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Effects of *Ophiocordyceps dipterigena* BCC 2073 β-Glucan as a Prebiotic on the *in vitro* Growth of Probiotic and Pathogenic Bacteria

Wai Prathumpai, Pranee Rachtawee, Sutamat Khajeeram, Pariya Na Nakorn

***Abstract*—**The β-glucan produced by *Ophiocordyceps dipterigena* BCC 2073 is a (1, 3)-β-D-glucan with highly branching O-6-linkedside chains that is resistant to acid hydrolysis (by hydrochloric acid and porcine pancreatic alpha-amylase). This β-glucan can be utilized as a prebiotic due to its advantageous structural and biological properties. The effects of using this β-glucan as the sole carbon source for the *in vitro* growth of two probiotic bacteria (*L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527) were investigated. Compared with the effect of using 1% glucose or fructo-oligosaccharide (FOS) as the sole carbon source, using 1% β-glucan for this purpose showed that this prebiotic supported and stimulated the growth of both types of probiotic bacteria and induced them to produce the highest levels of metabolites during their growth. The highest levels of lactic and acetic acid, 10.04 $g \cdot L^{-1}$ and 2.82 $g \cdot L^{-1}$, respectively, were observed at 2 h of cultivation using glucose as the sole carbon source. Furthermore, the fermentation broth obtained using 1% β-glucan as the sole carbon source had greater antibacterial activity against selected pathogenic bacteria (*B. subtilis* TISTR 008, *E. coli* TISTR 780, and *S. typhimurium* TISTR 292) than did the broths prepared using glucose or FOS as the sole carbon source. The fermentation broth obtained by growing *L. acidophilus* BCC 13938 in the presence of β-glucan inhibited the growth of *B. subtilis* TISTR 008 by more than 70% and inhibited the growth of both *S. typhimurium* TISTR 292 and *E. coli* TISTR 780 by more than 90%. In conclusion, *O. dipterigena* BCC 2073 is a potential source of a β-glucan prebiotic that could be used for commercial production in the near future.

***Keywords*—**β-glucan, *Ophiocordyceps dipterigena*, prebiotic, probiotic, antimicrobial.

I. Introduction

PREBIOTICS that cannot be digested by enzymes in the digestive system and/or cannot be metabolized by the host can support, stimulate and maintain the growth of probiotics in the small intestine and suppress the growth of non-probiotics, such as *Salmonella* spp. and *Escherichia coli* [1]. There are many types of prebiotics, such as alcohol sugars, non-resistant and resistant starches, inulin, and non-digestible oligosaccharides [2]-[4]. These compounds are found mainly in plants, such as chicory root, Jerusalem artichokes, dandelion greens, garlic, leeks, onions, raw asparagus, wheat bran and bananas [5]. Inulin and oligosaccharide probiotics are commonly found in plants [6]. Some prebiotics are produced by fungi and bacteria, including FOSs and galactooligosaccharides (GOSs) [5], [7], with mushrooms and entomopathogenic fungi being the main fungal producers of prebiotics. The fructan β-fructosidase produced by *Aspergillus niger* can be used to synthesize FOS [7]. Aida et al. [8] reported the potential applications of prebiotics obtained from mushrooms, which include carbohydrate-containing molecules such as chitin, hemicellulose, β-glucans, mannans, xylans and galactans. Most of the polysaccharides produced by mushrooms are linear or branched glucans that have different stuctures of (1, 3), (1, 6)- β-glucans and (1, 3)-α-glucans [8], [32]. β-glucooligosaccharides, α-glucooligosaccharides, β-GOSs, α-GOSs, and FOSs are examples of prebiotics that are produced by bacterial enzymes in the colon [9]. Splechtna et al. [10] reported that GOSs produced by *Lactobacillus reuteri* L103 and *L. reuteri* L461 through transgalactosylation reactions, catalyzed by β-galactosidases hydrolases (β-Gals) and using lactose as the substrate converted approximately 80% of this substrate, resulting in a yield of 38% GOSs [10]. In a study of the effects of two prebiotics [high-solubility inulin (HSI) and oligofructose (OF)] on the *in vitro* and *in vivo* growth of bifidobacteria and lactobacilli cultivated in human feces, these prebiotics were found to increase the bacterial growth rates [1].

