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Integration of genomic and proteomic data to identify candidate genes in HT-29 cells after incubation with *Bifidobacterium bifidum* ATCC 29521

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ABSTRACT

As the predominant group inhabiting the human gastrointestinal tract, bifidobacteria play a vital role in human nutrition, therapeutics, and health by shaping and maintaining the gut ecosystem, reducing blood cholesterol, and promoting the supply of nutrients. The interaction between bacterial cells and human intestinal epithelial cell lines has been studied for decades in an attempt to understand the mechanisms of action. These studies, however, have been limited by lack of genomic and proteomic database to aid in achieving comprehensive understanding of these mechanisms at molecular levels. Microarray data (GSE: 74119) coupled with isobaric tags for relative and absolute quantitation (iTRAQ) were performed to detect differentially expressed genes and proteins in HT-29 cells after incubation with *Bifidobacterium bifidum*. Real-time quantitative PCR, gene ontology, and Kyoto Encyclopedia of Genes and Genomes analyses were further conducted for mRNA validation, functional annotation, and pathway identification, respectively. According to the results of microarray, 1,717 differentially expressed genes, including 1,693 upregulated and 24 downregulated genes, were selected and classified by the gene ontology database. The iTRAQ analysis identified 43 differentially expressed proteins, where 29 proteins were upregulated and 14 proteins were downregulated. Eighty-two candidate genes showing consistent differences with microarray and iTRAQ were further validated in HT-29 and Caco-2 cells by real-time quantitative PCR. Nine of the top genes showing interesting results with high confidence were further investigated in vivo in mice intestine samples. Integration of genomic and proteomic data provides an approach to identify candidate genes that are more likely to function in ubiquitin-mediated proteolysis, positive regulation of apoptosis, membrane proteins, and transferase catalysis. These findings might contribute to our understanding of molecular mechanisms regulating the interaction between probiotics and intestinal epithelial cell lines.

Key words: HT-29 cells, *Bifidobacterium bifidum*, microarray, iTRAQ

INTRODUCTION

The microbial inhabitants of the gastrointestinal tract constitute the most complex ecosystem in the human body and form a closely integrated unit with host developmental processes (Hooper and Gordon, 2001; McFall-Ngai, 2002; Phillips, 2006; Candela et al., 2008; Yin, 2013). As the predominant group normally inhabiting the gastrointestinal tract (Modler, 1994; Guarner and Malagelada, 2003), *Bifidobacterium* are the most widely used probiotic bacteria (Macpherson and Harris, 2004), representing about 8 to 10% of the normal adult fecal flora and reaching concentrations of 10^{10} cfu/g in the intestine (Tannock, 1995; Gibson et al., 2004). During the past 2 decades, accumulating data have shown that *Bifidobacterium* play a vital role in human nutrition, as well as therapeutic and health benefits by shaping and maintaining the gut ecosystem (Chow and Lee, 2008), reducing blood cholesterol (Modler, 1994), preventing diarrheas (Salminen et al., 1996), increasing immunity (Gill et al., 2000; Turroni et al., 2014), and promoting the supply of nutrients. Consequently, *Bifidobacterium* have become an increasingly interesting bacteria for probiotic applications in pharmaceutical and dairy products (Candela et al., 2005).

During the last few years, the use of human intestinal epithelial cell line (**IEC**) HT-29 has increased dramatically in many research fields including the pharmaceutical sciences (Artursson et al., 2001). The interaction between *Bifidobacterium* and IEC have been studied for decades to understand the mechanisms of action, such as adhesion and colonization properties (Candela et al.,

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2005; Guglielmetti et al., 2008; Qin et al., 2010; Kainulainen et al., 2013; Turroni et al., 2014), antimicrobial properties (Gagnon et al., 2004; Zhang et al., 2010), and effect on expression profiles (Riedel et al., 2006) after interaction of *Bifidobacterium* strains with human IEC. Candela et al. (2008) showed that *Bifidobacterium* can modulate IL-8 production (Candela et al., 2008), which is an acute inflammatory response; however, the mechanism behind this is still not clearly. Therefore, the elucidation of function represents a significant step toward understanding those molecular mechanisms.

