Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Stability of spray-dried synbiotics containing *Lactobacillus plantarum* DSM 2648 and exopolysaccharide from *Pediococcus acidilactici* TISTR 2612 and its vivo effectiveness

Achara Chaiongkarn[1*], Jirapa Dathong[1], Wipaporn Phatvej[1], Premsuda Saman[1], Chutima Kuancha[1] and Siritorn Teetavet[1]

[1]Thailand Institute of scientific and Technological Research, Pathum-thani 12120, Thailand

ARTICLE INFO

Article history:

Received 31 July 2018

Received in revised form 31 December 2018

Accepted 08 January 2019

Keywords:

Isomalto-oligosaccharides

Prebiotic

Rice flour

Cassava starch

ABSTRACT

Spray drying is an economical process for dehydrating microorganisms into a powder form that preserves viability for use at the industrial scale. It is especially applicable for probiotics and other temperature sensitive functional foods. In this study, the survival and functionality of synbiotics were evaluated after spray drying. A mixture of stationary phase probiotic culture (*Lactobacillus plantarum* DSM 2648) and 2 % (w/v) of exopolysaccharide (EPS) produced from *Pediococcus acidilactici* TISTR2612 was spray-dried with outlet air temperature of 70 ±1°C. Results showed a probiotic survival rate decrease from 96.74 % (log 10.04 CFU/g) after spray-drying to 96.01, 96.00, 93.18 and 92.17 % during room temperature storage in a desiccator for 1, 2, 3 and 4 weeks, respectively. In simulated gastric juice (pH 2) and in 2 % of bile salts solution, probiotics survival rates were 97.94 and 91.56 % and EPS hydrolysis reached 3.26 and 7.23 %, respectively, after incubation at 37 °C for 12 h. *In vivo* testing of the impact of synbiotics on reducing colorectal cancer risk showed a significant decrease of *β*-glucuronidase bacterial enzyme activity, with 36.56 and 50.55 % reduction in male and female rats, respectively. Finally, the acute oral toxicity (LD50) testing was performed by feeding rats with the maximum dose of synbiotics (5 g per kg body weight). No mortality or organ abnormalities (from necropsy findings) were observed in any of the rats after oral administration for 15 days. These results suggest that synbiotics could potentially be a safe functional food for aiding disease prevention in the future.

* *Corresponding author*: Tel.: +66-02-577-9063; fax: +66-02-577-9058
E-mail address: achara@tistr.or.th

Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

Functional foods are well known for their abilities to improve functions in the body by reducing inflammation, fighting off disease, supporting good gut health, and improving nutrient absorption, among other benefits. Several food components, including carotenoids, dietary fiber, fatty acids, flavonoids, specific minerals, prebiotics and probiotics have been linked to functional benefits (FAO, 2007). Probiotics and prebiotics are two food components that can be used together for greater effect. Probiotics are living microorganisms that are beneficial to the digestive system, and prebiotics are nondigestible food ingredients that help stimulate probiotic growth and activity. The consumption of probiotics may help reduce fecal bacteria, intestinal inflammation, and incidence of colorectal cancer (CRC) (O'Mahony *et al*., 2001; Bibiloni *et al*., 2005; Gaudier *et al*., 2005). To confer these and other health benefits, probiotics should be ingested in adequate amounts, usually at 10^6-10^7 CFU/g of product (FAO/WHO, 2002; FAO/WHO, 2006). By improving the viability of probiotics, such as bifidobacteria and lactobacilli in the colon, and inhibiting the growth of pathogenic bacteria, prebiotics can further improve gut microbial balance (Fuller, 1989; Gibson and Roberfroid, 1994). When a mixture of probiotics and prebiotics are used in synergy, the result is a synbiotic formulation. Synbiotics ensure greater probiotic proliferation in the gut and more efficient implantation in the colonic microbiota by using complimentary prebiotics to improve the survival of probiotic bacteria during passage through the upper intestinal tract (Roberfroid, 1998; Goorbeyre *et al*., 2011). Saulnier *et al*. (2008) reported that synbiotics were more effective than probiotics or prebiotics alone in modulating the gut microflora by increasing the beneficial bacteria. In CRC biomarker investigation, the combination of *Bifidobacterium longum* and inulin was more efficient at decreasing β-glucuronidase activity on colonic aberrant crypt foci (ACF) than *B. longum* or inulin alone (Rowland *et al*., 1998). Moreover, dietary synbiotics significantly reduced colorectal proliferation, DNA damage and the capacity of fecal water to induce necrosis in polypectomized patients and favorably altered the colonic bacteria ecosystem in polyp and colon cancer patients (Rafter *et al*., 2007). Thus, synbiotics are gaining interest for dietary supplementation to reduce multiple factors associated with colon cancer risk in humans.

