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Characterization of exopolysaccharide from *Lactobacillus fermentum* TISTR 2514 and its potential prebiotic properties

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Abstract

Exopolysaccharide (EPS) is an alternative interest for functional food ingredients development. *Lactobacillus fermentum* TISTR 2514 is a lactic acid bacteria and able to produce EPS. The aims of this study were to characterize and investigate the prebiotic potential of EPS produced by *L. fermentum* TISTR 2514 from Thai-fermented food. The EPS showed a symmetrical sharp peak which separated using Gel permeation chromatography (GPC) technique and its molecular weight was 48.192 kDa. HPLC analyses presented that the EPS consisted of rhamnose, galactose, mannose and glucose. The prebiotic potential of EPS was studied. The results indicated that EPS could tolerate to artificial gastric juice (pH 1) for 2 h and α-amylase (pH 7) for 6 h which more than 89 and 82% , respectively. Furthermore, these EPS could stimulate the growth of probiotics (*L. rhamnosus* DSM 20021 and *L. casei* DSM 20011), higher than using FOS which is commercial prebiotics. Bacterial enzyme assessment in the combination of probiotics and EPS produced from *L. fermentum* TISTR 2514 (candidate prebiotic) revealed a greater reduction in β-glucuronidase activity than using probiotics or EPS alone. The ability of synbiotic (probiotics and EPS) is particularly interesting for potentially reducing the β-glucuronidase activity and for decreasing the risk of colorectal cancer, however further in-depth study is required.

Keywords: Exopolysacharide (EPS), Probiotics, Prebiotics, Synbiotics, Bacterial enzyme

1. Introduction

Traditional fermented food in Thailand varies from region to region and also serves as a source for Lactic Acid Bacteria (LAB) screening. LABs are associated with fermented food and are granted the status of 'generally recognized as safe' (GRAS) [1]. Some sources of LAB commonly found from Thai-Fermented food include kapi (fermented shrimp or small fish paste), fermented bamboo shoot, thua-nao (fermented soil bean), plara, plasom, and nampla (fermented fish) etc. Some strains of LAB are capable of secreting extracellular polysaccharides or exopolysaccharides (EPSs) [2]. EPSs have been widely used in the food industry as texturizers, viscosifiers, emulsifiers and synerisis-lowering agents [3]. In addition, EPSs are used as a functional food to modulate the composition of gut microbiota to stimulate human gastrointestinal health [4]. Potential prebiotic effects of EPSs on the human intestinal microbiota as a drug against colon cancer, immune stimulators etc. were reported [5]. In the gastrointestinal tract (GI), the population of bacteria colonization varies by dietary changes and host developments [6]. The colonic microbiota can play both beneficial and detrimental roles by controlling the epithelial cell proliferation and differentiation through host nutrition via metabolism [7 & 8]. On the other hand, the consumption of red meat and animal fat is associated with increased risk of colorectal cancer, which is due to an imbalance of the intestinal microbiota [9]. However, the previous experiments suggested that several probiotics and prebiotics are associated with reducing the risk of cancers in animal and human. Such as

the consumption of *L. acidophilus* in experimental animal models showed that the fecal bacteria enzyme activities such as β-glucuronidase, azoreductase and nitroreductase decreased. These enzymes are well known as they can catalyze the conversion procarcinogens to carcinogenic in colon cancer [10 & 11]. In human studies found that fecal bacteria enzyme activities decreased when healthy female adults ate yogurt containing viable *Lactobacillus* GG for four weeks [12]. Thus, several food ingredients have been gained interest as functional components for health, including probiotics and prebiotics. A prebiotic is a 'non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health' by stimulating particular beneficial bacteria while inhibiting the growth of pathogenic bacteria [13]. Prebiotics α-D-glucan from *L. plantarum* DM5 reveals the ability to stimulate probiotic growth and did not support the growth of *E. coli* [14]. EPS from microbial sources can be classified into two groups based on their monosaccharide composition and biosynthetic pathway [15]. Homosaccharides (HoPS) consist of identical monosaccharides, D-glucose or D-fructose while heterosaccharides contain regular repeating units of 3-8 different carbohydrate, D-glucose, D-galactose, L-rhamnose and their derivatives [16]. Recently, the application of EPS-producing LAB as a prebiotic has been widely investigated for applications in the food industry and as a feed additive. Thus, in the prebiotic material selection, we considered EPS-producing LAB and their prebiotic properties such as resistance to degradation by human gastrointestinal juice, mammalian enzyme, and fermentation by probiotic. We also considered stimulation of the growth or activity of beneficial microorganisms in the gut, and especially the reduction of β-*glucuronidase* activity produced by pathogen *in-vitro*. Finally, the aims of this study were to find and understand new types and sources of natural prebiotics to develop material products from lactic acid bacteria that have prebiotic potential.

