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In vitro digestibility and prebiotic potential of curdlan (1 → 3)-β-D-glucan oligosaccharides in *Lactobacillus* species

Yuqin Shi[a], Jun Liu[a], Qiaojuan Yan[b], Xin You[b], Shaoqing Yang[a], Zhengqiang Jiang[a,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, No.17 Qinghua Donglu, Haidian District, Beijing 100083, China

[b] Bioresource Utilization Laboratory, College of Engineering, China Agricultural University, No.17 Qinghua Donglu, Haidian District, Beijing 100083, China

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ABSTRACT

Prebiotic effects of curdlan (1 → 3)-β-D-glucan oligosaccharides (GOS) were examined. GOS was tolerant against simulated gastrointestinal digestion, as well as low pH, thermal, and Maillard reaction conditions likely occurred during food processing. Growth of tested *Lactobacillus* (*L.*) strains was improved by GOS except *L. brevis* NRRL B-4527. *E. coli* did not grow on GOS as the only carbon source. *In vitro* batch fermentation using human faecal microbiota showed that GOS significantly increased the population of *Lactobacillus* sp. followed by *Bifidobacterium* sp. and *Bacteroides* sp. Growth of *L.* strains on GOS produced lactic acid, acetic, and propionic acid with decreased culture medium pH. Utilization pattern of GOS by representative *L.* strains was strain dependent. GOS with degree of polymerization (DP) of 2 and 3 were readily consumed. Findings here indicated that curdlan GOS (DP = 2 and 3) are promising physiologically active prebiotics for improvement of human intestinal health.

1. Introduction

Currently, a considerable sector of the world's population has serious health problems related with gastrointestinal tract. Dietary approaches, such as prebiotics, have been increasingly accepted for improving large bowel health (Gullon et al., 2014). Based on definition, prebiotics should be firstly tolerant to low pH of stomach and resistant to digestive enzymes in small intestines. When reached colon, prebiotics could be fermented by gastrointestinal microbiota to stimulate the growth of certain indigenous gut bacteria, such as *Bifidobacteria* and *Lactobacilli* (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Meanwhile, metabolisms of short chain fatty acids (SCFAs), predominantly acetic, propionic, and butyric acids, are produced with lowered large bowel pH (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). Consequently, proliferation of less desirable bacterial groups such as species of *Clostridium* and *Bacteroides* is inhibited, resulting in altered composition of intestinal microbiota (Jacobs, Gaudier, Van Duynhoven, & Vaughan, 2009). Besides, metabolic activity of intestinal microbiota can be affected such as decreased microbial enzymatic activity (Wong & Jenkins, 2007). In total, fermentation of prebiotics can modulate large bowel environment to benefit the host's well-being and health (Hu, Nie, Li, & Xie, 2013). So far, only a small number of prebiotics are commercialized, such as fructo-oligosaccharides, inulin, galacto-oligosaccharides, and *gluco*-oligosaccharides (Roberfroid et al., 2010). It is of great interests to produce novel forms of prebiotics from readily available and renewable carbohydrate resources.

Curdlan, a microbial extracellular homo-polysaccharide, is composed of linear (1 → 3)-linked β-D-glucan without branching (Xu & Zhang, 2016). It has been approved by US FDA to be safe as food stabilizer, thickener, and/or texturizer based on its remarkable rheological and gelation behaviors (Spicer, Goldenthal, & Ikeda, 1999). Curdlan can also act as formulation aid or processing aid for food production such as low-fat meat (Funami, Yada, & Nakao, 1998; Funami, Yotsusuka, & Nakao, 1998). As an emerging prebiotic candidate, curdlan has been reported to demonstrate prebiotic effects for favoring the growth of *Bifidobacterium* sp. in rat *cecum*. When curdlan from the bacteria *Alcaligenes faecalis* was fed to rats for 4 weeks, population of *Bifidobacteria* in faecal was significantly improved with production of SCFAs and lactate (Shimizu et al., 2001). Similarly, dietary supplementation with 1% (1 → 3)-β-D-glucan (Laminarin) derived from algae has increased *Bifidobacterium* counts in the cecum of rats (Kuda, Yano, Matsuda, & Nishizawa, 2005). Feeding of (1 → 3)-β-D-glucan (Laminarin) reduced *E. coli* population in faeces of post weaning pigs, benefiting daily gain and gain to feed ratio (O'Doherty, McDonnell, & Figat,

Abbreviations: GOS, (1 → 3)-β-D-glucan oligosaccharides; MW, molecular weight; TLC, thin layer chromatography; SCFAs, short chain fatty acids; DP, degree of polymerization; HD, hydrolysis degree; SSF, simulated salivary fluid; SGM, simulated gastric medium; SIM, simulated intestinal medium; PI, prebiotic index; PAS, prebiotic activity score

* Corresponding author.
E-mail address: zhqjiang@cau.edu.cn (Z. Jiang).