The genome of *Bifidobacterium bifidum* has recently been sequenced and annotated (Turroni et al., 2010; Ferrario et al., 2015), revealing metabolic pathways for host-derived glycan foraging (Turroni et al., 2010); however, there are gaps in information about bifidobacterial physiology and ecology. The molecular mechanisms that allow bifidobacteria to interact with the host are not confined to the regulation of immune-related factors, the integrity of the intestinal mucosal barrier, and competition with binding sites of harmful bacteria. As only a few molecules have been described thus far, more investigations are necessary to elucidate the mechanisms between bifidobacteria and their host, such as the protein expressed by transaldolase gene (O'Connell Motherway et al., 2011; González-Rodríguez et al., 2012).

Microarray technology offers a high-throughput approach to determine the expression of thousands of positional candidate genes in samples (Buratti et al., 2013; Algahtani et al., 2014), and provides expression levels as absolute values computed as the number of transcripts observed for individual genes. Integration of gene expression information and pathway information together with functional enrichment information will help prioritize positional candidate genes (Hornshøj et al., 2009). However, the regulation at the mRNA level does not necessarily correlate with changes at the protein level (Pawar et al., 2013), and microarrays do not always estimate absolute expression levels accurately (Held et al., 2006; Fu et al., 2009). Hence, proteomics present another powerful tool for global investigation of a great multitude of differentially expressed proteins, and a complementary method to study steady-state gene expression and perturbation-induced changes. An isobaric tags for relative and absolute quantitation (**iTRAQ**)-based quantitative proteomics approach can achieve a greater depth of proteome coverage, including a broader range of protein classes, and has the potential to reveal underlying molecular mechanisms (Yun et al., 2011; Sun et al., 2012; Tse et al., 2013; Zhang et al., 2014).

To fully understand the function of probiotics, enhancing our comprehensive understanding of bifidobacteria and the role they play in the intestinal ecosystem, we analyzed genomic and proteomic data in enterocyte-like HT-29 cells after interaction with *B. bifidum* by coupling microarray with the iTRAQ-labeling technique. These technologies also provided information on the role of cells on *B. bifidum* influence in IEC, which is still poorly understood. Differentially expressed genes and proteins were picked out in accordance with the ratio of standard and real-time PCR for further validation. Gene ontology (**GO**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) analyses were also conducted for mRNA validation as well as functional annotation and pathway identification, respectively.

MATERIALS AND METHODS

Cells and Animals

Nontransformed human Caucasian colon adenocarcinoma cells HT-29 (ATCC: HTB-38) and Caco-2 (ATCC: HTB 37) were routinely cultured in 75-cm^2 flasks and maintained in Dulbecco's Modified Eagle's medium (Hyclone Laboratories Inc., Logan, UT) containing 25 m*M* glucose supplemented with inactivated 10% fetal bovine serum (Hangzhou Sijiqing Bioengineering Material Co. Ltd., Zhejiang, China), 1% sodium pyruvate, and antibiotics (penicillin 100 IU/mL and streptomycin 100 mg/mL) in a 5% CO_2 atmosphere with 90% relative humidity at 37°C. Cells were subcultured after 72 h to 80% confluence. Cells from passage number 37 to 70 were used for the experiments. BALB/C mice (6–8 wk old, 18 ± 2 g) were purchased from the Hebei Municipal Center for Disease Control and Prevention, Shijiazhuang, China.

Preparation of Samples

Bifidobacterium bifidum was isolated from humans and identified as ATCC 29521 by sequencing of 16S rDNA. Before the experiment, this microorganism was recovered through 2 successive transfers in de Man, Rogosa, Sharpe (Qingdao Hope Bio-Technology Co. Ltd., Qingdao City, China) agar and broth medium supplemented with 0.5 g of L-cysteine/L at 37°C for 24 h in an anaerobic incubator. For cell culture assays, the overnight culture of *B. bifidum* was transferred to a 50-mL centrifuge tube and harvested by centrifugation (6,000 × *g*, 10 min, 4°C), and washed twice with deionized water. The supernatant was gently discarded, and the bacterial pellet was resuspended to 1×10^8 cfu/mL of bacteria per milliliter with Dulbecco's Modified Eagle's medium for later use.

The HT-29 and Caco-2 cells were seeded in 75-cm^2 (250-mL) cell culture dishes (Greiner Bio-One, Monroe,