

Hydrolyzed Inulin with Different Degree of Polymerization as Prebiotic for *Lactobacillus plantarum*

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The study was conceived to investigate the utilization of hydrolyzed inulins from Jerusalem artichoke (JA) with different degree of polymerization (DP) on the growth and antibacterial nature of probiotic bacterium, *Lactobacillus plantarum*. High long chain inulin (InuL) and high short chain inulin (InuS) of acid hydrolyzed inulin were studied as carbon sources for the growth of *L. plantarum*, growth inhibition of *Escherichia coli* and *Salmonella enterica* serovar Typhi by *L. plantarum*, and inulin supplementation was evaluated by using co-culture and agar well diffusion methods. The results suggested that InuL and InuS support the growth of *L. plantarum* at significant level ($P < 0.05$) compared to control and it improves the bacterial inhibitory nature of *L. plantarum* against *E. coli* and *S. Typhi*. Whereas, influence of InuL and InuS were not observed on well diffusion based analysis of antibacterial activity. This is the preliminary study and more detailed explorations are required to address the inhibitory nature of InuL and InuS mediated *L. plantarum* against *E. coli* and *S. Typhi*.

Keywords: Inulin, *Lactobacillus plantarum*, Oligosaccharide, Prebiotic.

Intestinal microbiota (IM) consists of diverse, dynamic and complex populations and they have crucial role in overall health of the host¹. Positive regulation of IM leads to health promotion of host that can be achieved by prebiotics. Prebiotics are, low molecular weight, short-chained carbohydrates that are naturally found in plants, fermented food elements that positively alter the composition and/or activity of intestinal microbiota to facilitate the host health promotion². Jerusalem artichoke (JA) is known as inulin-rich, an innate polysaccharide, plant, approximately 14-20 % of fresh weight accounts for inulin content³⁻⁵. Prebiotics are officially recognized as natural food ingredient. It is classified as dietary fiber in most of the European countries⁶.

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Inulin and oligofructose are non-digestible carbohydrate consisting of linear fructose oligomers and polymers with β (2 \rightarrow 1) fructosyl-fructose links⁷ and a glucose molecule which typically resides at the end of each fructose chain connected by α (1 \rightarrow 2) bond. Inulin naturally occurs as series of oligo- and polysaccharides with different chain lengths from 2 to 60 units. Native inulin is a linear β (2 \rightarrow 1) fructan with degree of polymerization (DP) of 2 to 60, and its partial enzymatic hydrolysis yields oligofructose (DP of 2 to 8)⁸. Inulin and oligofructose have been identified as prebiotics due to their properties such as resistance against gastric acidity, hydrolysis by mammalian enzymes and fermentation by gut microflora, most desirably it selectively stimulates the growth and/or activity of beneficial intestinal bacteria. These oligosaccharides promote the colonization of probiotics, especially lactic acid

bacteria (LAB)⁹. Several studies have suggested that oligosaccharide with different DP have different fermentability in the colon^{3,10}.

The present study was conceived to analyze the acid hydrolyzed inulin, extracted from JA, with different DP (long chain inulin (InuL) and short chain inulin (InuS)) on growth of *Lactobacillus plantarum*, known probiotic LAB. Further, antibacterial ability of hydrolyzed inulin co culture with *L. plantarum* was assessed against major food pathogens, *Escherichia coli* and *Salmonella enterica serovar Typhi*.

MATERIALS AND METHODS

Partial acid hydrolysis of inulin

Jerusalem artichoke (JA) was purchased from Faculty of Agriculture, Khon Kaen University, Thailand. Washed JAs were chopped and blended into small pieces. Blended JAs were soaked in 0.01 or 0.1 % (v/v) lactic acid for 10 min to obtain inulin-long chain (InuL) and inulin-short chain (InuS), respectively. The samples were then filtered through filter cloth and dried at 60 °C for 48 h. Dried hydrolyzed inulins were powdered using grinder (Philips HR2102).