The cost of the production of prebiotics from plants is high because the process involves hot-water extraction, partial enzymatic hydrolysis, purification, evaporation and spray drying [11]. Additionally, the prebiotics produced from plants are limited by the growth season. In contrast, the process of fungal prebiotic production can be controlled in a close system, it is not dependent on the environmental conditions and can be completed in a shorter time period. The β-glucan produced by *O. dipterigena* BCC 2073, which has a molecular weight of 6.3×10^5 - 7.7×10^5 Da, is a (1, 3)-β-D-glucan with highly branching *O*-6-linked side chains and consists of 1.86% arabinose, 29.08% mannose, 25.86% galactose and 43.05% glucose [12], [13]. The *O. dipterigena* BCC 2073 β-glucan is resistant to hydrolysis (by hydrochloric acid and porcine pancreatic α-amylase) [14], [15]. Due to these properties, this β-glucan can be utilized as a potential prebiotic. The prebiotics produced on a commercial scale are generally

Wai Prathumpai*, Pranee Rachtawee, and Sutamat Khajeeram are with the Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Phahonyothin Rd., Klong Nueng, Klong Luang, Pathum Thani, 12120, Thailand (Corresponding author; phone: +66 2564 6700 ext. 3525-6, fax: +66 2564 6707, e-mail: wai.pra@biotec.or.th).

Pariya Na Nakorn is with the Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Klong Nueng, Klong Luang, Pathum Thani, 12120, Thailand.

obtained from plants, and there have been few reports of the production of a fungal prebiotic [1], [6]. The aims of this study were to investigate β-glucan production by *O. dipterigena* BCC 2073 and to evaluate the prebiotic properties of this compound. *O. dipterigena* BCC 2073 can produce β-glucan through submerged-culture fermentation with 60 $g \cdot L^{-1}$ of glucose and 14 $g \cdot L^{-1}$ of malt extract at a yield of 41.2 $g \cdot L^{-1}$ in a 5-L fermenter [16]. The levels of exopolysaccharide production by the fungi of other genera belonging to the group that includes *O. dipterigena* BCC 2073 were relatively lower, e.g., that of *Paecilomyces japonica* grown in a 2-L fermenter ranged from 8 to 34.5 $g \cdot L^{-1}$, depending on the type and quantities of carbon sources provided [17]-[19]. *Paecilomyces sinclairii* produced 2 $g \cdot L^{-1}$ of exopolysaccharide [20], *Paecilomyces tenuipes* produced 3.4 $g \cdot L^{-1}$ of exopolysaccharide [19] and *Cordyceps militaris* produced 10.3 $g \cdot L^{-1}$ of exopolysaccharide [21]. *O. dipterigena* BCC 2073 is a potential source of a β-glucan prebiotic, the structure of which provides advantages compared with those of plant prebiotics. Hence, the *in vitro* effects of this β-glucan prebiotic on the growth of various bacteria cultivated in a 1-L fermenter were compared with those of glucose and FOS.

II. Materials and Methods

A. Inoculum Preparation

O. dipterigena BCC 2073 was grown initially on potato dextrose agar at 25 °C for 5-7 days. An agar block (1 cm^3) containing the growing culture was cut into small pieces and the pieces were transferred to 25 mL of potato dextrose broth (Becton, Dickinson and company, MD, USA) in a 250-mL Erlenmeyer flask. The liquid seed culture was incubated for 5-7 days at 25 °C on a rotary shaker agitated at 200 rpm (New Brunswick, NJ, USA).