CRC is the third most commonly diagnosed cancer among men and women in the United States and the fourth leading cause of cancer-related deaths in the world (Alnold *et al*., 2016; Rebecca *et al*., 2017). In Thailand, CRC cases are increasing with average annual change of 4.1% and 3.3% in men and women, respectively (Virani *et al*., 2017). The factors with "Westernization" such as obesity, smoking, alcohol consumption, low fruit-vegetables intake and physical inactivity are associated with this considerable increasing rate (Center *et al*., 2009). Therefore, the decrease incidence of CRC is likely driven by changes in lifestyle and dietary patterns (Alnold *et al*., 2016) and development of functional food ingredients is an alternative of interest.

Spray drying is a dehydration process that can be used to produce viable probiotic powders for incorporation into food systems (Fu and Chen, 2011). This process offers high production rates and low operating costs (Corcoran *et al*., 2004). Process parameters of spray drying, including inlet and outlet air temperatures, feed flow rate, residence time at drying chamber, thermodynamic process, species and strains type, adaptation of the bacteria to heat or osmotic stress condition, growth state are associated with the survival of probiotics (Gardier *et al*., 2000; Ananta *et al*., 2005; Lapsiri *et al*., 2012; Behboudi-Jobbehdar *et al*., 2013; Bustos and Borquez, 2013). In addition, stationary phase cultures of bacteria are suitable for the spray drying process (Corcoran *et al*., 2004).

In a previous study, the combination of *Lactobacillus plantarum* DSM 2648 and exopolysaccharide (EPS) produced from *Pediococcus acidilactici* TISTR 2612 showed the highest potential for β-glucuronidase reduction (Chaiongkarn, *et al*., 2017). β-glucuronidase is associated with the conversion of procarcinogens to carcinogens in colon cancer (Goldin and Gorbach, 1976; Goldin and Gorbach, 1980). In the present study, spray drying with outlet air temperature of 70 ±1°C was used for incorporating the combination of *L. plantarum* and EPS as a synbiotic ingredient for further use in functional food or dietary supplements. The aims of this study were to examine the survival and stability of *L. plantarum* DSM 2648 after spray drying and during powder storage in the presence of 2 % w/v EPS produced by *P. acidilactici* TISTR 2612 as a probiotics carrier and to investigate the effectiveness of this synbiotic product for reducing the risk of CRC via *in vivo* assessment of fecal bacteria enzyme investigation (β-glucuronidase).

MATERIALS AND METHODS

Preparation of EPS from *P. acidilactici* TISTR 2612

A single colony of *P. acidilactici* TISTR 2612 was grown in 5 mL of MRS broth (Himedia, India) at 37 ºC under anaerobic condition for 16-18 h. Fresh overnight culture was scaled up to 100 and finally to 1,000 mL (at the rate 5% *P. acidilactici* v/v) in MRS broth containing 1% sucrose for EPS inducing at 37°C under anaerobic condition for 48 h (Dathong *et al*., 2016). The cells were removed after centrifugation at 8,000 rpm for 10 min. The supernatant was then collected and concentrated by rotary evaporator to one third of its volume. EPS was precipitated with two volumes of cold 95% ethanol. The mixture was stored at 0 ºC for 48 h and centrifuged at 8,000 rpm for 10 min. The supernatant was discarded and the EPS was resuspended in 10-50 mL of distilled water and then freeze-dried. A 2% EPS solution was prepared by dissolving EPS powder in 500 mL of distilled water, followed by autoclaving at 121°C for 15 min.

Preparation of probiotic culture for spray drying

In an initial experiment that defined the growth of *L. plantarum* DSM 2648 under broth culture conditions, the stationary phase was reached after 12-24 h of fermentation (Chaiongkarn *et al*., 2017). A single colony of *L. plantarum* DSM 2648 (DSMZ Bacteria collection, Germany) was grown in 5 mL MRS broth (Himedia, India) under anaerobic condition at 37° C for 16-18 h. One percent of the starter cell solution (with OD600 of 0.5) was added to 50 mL MRS broth and incubated at 37°C for 16-18 h. A 50 mL of the cell suspension was scaled up to 500 mL in MRS broth (at the rate 10 % v/v) and placed at 37°C for 24 h. Stationary phase *L. plantarum* DSM 2648 was harvested after centrifugation at 8,000 rpm for 10 min and resuspended in 200 mL of 0.85 % of sterile NaCl. The cell suspension was centrifuged and washed repeatedly at least 2 times. Finally, probiotics cell pellet was resuspened with 500 mL EPS solution (2% w/v). To validate the survival rate of probiotics during the drying process, cell counts were observed before and after spray drying.