Characterization of acid hydrolyzed inulin

DP profile of acid hydrolyzed inulins was determined by high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) using Dionex ICS 3000 (Sunnyvale, CA, USA) equipped with ED 40-pulsed electrochemical detector. Gold and silver chloride served as working and reference electrode, respectively. The hydrolyzed inulins were diluted to appropriate concentration with deionized water, and filtered through 0.45 µm Nylon membrane filter (Corning, NY, USA). The injection volume was 25 µL. A CarboPac PA200 column (3 mm x 250 mm) connected to the associated guard column was used with two gradient eluents at the flow rate of 0.20 ml/min. The mobile phase consisted of 150 mM sodium hydroxide (eluent A), 150 mM sodium hydroxide and 500 mM sodium acetate (eluent B). The elution gradient was 0-15 min with 100 % eluent A, 15-45 min with linear gradient from 0 to 60 % eluent B, 45-90 min with linear gradient from 60-90 % eluent B, 90-110 min with linear gradient from 90 to 100 % eluent B, and 110-120 min with linear gradient from 100 to 0 % eluent B.

Enzyme hydrolysis of inulin

Average degree of polymerization (DP_{av}) of acid hydrolyzed inulins was calculated. The inulins were hydrolyzed using the mixture of *exo*- and *endo*-inulinase to remove the fructose and glucose residues from inulin. 2.5 g of acid hydrolyzed inulins were mixed with 50 mL of 100 mM sodium acetate buffer pH 4.5 and 10 µL of 10-fold diluted solution of Fructanase (Megazyme, 2000 Unit/mL). The mixture was hydrolyzed at 45 °C for 12 h. The reactions were then heat inactivated.

Determination of fructose and glucose in enzyme hydrolyzed inulin

Fructose and glucose composition of enzyme hydrolyzed inulins were analyzed by using High Performance Liquid Chromatography (HPLC) (Thermo Scientific, Fremont, CA) that is equipped with ConstaMetric 4100 solvent delivery system, Water 410 Millipore-RI refractive index detector and Spectra SYSTEM AS3000 auto sampler. The data was acquired using the Clarity lite V 2.6.06.574 (DataApex, Prague, The Czech Republic). Shodex Asahipak NH2P-50 4E column (5 µm, 4.6 × 250 mm) was used with acetonitrile-water (75:25) as mobile phase for isocratic elution. The flow rate was 1.0 mL/min. The sugar and oligosaccharide standards were purchased from Wako (Osaka, Japan).

Prebiotic and probiotic preparation

Two forms of hydrolyzed inulin with different DP (InuL and InuS) were tested for prebiotic nature on probiotic strain, *L. plantarum* as per the guidelines of evaluation of probiotics in food¹¹. *L. plantarum* was received from Health Innovation Institute, Chiang Mai, Thailand.

Growth of *L. plantarum* on hydrolyzed inulin

L. plantarum was cultured at 37 °C for 24 h in MRS (de Man, Rogosa, Sharpe) broth and resuspended in PBS (phosphate buffer saline). Population density was determined by reference to an optical density curve to provide 10⁸ CFU/mL. The suspensions were incubated in MRS broth with 2 % of (w/v) dextrose (control), and MRS broth with InuL or InuS (1 % w/v) at 37 °C for 48 h. The fermentation broths were collected at 0, 6, 12, 18, 24 and 48 h, respectively. The growth of *L. plantarum* was determined by plating onto MRS agar and incubated at 37 °C.

Inhibition of pathogenic bacteria using co-culture technique

Bacterial pathogens such as *Escherichia coli* ATCC 25922 and *Salmonella* Typhi were provided by the Health Product Research and Development Unit, Faculty of Pharmacy, Chiang Mai University. *E. coli* and *S. Typhi* (10^5 CFU/mL) were incubated with or without (control) *L. plantarum* (10^6 CFU/mL) in MRS broth (2 % w/v dextrose) and MRS broth with InuL or InuS (1 % w/v) at 37 °C for 48 h. The samples were collected at 0, 3, 6, 9, 12, 18, 24 and 48 h of incubation. The growth of *E. coli* and *S. Typhi* were determined by plating onto EMB (Eosin methylene blue) agar and SS (*Salmonella Shigella*) agar, respectively and incubating at 35 °C for 48-72 h. Percentage of inhibition were calculated as follows.

$$\text{Percentage of inhibition} = \frac{F_{\text{in MRS}} - F_{\text{in MRS} + L. plantarum + \text{InuL or InuS}}}{F_{\text{in MRS}}} \times 100$$

Agar well diffusion technique

Cell free supernatant of *L. plantarum* cultured in MRS broth with 2 % w/v dextrose (control) and, MRS broth with InuL or InuS (1 % w/v) was tested for antibacterial activity against *E. coli* and *S. Typhi* by agar well diffusion method (12, 13). Moreover, aluminum rings (9 mm) were used to create wells on pre-swabbed TSA with test pathogens (Trypticase soy agar). 100 µL of cell free supernatant of *L. plantarum* was used and the plates were incubated at 37 °C for 24 h. After incubation, zone of clearance was measured and the results were interpreted according to M100-S18 document¹⁴.