2. Materials and methods

2.1 EPS-inducing from L. fermentum TISTR 2514

A single colony of *L. fermentum* TISTR 2514 was used as a starter in MRS broth (Himedia, India). Fermentations were incubated at 37ºC under anaerobic conditions for 48 h. Fermented samples were centrifuged at 8,000 rpm for 10 min and the supernatant was collected. Supernatant was then collected and concentrated by rotary evaporator to one third its volume. EPS was precipitated with two volumes of 95% cold ethanol. The mixture was stored at 0 ºC for 48 h and centrifuged at 8,000 rpm for 10 min. The precipitate was dissolved in dH_2O. The samples were freeze-dried and kept at -20ºC.

2.2 Determination of molecular weight distribution of exopolysaccharide and monosaccharide composition

The molecular weights of EPS samples were analyzed by high-performance size-exclusion chromatography (HPSEC) on agilent 1100 HPLC system equipped with a TSK-GEL G2000 PWxl column (7.8 mm × 300 nm, Tosoh Corp., Tokyo, Japan) and a refractive index detector (RID). EPSs were dissolved with 0.05M sodium bicarbonate buffer pH11 to 0.2% w/v and then all samples and standards were filtered through a 0.45 µl syringe filter. Aliquots of 100 µl sample solution were injected and eluted with 0.5 mg/mL of Na_2SO_4 solution at flow rate 0.5 mL/min. Series dextrans (MW 4,400-401,000) were used as standards for molecular weight measurement. Mono- and disaccharides were analyzed by High Performance Liquid Chromatography (HPLC).

2.3 Tolerance of α-amylase

The hydrolysis of EPS by α-amylase was determined following the method by Wichienchot (2010). The commercial prebiotics (Fructooligosaccharides or FOS) was used as a positive control. 400 mg aliquots were dissolved in 40 mL of 20 mM sodium phosphate buffer (pH 5, 6, 7) and incubated with 2U/mL α-amylase at 37ºC for 6h. The reaction mixtures were collected and the reducing sugar and total sugar content was determined using DNS method [17 - 19] and phenol-sulfuric acid method, respectively, after incubation for 0, 1, 2, 4, 6 h. The hydrolysis degrees of samples were calculated according to the formula [20] as following:
Hydrolysis degree (%)

$$= \frac{reducing\ sugar\ released}{total\ sugar - initial\ reducing\ sugar} \times 100$$

Where reducing sugar released is the difference between reducing sugar content at specified time and initial reducing sugar content.

2.4 Effect of artificial human gastric juice on EPS

One percent (w/v) of EPS and FOS were dissolved in artificial gastric juice according to the method described by Wichienchot [21]. Artificial human gastric juice was prepared as following: $Na_2HPO_4 \cdot H_2O$ (8.25 g), NaH_2PO_4 (14.35 g), NaCl (8 g), KCL (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.1 g) and $MgCl_2 \cdot 6H_2O$ (0.18 g) were dissolved and filled to 1,000 mL with distilled water. The solution of artificial human gastric juice was adjusted to pH 1, 2, 3, 4 and 5, respectively. Sample solutions were incubated at 37ºC for 6 h and reducing sugar measurements were taken at 0, 1, 2, 4 and 6 h using DNS method and total sugar was determined with phenol-sulfuric acid method using glucose as a standard. The percentage hydrolysis of sample was calculated as mentioned in earlier section.

