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RESEARCH ARTICLE

Production of Xylooligosaccharides from Corncob Using a Crude Thermostable Endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 and Prebiotic Properties

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Abstract Production of xylooligosaccharides (XOs) from corncob using the thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 was investigated using KOH pretreatment, followed by enzymatic hydrolysis. The optimal reaction time for production of XOs was 12 h, after which xylobiose comprised a majority of products, and a low xylose content was observed. The optimal conditions for production of XOs were studied using a central composite design. At an enzyme concentration of 129.43 U/g of substrate, 53.80°C, and pH 6.17, the yield of XOs reached 162.97 mg/g of substrate or 752.15 mg/g of hemicellulose in KOH-pretreated corncob. The prebiotic properties of XOs derived from corncob were also investigated using *in vitro* fermentation of those XOs with the known probiotic strains *Lactobacillus casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465. XOs derived from corncob were comparable to commercial XOs for an ability to enhance the growth of the specified probiotic lactobacilli.

Keywords: alkali pretreatment, corncob, prebiotics, *Streptomyces thermovulgaris* TISTR1948, xylooligosaccharide

Introduction

Xylooligosaccharides (XOs) are a type of non-digestible oligosaccharide occurring naturally in fruits, vegetables, bamboo shoots, honey, and milk (1). Notably, they are known to have beneficial effects on gastrointestinal health by increasing the probiotic population, providing short-chain fatty acids (SCFAs), and reducing the colonic pH (2). Moreover, XOs have several health benefits including, 1) reducing diabetes, arteriosclerosis, and gastrointestinal tract disease symptoms, 2) biological activities of antioxidant, antibacterial, and immunomodulatory actions, and 3) reduction of the risk of colon cancer (3,4). Recent studies have focused on use of XOs by bifidobacteria (3,5-7), while a few lactobacilli have also been reported (3,7). Almost all *Bifidobacterium* spp. are capable of using XOs as a prebiotic (3,5-7). However, the effects of XOs on promoting the growth of lactobacilli have not been well studied. This study investigated the effects of XOs on the growth of the well-known probiotic lactic acid bacteria *Lactobacillus plantarum,* one of the few lactobacilli that is widely used for the prebiotic properties of XOs (the probiotic lactobacilli species *L. casei* and *L. lactis* are not widely used) to determine new lactobacilli that can use corncob derived XOs.

XOs can be produced commercially from xylan-containing lignocellulosic materials (LCMs) using chemical hydrolysis, enzymatic hydrolysis, and chemical pretreatments combined with enzymatic hydrolysis (4). Enzymatic hydrolysis is the most commonly used method because of the specificity and controllability of the reaction, as well as limited formation of byproducts. Production of XOs from LCMs requires alkali or acid pretreatments prior to enzymatic hydrolysis in order to limit corrosion effects and generation of unwanted products from polysaccharides and lignin, as well as to improve the effectiveness of enzymatic hydrolysis (8). Specifically, an alkali pretreatment acts to solubilize hemicellulose and cellulose to a lesser degree than acid

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pretreatments or hydrothermal processes, and results in less inhibitor formation. Alkali pretreatment is more effective for LCMs that have a low lignin content (<26%), such as corn stover, switchgrass, sugar cane bagasse, wheat bran, softwood, and rice straw (9). Different alkali solutions can be used for pretreatment of LCMs prior to production of XOs, including NaOH, KOH, $Ca(OH)_2$, and NH_4OH (10,11). Xylanases are critical for xylan biodegradation. Endo-xylanase is a type of glycoside hydrolase that hydrolyzes the internal linkages of xylan by a random attack mechanism to cleave the xylan backbone, producing XOs (12). XOs produced using different alkali-pretreated LCMs catalyzed using different xylanases have been reported, including corncob (13,14), wheat bran (3), natural grass (10), oil palm frond fiber (15), and sugarcane bagasse (11). Thailand generates more than 29 million tons of agricultural waste per year. Due to lack of a market demand for these materials, most farmers eliminate this waste by burning. Corncob is the most abundant low-cost local agricultural LCM waste product in Thailand. The main type of hemicellulose in corncob is xylan, making it a suitable starting material for production of XOs.

Streptomyces sp. is a dominant xylanolytic bacterium that mainly produces endo-type xylanase (16). *S. thermovulgaris* TISTR1948 was previously reported to produce thermostable endo-xylanase with a high level of xylanase activity (274.49 U/mL) (17). The advantage for production of XOs using a cellulase-free endo-xylanase from strain TISTR1948 includes the highest level of enzyme activity and a higher yield of XOs than with use of other thermotolerant *Streptomyces* sp. (13,14). Moreover, using a cellulase-free xylanase at a high temperature can prevent contamination with glucose and microbial growth in production of XOs. Thus, the main purpose of the present study was to optimize the chemo-enzymatic process for production of XOs from corncob using economically generated thermostable endo-xylanase from *S. thermovulgaris* TISTR1948. Moreover, the prebiotic properties of corncob derived XOs were studied using *in vitro* fermentation of XOs derived from corncob with probiotic lactobacilli strains.

Materials and Methods

Microorganisms *S. thermovulgaris* TISTR1948 (isolated from soil samples under canopies of teak trees collected in Chiang Mai, Thailand in January of 2010) was used to produce thermostable endo-xylanase. The probiotic lactic acid bacteria *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 were provided by the Thailand Institute of Scientific and Technological Research (TISTR) and these 3 strains were used to study prebiotic activity. All microorganisms were maintained at –20°C in glycerol stock.