Statistical analysis

Values are represented as mean ± standard deviation (SD). Data were analyzed using SPSS 17.0 for windows® (2009 SPSS, Inc.). One-way analysis of variance (ANOVA) followed by Duncan student range test was performed. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Partial hydrolysis of inulin and determination of sugars and oligosaccharides

The DP profile of acid hydrolyzed inulins was determined by HPLC (Fig. 1). The levels of monosaccharide, disaccharide and oligosaccharides were determined and average DP

(DP_{av}) were calculated¹⁵ (Table 1). DP_{av} was calculated using the following equation,

$$DP_{av} = \frac{F_{end} - F_{start}}{G_{end} - G_{start}} + 1$$

Whereas F_{end} , F_{start} , G_{end} and G_{start} represented the concentration of fructose after hydrolysis, concentration fructose before hydrolysis, concentration of glucose after hydrolysis and concentration glucose before hydrolysis, respectively. Significant level of differences was observed in monosaccharides, oligosaccharides content and DP_{av} value between InuL and InuS (Table 1). The amount of monosaccharides and oligosaccharides (DP 3-10) in InuS was found as higher than InuL.

Effect of hydrolyzed inulin on growth of *L. plantarum*

The growth of *L. plantarum* in the presence of InuL or InuS (1% w/v) was determined. Gradual bacterial multiplication was observed in all the conditions up to 18 h of incubation followed by the decline phase at the latter incubation. As per the data gathered after 18 h of incubation, the concentration of bacterial load, in terms of log CFU, was slightly higher in InuS (9.26 ± 0.10 log CFU/

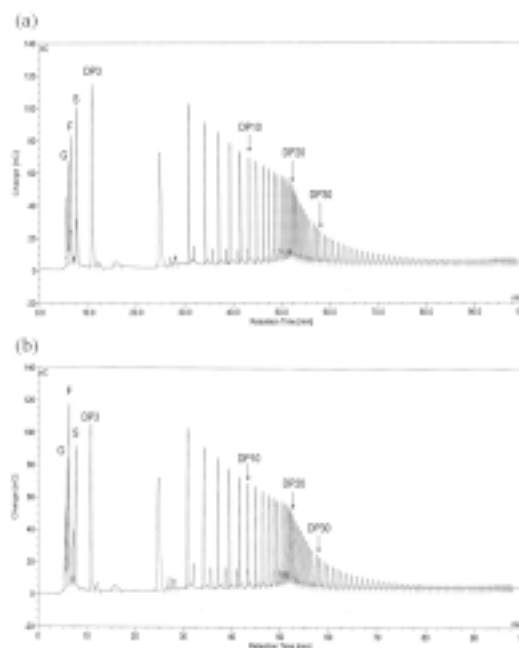


Fig. 1. Chromatograms of InuL (a) and InuS (b). G, F and S represent the glucose, fructose and sucrose, respectively

mL) than InuL (8.06 ± 0.20 log CFU/mL). Whereas, after 48 h of incubation, the data suggested that equal rate of decline has been occurred in all the conditions (Fig. 2) and also revealed that hydrolyzed inulin, both InuS and InuL, can be used as the carbon source by the selected probiotic strain. The significant difference was not observed between control and InuS supplemented sample, may be due to the monosaccharide content of InuS, which can be directly utilized by bacteria for

growth. However, slight differences were observed between InuS and InuL, due to the high content of mono and disaccharides in InuS (Table 1). The efficiency of prebiotic inulin depends on DP³. The high DP provides more resistant against saccharolytic fermentation, which facilitates the effective attaining of prebiotics to distal colon^{16,10}. The growth of *L. plantarum* in MRS + InuL medium was found to be slower but consistency in growth was observed. This data suggested that inulin,

Table 1. Composition, DP distribution and DP_{av} of acid hydrolyzed inulins (%).

Inulin	Monosaccharide	Sucrose	DP 3-10	DP 11-20	DP 21-30	DP > 30	DP _{av}
InuL	5.89	4.57	42.55	21.38	13.33	12.27	23
InuS	8.19	4.04	56.54	14.37	8.92	7.66	18

especially InuL, could be the potent prebiotic constituent.

