congestion were greatly reduced in liver tissue after the administration of *E. hirae* WEHI01.

Effect of *E. hirae* WEHI01 on Total Bile Acid Levels in Rats

An increase in the expression of *PPARA* and *CYP7A1* in the obese model (Figure 3) might have promoted the conversion of cholesterol into bile acid, reducing cholesterol content; as shown in Figure 6, *E. hirae* WEHI01 significantly reduced serum total bile acid levels ($P < 0.001$) compared to the Dm group. This finding was supported by a similar study (Jiang et al., 2018). High bile acid levels are risk factors for T2DM (Sheoran and Tiwari, 2019), and their regulation in vivo may be a potential target for the treatment of metabolic diseases (Thomas et al., 2008).

Effect of *E. hirae* WEHI01 on Intestinal Microbiota of Rats

Prawitt et al. (2011) proposed that the pathogenesis of T2DM might be due to bile-acid-mediated control, which in turn is related to changes in microflora. To assess whether *E. hirae* WEHI01 changed the gut microbial community in rats with T2DM, we sequenced the 16S rRNA genes from variable regions V4 of feces in rats using Illumina HiSeq/MiSeq platforms. As shown in Figure 7A, the intestinal microbiota among the 3 study groups were obviously different. The distance of 2 data points in the 2-dimensional PCA plot is a visual representation of the similarity between different microbiota compositions (Tian et al., 2016). The Dm group had a distinct microbiota composition that was clustered differently from the other 2 groups. As shown in Figure 7B, the community richness of the Dm group measured by α diversity was increased (Shannon index) and decreased (Simpson index) at the OTU level compared with the NDm group, and the WEHI01 group showed a lower tendency toward an increase in diversity of intestinal microbiota.

To profile specific changes in the gut microbiota, we also analyzed the relative abundance of predominant taxa in the 3 groups. Figure 8 shows a detailed overview of the cecal microbiota composition at the level of phylum, class, order, and family. Compared with the Dm group, we found a nonsignificant difference in dominant phyla, including *Firmicutes* and *Bacteroidetes*, for the WEHI01 group ($P > 0.05$), but the abundance of *Actinobacteria* and *Verrucomicrobia* were significantly decreased ($P < 0.01$ and $P < 0.05$, respectively). At

the class level, *Bacilli* and *Verrucomicrobiae* were significantly increased and decreased in the WEHI01 group, respectively ($P < 0.05$). At the order level, the abundance of *Lactobacillales* was significantly increased in the WEHI01 group ($P < 0.05$). At the family level, the abundance of *Lactobacillaceae* was significantly increased, and that of *Ruminococcaceae* was significantly decreased for WEHI01 group ($P < 0.05$).

Gut microbiota play an important role in the development of T2DM (Cani et al., 2012). To the best of our knowledge, the phyla that account for the vast majority of all gut microbes include the gram-negative *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia*, and the gram-positive *Firmicutes* and *Actinobacteria* (Kootte et al., 2012). Type 2 diabetes mellitus is characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens (Qin et al., 2012). For ex-

Figure 6. Effect of *Enterococcus hirae* WEHI01 on total bile acid (TBA) levels in the serum of each group. Values are expressed as mean $\pm$ SEM ($n = 5$ per group). *** $P < 0.001$. Dm = rats with diabetes mellitus; NDm = rats without diabetes mellitus; WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01.

ample, several studies have suggested that obesity and T2DM are associated with an increase in *Firmicutes* and a lower abundance of *Bacteroidetes* (Turnbaugh et al., 2009; Zhang et al., 2013). The antidiabetic ef-

fect of *E. hirae* WEHI01 may be partially mediated by regulating the composition of gut microbes. In the current study, *E. hirae* WEHI01 could have improved T2DM by changing the proportion of *Bacteroidetes* to

Figure 7. Effect of *Enterococcus hirae* WEHI01 on intestinal microbiota. (A) Principal component analysis (PCA), and (B) Shannon diversity and Simpson diversity. Boxplots were used to display the differences in α -diversity among groups. The 5 horizontal lines, from bottom to top, are the minimum value, the first quartile, median, the third quartile, and the maximum value, respectively. Circles indicate outliers. Dm = rats with diabetes mellitus; NDm = rats without diabetes mellitus; WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01.

Figure 8. Effect of *Enterococcus hirae* WEHI01 on relative abundance at phylum, class, order, and family levels of the intestinal microbiota. * $P < 0.05$; ** $P < 0.01$ compared with Dm group. Dm = rats with diabetes mellitus (M1H–M5H on x-axis of figures); NDm = rats without diabetes mellitus (C1H–C5H); WEHI01 = rats with diabetes mellitus and supplemented with *E. hirae* WEHI01 (011H–015H). Error bars indicate SD.

Firmicutes and increasing the abundance of *Lactobacillales*. Similarly, *L. casei* CCFM419 improved T2DM by changing the ratio of *Bacteroidetes* to *Firmicutes* and enriching the abundance of *Lactobacillales* (Zhang et al., 2013). Moreover, information in the literature varies with respect to the benefits of *Lactobacillaceae* (such as improving T2DM, anti-inflammatory effects, and reduced BW; Yadav et al., 2007; Kang et al., 2010), by producing short-chain fatty acids, which could be helpful for energy metabolism, maintaining intestinal integrity, preventing endotoxemia, and intestinal gluconeogenesis (Zhang et al., 2017b).

Some publications have reported on the administration of non-*E. hirae* strains. For instance, *L. casei* reduced obesity in rats with obesity and T2DM (Zhang et al., 2014). To the best of our knowledge, we have reported for the first time that *E. hirae* from an infant improved obesity and T2DM in rats.

CONCLUSIONS

Our study demonstrated that the administration of *E. hirae* WEHI01 could lead to recovery of BW loss and improvements in serum lipids, regulate glycolipid metabolism in the liver associated with HFS-induced obesity, and improve glucose tolerance in a T2DM rat model. It also reduced levels of inflammation and bile acids in serum, and restored the morphology of the pancreas, kidney, and liver. Moreover, *E. hirae* WEHI01 dramatically modified the gut microbiota and increased the abundance of *Lactobacillales*. Our findings suggest that *E. hirae* WEHI01 isolated from a Chinese healthy infant has potential as a therapeutic probiotic for treating obesity and even T2DM by altering the gut microbiome.

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ORCIDS

Nagendra P. Shah <https://orcid.org/0000-0003-4746-3232>
Hua Wei <https://orcid.org/0000-0003-4341-8290>