A 0.5 mL cell suspension was collected for serial dilution in 4.5 mL of normal saline while 0.5 g of spray-dried sample was rehydrated in 5 mL of normal saline and was serially diluted and plated on MRS agar+0.5% CaCO3 under anaerobic conditions at 37°C for 48 h. The growth of the probiotics was enumerated as CFU/g (of dry matter) and cell counts were expressed as mean of duplicate measurement.

Spray-dying process and storage

A laboratory scale spray dryer (Buchi mini spray dryer, model B290, Flawil, Switzerland) was used to process synbiotic microparticles at an air inlet and outlet temperature of 135-140°C and 70±1°C, respectively. The feed solution was atomized into droplets with 0.7 mm i.d. nozzle, at aspirator pressure of 100 %, atomizer pressure of 650 NI/h and flow rate of 12 mL/min. The percentage of bacteria survival was calculated as following; % survival = (N/N_0) x 100, where N was the number of bacteria in the spray-dried powder (CFU/g) and N_0 represented the number of bacteria in the cell solution before drying (described above). Aliquot powders of synbiotics were placed in sterile bottles sealed with parafilm and stored in a desiccator at room temperature. Viability of probiotics was determined at 1, 2, 3 and 4 weeks. For long-term investigation, synbiotics powders were kept in vacuum sealed aluminum foil bags and stored at 4°C and room temperature for 8 month.

Simulated gastrointestinal and bile salts solution tolerance test

Simulated gastric juice (SGJ) was prepared as described by Lian *et al*. (2003). Briefly, 0.3% of pepsin was dissolved in 0.5% (w/v) NaCl solution, adjusted to pH 2.0 with 1 M HCl and 1 M NaOH and sterile-filtered through a membrane with 0.2 μm (Life Sciences, Ann Arbor, MI, USA). The solution should be used immediately and could be kept in the fridge (not longer than 24 hours). Bile salt (Himedia, India) was dissolved in distilled water to a concentration of 2 % (w/v) and the solution was sterilized at 121°C for 15 minutes. One gram of spray dried synbiotics microparticles was separately dissolved in 10.0 mL of SGJ (pH 2) and 2% of bile salts solutions. The suspensions were taken at 0 h and 12 h after incubation at 37°C to determine viability of *L. plantarum* DSM 2648. EPS hydrolysis was determined following the methods by Nelson N, (1944), Dubois *et al*. (1956), Krasse and Carlsson (1970) and Wichienchot *et al*. (2010). The hydrolysis degrees of samples were calculated according to the formula as following, where reducing sugar released is the difference between reducing sugar content at specified time and initial reducing sugar content.

Hydrolysis degree (%) = (Reducing sugar released / Total sugar-initial reducing sugar) × 100

Animals

This study was approved by the TISTR Animal Ethical Committee (No.59001). Wistar rats (7 weeks old) weighting 267-304 g and 181-210 g (male and female) were purchased from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornpathom. The rats were allowed to acclimatize to the housing condition with standardized conditions according to animal experiment guidelines at 24 ± 2°C, 50 ± 10% humidity, 12 h light/dark cycle. The rats were housed in cages bedded with dried corncob and dried water hyacinth, and fed standard laboratory animal food pellets and RO drinking water *ad libitum*.

Treatments and β -glucuronidase assay

Rats were randomly divided into 2 groups of each gender to experiment and control group (n=5/each group). In treatment group; rats were orally administrated with UHT milk containing 2% synbiotic products (5 mL/ kg body weight) with containing log 9 to log 10 CFU/g of probiotics. For the control group; the rats were fed UHT milk only. The rats were fed via oral gavage daily for 4 weeks. After dosing, all animals were observed once per day and their body weights were recorded weekly and at the end of the test. The mean body weight gain of the test group animals was calculated and compared to that of the control group rats using ANOVA (p-value ≤ 0.05). At the end of the test, all survivors were humanely killed by CO2 asphyxiation. Likewise, the fresh feces of each rat in the treatment group was taken from the large intestinal part of the caecum after death and placed in sterile aluminum foil and then mixed until homogenization. The homogenized-fecal samples (1.5 g) were dissolved in 30 mL phosphate buffer at pH 7.0 and sonicated for 2 min (4 cycles, 30 sec, 0°C). The suspension of feces was centrifuged at 8,000 rpm for 10 min at 4°C. The supernatant was collected to a sterile tube and kept at -80°C until it was carried out for the assessment of β-glucuronidase. The β-glucuronidase assay was conducted as described by Goldin and Gorbach (1976). The protocol for enzymatic assay of β-glucuronidase (EC 3.2.1.31, Sigma Chemical Co., St. Louis, Mo.) was based on the determination of phenolphthalein released from phenolphthalein β-D-glucuronide and the results were displayed as units/g solid ±SD.

