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Fermentation properties of isomaltooligosaccharides are affected by human fecal enterotypes

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ABSTRACT

Isomaltooligosaccharides (IMOs) are enzymatically synthesized oligosaccharides that have potential prebiotic effects. Five IMO substrates with 2–16° of polymerization (DP) were studied for their fermentation capacities using human microbiomes in an *in vitro* batch fermentation model. Eleven fecal slurries belonging to three enterotypes, including the *Bacteroides*-, *Prevotella*- and Mixed-type, exhibited different degradation rates for long chain IMOs (DP 7 to 16). In contrast, the degradation rates for short chain IMOs (DP 2 to 6) were not affected by enterotypes. Both 16S rRNA gene sequencing and quantitative PCR demonstrated that, after fermentation, the *Bifidobacterium* growth with IMOs was primarily detected in the *Bacteroides*- and Mixed-type (non-*Prevotella*-type), and to a lesser degree in the *Prevotella*-type. Interestingly, the *Prevotella*-type microbiome had higher levels of propionic acid and butyric acid production than non-*Prevotella*-type microbiome after IMOs fermentation. Moreover, principal coordinate analysis (PCoA) of both denaturing gradient gel electrophoresis (DGGE) profiling and 16S rRNA sequencing data demonstrated that the microbiome community compositions were separately clustered based on IMO chain length, suggesting significant impact of DP on the bacterial community structure. The current results clearly demonstrated that the IMO chain length could modulate the structure and composition of the human colonic microbiome. Different responses to short and long chain IMOs were observed from three human enterotypes, indicating that IMOs may be used as therapeutic substrates for directly altering human colonic bacteria.

1. Introduction

The human large intestine is inhabited by a complex and metabolically active microbial community (>10^{11} cells/g digestive contents), which is composed of anaerobic bacteria that are mainly classified as Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia [1,2]. Despite of substantial inter-individual variation between microbiomes, *Bacteroides, Prevotella* and *Ruminococcus* have been identified as the major genus to distinguish three enterotypes common to all humans, regardless of host nationality or continent-specificity, of which the composition is influenced by long-term dietary regimes [3,4]. The complex dietary residues that escape the digestion by enteric enzymes are the primary energy source for microbial fermentation, which are mainly non-starch polysaccharides. *Bacteroides* species are found in people that consume diets high in protein and fats, and mainly live in urban environments or have a Western lifestyle. In contrast, *Prevotella* species are present in people living in rural areas [5]. Short chain fatty acids (SCFAs) - such as acetate, propionate and butyrate - are the major metabolites generated during colonic carbohydrate fermentation. Among these fatty acids, butyrate serves anti-inflammatory and anti-carcinogenesis roles in the gut [6]. A healthy colonic microbiome is characterized as having a high abundance of butyrate-producing bacteria, mucin degraders, and *Bifidobacteria* [7].

Prebiotics are non-digestible carbohydrates that are selectively

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fermented in the colon by desirable bacterial species, such as *Bifidobacteria* and Lactobacilli [8], and the beneficial impacts of prebiotics on the human health have been intensely-studied in recent decades. Prebiotics promote positive health by stimulating bifidobacterial growth, increasing butyrate production and improving lipid metabolism, all of which have been confirmed by measuring fructooligosaccharides, galactooligosaccharides and inulin [9]. In addition, the chemical structures and the degree of polymerization (DP) of prebiotics affect fermentation properties in the human gut [10]. For example, long chain inulin-like fructans can ameliorate type I diabetes symptoms in mice, in contrast to short chain fructans [11]. This therapeutic property could be ascribed to the different fermentation rates of inulin-like fructans inside the colon. In general, the short chain inulin-like fructans are rapidly fermented in the left side of colon, while long chain fructans are associated with slow fermentation rates, often allowing for activity to reach the right side colon or the rectum [12].

Isomaltooligosaccharides (IMOs) are natural oligosaccharides that are found in various fermented foods, such as miso, sake and soy sauce [13]. Commercial IMOs are synthesized using industrial enzymatic processes, as extraction from natural sources is not economical [14]. IMOs consist of α-D-o-glucose residues that are linked by α (1–6) glycosidic bonds [15] and can be produced industrially in large batches from starch hydrolysates using α-transglucosidase [16] or by α-amylase with α-glucosidase, either in combination with pullulanase [17]. The final IMO product is a mixture that is characterized by a DP that varies from 2 to 10 and several linkage types (α-1-2, 3, 4, or 6), as well as the proportion and position of each linkage type (only α-(1–6) or combined types). Therefore, IMOs with varied DP values may exert different impacts on human colonic microbiota.

In this study, the effects of five IMO substrates with different chain lengths on the human fecal microbiome were studied by *in vitro* batch fermentation. In addition to different fermentation properties, the microbiome compositions with different enterotypes were significantly altered when incubated with IMOs that had long or short chain length in polymerization, as determined by both PCR-DGGE profiling analysis and 16S rRNA gene sequencing.