Alkali pretreatment of corncob Corncob and rice straw were obtained from local farmers in Chiang Mai and Phayao provinces, Thailand in December of 2012. They were sundried for 5 days, cut into 10.0 cm sections, and kept at 4°C until used. The alkali pretreatment step used in this study was modified from the method first described by Jayapal *et al.* (11). Dried corncob was ground with a hammer mill (Munson, Utica, NY, USA) and filtered through a sieve with <100 mesh size, then subjected to alkali pretreatment by soaking in 10.0% (w/v) KOH at 100°C for 1 h, followed by adjustment of the pH to 7.0 using addition of 5.0% (w/v) H_2SO_4. The resulting product was washed with tap water, filtered through a filter cloth with <200 mesh size, then the filtrate was dried at 80°C in a hot air oven (FN 500; NÜVE, Ankara, Turkey) for 48 h.

Thermostable endo-xylanase production Crude thermostable endo-xylanase was produced in a basal medium (17). Strain TISTR1948 was cultivated in a 250 mL Erlenmeyer flask containing 50 mL of a basal medium and incubated in a shaking incubator (Kühner, Basel, Switzerland) offset to 250 rpm at 50°C for 96 h (17). The highest endo-xylanase activity observed was 270.41 U/mL.

Enzyme assays The xylanase activity was measured using a 1.0% (w/v) beechwood xylan (Sigma, St. Louis, MO, USA) solution in 0.1 M potassium-phosphate (K-P) buffer (pH 6.5) as substrate. The clear supernatant from the culture medium was diluted using 0.1 M K-P buffer (pH 6.5) and incubated at 55°C with 1.0% (w/v) beechwood xylan for 10 min. Release of reducing sugars was measured using the dinitrosalicylic acid (DNS) method (18). An amount of 1 unit of xylanase activity (U) was defined as the amount of enzyme liberating 1 μmol of reducing sugar per min under assay conditions (17).

Production of XOs derived from corncob Corncob pretreated with KOH was used as a substrate for production of XOs. The substrate was subjected to enzymatic hydrolysis by mashing in 10 mM K-P buffer, pH 6.5 (15.0% w/v). Then, 100 U/g of a substrate of crude thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 was added, and the reaction was carried out at 55°C under static conditions for 24 h. Corncob samples pretreated with KOH and beechwood xylan were periodically taken and analyzed using thin-layer chromatography (TLC, silica gel 60 F_{254}; Merck, Darmstadt, Germany) as described by Kubata *et al.* (19). Results were compared to results for the standards xylose and arabinose (Sigma), and xylobiose, xylotriose, xylotetraose, and xylopentaose (Megazyme, Wicklow, Ireland).

Analysis of corncob composition and morphology For analytical purposes, raw corncob, KOH-pretreated corncob, and hydrolyzed KOH-pretreated corncob were analyzed using standard methods for lignin content (TAPPI T-222-om-11), cellulose content (TAPPI T-203-cm-09), hemicellulose content (TAPPI T-203-om-10), and ash content (T-211-om-93) (8). Different corncob samples were viewed under a scanning electron microscope (SEM, JEOL 5410-LV; JEOL, Tokyo, Japan) for observation of morphology, surface area, and physical structure. Dried samples of raw corncob, KOH-pretreated corncob, and hydrolyzed KOH-pretreated corncob were mounted on stubs, placed on conductive carbon tape, and coated with gold using a sputter coater (JEOL JFC-1200; JEOL) at 15 mA for 150 s.

Optimization of production of XOs from corncob using RSM The optimal conditions for production of XOs using KOH-pretreated corncob were studied using response surface methodology (RSM) with a central composite design (CCD). The statistical software package Design Expert, version 6.0.10 (Stat-Ease Inc., Minneapolis, MN, USA) was used. The enzyme dosage, pH, and temperature were the most important conditions affecting the yield of XOs. A range of thermostable endo-xylanase concentrations (X_1) between 100 and 150 U/g of substrate with a boundary of 82-168 U/g of substrate for ±α, the pH level (X_2) between 5.5-7.5 with a boundary of 4-9 for ±α, and the temperature (X_3) between 45 and 65°C with a boundary of 38-72°C for ±α were used in the experimental design. In total, 17 experiments with 3 replicates for center points were performed. The CCD contained an imbedded factorial or fractional factorial matrix with center points and star points around the center point that allowed estimation of the curvature. The precise value of α depended on properties needed for the design and the number of factors used (in this case, α=1.68). The CCD always contains twice as many star points as factors in the design. The star points represent new extreme values (low and high) for each factor in the design. To maintain the ability to rotate the CCD, the value of α depended on the number of experimental runs in the factorial portion of the CCD (20). Significant values of the model equation and model terms were evaluated using Fisher's Exact test as expressed as an *F* ratio:

$$Y=\beta_0+\Sigma\beta_i X_i+\Sigma\beta_{ii}X_i^2+\Sigma\beta_{ij}X_iX_j \quad (1)$$

where Y represents the response variable, β_0 is the interception coefficient, β_i the coefficient for the linear effect, β_{ii} is the ij^{th} coefficient of the interaction effect, and X_iX_j are input variables that influence the response variable Y. The response variable in each trial was the mean value of 3 replicates.