***L. plantarum* and inulin inhibits the growth of bacterial pathogens**

Bacterial growth inhibiting nature of *L. plantarum* combined inulin was assessed against *E. coli* and *S. Typhi* by co-culture method. No significant changes were observed in control and MRS + inulin formulas, with respect to the growth of *E. coli*. Whereas, slight reduction in the log CFU value of *E. coli* was noticed during co-culturing with *L. plantarum*. Surprisingly, no live *E. coli* cells were recovered from MRS + *L. plantarum* + inulin (InuS and InuL) based co-culturing flask after 48 h of incubation. Gradual reduction in the live bacterial load was clearly

recorded (Fig. 3a). Similar pattern was observed against *S. Typhi* also; however, more surprisingly complete eradication of live *S. Typhi* cells were documented in co-culturing with *L. plantarum*. In the presence of hydrolyzed inulin, the inhibitory nature of *L. plantarum* was found to be improved against *S. Typhi* (Fig. 4a).

Hundred percentage of inhibition of *E. coli* was found to after 18 h of incubation in all the co-culturing formulas. The specific growth rate of *E. coli* in control was found to be 0.93 ± 0.02 log CFU per h with average doubling time of 44.58 ± 0.88 min. There was no significant difference ($P > 0.05$) in *E. coli* growth in both control and MRS with InuL or InuS. Specific growth rate of *E. coli* co-cultured with *L. plantarum* in all media was

Table 2. Specific growth rate (μ) and doubling time (t_d) of *E. coli* or *E. coli* co-cultured with *L. plantarum* in MRS broth (control) and MRS broth with InuL or InuS.

Treatment	Specific growth rate (μ) (h ⁻¹)	Doubling time (td) (min)
<i>E. coli</i> in MRS	$0.93 \pm 0.02A$	$44.58 \pm 0.88C$
<i>E. coli</i> in MRS + InuL	$0.92 \pm 0.05A$	$45.35 \pm 2.62BC$
<i>E. coli</i> in MRS + InuS	$0.90 \pm 0.05AB$	$46.55 \pm 2.60BC$
<i>E. coli</i> in MRS with <i>L. plantarum</i>	$0.76 \pm 0.03BC$	$55.10 \pm 2.36AB$
<i>E. coli</i> in MRS + InuL with <i>L. plantarum</i>	$0.75 \pm 0.10C$	$56.55 \pm 8.00A$
<i>E. coli</i> in MRS + InuS with <i>L. plantarum</i>	$0.73 \pm 0.07C$	$57.15 \pm 5.38A$

Values are represented as mean \pm SD. Alphabets indicates the significant differences ($P < 0.05$) between treatments.

found to be significantly lower ($P < 0.05$) than *E. coli* cultured without *L. plantarum* (Fig. 3b). The specific growth rate of *S. Typhi* in control was found to be 1.33 ± 0.02 log CFU per h with average

doubling time of 31.39 ± 0.39 min. The rest of the results were similar as *E. coli* (Fig. 4b).

As detailed in materials and methods, antibacterial activity of cell free supernatant of *L.*

Table 3. Specific growth rate (μ) and doubling time (t_d) of *S. Typhi* or *S. Typhi* co-cultured with *L. plantarum* in MRS broth (control) and MRS broth with InuL or InuS.

Treatment	Specific growth rate (μ) (h ⁻¹)	Doubling time (t_d) (min)
<i>S. Typhi</i> in MRS	$1.33 \pm 0.02A$	$31.39 \pm 0.39C$
<i>S. Typhi</i> in MRS + InuL	$1.03 \pm 0.03B$	$40.43 \pm 1.00B$
<i>S. Typhi</i> in MRS + InuS	$1.03 \pm 0.01B$	$40.33 \pm 0.32B$
<i>S. Typhi</i> in MRS with <i>L. plantarum</i>	$0.89 \pm 0.01C$	$48.04 \pm 0.30A$
<i>S. Typhi</i> in MRS + InuL with <i>L. plantarum</i>	$0.87 \pm 0.02C$	$47.48 \pm 1.06A$
<i>S. Typhi</i> in MRS + InuS with <i>L. plantarum</i>	$0.88 \pm 0.02C$	$46.77 \pm 0.83A$

Values are represented as mean \pm SD. Alphabets indicates significant differences ($P < 0.05$) between treatments.