2.5 Effect of EPS-producing LAB on growth of probiotics

One percent of inoculums (with OD_{600} of 0.5) were inoculated in basal medium broth (*L. plantarum* DSM 2648, *L. rhamnosus* DSM20021, *L. casei* DSM20011), using different carbon sources supplemented with 1% w/v of glucose, FOS and EPS then incubated at 37°C for 48 h in anaerobic condition. The basal culture medium consisted of peptone water 2.00 g, yeast extract 2.00 g, NaCl 0.10 g, K_2HPO_4 0.04 g, KH_2PO_4 0.04 g, $MgSO_4 \cdot 7H_2O$ 0.01 g, $CaCl \cdot 2H_2O$ 0.01 g, $NaHCO_3$ 2.00 g, L-cysteine HCl 0.50 g, bile salts 0.50 g, hemin 0.05 g and following with liquid addition; Resazurin (0.05 g/L) 4.00 mL, vitamin k (1,000 µg/mL) 10 µl, Tween-80 2.00 mL and adjust volume to 1,000 mL, sterilization at 121°C for 15 minutes. The growth of probiotics was enumerated as CFU/mL counting colonies which appeared on MRS agar+ 0.5% $CaCO_3$ using drop plate technique and incubated in anaerobic condition at 37 ºC for 48 h (samples taken every 3 h for 24 h and then every 6 h afterward).

2.6 Effect of EPS for β -glucuronidase –fecal bacteria enzyme activities reducing in-vitro

One percent of *E. coli* (measuring 0.5 at OD_{600} on a spectrophotometer) was cultured in basal medium broth using different carbon sources supplemented with 2% w/v of glucose, FOS and EPS + 1% of *L. casei* at OD_{600}=0.5 and EPS +1% of *L. rhamnosus* at OD_{600}=0.5 then incubated at 37°C for 48 h in anaerobic condition. Cell solution was centrifuged at 8,000 rpm for 10 minutes at 4 ºC. The supernatant was discarded and homogenized in 30 mL of phosphate buffer at pH 7.0. The homogenized cell was sonicated (4 cycles, 30 sec, 0ºC) and centrifuged at 8,000 rpm for 10 minutes at 4 ºC. The supernatant was collected to a clean tube and kept in -80ºC until it was carried out for β-glucuronidase enzymatic assay. Fecal β-glucuronidase activity was assayed in triplicate looking for the release of phenolphthalein from phenolphthalein mono-β-glucuronide (Sigma Chemical Co., St. Louis, Mo.) and was measured at 540 nm in a spectrophotometer (Milton Roy Co, Rochester, NY). The results were displayed as units/mL (mean ± SD).

2.7 Statistical analysis

In this study, the average results of three replicates were displayed as mean ± SD**.** Statistical analysis was performed by SPSS (version 18). The difference between the means was analyzed by Turkey's method and the level of significance was defined at *p-value* < 0.05.

3. Results

3.1 Molecular weight distribution of EPS-Producing L. fermuntum TISTR 2514

The molecular weight (MW) of EPS is an important characteristic of its biological activity. Size exclusion chromatography was used to estimate the Mw of EPS. A calibration curve was constructed based on retention time of dextran with different Mw. The results were shown in figure 1. The profile of EPS as a symmetrical sharp peak with only one peak indicated that EPS fraction was the homogeneous polysaccharide. According to the calibration curve of standards, the Mw of EPS was estimated to be 48.192 kDa. HPLC analysis showed that monosaccharide compositions of EPS were 28.81% galactose, 25.68% rhamnose, 23.21% glucose and 22.30% mannose, which was similar to patterns in TLC (data not show).