Acute oral toxicity testing

This experiment was approved by TISTR Animal Ethical Committee no.T58001. The acute oral toxicity determination (LD50) of synbiotics was carried out with slight modification to the Test Guideline No. 423: Acute oral toxicity-Acute toxic class method of the OECD Guidelines for Testing of Chemicals (2001). Rats were randomly divided into 3 groups of each gender (n=5/each group). All animals were fasted for 16 h with free access to water. After fasting, the rats were administered synbiotics orally at dose 2 g/kg and 5 g/kg. Distilled water was given to the control group. After administration of the test samples, mortality and clinical signs which included changes in skin, fur, eyes and mucous membranes were noted at the first 4 h subsequently for 72 h and thereafter for 15 days. The body weight was recorded at day-1 (after fasting), day-8 and day-15. At the end of the test (day-15), all survivors were weighed and humanely killed by CO_2 asphyxiation (AVMA Guidelines for the Euthanasia of Animals, 2013) and any gross pathological changes were recorded for each rat. The mean body weight gains of the treatment groups were calculated in comparison to the control group.

Scanning electron microscopy (SEM)

EPS and synbiotics microparticles were sent to National Metal and Materials Technology Center (MTEC) for morphology analysis. The samples were sputter coated with a thin layer of gold. Scanning electron microscopy (SEM) micrographs were acquired using a scanning electron microscope (HITACHI, S-3400N, Japan) at an accelerating voltage of 20 kV.

Statistical analysis

The results of three replicates were displayed as mean ±SD. Statistical analysis was performed by SPSS (IBM SPSS Statistics Software version 18, Co., Ltd., Thailand). The difference between the means was analyzed by Turkey's method at *p-value* < 0.05.

RESULTS AND DISCUSSION

Survival and stability of probiotics after spray drying process

After spray drying, the yields of synbiotic microparticles were between 65 and 80 % with probiotics survival at 96.74% as shown in Figure 1(a) N0 =log 10.38±0.08 and N=log 10.04±0.11 CFU/g. Results indicated that EPS was suitable for probiotics encapsulation with high survival rate during spray drying process. Jantzen *et al* (2013) found that survival rate of *L. reuteri* decreased by log 2 cycles after drying and log 1 cycle during 4 weeks of storage when bacteria was encapsulated with whey from slurry fermentation. Carrier media can improve survival rates after the spray drying process and enhance survival in simulated gastrointestinal conditions (Hamsupo *et al*., 2005; Paez *et al*., 2012). To investigate product stability during storage at RT, the viable numbers of *L. plantarum* DSM 2648 significantly decreased after a storage period for 4 weeks (*p-value* < 0.05), according for the survival of 96.01, 96.00, 93.18 and 92.17 %, respectively. Although the survival of probiotics tends to decrease during storage at RT, the viability of probiotics did not decrease from the beginning of storage at log 10.04 CFU/g when the synbiotic microparticle was stored under vacuum seal at RT and 4°C for 8 month (showing 110.25 and 110.57 % survival, respectively). The results suggested that for prolonged shelf-life probiotic products should be kept at vacuum seal due to oxygen content as an important factor affecting probiotic survival and growth during the storage period (Tripathi *et. al*. 2014;).

For the morphological analysis of spray-dried microparticles, EPS had smooth spherical shape Figure 2 (a) while *L. plantarun* DSM 2648-encapsulated with EPS displayed bacteria cells covered with EPS Figure 2 (b). Thus, the high survival of probiotics during the spray drying was probably due to thermal protection of probiotics cells with EPS (Fritzen-Freire *et al*., 2012).