For optimization purposes, the reaction was carried out according to a CCD. Samples were periodically taken at preset times (0, 3, 6, 9, 12, 18, and 24 h), and centrifuged in a micro-centrifuge (MIKRO 120; Hettich, Tuttlingen, Germany) at 18,625×*g*, and analyzed using HPLC with an Aminex HPX 87H column (300×7.8 mm; Bio-Rad, Hercules, CA, USA). To confirm the applicability of the CCD optimization model, production of XOs was carried out using hydrolysis of KOH-pretreated corncob under the optimal conditions of an enzyme concentration of 129.43 U/g of substrate and a pH of 6.17 at 53.80°C. Samples were taken at 0, 6, 12, 18, and 24 h and analyzed using HPLC (Bio-Rad).

Analysis of XOs from corncob and other sugars Hydrolysis products were filtered through a cellulose-acetate membrane (0.2 μm; Sartorius, Göttingen, Germany) and subjected to HPLC (Bio-Rad) analysis. The hydrolysis products were analyzed using a modified HPLC (Bio-Rad) method described by Akpinar *et al.* (21). The mobile phase consisted of 5.00 mM H_2SO_4 as an eluent at a flow rate of 0.45 mL/min, and the column thermostat was set at 40°C. Sugar was detected using an RI detector (refractive index detector RID-10A) in a linear gradient over 20 min, and glycerol was used as an internal standard.

Preparation of the powder of XOs from corncob After 12 h of enzymatic hydrolysis, crude XOs derived from corncob were separated by filtering through filter paper (Whatman No. 4). The liquid hydrolysate was demineralized by mixing with DEAE-cellulose (10% w/v) for 30 min at 4°C, followed by centrifugation at 18,625×*g* for 15 min. The clear hydrolysate solution was concentrated in a rotary vacuum evaporator at 55°C, and 5.0% (w/v) maltodextrin was added before processing by spray drying to recover XOs derived from corncob in a powder form.

***In vitro* fermentation of corncob derived XOs** The probiotic lactic acid bacteria *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 were used to study the prebiotic activity of corncob derived XOs. An inoculum was prepared using pre-culture in MRS medium at 30°C under anaerobic conditions in an anaerobic jar (Schuett-Biotec GmbH, Göttingen, Germany) for 24 h. Subsequent probiotic cultures were grown at 30°C statically under anaerobic conditions in anaerobic jars on MRS medium according to Chapla *et al.* (22) supplemented with either glucose or xylose (as a non-prebiotic control), maltodextrin (Sigma), commercial XOs (Wako, Osaka, Japan), or corncob derived XOs (20.0 g/L) as a sole carbon source, and compared with a control (without sugar). The medium was inoculated with 1.0% (v/v) of a pre-culture solution at 30°C under anaerobic conditions. Culture broth samples were periodically taken (0, 6, 12, 18, 24, 36, and

48 h) to monitor growth and use of XOs by probiotic lactobacilli using viable counts on MRS plates. Colony forming units (CFU) were counted on plates containing 30 to 300 colonies, and the cell concentration was expressed as log CFU/mL.

Results and Discussion

Alkali-pretreatment of corncob The recovery yield after alkali pretreatment with 10% KOH (w/v) was 43.69±1.30% (w/w). The major components of raw corncob and KOH-pretreated corncob are shown in Table 1. After alkali pretreatment, the hemicellulose and lignin contents in KOH-pretreated corncob decreased because the alkaline solution probably caused swelling, leading to an increase in the internal surface area, a reduction in the degree of polymerization (DP) and crystallinity, and disruption of the crystalline structure by separation of structural linkages between lignin, hemicellulose, and cellulose (9). SEM results revealed that the KOH solution increased the surface area of the rigid raw corncob structure (Fig. 1A-1D). Therefore, the amount of hemicellulose and lignin that was soluble in the alkaline solution resulted in an increase in the cellulose content in KOH-pretreated corncob. Although alkali pretreatment resulted in a decreased hemicellulose content, the treatment facilitated the enzymatic hydrolysis reaction by making LCMs suitable substrates for enzymatic hydrolysis and more accessible to endo-xylanase (9).

Production of corncob derived XOs After periodical sampling, hydrolysis products from KOH-pretreated corncob were analyzed using TLC (Fig. 2A), and compared with the hydrolysis products of commercial xylan (beechwood xylan) (Fig. 2B). The product from enzymatic hydrolysis of KOH-pretreated corncob contained different types of sugar, including arabinose, xylose, xylobiose, xylotriose, xylotetraose, xylopentaose, and other XOs (X6). KOH-pretreated corncob and beechwood xylan showed different hydrolysis patterns because the different types of xylan in the corncob (annual plant) and the beechwood (hardwood) are different (9). For KOH-pretreated corncob, the spots of samples on the origin in Fig. 2A became darker when the

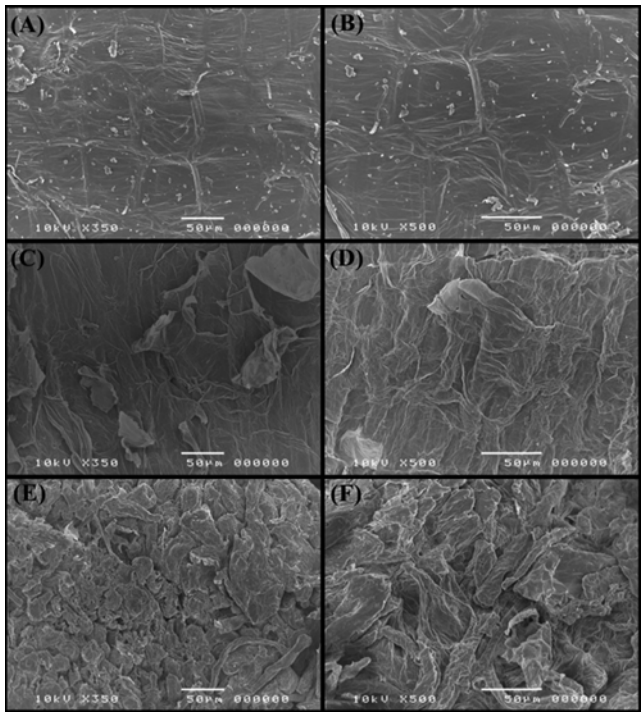

Fig. 1. SEM photomicrograph of the surface of raw corncob (A and B), KOH-pretreated corncob (C and D), and hydrolyzed KOH-pretreated corncob (E and F).