plantarum cultured with the supplementation of InuL and InuS was assessed by the comparison with gentamicin. Clear zone formation (16-17 mm) due to antibacterial nature of the supernatant was measured after 24 h. No significant differences ($P > 0.05$) were observed among the control and InuL and InuS supplemented samples with respect to the inhibitory zone against tested pathogens. The

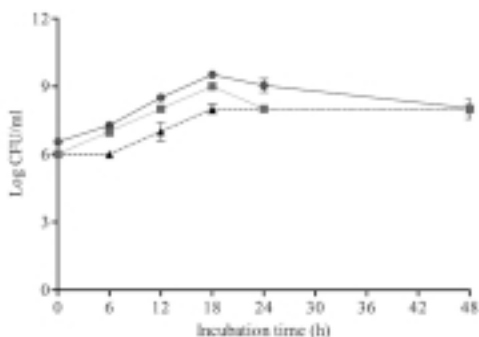


Fig. 3. (a) The growth inhibition of *E. coli* co-cultured with *L. plantarum* in MRS broth and MRS broth with InuL or InuS after 48 h of incubation (*E. coli* in MRS 2×10^6 , *E. coli* in MRS + InuL 1×10^6 and *E. coli* in MRS + InuS 1×10^6 ; *E. coli* in MRS with *L. plantarum* 1×10^6 , *E. coli* in MRS + InuL with *L. plantarum* 1×10^6 and *E. coli* in MRS + InuS with *L. plantarum* 1×10^6). (b) Percent inhibition of *E. coli* co-cultured with *L. plantarum* in MRS broth and MRS broth with InuL or InuS after 48 h of incubation (*E. coli* in MRS with *L. plantarum* 1×10^6 , *E. coli* in MRS + InuL with *L. plantarum* 1×10^6 and *E. coli* in MRS + InuS with *L. plantarum* 1×10^6).

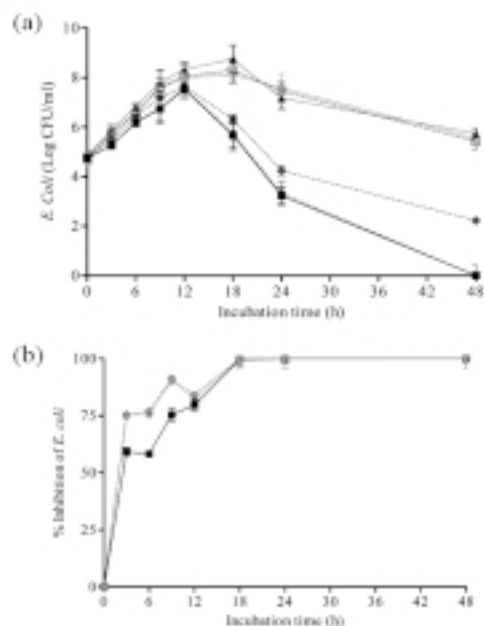


Fig. 4. (a) The growth inhibition of *S. Typhi* co-cultured with *L. plantarum* in MRS broth and MRS broth with InuL or InuS after 48 h of incubation (*S. Typhi* in MRS 2×10^6 , *S. Typhi* in MRS + InuL 1×10^6 and *S. Typhi* in MRS + InuS 1×10^6 ; *S. Typhi* in MRS with *L. plantarum* 1×10^6 , *S. Typhi* in MRS + InuL with *L. plantarum* 1×10^6 and *S. Typhi* in MRS + InuS with *L. plantarum* 1×10^6). (b) Percent inhibition of *S. Typhi* co-cultured with *L. plantarum* in MRS broth and MRS broth with InuL or InuS after 48 h of incubation (*S. Typhi* in MRS with *L. plantarum* 1×10^6 , *S. Typhi* in MRS + InuL with *L. plantarum* 1×10^6 and *S. Typhi* in MRS + InuS with *L. plantarum* 1×10^6).

result demonstrated that metabolites of *L. plantarum* and hydrolyzed inulin displayed the antibacterial activity. Moreover, inulin supplementation will not affect the efficacy.