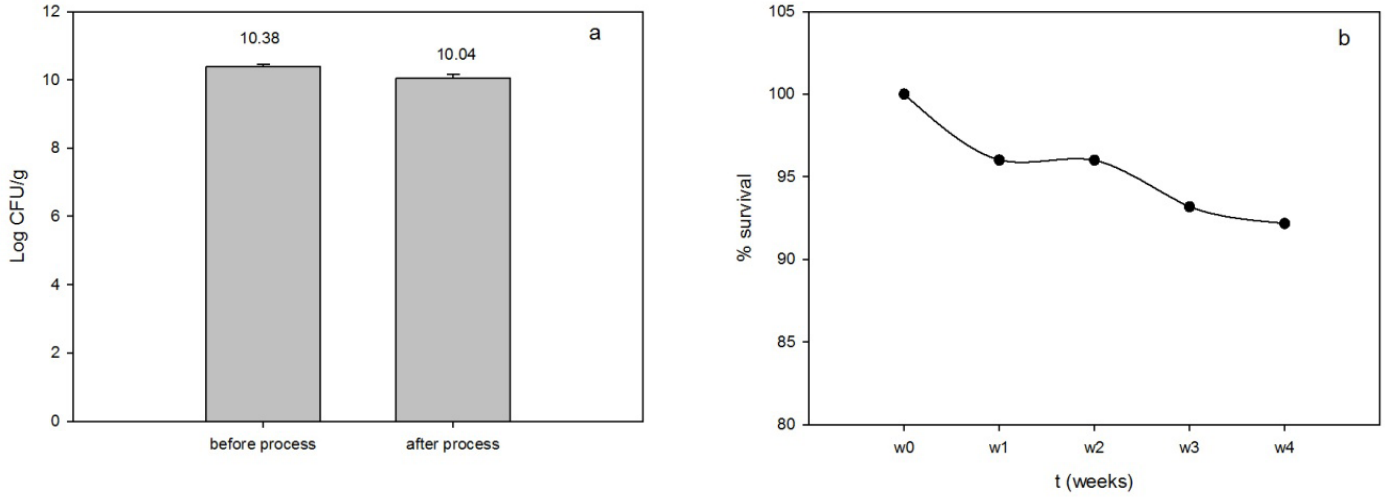

Figure 1. Probiotics survival after spray drying (a) and during storage at RT for 4 weeks (b)

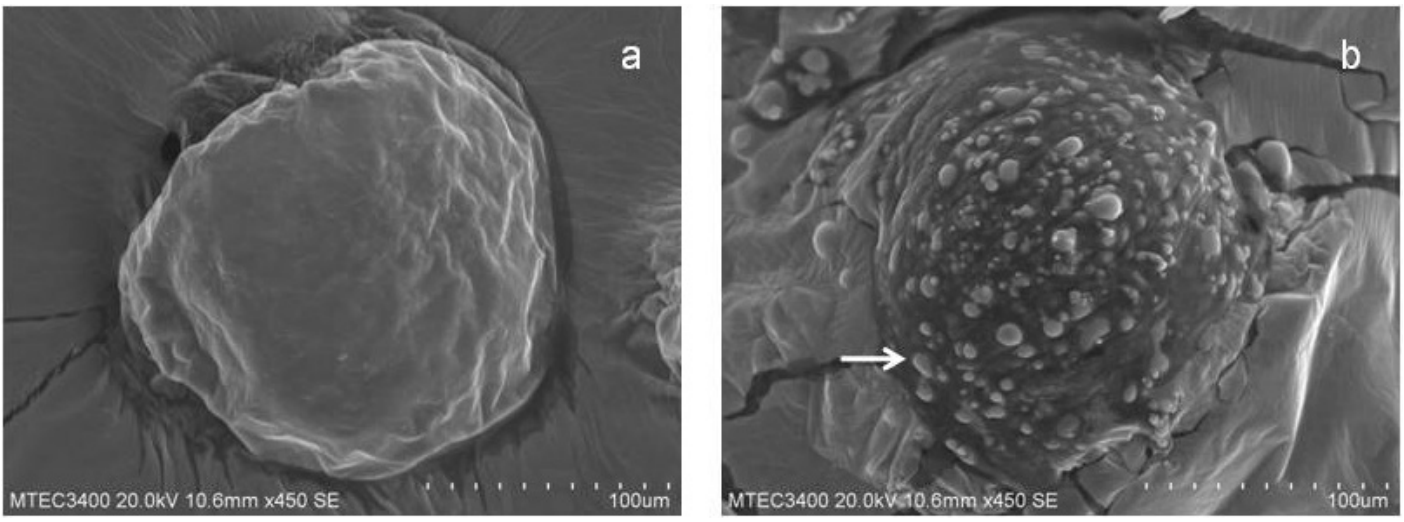

Figure 2. SEM of EPS without probiotics (a) and powder of synbiotics (b) using high vacuum technique (scale bar 100 μ m). The arrow indicated the *L. plantarum* DSM 2648 was covered with EPS.

Tolerance to simulated gastrointestinal (SGI) and bile salts solution

The survivals of microencapsulated cells of *L. plantarum* DSM 2648 were 97.94 % and 91.56 % after 12 h exposure to SGJ pH 2 and 2 % bile salts solution, respectively, while the EPS was hydrolyzed by 3.26 % and 7.23 %, respectively (Table 1). The results suggested that *L. plantarum* DSM 2648 and EPS produced by *P. acidilactici* TISTR 2612 were more susceptible to 2% bile salts than SGJ (pH 2). More than 80 % of viable probiotics and more than 90 % of EPS were resistant to SGI (pH2) and 2% bile salts solution.