B. Production and Preparation of the β-glucan of O. dipterigena BCC 2073

The β-glucan used in this study was obtained from the supernatants of *O. dipterigena* BCC 2073 cultures. β-glucan is an extracellular product that is secreted into the fermentation medium. The fungus was cultivated in a 10-L fermenter with a working volume of 8 L for β-glucan production. The culture medium (Marubishi, Pathum Thani, Thailand) contained 60 $g \cdot L^{-1}$ of glucose, 14 $g \cdot L^{-1}$ of malt extract (Becton, Dickinson and company, MD, USA), 0.5 $g \cdot L^{-1}$ of KH_2PO_4, 0.2 $g \cdot L^{-1}$ of K_2HPO_4, 0.2 $g \cdot L^{-1}$ of $MgSO_4 \cdot 7H_2O$, 0.14 $g \cdot L^{-1}$ of $MnSO_4 \cdot H_2O$, 1 $mL \cdot L^{-1}$ of a vitamin solution (Blackmores, NSW, Australia) and 1 $mL \cdot L^{-1}$ of a trace element solution (trace elements consisted of 14.3 $g \cdot L^{-1}$ $ZnSO_4 \cdot H_2O$, 2.5 $g \cdot L^{-1}$ $CuSO_4 \cdot 5H_2O$, 0.5 $g \cdot L^{-1}$ $NiCl_2 \cdot 6H_2O$ and 13.8 $g \cdot L^{-1}$ $FeSO_4 \cdot H_2O$). The culture was agitated at 300 rpm with an aeration rate at 1 vvm, but the pH was not controlled. The culture broth was centrifuged at 10,000 rpm for 10 min, after which the supernatant was removed from the mycelia. The culture filtrate was mixed with four volumes of 95% ethanol, stirred vigorously for 10 to 15 min, and stored at 20 ºC for at least 12 h. The precipitated polymer was pelleted by centrifugation at 10,000 rpm for 20 min and then was lyophilized. The β-glucan was re-dissolved in distilled water, and any insoluble material was removed by centrifugation at 10,000 *g* for 20 min. The supernatant was then placed in a dialysis membrane (2-kDa cut off, Spectrum Laboratories, CA, USA), dialyzed against 4 L of distilled water for 24 h, and then lyophilized [12], [13].

C. Bacterial Inoculum Preparation

Lactobacillus acidophilus BCC 13938 and *Bifidobacterium animalis* ATCC 25527 were pre-grown in Man Rogosa Sharpe (MRS) medium (Merck, Germany) and Reinforced Clostridial Medium (Difco™, Becton, Dickinson and company, MD, USA), respectively, at 37 °C for 24-48 h.

Salmonella typhimurium TISTR 292, *Bacillus subtilis* TISTR 008 and *E. coli* TISTR 780 were grown in nutrient broth incubated at 37 °C for 24 h on a rotary shaker shaking at 200 rpm.

D. Cultivation of L. acidophilus BCC 13938 and B. animalis ATCC 25527

The two probiotic strains, *L. acidophilus* BCC 13839 and *B. animalis* ATCC 25527 were cultivated in a 1-L (Biostat Q, B. Braun, Germany) with a working volume of 700 mL. The cultivation conditions were as follows: 37 ºC, agitation at 150 rpm, no aeration, pH not controlled, and cultivation period of 48 h. *L. acidophilus* BCC 13938 was cultivated in MRS medium and *B. animalis* ATCC 25527 was cultivated in Reinforced Clostridial medium that lacked a carbon source. In this study, these media were supplemented with glucose, FOS or β-glucan at a oncentration of 1% (w/v) as the carbon source. The fermentation broth was collected at 0, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 36 and 48 h of cultivation. The growth rate of each strain was monitored by measuring the optical density at 600 nm, and the viability rate was monitoring using the total plate count method, using MRS agar medium for *L. acidophilus* BCC 13938 and Reinforced Clostridial agar medium for *B. animalis* ATCC 25527. The glucose, lactic acid and acetic acid contents of the culture supernatant were determined using HPLC.

E. Determination of the Biomass and the Viability Rate

The culture broth was collected to determine the turbidity level using spectrophotometry at a wavelength of 600 nm. Fresh culture medium was used as the blank. Then, the viability rates were determined by serially diluting the cultures using fresh medium. The media used for the plate counts were MRS agar (Merck KGaA, Darmstadt, Germany) and Reinforced Clostridial medium agar (Difco™, Becton, Dickinson and company, MD, USA) for *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527, respectively. The plates were incubated at 37 ºC for 24-48 h, and the number of bacterial colonies on petri dishes containing 30 to 300 colonies was counted. The number of colony-forming units was calculated by multiplying the number of colonies/plate by the dilution rate.