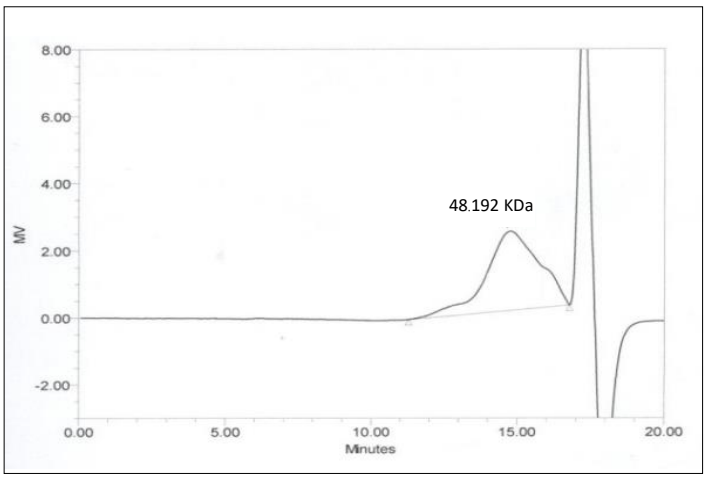

Figure 1 Molecular weight distribution of EPS obtaining from *L. fermentum* TISTR 2514 (estimated by GPC).

3.2 Effect of artificial human gastric juice on EPS

EPS were tested for prebiotic properties; tolerance to artificial human gastric juice and α-amylase hydrolysis, to understand movement through the gastrointestinal (GI) tract. After incubation at pH 1, 2, 3, 4 and 5 for 6 h, the percentage of hydrolysis increased with decreasing pH. The percentage of hydrolysis of pH 1 had a significantly higher effect compared to other pH (*p-value* < 0.01) (figure 2). The hydrolysis degree of EPS was 10.88, 5.77, 1.43, and 0.32, while percentage of hydrolysis of FOS was 46.57, 10.52, 1.16 and 1.13 at pH 1, 2, 3 and 4, respectively, after incubation for 2 h. However the maximum of hydrolysis degree of EPS did not exceed 18% whereas the maximum hydrolysis of FOS was more than 120% after incubation for 6 h. The results indicated that EPS had more than 89% resistance to gastric juice at pH1 for 2 h and foods usually pass through the human stomach within 2-4 h [22].

3.3 Tolerance of α-amylase

The hydrolysis of EPS and FOS by α-amylase at pH 5, 6 and 7 for 6 h indicated that the percentage of hydrolysis was not significant (*p-value* > 0.05) at different pH, however the trends of hydrolysis degree increased with incubation time and pH. Percentage of EPS hydrolysis after incubation for 6 h was 12.84, 14.38 and 17.29 at pH 5, 6 and 7, respectively. While the hydrolysis of FOS was lower than EPS, 0.44, 0.62 and 0.68% were estimated at pH 5, 6 and 7 after 6 h of incubation, respectively. The results suggested that FOS showed significantly higher α-amylase tolerance; however EPS resistance to α-amylase at more than 82 % indicated that EPS had better α-amylase tolerance than inulin, which had a degree of hydrolysis at more than 25% at pH 7 after 2 h of incubation [21]. Although the percentage of hydrolysis of EPS was higher than FOS, the acidity tolerance of FOS was significantly lower than EPS after incubation for 2 h afterward. From the hydrolysis of EPS by gastric juice and α-amylase the results suggested that at least 60% of EPS had remained able to reach the colon through human consumption.