The obtained results indicated that EPS produced from *P. acidilactici* TISTR 2612 could protect *L. plantarum* DSM 2648 during the spray drying process, SGJ pH 2 and 2% of bile salts solution.

Table 1. Survival rate of *L. plantarum* DSM 2648 and EPS hydrolysis after 12 h exposure to SGJ pH 2 and 2% bile salts solution

	% Survival of *L. plantarum* DSM 2648	% Hydrolysis of EPS
SGJ (pH 2)	97.94	3.26
2% bile salts	91.56	7.23

The effectiveness on *β*-glucuronidase reduction

The effectiveness of synbiotic microparticles on reducing the risk of CRC *in-vivo* was investigated via β -glucuronidase enzyme assessment. Five grams of synbiotics per kg of rat body weight per day was used in all periods of experimentation. After a period of 4 weeks, there were no significant differences in final body weight between dietary group in the same gender, showing 406.4 ± 10.8 and 404.0 ± 9.0 in male rats and 250.6 ± 11.5 and 246.60 ± 6.1 in female rats of the control and treatment group, respectively. On the other hand, the β-glucuronidase enzyme activity of rats treated with synbiotics was significantly lower than the control (p-value =0.000) showing 36.56 % and 50.55 % reduction in male and female rats, respectively.

Fecal bacteria enzymes such as β -glucuronidase, nitroreductase and azoreductase commonly produced by Bacteroides, Clostridium, and Enterobacteriaceae are associated with the risk of colon cancer (Raman *et al*., 2013), as their activities generate toxic and carcinogenic substances. Fecal enzyme activity can be influenced by diet (Goldin and Gorbach, 1976). Hylla *et al*. (1998) found that the consumption of resistance starch significantly decreased total concentrations of fecal bile acids in healthy volunteers that may be relevant for cancer prevention. Results from the present study suggested that synbiotics microparticles have potentially important effects on decreasing the risk of CRC in in-vivo experiments. In the previous study, oligofructose-based prebiotics such as fructooligosaccharides and inulin were emphasized for colon cancer protection. In DMH-induced CRC in rats study, Hughes and Rowland (2001) found that oligofructose and long chain inulin had protective effects at an early stage in the onset of cancer. An administration of *L. rhamnosus* GG and *L.acidophilus* to DHM-treated Sprague Dawley rats was more efficient at decreasing aberrant crypt foci (ACF) counts and β-glucuronidase activity (Verma and Shukla, 2012). In this experiment, we found a new source of prebiotics (EPS produced from *P. acidilactici* TISTR 2612) that had a potential effect to reduce the risk of CRC.

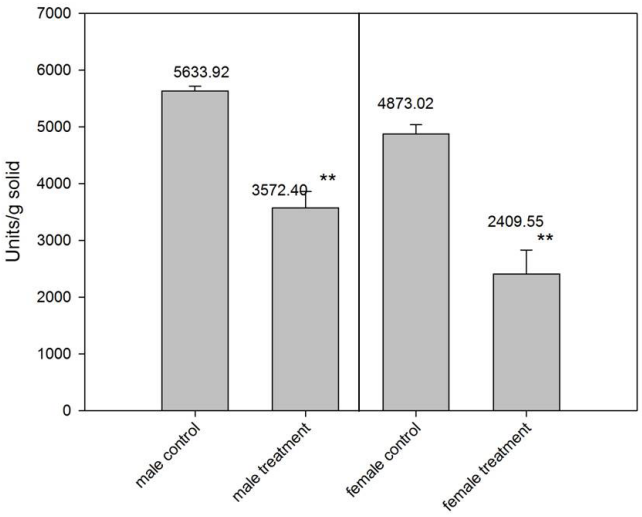

Figure 3. β -glucuronidase enzyme activity of investigated rats after 4 weeks of feeding with synbiotics for 4 weeks. ** *p-value* < 0.05

Table 2. Summary data from acute oral toxicity (LD50) in rats

Gender	Groups	[a] Body weight increasing (g)		Gross pathology after treatment
		Days 8	Days 15	
Male rats	Control	9.60 0.60	61.00 3.08	normality
	2 g/kg body weight	9.60 0.81	71.20 2.80*	normality
	5 g/kg body weight	9.40 0.68	58.20 3.42	normality
Female rats	Control	13.40 2.15	33.40 2.63	normality
	2 g/kg body weight	14.80 2.41	34.40 2.01	normality
	5 g/kg body weight	10.20 0.58	24.40 1.47	normality

[a] body weight increasing (mean SE), mean comparison of final body weight using ANOVA (* indicate p-value < 0.05)

Acute oral toxicity (LD50)

Acute toxicity determination is a method for assessing acute oral toxicity that involves the recognition of a dose level that causes mortality (Subhal and Geetha, 2017). The mortality and clinical signs which included changes in skin, fur, eyes and mucous membranes, weight were noted after rats were fed with synbiotics for the first 4 h, 8 days and thereafter for 15 days, respectively. The results showed that synbiotics did not significantly affect female rats body weight (*p-value*=0.687 at days 15) while male rats fed with 2 g of synbiotics had significant body weight increase from control and 5 g feeding rats at day 15. The results of acute oral toxicity test showed no mortality or abnormal organs from necropsy findings in any male or female rats (Table 2).