F. Determination of the Sugar and Metabolite (Lactic and Acetic Acid) Contents

The culture supernatants were centrifuged at 10,000 rpm for 10 min and then were filtered through 0.22-µm filter paper. The filtrates were analyzed by HPLC using an Aminex column (Bio-Rad, Hercules, Calif.) at 65 °C, using 5 mM H_2SO_4 as the mobile phase, delivered at a flow rate of 0.6 mL·min^{-1} and at a pressure of 1000 to 1200 psi. Glucose, lactic acid and acetic acid were detected using a refractive index detector (Waters 410 Differential Refractometer, Millipore Corp., Milford, MA, USA).

G.In vitro Test for Anti-Bacterial Properties

L. acidophilus BCC 13938 and *B. animalis* ATCC 25527 were cultivated on different carbon sources at a concentration of 1% (β-glucan, glucose, and FOS) in a 1-L fermenter with a working volume of 700 mL for 48 h. The culture broths were then centrifuged at 13,000 rpm at 4 °C for 10 min. The supernatants were then filtered through 0.22-µm filter paper, and the pH of the filtrates was adjusted to 7.0. 50 µl of the supernatants was added to 96-well plates to test their antibacterial activities. 50 µl of *S. typhimurium* TISTR 292, *B. subtilis* TISTR 008 or *E. coli* TISTR 780 was then added to the wells, and the plates were incubated at 37 °C for 15 h. Viability counts were then performed by serially diluting the cultures using nutrient broth. The experimental viability rates were calculated by normalization to the viability rates observed under the control conditions.

III. Results and Discussion

The effects of different carbon sources on the growth patterns of two selected probiotic bacteria, *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527, in medium containing 1% (w/v) glucose, FOS or β-glucan, and their levels of metabolic production in these media were investigated. This goal of the study was to evaluate the potential of *O. dipterigena* BCC 2073 β-glucan as a potential prebiotic. The patterns of growth of *L. acidophilus* BCC 13938 cultivated using each of tested sole carbon sources were similar, in which after a lag phase at 3 h, exponential growth occurred between 3 and 6 h of cultivation. After 6 h of cultivation, the viability level of this strain stabilized (Figs. 1 A, B). *L. acidophilus* BCC 13938 grew most rapidly in glucose-containing medium. The observation of the stable growth of *L. acidophilus* BCC 13938 in β-glucan-containing medium compared with its growth in glucose- and FOS-containing media indicated that β-glucan was the appropriate prebiotic for the growth of this strain. *O. dipterigena* BCC 2073 β-glucan, which has a molecular weight of 6.3 x 10^5 - 7.7 x 10^5 Da, is a (1, 3)-β-D-glucan with highly branching *O*-6-linked side chains that contain 1.86% arabinose, 29.08% mannose, 25.86% galactose and 43.05% glucose [12], [13]. The β-glucan of *O. dipterigena* BCC 2073 is resistant to hydrolysis (by hydrochloric acid and porcine pancreatic α-amylase) [14], [15]. These properties of β-glucan promoted the stable growth of *L. acodiphilus* BCC 13938 and *B. animalis* ATCC 25527 after 6 h of cultivation.

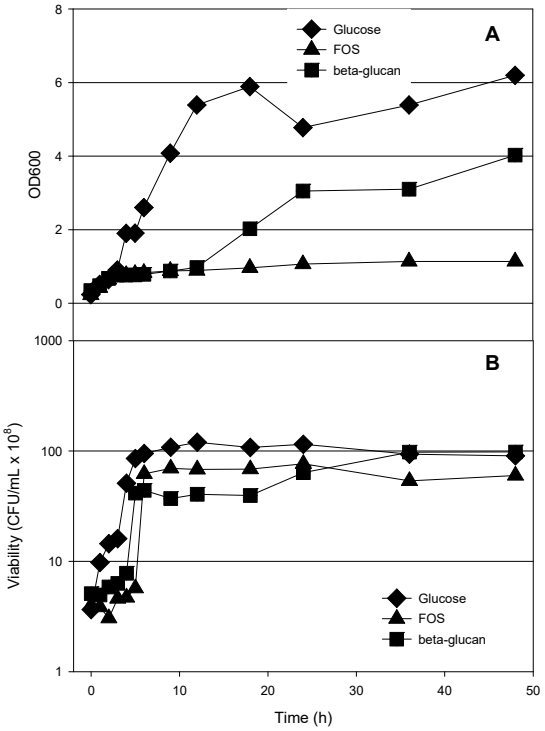

Fig. 1 Growth patterns of *L. acidophilus* BCC 13938 grown in the presence of different soles carbon sources, A: OD 600 values, B: Viability rate