3.4 Ability for growth-stimulation of probiotics

In this study, we used 2% w/v of glucose, EPS and FOS as a carbon sources for comparing data. The effect of EPS on probiotic growth in basal medium was investigated. The results showed that EPS was able to promote the growth of probiotic organisms including *L. rhamnosus* DSM 20021 and *L. casei* DSM 20011 from log 6.02 $\pm$ 0.08 to log 8.08 $\pm$ 0.06 CFU/mL and from log 6.03 $\pm$ 0.05 CFU/mL to log 8.17 $\pm$ 0.02 which significantly higher more than FOS at 24 and 48 h. Glucose significantly promoted the growth of *L. rhamnosus* DSM 20021 and *L. casei* DSM 20011 from EPS and FOS (*p-value* < 0.05) within 24 h while FOS stimulated the growth of *L. rhamnosus* DSM 20021 and *L. casei* DSM 20011 from log 6.14 $\pm$ 0.02 to log 7.60 and from log 6.01 $\pm$ 0.09 to log 7.59 $\pm$ 0.02 (within 48 h), respectively. The growth of *L. plantarum* DSM 2648 did not significant when cultured with a different carbon source at 24 h (Table 1). The results suggested that EPS could stimulate the growth of *L. rhamnosus* DSM 20021 and *L. casei* DSM 20011 (from log 6 to log 8 CFU/mL) within 24 h. Thus, in the next study we emphasized on the independent effect of EPS comparing with EPS-dependent with *L. casei* DSM 20011 and *L. rhamnosus* DSM 20021 for β-glucuronidase enzyme activity reducing *in vitro*.

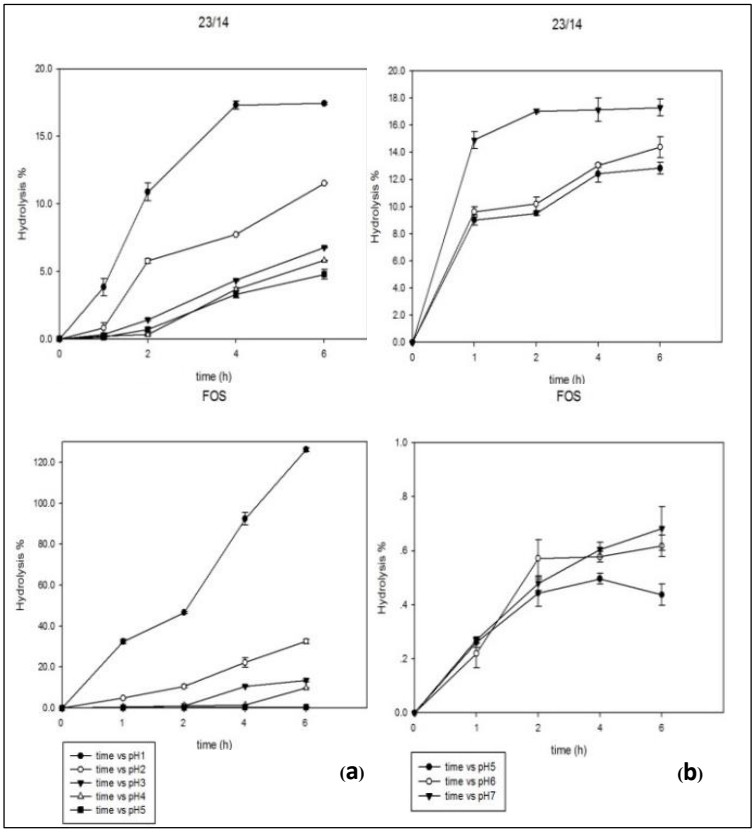

Figure 2 The hydrolysis degrees of EPS-producing strain of *L. fermentum* compared with FOS to artificial human gastric juice at pH 1, 2 3, 4 and 5 (a) and to α -amylase (2U/mL) at pH 5, 6 and 7 (b) for 6 h.

Table 1 Growth profile of probiotic bacteria shows as $\log_{10}$ CFU/ mL when cultured in basal medium which supplemented with different carbon source (Glucose, EPS-producing *L. fermentum*, and FOS)