CONCLUSIONS

The spray drying process was suitable for production and preservation of the synbiotics products. The survival of stationary phase probiotics after spray drying at outlet air temperature of 70 ± 1°C in the presence of 2% (w/v) of EPS was high (96.74%). So, EPS produced by *P. acidilactici* had the capacity to protect *L. plantarum* DSM 2648 during spray drying process and storage. The probiotic survival rates of 96.01%, 96.00%, 93.18% and 92.17% were achieved after 1, 2, 3 and 4 weeks of storage in a dessicator, respectively. The survival rate of probiotics in stimulated gastrointestinal pH 2 and 2% of bile salts at 37°C for 12 h were 97.94% and 91.56% while the hydrolysis of EPS was reached 3.26% and 7.23%, respectively. The effectiveness of synbiotics for reducing the risk of colorectal cancer in-vivo suggested that the activity of *β*-glucuronidase significantly decreased in rats orally administered with 5 g of synbiotics per kg of body weight/day for 4 weeks. Further study of synbiotics indicated that no acute toxicity effects were observed in the group of rats fed with synbiotics in the maximum dose after 15 days. From these results, synbiotic microparticles could contribute to functional food ingredient development in the future.

ACKNOWLEDGEMENTS

This study was supported by TISTR culture collection center and National Research Council of Thailand (NRCT)..

REFERENCES

Ananta, E., Volkert, M. and Knorr, D. 2005. Cellular injuries and storage stability of spray dried *Lactobacillus rhamnosus* GG. International Deitary Journal.15: 399-409.

Arnold, M., Sierra M. S., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F. 2006. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 0:1-9.

Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L. and Fisk,I. 2013. Optimization of spray-drying process conditions of maimally Vvable microencapsulated *L. acidophilus* NCIMB 701748. Drying Technology. 31:1274-1283.

Bibiloni, R., Fedorak, R.N., Tannock, G.W., Madsen, K.L., Gionchetti, P., Campieri, M., De Simone, C. and Sartor, R.B. 2005. VLS#3 probiotics-mixture induces remission in patients with active ulcerative colitis. American Journal Gastroenterology.100: 1539-1546.

Bustos, P. and Borquez, R. 2013. Influence of osmotic stress and encapsulating materials on the stability of autochthonose *Lactobacillus plantarum* after spray drying. Drying Technology. 31: 57-66.

Center, M.M., Jemal, A., Smith, R.A. and Ward, E. 2009. Worldwide variations in colorectal cancer. Cancer Journal for Clinicians. 59: 366-378.

Chaiongkarn, A., Dathong, J., Saman, P., Moonmangmee, S., Kuancha, C., Chatanon, L. and Teetavet, S. 2017. Proceeding of 7th International conference on Fermentation Technology for Value Added Agricultural Products (FerVapp2017), Khon Kaen, Thailand, 137-145.

Chaiongkarn, A., Dathong, J., Moonmangmee, S., Saman, P., Lakhavat, S. and Sitdhipol, J. 2017. Research and development on symbiotic products for risk reduction of colon cancer. Final report of Grant (E) Research Project No. 57-50, TISTR, Pathum Thani, Thailand.

Corcoran, B.M., Ross, R.P., Fitzgerald, G.F. and Stanton, C. 2004. Comparative survival of probiotic Lactobacilli spray-dried in the presence of prebiotic substances. Journal of Applied Microbiology. 96:1024-1039.

Dathong, J., ThangTham, P., Lapromma, N., Saman, P., Moonmangmee, S., Ritthisorn,s. and Chaiongkarn, A. 2016. Poster presentation, The 28th Annual Meeting of the Thai Society for Biotechnology and International Conference 28-30 November 2016, Chiang Mai, Thailand

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28: 350-356.

FAO. 2007. Report on functional foods, food quality and standards service (AGNS). URL (file:///C:/Users/Admin/Downloads/Functional_Foods_Report_Nov2007.pdf) (accessed on 23 May 2018).

FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food and Agriculture Organization of the United Nations and World Health Organization Working Group Report, London Ontario, Canada.

FAO/WHO. 2006. Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper No. 85, FAO, Rome, Italy.

Ferlay, J. and Soerjomataram, E. I. 2012 GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC cancer base No. 11.: International Agency for Research on Cancer.