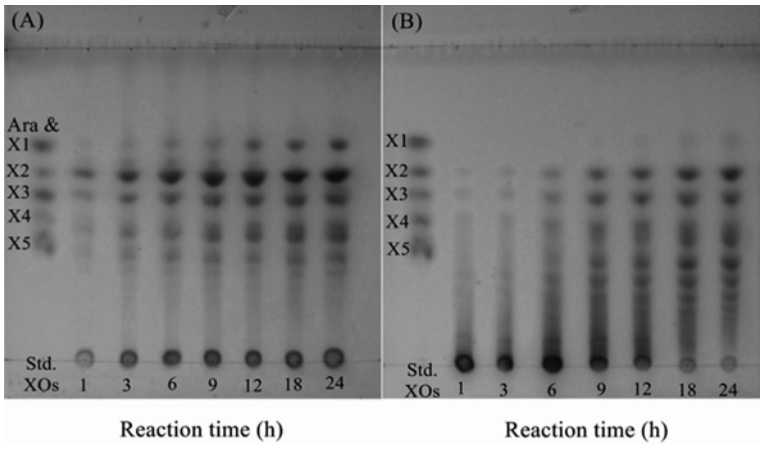

Fig. 2. TLC chromatogram of the time course for production of XOs from corncob (A) and beechwood xylan (B) catalyzed using the thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948. X1, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose; Ara, arabinose

Table 1. The composition of raw corncob, KOH-pretreated, and hydrolyzed KOH-pretreated corncob

Composition (%)	Raw corncob	KOH-pretreated corncob	Hydrolyzed KOH-pretreated corncob
Cellulose	40.38±1.02	65.21±1.41	84.81±1.10
Hemicellulose	41.45±1.23	24.67±0.71	5.17±0.17
Lignin	7.26±1.17	4.29±0.40	5.27±0.25
Ash	1.37±0.03	0.47±0.03	0.04±0.03
Other components (by difference)	9.54±1.61	5.36±0.64	4.71±0.39

reaction time was longer, perhaps due to accumulation of partially hydrolyzed products obtained from KOH-pretreated corncob. Beechwood xylan is a soluble polymer constructed from β-D-xylopyranose. After a long reaction time, that polymer was mostly hydrolyzed into shorter oligomers (XOs) and the spots on the origin in Fig. 2B were lighter. The optimal hydrolysis time for production of XOs from KOH-pretreated corncob was 12 h. Xylobiose was the main product, while the xylose content was low. Therefore, 12 h of reaction time was determined to be optimal for production of corncob derived XOs using RSM with a CCD.

Analysis of the corncob composition and morphology The composition of raw corncob, KOH-pretreated corncob, and hydrolyzed KOH-pretreated corncob were analyzed using the TAPPI method (8) (Table 1). The hemicellulose content in xylanase-hydrolyzed KOH-pretreated corncob was 5.17%, which was less than the 24.67% in KOH-pretreated corncob, indicating that hemicellulose was enzymatically hydrolyzed to XOs.

Moreover, SEM results showed that different corncob samples had different surface morphologies. Raw corncob was rigid with a smooth lignocellulose structure and no pores (Fig. 1A, 1B). KOH-pretreated corncob (Fig. 1C, 1D) showed a flaky and rough surface. Thus, KOH increased the surface area accessibility of the enzyme, breakdown of the rigid structure, and partial decrystallization of the corncob. After enzymatic hydrolysis using the thermostable endo-xylanase from *S. thermovulgaris* TISTR1948, the corncob surface had more surface area, a porous structure, and a rough surface (Fig. 1E, 1F). Thus, endo-xylanase could breakdown the amorphous structure of hemicellulose to release XOs and other sugars, and retain cellulose in the rough corncob.

The content of XOs was approximately 16.30%. Other products of xylose and arabinose were 3.28% (by weight of the hemicellulose content in KOH-pretreated corncob), which was similar to the decreased content of hemicellulose in enzymatically hydrolyzed KOH-pretreated corncob (19.58%).

Optimal conditions for corncob derived production of XOs In this study, the 3 factors of enzyme dosage, pH, and temperature were used for optimization of production of XOs. The CCD experiment led to a total of 17 sets of experiments. Respective yields of XOs at 6, 12, 18, and 24 h of reaction time are shown in Table 2. The CCD generated a quadratic equation for corncob derived yields of XOs (Y) as follows:

$$Y=+150.58+4.39X_1-13.90X_2-8.15X_3-3.47X_1^2-16.34X_2^2 -18.74X_3^2-6.83X_1X_3 \quad (2)$$

Results obtained using the CCD were analyzed using an analysis of variance (ANOVA). Values of "p value>F" of less than 0.0001 indicated that model terms were significant. In this case X_1, X_2, X_3, X_1^2, X_2^2, X_3^2, and X_1X_3 were significant model terms. An F value of 48.59 was interpreted to indicate that the model was significant. An F value for "Lack of fit" of 4.40 implied there was a 19.55% chance that a "Lack of fit" this large could occur due to noise. The quality of the model was expressed in terms of the R^2 value. The predicted values matched the experimental values at R^2=0.9842, adj-R^2=0.9640, and pred-R^2=0.8810. The results showed that all factors significantly influenced the yield of XOs.