Probiotics	**Carbon source**	**Log CFU/mL**			
		0 h	**12 h**	**24 h**	**48 h**
L. plantarum DSM 2648	Glucose	6.34 ± 0.04	7.80 ± 0.04	7.41 ± 0.06^{a}	7.01 ± 0.06^{a}
	EPS	6.23 ± 0.08	7.48 ± 0.01	7.51 ± 0.01^{a}	7.40 ± 0.02^{b}
	FOS	6.32 ± 0.04	6.67 ± 0.06	7.44 ± 0.07^{a}	7.13 ± 0.05^{c}
L.Rhamnosus DSM 20021	Glucose	6.05 ± 0.06	8.19 ± 0.02	9.23 ± 0.05^{a}	9.00 ± 0.04^{a}
	EPS	6.02 ± 0.08	7.01 ± 0.02	8.08 ± 0.06^{b}	8.10 ± 0.08^{b}
	FOS	6.14 ± 0.02	6.11 ± 0.06	6.06 ± 0.06^{c}	7.60 ± 0.02^{c}
L. casei DSM 20011	Glucose	6.04 ± 0.15	7.52 ± 0.06	8.62 ± 0.04^{a}	8.60 ± 0.02^{a}
	EPS	6.03 ± 0.05	7.18 ± 0.09	8.17 ± 0.02^{b}	7.90 ± 0.28^{b}
	FOS	6.01 ± 0.09	6.09 ± 0.08	7.41 ± 0.07^{c}	7.59 ± 0.02^{b}

***** All the experiment were repeated in three replicates ($n = 3$) and the results are expresses as mean $\pm$ SD. The difference between the means was analyzed by Turkey's method and the level of significance was defined at *p-value* < 0.05 ($^{a, b}$ and ^{c} refer to the groups at difference mean)

3.5 Effect of synbiotic for β -glucuronidase enzyme activity reducing

The role of EPS for reducing the activity of β -glucuronidase enzyme was investigated *in-vitro*. The activity of β -glucuronidase enzyme was measured following the sigma protocol (EC.3.2.1.31). The results suggested that the activity of β -glucuronidase enzyme did not significantly decrease (*p-value* > 0.05) when *E. coli* was cultured with different carbon sources, showing 208.75 ± 14.07, 228.13 ± 2.86 and 235.00 ± 24.97 units/mL

enzyme of glucose, FOS and EPS, respectively. The activity of β-glucuronidase enzyme significantly decreased (*p-value* < 0.01) when *E.*coli was cultured with EPS + *L. casei* DSM 20011 and EPS + *L. rhamnosus* DSM 20021, showing 130.00 ± 19.69 and 100.00 ± 21.07 units/mL enzyme, respectively. Conversely, when *E. coli* was cultured with *L. casei* DSM 20011 or *L. plantarum* DSM 20021 alone, supplemented with glucose in the same conditions for 48 h, the data exhibited that activity of β-glucuronidase enzyme did not significantly decrease from the control (data not shown) . The evidence suggested that the activity of β-glucuronidase enzyme significantly decreased when *E.coli* was cultured with the combination of probiotics (strains of *L.casei* DSM 20011 or *L. rhamnosus* DSM 20021) and EPS-producing *L. fermentum* TISTR 2514 *in-vitro* for 48 h. Nevertheless, *in-vivo* experiment when prebiotic (inulin) or probiotic (*B. longum*) were fed in rat (azoxymethane-induced ACF) for 12 weeks, showed β-glucuronidase enzyme activity significantly decreased by feeding inulin or *B. longum* or both. However, animals fed *B. longum* alone exhibited a smaller decrease (30%) while rats fed with inulin+ *B. longum* showed the greatest decrease (55%) of β-glucuronidase enzyme activity [23].