2. Materials and methods

2.1. Fecal sample origins

A total of eleven healthy human volunteers living in Hangzhou, China, ranging from 22 to 50 years old, were recruited for the study. All volunteers consumed traditional Chinese food and none one claimed as vegetarian. The donors had received neither antibiotics, nor pro- or prebiotic treatments for at least three months prior to sample collection. All volunteers were provided with informed, written consent, and the study was approved by the Ethics Committee of the Zhejiang Academy of Agricultural Sciences.

2.2. IMOs

Five IMO substrates with different DP and from three manufacturers were used. IMO-S was composed of 90% IMOs with DP 2 to 4 and was obtained from Bailong Chuangyuan Biotechnology Co., Ltd. (Yucheng Shandong, China). IMO-B was composed of 90% IMOs with DP 2 to 5 and was obtained from Baolingbao Biotechnology, Co., Ltd. (Yucheng Shandong, China). IMO-H1, IMO-H2, IMO-H3 were obtained from Herbon International Holdings Co., Ltd. (Hongkong, China), and they were composed of 20% DP 4, 60% DP 4–6 and 20% DP 6; 20% DP 7, 60% DP 7–15 and 20% DP 15; and 20% DP 8, 60% DP 8–16 and 20% DP 16, respectively. For thin-layer chromatography (TLC) analysis, IMO standards composed of DP of 2 and 3 were obtained from Tokyo Kasei Kogyo Co., Ltd. (TCI). DP 4, 5 and 6 were obtained from the Seikagaku Biobusiness Corporation (Tokyo, Japan).

2.3. Batch culture fermentation of IMOs with human fecal slurries

Batch culture fermentation was conducted using the procedure described by Lei et al. [18]. The basic growth medium VI contained the following: 4.5 g/L yeast extract; 3.0 g/L tryptone; 3 g/L peptone; 0.4 g/L bile salts No. 3; 0.8 g/L L-cysteine hydrochloride; 4.5 g/L NaCl; 2.5 g/L KCl; 0.45 g/L $MgCl_2 \cdot 6H_2O$; 0.2 g/L $CaCl_2 \cdot 6H_2O$; 0.4 g/L KH_2PO_4; 1 mL of Tween 80; 1 mL of Resazurin and 2 mL of a solution of trace elements [19]. In order to assess the degradation and utilization of IMOs by the human fecal microbiome, 8.0 g each of IMO-S, IMO-B, IMO-H1, IMO-H2 and IMO-H3 were added into the growth medium as the sole carbon source. The medium was adjusted to pH 6.5 before sterilization via autoclave. Five mL of test medium was dispensed into a 10 mL-bottle under anaerobic conditions.

Fresh fecal samples (0.8 g) were homogenized with 8 mL of 0.1 M anaerobic phosphate-buffered saline (pH 7.0) using an automatic fecal homogenizer (Halo Biotechnology Co. LTD., Jiangsu, China) to make a 10% (w/v) slurries. The batch fermentation was performed using 1% fecal slurry in anaerobic growth media at 37 °C for 48 h. One mL aliquots were removed at 0, 24 and 48 h for further analysis. The cultures were centrifuged and the precipitates were collected and stored at −30 °C for DNA extraction.

2.4. Thin-layer chromatography

The IMO degradation products were detected by TLC analysis. Briefly, samples (0.2 μL) were loaded onto a pre-coated silica gel-60 TLC aluminum plate (Merck, Germany). After being developed with a solvent system consisting of formic acid/n-butanol/water (6:4:1, v:v:v), the plate was soaked in orcinol reagent (Sigma-Aldrich Co. LLC) and visualized at 120 °C for 1 min [20].

The scanned TLC profiles were analyzed by Quantity One (Bio-Rad, USA) and the amounts of IMO in each samples were quantified. Degradation rates were calculated as the percentage of total amounts at 0 h subtracted from that at 48 h after fermentation.

2.5. DNA extraction

Bacterial genomic DNA was isolated from fermentation samples obtained at 0 and 48 h was extracted using a QIAamp DNA Stool Mini Kit according to the manufacturer's instructions (Qiagen, Germany). The concentration of extracted DNA was determined using a NanoDrop ND-2000 (NanoDrop Technologies, U.S.), and its integrity and size were confirmed by agar gel electrophoresis (1.0%) [20] and stored at −20 °C.

2.6. Denaturing gradient gel electrophoresis analysis (DGGE)

In order to analyze the microbial communities, the V3 region of the 16S rRNA gene (positions 341–534 of the *Escherichia coli* gene) in the stored DNA samples were amplified by the primers 341f (5′-ATTACCGCGGCTGCTGG-3′) and 534r with GC clamps (5′-CGC-CCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGG-AGGCAGCAG-3′). The amplicons were analyzed using DGGE as described previously [18]. After electrophoresis, DNA bands were photographed under UV (Gel Doc XR, Bio-Rad) after staining with SYBR Green I. The gel profile was analyzed by Quantity One (Bio-Rad, USA).

2.7. Quantitative PCR (qPCR)

The number of *Bifidobacterium* and *Lactobacillus* species in the