The probability (p) value of the model was relatively low (<0.0001), indicating that the model was statistically significant. The coefficient of variation for the original model (R^2=0.9842) and the modified model (R^2=0.9769) indicated a high degree of correlation between the experimental observations. As such, both models were used to predict production of XOs from KOH-pretreated corncob using the thermostable endo-xylanase from *S. thermovulgaris* TISTR1948. The interaction term between the enzyme dosage and the temperature (X_1X_3) had a relatively low p value of less than 0.05 (0.0048). Based on the p value, xylanase dosages and temperature levels both influenced production of XOs.

The yield of XOs was strongly affected by both enzyme dosage and temperature (Fig. 3). The yield of XOs increased when the thermostable endo-xylanase dosage was increased from 137.98 mg/g of substrate at 82.96 U/g of substrate (low point-α) to 153.55 mg/g of substrate at 125.00 U/g of substrate (center point). The thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 may be more effective enzyme for production of XOs from corncob than xylanase from other *Streptomyces* spp. (13,14). However, at an enzyme

Table 2. Experimental values for the yield of XOs (%) from KOH-pretreated corncob

Run order	Code levels			Production yield of XOs (%)				
				Predicted values	Actual values			
	X_1 Enzyme dosage (U/g substrate)	X_2 pH	X_3 Temperature (°C)	(12 h)	(6 h)	(12 h)	(18 h)	(24 h)
1	82.96	6.50	55.00	13.34	9.12	13.80	10.98	10.17
2	125.00	6.50	38.18	11.13	7.51	11.20	9.47	9.28
3	100.00	7.50	65.00	9.52	3.70	9.20	8.87	8.61
4	100.00	5.50	65.00	11.74	4.21	11.90	10.55	7.87
5	167.04	6.50	55.00	14.82	9.04	14.30	13.97	13.23
6	150.00	7.50	45.00	11.47	6.62	11.40	8.49	7.94
7	125.00	6.50	55.00	15.06	9.36	15.00	13.07	11.89
8	100.00	7.50	45.00	9.83	6.01	9.80	7.28	6.34
9	125.00	4.82	55.00	12.78	9.93	12.60	10.99	9.95
10	150.00	7.50	65.00	8.43	6.40	8.90	8.73	7.56
11	150.00	5.50	65.00	11.86	8.56	12.00	8.42	8.22
12	125.00	6.50	55.00	15.06	9.28	14.90	10.38	9.16
13	125.00	8.18	55.00	8.10	5.87	8.10	7.86	7.41
14	125.00	6.50	55.00	15.06	8.76	15.40	14.54	14.29
15	150.00	5.50	45.00	14.81	10.64	15.30	15.31	14.89
16	100.00	5.50	45.00	11.96	4.02	11.50	8.85	8.55
17	125.00	6.50	71.82	8.39	4.18	8.20	6.90	6.92

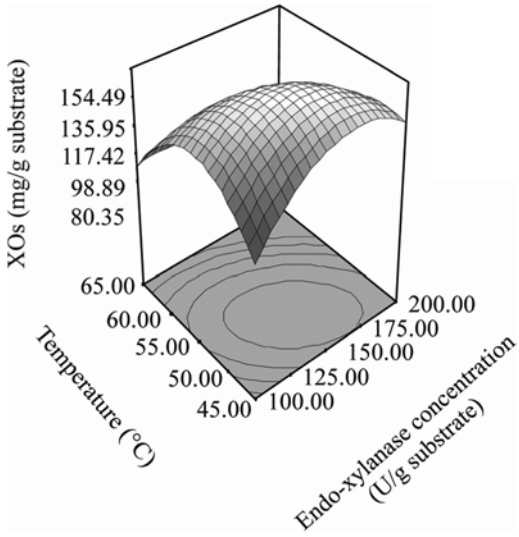

Fig. 3. Corncob derived production of XOs in a 3-dimensional graph for quadratic response surface optimization. The factors of enzyme dosage and temperature were compared.

dosage higher than 150 U/g of substrate, the yield of XOs decreased. A similar result was observed by Jayapal *et al.* (11) where the concentration of xylobiose decreased from 1.18 to 0.83 mg/mL when the xylanase dosage was increased from 2.65 to 13.25 U. Moreover, Brienzo *et al.* (23) reported that *Thermoascus aurantiacus* xylanase dosages higher than 120 U/g led to drastic decreases in yields of XOs. A possible explanation for this effect is that a reduction of DP in xylan generates high amounts of xylose at higher enzyme dosages. Temperature is reported to be an important factor for xylanase activity (24). A high temperature (71.82°C, high point+α) significantly decreasing yields of XOs was observed (Fig 3). It might be an inactivation of the enzyme at higher temperatures during longer reaction times (22). The maximum corncob derived yield of XOs (153.55 mg/g of substrate) was obtained at the center point (55°C) and a pH value of 6.17. The positive effect on production of XOs at a high temperature included dissolution of a high concentration of xylan, preventing microbial contamination, and increasing the reaction rate (25). While, the optimal pH for production of XOs is required for binding of the enzyme to the substrate, catalytic activity of the enzyme, ionization of the substrate, and stabilization of the 3 dimensional structure of enzyme are also important (26).