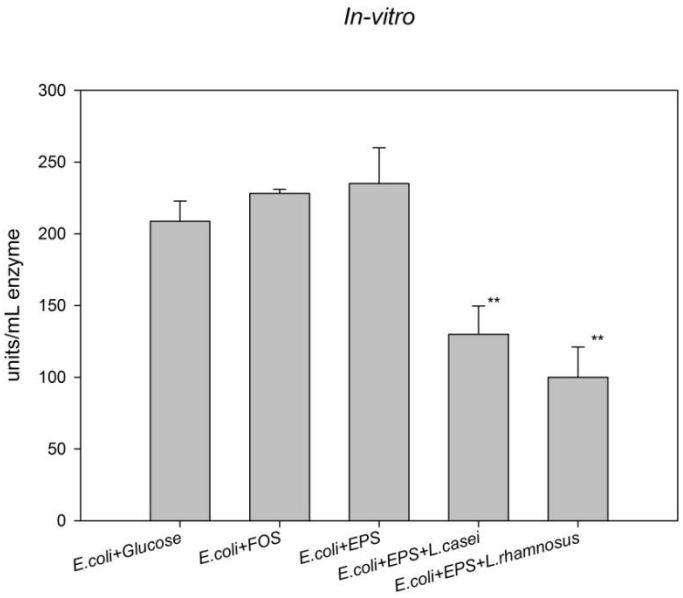

Figure 3 the units/mL enzyme of β-glucuronidase enzyme activity of *E.coli* when cultured in basal medium with different carbon sources *in-vitro*, comparing with control *(E.coli + Glucose)*, ***the star refer to p-value <0.01.*

4. Discussion

EPS-producing LAB is widely interesting for food additive application, especially focusing on prebiotic properties. Prebiotics are non-digestible carbohydrates associated with health and wellbeing that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon [13]. EPS strain of *L. fermentum* TISTR 2514 showed important prebiotic properties, including resistance to gastric acidity and enzymatic hydrolysis, and can therefore pass through the gastrointestinal tract to the colon. At least 60% of EPS had reached the colon through human consumption**.** The present study provides evidence that EPS could also stimulate the growth of *L. rhamnosus* DSM 20021 and *L. casei* DSM 20011 from log 6 to 8 CFU/mL within 24 h. EPS did not stimulate the growth of *L. plantarum* DSM 2648. According to previous research, the effect of prebiotics on an animal model found that oligofructose enriched inulin (100g/kg) could stimulate IL-10 production in Peyers patches (PP) in AOM-induced rat [24]. Inulin and oligofructose reduced the severity of DMH-induced CRC in rats [25]. In a human study, the number of *Bifidobacterium adolescentis* increased the most when 12 volunteers ingested 10g inulin/day for 16 days, compared with a control period without any supplement intake [26]. However, *in vitro* studies have demonstrated that the combination between probiotic and prebiotics were more effective than prebiotics or probiotics alone in modulating the gut microflora [27]. Results showed that the combination of maltodextrin and *Lactobacillus paracasei* decreased the number of *E. coli* colonizing the jejunal mucosa of gnotobiotic piglets by 1 logarithm from control. When *Lactobacillus paracasei* was cultured in combination with FOS, counts of *Lactobacillus* spp. and *Bifidobacterium* spp. significantly increased [28] . In addition, assessment of bacterial enzyme activity *in-vivo* showed that the

combination of inulin (prebiotic) and *Bifidobacterium longgum* (probiotic) was more successful at decreasing azoxymethane-induced ACF than either compound alone in rat [23] . Recent *in-vitro* preliminary study confirmed that the combination between EPS produced from *L. fermentum* TISTR 2514 (candidate prebiotic) and probiotics have more efficiency for decreasing activity of β-glucuronidase enzyme when compared with control**.**

5. Conclusions

A study of the potential prebiotic properties of EPS indicated that EPS could stimulate the growth of probiotics (*L. rhamnosus* DSM 20021 and *L. casei* DSM 20011from log 6.02 ± 0.08 to log 8.08 ± 0.06 CFU/mL and from log 6.03 ± 0.05 CFU/mL to log 8.17 ± 0.02 in 24h, respectively) higher than using FOS. In addition, EPS could resist artificial gastric juice, with more than 89% tolerance at pH 1 for 2 h and more than 82% tolerance to α-amylase at pH 7 for 6 h. A bacterial enzyme assessment revealed that the combination of probiotics and EPS-producing strain of *L. fermentum* TISTR 2514 (candidate prebiotic) significantly decreased β-glucuronidase activity enzyme. However, further study of the potential prebiotic properties of this EPS is required.