Kunova et al. [22] reported that compared with raffinose, lactulose and GOSs, FOS at a final concentration of 0.2% was the best carbon source for the growth of *Lactobacillus*, which achieved its highest viability rate of 10^8 CFU·mL^{-1} at 24 h of cultivation. Fig. 3 A shows the rate of lactic acid production by *L. acidophilus* BCC 13938 grown in the presence of each of the tested carbon sources. In this study, the highest level of lactic acid production of 10.04 g·L^{-1} was achieved by *L. acidophilus* BCC 13938 grown in glucose-containing medium. Mandadzhieva et al. [23] reported similar results, showing that the levels of lactic acid production of *L. acidophilus* L.a.504, *L. acidophilus* L.d.B7, *L. acidophilus* L.f.B5 and *L. acidophilus* L.sS161 cultured in the presence of 3% glucose were 48.0, 37.5, 23.5 and 21.3 µmol·mL^{-1}, respectively. Su et al. [4] studied the effect of various carbon sources, FOS, inulin, arabinoglucan, soybean oligosaccharide (SOS), β-glucan, D(+)-raffinose and glucose (the latter as the positive control), at different concentrations (w/v) on the growth of the probiotic *L. acidophilus* L10, finding that the highest growth rate was achieved using them at a concentration of 1.5%. Nazzaro et al. [24] reported that the highest level of viability of *L. plantarum* subsp. *plantarum* (10.28x10^{10} CFU·mL^{-1}) was achieved using 2% inulin as the carbon source compared with those obtained using glucose or pectin. Results similar to those of the present study were obtained in an investigation of the growth rates and lactic-acid production rates of *L. acidophilus*

ATCC 43121 grown *in vitro* in the presence of different concentrations (0.25, 0.50, 0.75, 1.00 or 1.5%) of seven prebiotics (inulin, xylitol, sorbitol, mannitol, FOS, lactulose and raffinose) [25]. Rycroft et al. [26] reported that FOS and XOS were both good carbon sources for *L. acidophilus* and *L. plantarum* and improved their growth rates, as well as protecting these strains from the adverse effects of acidity level in the gastrointestinal tract. Many non-digestible oligosaccharide (NDO) prebiotic compounds that resist digestion and absorption in the small intestine can be produced in the large intestine and can facilitate the maintenance of colonic functional regularity and reduce the risk of developing colonic diseases [2].

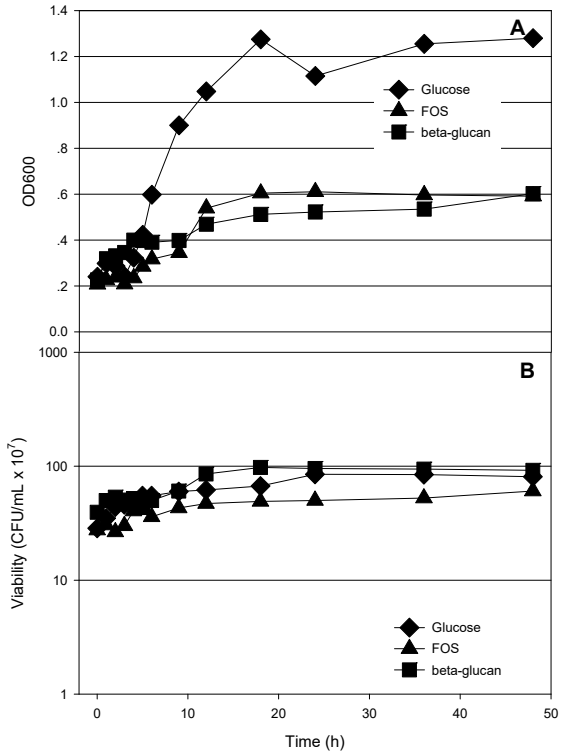

Fig. 2 Growth patterns of *B. animalis* ATCC 25527grown in the presence of different sole carbon sources, A: OD 600 values, B: Viability rate