To confirm applicability of the CCD optimization model, production of XOs was carried out using hydrolysis of KOH-pretreated corncob under the optimal conditions for maximum yield of XOs of an endo-xylanase dosage of 129.43 U/g of substrate and a pH of 6.17 at 53.80°C. A yield of XOs of 162.97 mg/g of substrate, or 752.15 mg/g of hemicellulose (by calculation) was obtained, a value that was 5.10% higher than predicted. The model generated using the CCD could be used to predict the maximum yields of XOs.

The composition of XOs derived from corncob The composition of corncob derived samples of XOs is shown

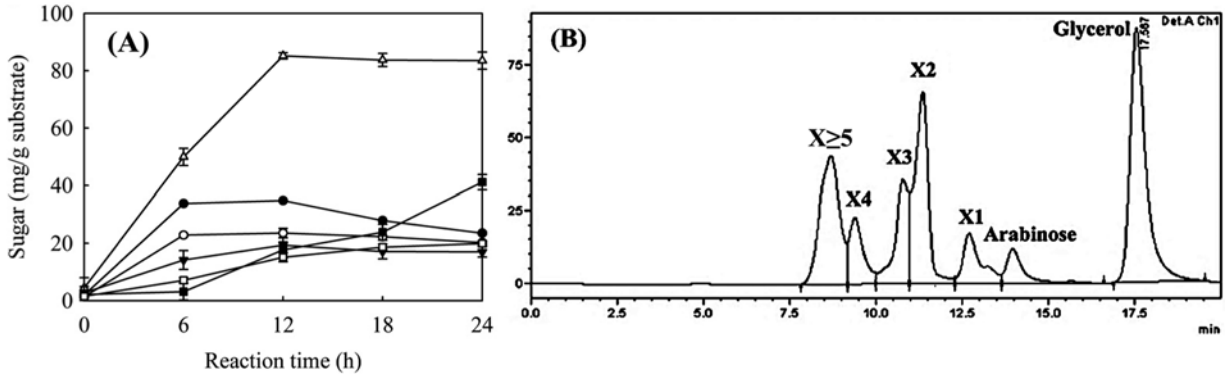

Fig. 4. Time course of corncob derived production of XOs from validation of the CCD experimental model. (A) An HPLC chromatogram of corncob derived XOs at 12 h (B). △, xylobiose; ●, xylopentaose and other XOs (X>5); ○, xylotetraose; ▼, xylotriose; ■, xylose; □, arabinose

in Fig. 4A. As a general trend, the concentration of XOs increased sharply after 12 h. The HPLC chromatogram (Fig. 4B) at 12 h indicated a maximum yield of XOs of 162.97±2.85 mg/g of substrate, with a concentration of xylobiose of 85.15±1.18 mg/g of substrate, xylotriose of 19.34±0.55 mg/g of substrate, xylotetraose of 23.58±1.62 mg/ g of substrate, and xylopentaose and other XOs (X5) of 34.90±0.31 mg/g of substrate. A low xylose content of 17.70±0.80 mg/g of substrate and an arabinose content of 15.14±1.55 mg/g of substrate were observed.

After 12 h, contents of XOs continuously decreased and the yield of XOs dropped to 143.76±1.54 mg/g of substrate at 24 h. The xylose content reached 41.16±1.65 mg/g of substrate. Based on these results, and on a report by Javier *et al.* (12) that showed that endo-xylanase is still active on xylooligomers with a degree of polymerization >2, endo-xylanase can hydrolyze xylotriose to xylobiose and xylose after prolonged incubation.

Comparison of yields of XOs between chemo-enzymatic conversion using the thermostable endo-xylanase from *S. thermovulgaris* TISTR1948 and other methods Li *et al.* (13) reported that purified endo-xylanase from *S. rameus* L2001 could hydrolyze NaOH-pretreated corncob to produce XOs with a yield of 150 mg/g of xylan. Furthermore, Ai *et al.* (14) revealed that the immobilized xylanase from *S. olivaceoviridis* E-86 produced XOs from the same substrate with a yield of 387.5 mg/g of xylan. However, the yield of XOs obtained from both *Streptomyces* strains previously reported was relatively low, compared with TISTR1948 used in this study (752.15 mg/g of hemicellulose). Jayapal *et al.* (11) described a chemo-enzymatic process for conversion of sugarcane bagasse to XOs using NaOH pretreatment and enzymatic hydrolysis with a commercial xylanase from *Trichoderma viridae* (Sigma) in which, under optimum conditions, a total reducing sugar yield of 367.79 mg/g of xylan was reported (11). Similarly, use of commercial xylanase (Shearzyme 500L and Veron 191) has been shown to produce only 143 mg of XOs/g of xylan when KOH combined with $NaBH_4$-pretreated corncob was used as a substrate.

In this study, a high content of XOs of 752.15 mg/g of hemicellulose using KOH-pretreated corncob was obtained within 12 h, with only small amounts of xylose, resulting in an improvement in the enzymatic method for economical production of XOs from corncob.