6. Acknowledgements

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7. References

[1] Laws A, Gu Y, Marshall V. Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. Biotechnol Adv. 2001;19:597-625.
[2] Harutoshi T. Exopolysaccharides of Lactic Acid Bacteria for Food and Colon Health, Applications. Lactic Acid Bacteria – Intech. 2013;515-38.
[3] Patel S, Goyal A. The current trends and future perspectives of prebiotics research: a review. 3 Biotech. 2012;15:115–25.
[4] Fooks LJ, Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr. 2002;88:39-49.
[5] Grosu-Tudor SS, Zamfir M, Meulen RVD, Falony G, Vuyst LD. Prebiotic potential of some exopolysaccharides produced by lactic acid bacteria. Rom Biotechnol Lett. 2003;18:8666-76.
[6] Compare D, Nardone G. The bacteria-hypothesis of colorectal cancer: pathogenetic and therapeutic implication. Transl Gastrointest Cancer. 2014;3: 44-53.
[7] Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol 2015;6: 1-9.
[8] Cipe G, Idiz UO, Firat D, Bektasoglu H. Relationship between intestinal microbiota and colorectal cancer. World J Gastrointest Oncol. 2005;7:233-40.
[9] Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int J Cancer. 2006;119:2657-64.
[10] Goldin BR, Gorbach SL. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. J Natl Cancer Inst. 1976;57:371-75.
[11] Goldin BR, Gorbach SL. Effect of *Lactobacillus acidophilus* dietary supplementation on 1, 2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. J Natl Cancer Inst. 1980;64:263-5.
[12] Ling WH, Korpela R, Mykkanen H, Salminen S, Hannine O. Lactobacillus strains GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J Nutr. 1994;124:18-23.
[13] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr. 1994;125:1401-12.
[14] Das D, Baruah R, Goyal A. A food additive with properties of an α-D-glucan from *Lactobacillus plantarum* DM5. Int J Biol Macromol. 2014;69: 20-6.
[15] Jolly L, Vincent SJ, Duboc P, Neeser JR. Exploiting exopolysaccharides from lactic acid bacteria. Antonie Van Leeuwenhoek. 2002;82:367–74.
[16] Monsan P, Bozonnet S, Albenne C, Joucla G, Willemot R, Remaud-Simeson M. Homopolysaccharides from lactic acid bacteria. Int Dairy J. 2001;11:675–85.
[17] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric Method for Determination of sugars and Related Substances. Anal Chem. 1956;28:350-6.

[18] Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem. 1944;153:375-80.
[19] Somogyi M. A new reagent for the determination of sugar. J Biol Chem. 1945;160:61-8.
[20] Korakli M, Ganzle MG, Vogel RF. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*. J Appl Microbiol. 2002;92:958-65.
[21] Wichienchot S, Jatupornpipat M, Rastall RA. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. Food Chem. 2010;120:850-57.
[22] Wang X, Huang M, Sun FYH, Zhou X, GuoY, Wang X, et al. Rapeseed polysaccharides as prebiotics on growth and acidifying activity of probiotics *in vitro*. Carbohydr Polym. 2015;125:232-40.
[23] Rowland IR, Rumney CJ, Coutts JT, Lievense LC. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rat. Carcinogenesis. 1998;19:281-5.
[24] Roller M, Femia PA, Caderni G, Rechkemmer G, Watzl B. Intestinal immunity of rats with colon cancer is modulated by oligofructose-enriched inulin combined with *Lactobacillus rhamnosus* and *Bifidobacterium lactis*. Br J Nutr. 2014;92:931-8.
[25] Hughes R, Rowland IR. Stimulation of apoptosis by two prebiotic chicoryfructans in the rat colon. Carcinogenesis. 2001;22:43-7.
[26] Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut micribiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii.* Br J Nutr. 2008;101:541-50.
[27] Saulnier DMA, Gibson GR, Kolida S. *In vitro* effects of selected synbiotic on the human feacal microbiota consumption. FEMS Microbiol Ecol. 2008;66:516-27.
[28] Bamba A, Nemcova R, Gancarcikova S, Herich R, Guba P, Mudronova D. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br J Nutr. 2002;88:S95-9.