Figs. 2 A and B show the growth patterns of *B. animalis* ATCC 25527 cultivated using the tested carbon sources at a concentration of 1%. When this strain was grown in the presence of 1% glucose, the culture medium achieved a higher OD 600 value compared with that achieved using FOS or β-glucan at the same concentration. The patterns of *B. animalis* ATCC 25527 growth observed in the presence of all three of the tested carbon sources were similar and this strain reached a stationary phase that lasted from 12 to 48 h of cultivation in the presence of each of these compounds. In contrast, *B. animalis* ATCC 25527 achieved its highest growth rate when β-glucan was used as the sole carbon source, which might be due to the properties of β-glucan that promoted its growth. The highest level of acetic-acid production (Fig. 3B) observed in this study was obtained using glucose rather than FOS or β-glucan as the sole carbon source. The highest level of acetic-acid production, 2.82 $g \cdot L^{-1}$, was reached at 2 h of cultivation in the presence of glucose. Some *Bifidobacterium* species can degrade OF and inulin, and a variant β-fructofuranosidase gene has been found in *Bifidobacterium* [27]. Palframan et al. [28] reported that *B. bifidum* produced acetic acid and a monosaccharide (xylose) when grown using glucose as the carbon source. Gibson et al. [29] reported that lactic-acid bacteria and bifidobacteria are lactate and acetate producers, respectively, which is consistent with our finding that lactic acid and acetic acid were present in the cultures of lactic-acid and bifidobacteria, respectively.

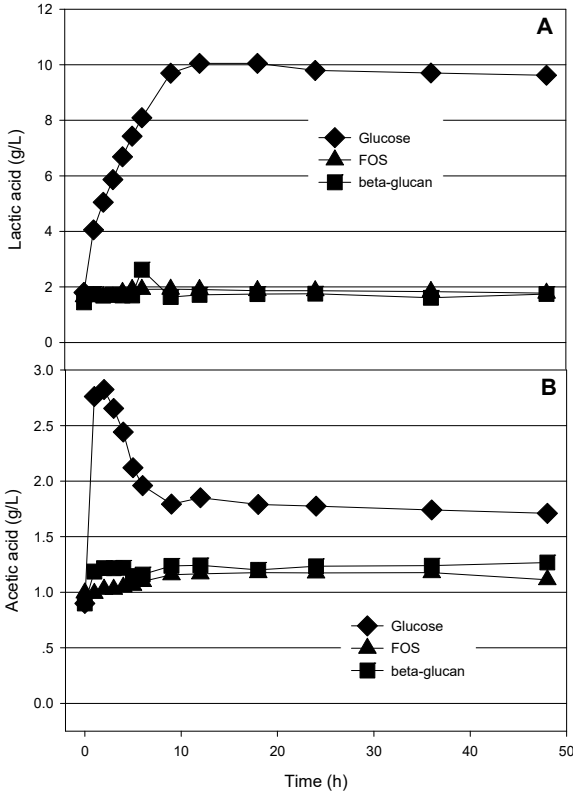

Fig. 3 Lactic (A) and acetic acid (B) production by *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527, respectively, grown in the presence of different sole carbon sources

A. Anti-Bacterial Activities of the Fermentation Broths of L. Acidophilus BCC 13938 and B. animalis ATCC 25527 Grown in the Presence of Various Single Carbon Sources at a 1%

The antibacterial activities of the fermentation broths of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 strains that were cultivated with different compounds at 1% as the sole carbon sources (glucose, FOS and β-glucan) were evaluated using model pathogenic bacteria as the test subjects (*E. coli* TISTR 10675, *S. typhimurium* TISTR 10747 and *S. aureus* TISTR 10908). Fig. 4A shows the inhibitory activities of the fermentation broths of *L. acidophilus* BCC 13938 that

were cultivated for various periods in the presence of different carbon sources against the growth of *B. subtilis* TISTR 008. The highest level of growth inhibition of *B. subtilis* TISTR 008 was observed using the fermentation medium of FOS-cultivated *L. acidophilus* BCC 13938 that had been grown for 3 h, which resulted in 80% growth inhibition. The fermentation broth of *L. acidophilus* BCC 13938 grown using β-glucan as the carbon source inhibited the growth of *B. subtilis* TISTR 008 by more than 70%, which was lower than the inhibitory rate achieved using its glucose-containing fermentation broth. The fermentation broth of *B. animalis* ATCC 25527 that was grown in the presence of each of the three tested carbon sources inhibited the growth of *B. subtilis* TISTR 008 by more than 70% (Fig. 4B).