Probiotic bacteria have been reported to possess antagonistic activity against food-borne pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *E. coli* and *Clostridium perfringens*¹⁷⁻¹⁹. *L. plantarum* has been reported as antagonist for *E. coli*²⁰ as well as inhibit food pathogens such as *Bacillus cereus*, *E. coli* and *S. enterica*²¹. In the present study, significant growth reduction was observed against *E. coli* and *S. Typhi* upon co-culturing with *L. plantarum*. The reduction in the bacterial cell might be due to the production of organic acids, liberated secondary metabolites and some bacteriocin as well as nutrient competition²². Some scientific reports have revealed that *L. plantarum* has anti bacterial impact against *Salmonella* spp., *E. coli* and *Cl. perfringens* by *in vitro*²³⁻²⁵ and *in vivo* studies²⁶.

Various *in vitro* and *in vivo* studies revealed that inulin and/or fructooligosaccharide (FOS) supplements promote the growth of bifidobacteria and lactobacilli, moreover, it selectively suppresses the growth of pathogens²⁷.²⁸ Short chain fatty acids (SCFAs) are proven as prebiotic by *in vitro* and *in vivo* studies²⁹. Significant level of increased SCFAs production was observed in the cecum ($P < 0.05$) of the rats fed on inulin compared to other fibers tested³⁰.

CONCLUSIONS

Hydrolyzed Inulin of JA by lactic acid has been proved as potent prebiotic ingredient. The current study suggested that InuL and InuS isolated from JA, enhances the antibacterial and probiotic ability of *L. plantarum*. Results of well diffusion assay suggested that mere antibacterial activity of *L. plantarum* has not been influenced by inulin. Thus, further detailed investigations are needed to address the inhibitory nature of InuL and InuS mediated *L. plantarum* against *E. coli* and *S. Typhi*.

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REFERENCES

1. Sekirov, I., Russell, S.L., Antunes, L.C., Finlay, B.B. Gut microbiota in health and disease. *Physiol. Rev.*, 2010; **90**(3): 859–904.
2. Gibson, G.R., Scott, K.P., Rastall, R.A., Tuohy, K.M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy E.F., Saulnier, D., Loh, G., Macfarlane, S., Delzenne, N., Ringel, Y., Kozianowski, G., Dickmann, R., Lenoir-Wijnkook, I., Walker, C., Buddington, R. Dietary prebiotics: Current status and new definition. *Food Sci. Technol. Bull. Funct. Foods.*, 2010; **7**:1–19.
3. Van Loo, J. The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. *Nutr. Res. Rev.*, 2004; **17**(1): 89–98.
4. Judprasong, K., Tanjor, S., Sungpuag, P., Puwastien, P. Investigation of Thai plants for potential sources of inulin-type fructans. *J. Food Comp. and Anal.*, 2011; **24**: 642–649.
5. Tanjor, S., Judprasong, K., Chaito, C., Jogloy, S. Inulin and Fructooligosaccharides in Different Varieties of Jerusalem Artichoke (*Helianthus tuberosus* L.). *KKU Res. J.*, 2012; **17**(11): 25–34.
6. Roberfroid, M., Slavin, J. Nondigestible oligosaccharides. *Crit. Rev. Food Sci. Nutr.*, 2000; **40**(6): 461–480.
7. Flamm, G., Glinsmann, W., Kritchevsky, D., Prosky, L., Roberfroid, M. Inulin and oligofructose as dietary fiber: a review of the evidence. *Crit. Rev. Food Sci. Nutr.*, 2001; **41**(5):353–362.
8. Roberfroid, M.B. Introducing inulin-type fructans. *Br. J. Nutr.*, 2005; **93**(Suppl 1):S13–S25.
9. Ooi, L.G., Liong, M.T. Cholesterol-lowering effects of probiotics and prebiotics: a review of *in vivo* and *in vitro* findings. *Int. J. Mol. Sci.*, 2010; **11**(6):2499–2522.
10. Van de Wiele, T., Boon, N., Possemiers, S., Jacobs, H., Verstraete, W. Inulin-type fructans of longer degree of polymerization exert more pronounced *in vitro* prebiotic effects. *J. Appl. Microbiol.*, 2007; **102**(2):452–60.
11. The United Nations Food and Agriculture Organization and the World Health Organization