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2010). After digestion, (1 → 3)-β-D-glucan can be fermented to metabolize SCFAs, particularly butyrate, with a reduced intestinal pH. Meanwhile, mucus composition has also been affected, thus, modulate intestinal metabolism (Deville, Gharbi, Dandrifosse, & Peulen, 2007). Besides, immunomodulatory effects of dietary (1 → 3)-β-D-glucan (Laminarin) have been related to its modulation on intestinal microbiota (Neyrinck et al., 2007). However, curdlan is water insoluble because of its high molecular weight (MW) and unique gelation property in solution (Xiao et al., 2017). This becomes a hindrance for expanding its applications particularly in food industry. Curdlan (1 → 3)-β-D-glucan oligosaccharides (GOS), the hydrolysate of curdlan, have shorter chain length, lower MW, and improved water solubility. GOS have demonstrated improved biological activities, such as induction of monocytes to produce tumor necrosis factor alpha (TNF-α) (Miyanishi, Iwamoto, Watanabe, & Oda, 2003; Pang, Otaka, Suzuki, Goto, & Ohnishi, 2004), stimulation of interleukin 1β secretion (Jamois et al., 2005), and initiating defense responses on tobacco plants (Fu et al., 2011; Klarzynski et al., 2000). Theoretically, GOS can be easy fermentable for diverse intestinal probiotics with improved prebiotic activities.

To verify the putative prebiotic potential, GOS have been prepared by enzymatic hydrolysis of curdlan using a GH family 64 β-(1 → 3)-glucanase and the structural features were characterized (Qin et al., 2017; Zhou et al., 2013). *In vitro* digestibility of GOS was tested under simulated gastrointestinal conditions. Stability of curdlan GOS against low pH, thermal, and Maillard reaction conditions likely occurred during food processing was investigated. Fermentation of GOS was performed with nine selected probiotic *Lactobacillus* (*L.*) strains and enteric bacteria of *E. coli* ATCC 11775. Findings from this study will tell mechanisms involved in prebiotic potential of GOS. This may benefit the utilization of GOS as food prebiotics for nutraceutical market.

2. Materials and methods

2.1. Materials

Curdlan (Food grade) from *Alcaligenes faecalis* was purchased from Jiangsu Yiming Biological Technology Co., Ltd. (Jiangsu, China). Alpha-amylase from human saliva (Type IX-A, 1000–3000 units/mg protein), pepsin from porcine gastric mucosa (35 kDa, 3200–4500 units/mg protein), and pancreatin from porcine pancreas (8 × USP specifications, contains enzymatic components including trypsin, amylase and lipase, ribonuclease, and protease) were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Sulfuric acid, phenol, 3,5-dinitrosalicylic acid (DNS) were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Beef extract, protease peptone, and yeast extract were obtained from AOBOX Biotechnology Co., Ltd. (Beijing, China). Chromatographical grade organic acid standards (≥99%), including formic acid, acetic acid, propionic acid, butyric acid, and lactic acid were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. All other chemicals were of analytical grade and used as received. Prebiotic potential of GOS was tested on nine selected *L.* strains as follows: *L. acidophilus* NRRL B-4495 (ATCC 4356), *L. brevis* NRRL B-4527 (ATCC 14869), *L. coryniformis* subsp. *coryniformis* NRRL B-4391 (ATCC 25602), *L. delbrueckii* subsp. *bulgaricus* NRRL B-548 (ATCC 7993), *L. casei* subsp. *casei* NRRL B-1922 (ATCC 393), *L. delbrueckii* subsp. *Lactis* AS 1.2132 (JCM 1248), *L. casei* AS 1.62 (BNCC 137633), *L. reuteri* ATCC 23272, and *L. rhamnosus* AS 1.2466 (ATCC 7469). *E. coli* ATCC 11775 was used as enteric bacteria. All selected bacteria strains were kept at −80 °C in MRS broth supplemented with 30% (v/v) glycerol.