***In vitro* fermentation of XOs derived from corncob** In this study, all 3 described lactobacilli strains namely; *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 were able to use XOs derived from corncob obtained from enzymatic hydrolysis of KOH-pretreated corncob for growth, based on an increase in viable cell counts on MRS medium. Maximum viable cell counts (log CFU/mL) and maximum specific growth rates (μ_{max}) in MRS medium supplemented with different carbon sources for *L. casei* TISTR1463 (Fig. 5A), *L. lactis* TISTR1464 (Fig. 5B), and *L. plantarum* TISTR1465 (Fig. 5C) are shown in Fig. 5. After a fermentation period of 48 h, all probiotic lactobacilli exhibited the highest capacity to grow on MRS medium supplemented with glucose, similar to observations previously reported by Madhukumar and Muralikrishna (27) and Rycroft *et al.* (28). However, maximum viable cell counts for *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 on XOs derived from corncob were higher than for a control (without a carbon source) at 1.50, 1.94, and 1.08 log CFU/mL, respectively. MRS medium supplemented with commercial XOs (Wako) resulted in higher maximum viable cell counts than the control for *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 (1.51, 1.99, and 1.03 log CFU/mL, respectively). Viable cell counts and maximum specific growth rates (μ_{max}) for *L. lactis* TISTR1464 on MRS supplemented with corncob derived XOs were markedly higher than for the other taxa (Fig. 5A, 5B, 5C), indicating a higher capacity to use XOs derived

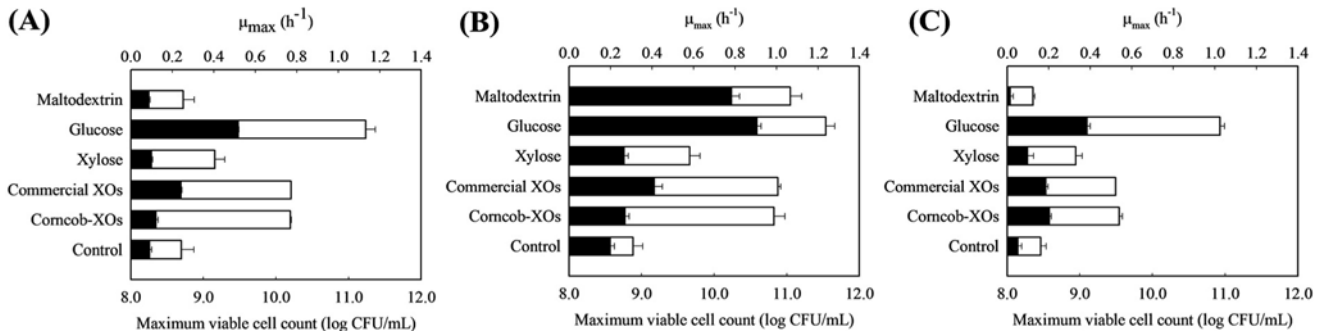

Fig. 5. Maximum viable cell counts (white bar) and maximum specific growth rates (black bar) of *Lactobacillus casei* TISTR1463 (A), *L. lactis* TISTR1464 (B), and *L. plantarum* TISTR1465 (C) in MRS medium supplemented with different carbon sources.

from corncob and commercial XOs, compared to *L. casei* TISTR1463 and *L. plantarum* TISTR1465. The different efficiencies for use of corncob derived XOs may depend on specific oligosaccharide use mechanisms for each strain (29).

Similar to the results for other *Lactobacillus* spp., such as *L. fermentum* (7), *L. acidophilus* (22), *L. fermentum* (22), *L. brevis* (27), and *L. plantarum* (27), the lactobacilli strains used in this study could not use XOs and glucose with similar degrees of efficiency. Kontula *et al.* (30) reported that *L. plantarum* can use oat bran-XOs. Moreover, Manisseri and Gudipati (3) and Madhukumar and Muralikrishna (27) reported that *L. plantarum* NDRI strain 184 is able to use XOs from wheat bran but grows poorly on Bengal gram husk XOs. The novel results from this study showed that *L. lactis* TISTR1464 had a greater ability to use corncob derived XOs than *L. plantarum* TISTR1465. Moreover, corncob derived XOs was a good carbon source for *L. casei* TISTR1463. The growth characteristics of *L. casei* TISTR1463, *L. lactis* TISTR1464, and *L. plantarum* TISTR1465 on corncob derived XOs were comparable to commercial XOs and demonstrated the prebiotic properties of corncob derived XOs.

Corncob, an abundant agricultural waste product in Thailand, has been shown to be a good cost-effective raw material for production of XOs using a thermostable endo-xylanase from *S. thermovulgaris*. Moreover, the prebiotic properties of corncob derived XOs were comparable with commercial XOs based on similar enhancement of the growth of probiotic lactobacilli strains.