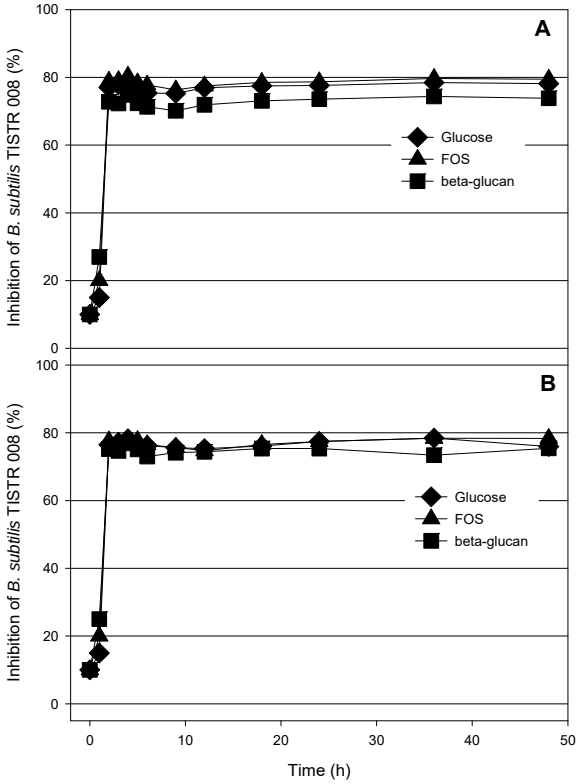

Fig. 4 Inhibition of the growth of *B. subtilis* TISTR 008 by the fermentation broth of A., *L. acidophilus* BCC 13938 and B., *B. animalis* ATCC 25527 grown using different sole carbon sources

Fig. 5A shows the growth-inhibitory effects of the fermentation broths of *L. acidophilus* BCC 13938 grown using each of the tested carbon sources against *E. coli* TISTR 780, which revealed that the fermentation broth of *L. acidophilus* BCC 13938 obtained at 3-48 h of cultivation in the presence of glucose, FOS or β-glucan inhibited the growth of *E. coli* TISTR 780 by approximately 90%. Fig. 5B shows that the fermentation broth of *B. animalis* ATCC 25527 that was cultivated for 3-48 h using glucose, FOS or β-glucan as the sole carbon source had a similar inhibitory effect on the growth of *E. coli* TISTR 780, resulting in approximately 90% inhibition of its growth.

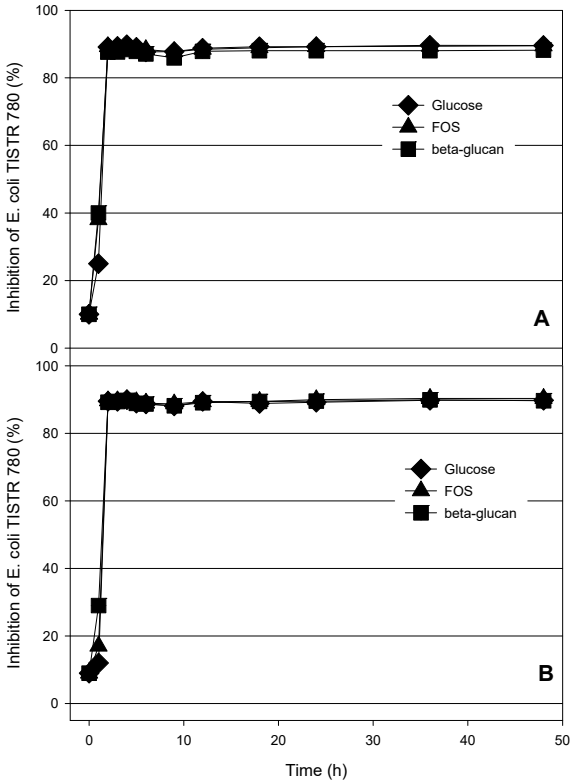

Fig. 5 Growth inhibition of *E. coli* TISTR 780 grown on different carbon sources by the culture broth of A., *L. acidophilus* BCC 13938 and B., *B. animalis* ATCC 25527