2.2. Preparation of GOS

Enzymatic hydrolysis of curdlan was performed by a GH family 64 β-(1 → 3)-glucanase (*Rm*Lam81A) for GOS preparation. Briefly, accurately weighed (2,000 g) curdlan powder was dispersed in 50 L deionized water for 3 h at room temperature (RT, 22–23 °C). The suspension was magnetically stirred at 2,000 rpm (Jieruier, Jintan, China) with pH be adjusted to 5.5 by glacial acetic acid. Then, *Rm*Lam81A dispersion (5.0 L) was added in a proportion of 2.0 U/mL and enzymatic reaction was performed at 42 °C for 16 h under constant stirring (100 rpm). Hydrolyzed curdlan suspension was then boiled at 100 °C in a water bath for 10 min to terminate the reaction. Insoluble fraction of hydrolysates were removed through frame filtration (WBG-1 Frame Filter, Guangzhou hundom Machinery Co., Ltd.). The filtrates were collected and subjected for spray drying (QPG-1.5 Spray Drier, Changzhou Jiafa Granulating Equipment Co., Ltd.). Dried GOS were kept in a desiccator at RT for subsequent analysis.

2.3. Structural feature characterization on GOS

2.3.1. Analysis of monosaccharides by HPLC

GOS were totally hydrolyzed by 1.0 M sulfuric acid at 100 °C for 3.0 h. Monosaccharides were analyzed by HPLC (Agilent 1260 series) equipped with a refractive index detector (G1362A). Separation of monosaccharides, including standards of glucose, xylose, galactose, and mannose, was performed on a BP-800 Pb^{2+} column operated at 80 °C. Deionized water was used as mobile phase at a flow rate of 0.8 mL/min.

2.3.2. Analysis of oligosaccharides by HPAEC

Oligosaccharide composition of GOS was analyzed by high performance anion-exchange chromatography (HPAEC) with pulsed amperometric detector (ICS-5000 $^+$, Thermo, USA). Analytes were gradient eluted (1.0 mL/min) with sodium acetate in 100 mM NaOH (from 0 to 350 mM within 20 min) on a CarboPac™ PA1 column (4 × 250 mm, Thermo, USA) held at 30 °C.

2.3.3. Molecular weight distribution of GOS

MW distribution of GOS was determined by gel permeation chromatography (GPC) on an Agilent 1260 Series HPLC system equipped with a refractive index detector. Each sample (0.5 mg/mL) was filtered (0.45 μm) and injected into TSKgel $GMPW_{XL}$ column (7.8 × 300 mm, Tosoh Biosep, Japan). The mobile phase was deionized water with a flow rate of 0.6 mL/min and the column temperature was 60 °C. Before measurement, the system was calibrated with dextran standards within an average MW range of 5.05×10^2–2.76×10^5 Da. MW of GOS was calculated by comparison with the calibration curve.

2.4. In vitro digestibility of GOS

In vitro digestibility of GOS was tested using the method previously described by Minekus et al. (2014) with small modifications. Stock solution was prepared by dissolving accurately weighed GOS in deionized water to make a concentration of 10.0 mg/mL. Before and after each *in vitro* digestion treatment, total carbohydrate content and reducing sugar content was determined by phenol-sulfuric acid method (Saha & Brewer, 1994) and DNS method (Saqib & Whitney, 2011), respectively. Glucose was used as internal standard to construct standard curve for sugar content calculation. Hydrolysis degree (*HD*, %) of GOS under each simulated digestion process was calculated using the following equation (Eq. (1)):

$$HD\ (\%) = \frac{R_S - R_O}{T_S - R_O} \times 100\% \quad (1)$$

Where R_S and T_S is the reducing sugar content and total sugar content after *in vitro* digestion treatment, respectively. R_O is the reducing sugar content before treating with simulated digestion medium.

2.4.1. Simulated oral digestion

Digestibility of GOS in oral phase was investigated using simulated salivary fluid (SSF) (pH 7.0) containing KCl (15.1 mM), KH_2PO_4 (3.70 mM), $NaHCO_3$ (13.6 mM), $MgCl_2$ (0.15 mM), $(NH_4)_2CO_3$