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References

1. Mäkeläinen H, Juntunen M, Hasselwander O. Prebiotic potential of xylo-Oligosaccharides. pp. 245-258. In: Prebiotics and Probiotics Science and Technology. Charalampopoulos D, Rastall R (eds). Springer, New York, NY, USA (2009)
2. Hsu CK, Liao JW, Chung YC, Hsieh CP, Chan YC. Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. J. Nutr. 134: 1523-1528 (2004)
3. Manisseri C, Gudipati M. Bioactive xylo-oligosaccharides from wheat bran soluble polysaccharides. LWT-Food Sci. Technol. 43: 421-430 (2010)
4. Aachary AA, Prapulla SG. Xylooligosaccharides (XOS) as an emerging prebiotic: Microbial synthesis, utilization, structural characterization, bioactive properties, and applications. Comp. Rev. Food Sci. F. 10: 2-16 (2011)
5. Rycroft C, Rastall R, Gibson G. The role of prebiotics in human gut microbiology. pp. 411-428. In: Novel Frontiers in the Production of Compounds for Biomedical Use. Broekhoven A, Shapiro F, Anné J (eds). Springer, Dordrecht, the Netherlands (2002)
6. Gobinath D, Madhu AN, Prashant G, Srinivasan K, Prapulla SG. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. Brit. J. Nutr. 104: 40-47 (2010)
7. Moura P, Barata R, Carvalheiro F, Gírio F, Loureiro-Dias MC, Esteves MP. *In vitro* fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium* and *Lactobacillus* strains. LWT-Food Sci. Technol. 40: 963-972 (2007)
8. Romaní A, Garrote G, Parajó JC. Bioethanol production from autohydrolyzed *Eucalyptus globulus* by simultaneous saccharification and fermentation operating at high solids loading. Fuel 94: 305-312 (2012)
9. Brodeur G, Yau E, Badal K, Collier J, Ramachandran KB, Ramakrishnan S. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. Enzyme Res. 2011: 787532 (2011)
10. Samanta AK, Jayapal N, Kolte AP, Senani S, Sridhar M, Suresh KP, Sampath KT. Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (*Sehima nervosum*). Bioresource Technol. 112: 199-205 (2012)
11. Jayapal N, Samanta AK, Kolte AP, Senani S, Sridhar M, Suresh KP, Sampath KT. Value addition to sugarcane bagasse: Xylan extraction and its process optimization for xylooligosaccharides production. Ind. Crops Prod. 42: 14-24 (2013)
12. Javier PI, Óscar G, Sanz-Aparicio J, Díaz P. Xylanases: Molecular properties and applications. pp. 65-82. In: Industrial Enzymes. Polaina J, MacCabe A (eds). Springer, Dordrecht, the Netherlands (2007)
13. Li X, Li E, Zhu Y, Teng C, Sun B, Song H, Yang R. A typical endo-xylanase from *Streptomyces rameus* L2001 and its unique

characteristics in xylooligosaccharide production. Carbohydr. Res. 359: 30-36 (2012)
14. Ai Z, Jiang Z, Li L, Deng W, Kusakabe I, Li H. Immobilization of *Streptomyces olivaceoviridis* E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production. Process Biochem. 40: 2707-2714 (2005)
15. Sabiha-Hanim S, Noor MAM, Rosma A. Effect of autohydrolysis and enzymatic treatment on oil palm (*Elaeis guineensis* Jacq.) frond fibres for xylose and xylooligosaccharides production. Bioresource Technol. 102: 1234-1239 (2011)
16. Li N, Shi P, Yang P, Wang Y, Luo H, Bai Y, Zhou Z, Yao B. Cloning, expression, and characterization of a new *Streptomyces* sp. S27 xylanase for which xylobiose is the main hydrolysis product. Appl. Biochem. Biotechnol. 159: 521-531 (2009)
17. Chaiyaso T, Kuntiya A, Techapun C, Leksawasdi N, Seesuriyachan P, Hanmoungjai P. Optimization of cellulase-free xylanase production by thermophilic *Streptomyces thermovulgaris* TISTR1948 through Plackett-Burman and response surface methodological approaches. Biosci. Biotech. Bioch. 75: 531-537 (2011)
18. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428 (1959)
19. Kubata BK, Suzuki T, Horitsu H, Kawai K, Takamizawa K. Purification and characterization of *Aeromonas caviae* ME-1 xylanase V, which produces exclusively xylobiose from xylan. Appl. Environ. Microbiol. 60: 531-535 (1994)
20. Haaland PD. Experimental Design in Biotechnology. Marcel Dekker Inc., New York, NY, USA. pp. 1-18 (1989)
21. Akpinar O, Erdogan K, Bostanci S. Production of xylooligosaccharides by controlled acid hydrolysis of lignocellulosic materials. Carbohyd. Res. 344: 660-666 (2009)
22. Chapla D, Pandit P, Shah A. Production of xylooligosaccharides from corncob xylan by fungal xylanase and their utilization by probiotics. Bioresource Technol. 115: 215-221 (2012)
23. Brienzo M, Carvalho W, Milagres AF. Xylooligosaccharides production from alkali-pretreated sugarcane bagasse using xylanases from *Thermoascus aurantiacus*. Appl. Biochem. Biotechnol. 162: 1195-1205 (2010)
24. Techapun C, Poosaran N, Watanabe M, Sasaki K. Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review. Process Biochem. 38: 1327-1340 (2003)
25. Aragon CC, Mateo C, Ruiz-Matute AI, Corzo N, Fernandez-Lorente G, Sevillano L, Díaz M, Monti R, Santamaría RI, Guisan JM. Production of xylo-oligosaccharides by immobilized-stabilized derivatives of endo-xylanase from *Streptomyces halstedii*. Process Biochem. 48: 478-483 (2013)
26. Kumar Sohpal V, Dey A, Singh A. Investigate of process parameters on xylanase enzyme activity in *Melanocarpus Albomyces* batch culture. In: The World Congress on Engineering. June 30-July 2. WCE, London, UK (2010)
27. Madhukumar MS, Muralikrishna G. Fermentation of xylo-oligosaccharides obtained from wheat bran and Bengal gram husk by lactic acid bacteria and bifidobacteria. J. Food Sci. Technol. 49: 745-752 (2012)
28. Rycroft CE, Jones MR, Gibson GR, Rastall RA. A comparative *in vitro* evaluation of the fermentation properties of prebiotic oligosaccharides. J. Appl. Microbiol. 91: 878-887 (2001)
29. Holt SM, Miller-Fosmore CM, Côté GL. Growth of various intestinal bacteria on alternansucrase-derived oligosaccharides. Lett. Appl. Microbiol. 40: 385-390 (2005)
30. Kontula P, von Wright A, Mattila-Sandholm T. Oat bran β-gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. Int. J. Food Microbiol. 45: 163-169 (1998)