Figs. 6 A and B demonstrate the levels of antibacterial activity of the fermentation broth of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 on the growth of *S. typhimurium* TISTR 292. The fermentation broths of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 grown using β-glucan as the sole carbon source more strongly inhibited the growth of *S. typhimurium* TISTR 292 compared with the fermentation broths obtained using these strains grown on glucose or FOS, with an approximately 90% growth-inhibition rate observed. These results suggested that the fermentation broths of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 that were grown using β-glucan as the sole carbon source compared with using glucose or FOS as the sole carbon source had superior antibacterial activities toward the model pathogenic bacteria tested, *B. subtilis* TISTR 008, *E. coli* TISTR 780, and *S. typhimurium* TISTR 292. These results showed that the prebiotic *O. dipterigena* BCC 2073 β-glucan investigated in this study possesses antimicrobial activities. Similar to our results, Ho et al. [30] reported that the fermentation broth of *L. acidophilus* NBM-01-07-002 possesses antimicrobial activities because it inhibited the growth of the pathogenic bacteria *E. coli* BCRC 10675, *S. typhimurium* BCRC 10747 and *S. aureus* BCRC 10908. Likotrafiti et al. [31] reported that the antimicrobial activities

of the supernatants of *L. fermentum* and *B. longum*culture broths against *E. coli* 086 were due to their contents of isomaltooligosaccharide (IMO). The antimicrobial activities of the fermentation broths of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 grown using β-glucan as the sole carbon source that were observed in this study might be due to their demonstrated contents of certain metabolites, such as lactic acid and acetic acid.

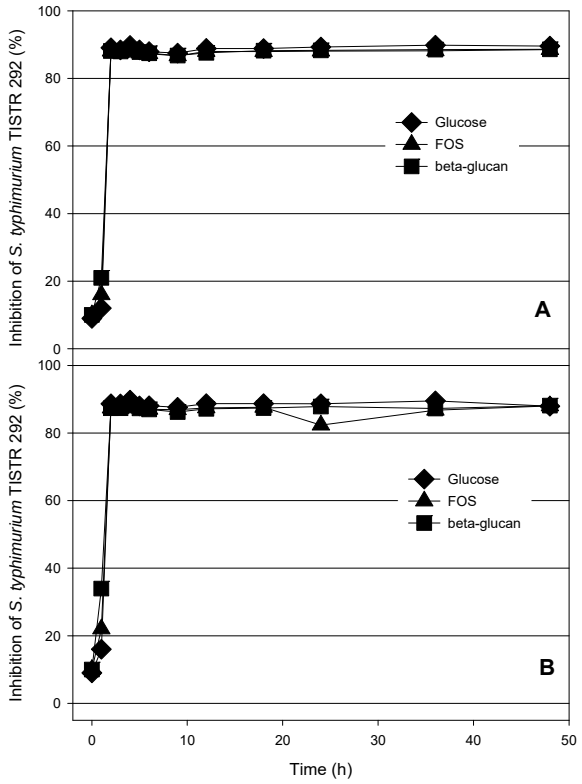

Fig. 6 Growth inhibition of *S. typhimurium* TISTR 292 grown on different carbon sources by the culture broth of A., *L. acidophilus* BCC 13938 and B., *B. animalis* ATCC 25527

IV. Conclusion

The growth patterns of the probiotic bacterial strains *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 grown in the presence of different sole carbon sources, including glucose, FOS and β-glucan, were studied. Compared with glucose and FOS, *O. dipterigena* BCC 2073 β-glucan, a potential prebiotic, most strongly stimulated the growth of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527. The antibacterial activities of the fermentation broths of *L. acidophilus* BCC 13938 and *B. animalis* ATCC 25527 cultivated in the presence of 1% glucose, FOS and β-glucan were investigated. The fermentation broth of *L. acidophilus* BCC 13938 grown in the presence of β-glucan inhibited the growth of *B. subtilis* TISTR 008 by more than 70% and inhibited the growth of *S. typhimurium* TISTR 292 and *E. coli* TISTR 780 by more than 90%. These results demonstrated that the β-glucan produced by *O. dipterigena* BCC 2073 could be used as a prebiotic due to its high molecular weight, highly branched structure, resistance to acid hydrolysis, capacity to support the growth of probiotic bacteria and its antimicrobial activities against pathogenic bacteria.

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