- (FAO/WHO) (2001) Probiotics in foods: Health and nutritional properties and guidelines for evaluation. (<http://www.fao.org/es/ESN/probio/probio.htm>).
12. Valgas, C., de Souza, S. M., Smânia, E. F. A., Smânia, Jr A. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.*, 2007; **38**: 369-80.
 13. Bonev, B., Hooper, J., and Parisot, J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *J. Antimicrob. Chemother.*, 2008; **61**: 1295–1301.
 14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 3rd ed., approved standard M31-A3. CLSI, Wayne, PA, 2008.
 15. Ronkart, S.N., Blecker, C.S., Fourmanoir, H., Fougny, C., Deroanne, C., Van Herck, J.C., Paquot, M. Isolation and identification of inulooligosaccharides resulting from inulin hydrolysis. *Anal Chim Acta.*, 2007; **604**(1): 81–7.
 16. Hughes, R., Rowland, I.R. Stimulation of apoptosis by two prebiotic chicory fructans in the rat colon. *Carcinogenesis.*, 2001; **22**(1): 43–7.
 17. Kasimoglu, A., Akgun, S. Survival of *Escherichia coli* O157:H7 in the processing and post-processing stages of acidophilus, yogurt. *Int. J. Food Sci. Technol.*, 2004; **39**(5): 563–8.
 18. Millette, M., Luquet, F.M., Lacroix, M. *In vitro* growth control of selected pathogens by *Lactobacillus acidophilus*- and *Lactobacillus casei*-fermented milk. *Lett. Appl. Microbiol.*, 2007; **44**(3): 314–9.
 19. Arias, O.A.B., Reyes, M.M.I.L., Navarro, V.M.L., Solis, C.Y.B., Marquez, G.M., Sanchez, S.G., Snell, C.R., Zuniga, R.R. Antagonistic effect of probiotic strains against two pathogens: *Salmonella Typhimurium* and *E. coli* O157:H7 resistant to antibiotics. *e-Gnosis.*, 2013; **11**:1–16.
 20. Lewus, C.B, Kaiser, A., Montville, T.J. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.*, 1991; **57**(6):1683–8.
 21. Castellano, P., Belfiore, C., Fadda, S., Vignolo, G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat. Sci.*, 2008; **79**(3):483–499.
 22. Omar, N.B., Abriouel, H., Lucas, R., Martinez-Canamero, M., Guyot, J.P., Galvez, A. Isolation of bacteriocinogenic *Lactobacillus plantarum* strains from ben saalga, a traditional fermented gruel from Burkina Faso. *Int. J. Food Microbiol.*, 2006; **112**(1): 44–50.
 23. Tshabalala, P.A., de Kock, H.L., Buys, E.M. Survival of *Escherichia coli* O157:H7 co-cultured with different levels of *pseudomonas fluorescens* and *lactobacillus plantarum* on fresh beef. *Braz. J. Microbiol.*, 2012; **43**(4): 1406–13.
 24. Prema, P. In vitro antagonistic activity of a probiotic *Lactobacillus plantarum* against water borne pathogens. *Int. J. Pharm. Phar. Sci.*, 2013; **5**(4): 175-8.
 25. Geria, M., Dambrosio, A., Normanno, G., Lorusso, V., Caridi, A. Antagonistic activity of dairy lactobacilli against gram-foodborne pathogens. *Acta. Sci. Technol.*, 2014; **36**(1):1–6.
 26. Murry, A.C, Hinton, A., Morrison, H. Inhibition of growth of *Escherichia coli*, *Salmonella typhimurium*, and *Clostridia perfringens* on chicken feed media by *Lactobacillus salivarius* and *Lactobacillus plantarum*. *Int. J. Poult. Sci.*, 2004; **3**(9): 603– 7.
 27. Gibson, G.R., Beatty, E.R., Wang, X., Cummings, J.H. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology.*, 1995; **108**(4):975–982.
 28. Cummings, J.H., Roberfroid, M.B., Andersson, H., Barth, C., Ferro-Luzzi, A., Ghos, Y., Gibney, M., Hermansen, K., James, W.P., Korver, O., Lairon, D., Pascal, G., Vorgen, A.G. A new look at dietary carbohydrate: chemistry, physiology and health. Paris Carbohydrate Group. *Eur. J. Clin. Nutr.*, 1997; **51**(7):417–423.
 29. Cummings, J.H., Macfarlane, G.T., Englyst, H.N. Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.*, 2001; **73**(2 Suppl):415S–420S.
 30. Roland, N., Nugon-Baudon, L., Andrieux, C., Szylit, O. Comparative study of the fermentative characteristics of inulin and different types of fibre in rats inoculated with a human whole faecal flora. *Br. J. Nutr.*, 1995; **74**(2): 239–49.