Fritzen-Freire, C.B.; Prudencio, E.S., Amboni, R.D.M.C., Pinto, S.S., Negrao-Murakami, A.N. and Murakami, F.S. 2012. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. Food Research International. 45: 306-312

Fu, N. and Chen, X.D. 2011. Toward a maximal cell survival in convective thermal drying process. Food Research International. 44:1127-1149.

Fuller, R. 1989. Probiotics in man and animals. Journal of Applied Bacteriology. 66: 365-378.

Gardier, G.E., O'Sullivan, E., Kelly, J., Auty, M.A.E., Fitzgerald, G.F., Collins, J.K., Ross, R.P. and Stanton, C. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Applied and Environmental Microbiology. 66: 2605-2615.

Gaudier, E., Michel, C., Segain, J.P., Cherbut, C. and Hoebler, C. 2005. The VLS#3 probiotics mixture modifies microflora but does not heal chronic dextran-sodium sulfate-induced colitis or reinforce the mucus barrier in mice. The Journal of Nutrition. 135: 2753-2761.

Gibson, G.R. and Roberfroid, M.B. 1994. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. The Journal of Nutrition. 25: 1401-1412.

Goldin, B.R. and Gorbach, S L. 1976. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. Journal of the National Cancer Institute. 57: 371-375.

Goldin, B.R. and Gorbach, S.L. 1980. Effect of *Lactobacillus acidophilus* dietary supplementation on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. Journal of National Cancer Institute .1980 64: 263-265.

Gourbeyre, P., Denery, S. and Bodinier, M. 2011. Probiotics, prebiotics, and synbiotics: Impact on the gut immune system and allergic reactions. Journal of Leukocyte Biology. 89: 685-695.

Hamsupo, K., Sukyai, P. and Nitisinprasert, S. 2005. Different growth media and growth phase affecting on spray drying of *Lactobacillus reuteri* KUB-AC5. Kasetsart Journal (Natural Science) (Thailand). 39: 718-724.

Hughes, R. and Rowland, I.R. 2001. Stimulation of apoptosis by two prebiotic chicoryfructans in the rat colon. Carcinogenesis. 22: 43-47.

Hylla, S., Gostner, A., Dusel, G., Anger, H., Bartram, H-P., Christl, S U., Kasper, H. and Scheppach, W. 1998. Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention[1-3]. The American Journal of Clinical Nutrition. 67: 136-42.

Jantzen, M., Gopel, A. and Beermann. 2013. Direct spray drying and microencapsulation of probiotic *Lactobacillus reuteri* from slurry fermentation with whey. Journal of Applied Microbiology. 115: 1029-1036.

Korakli, M., Ganzle, M.G. and Vogel, R.F. 2002. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*. Journal of Applied Microbiology. 92(5):958-965.

Krasse, B. and Carlsson, J. 1970. Various types of streptococci and experimental caries in hamsters. Archives of Oral Biology. 15: 25-32.

Lapsiri, W., Bhandari, B. and Wanchaitawawong, P. 2012. Viability of *Lactobacillus plantarum* TISTR 2075 in different protectans during spray drying and storage. Drying Technology. 30: 1407-1412.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. The Journal of Biological Chemistry. 153, 375-380.

O'Mahony, L., Feeney, M., O'Halloran, S., Murphy, L., Kiely, B., Fitzgibbon, J., Lee, G., O'Sullivan, G., Shanahan, F. and Collins, J.K. 2001. Probiotics impact on microbial flora, inflammation and tumor development in IL-10 knockout mice. Alimentary Pharmacological &Therapeutics. 15:1219-1225.

Paez, R., Lavari, L., Vinderola, G., Audero, G., Cuatrin, A., Zaritzky, N. and Reinheimer, J. 2012. Effect of heat treatment and spray dying on lactobacilli viability and resistance to simulated gastrointestinal digestion. Food Research International. 48: 748-754.

Rafter, J., Bennett, M., Caderni, G., Clune, Y., Hughes, R., Karlsson, P. C., Klinder, A., O'Riordan, M., O'Sullivan, C., Pool-Zobel, B., Rechkemmer, G., Roller, M., Rowland, I., Salvadori, M., Thisjs, H., Loo, J. V., Watzl, B. and Collins, J. K. 2007. Dietary synbiotics reduce cancer risk factors in polypectomizied and colon cancer patients. The American Journal of Clinical Nutrition. 85:488-496.

Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., Purama, R. K., Dave, J. M. and Vyas, B. R. 2013. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes. 4(3): 181-192.

Rebecca, L. S., Miller, K. D., Fedewa, S A., Ahnen, D.J., Meester, R.G.S., Barzi, A. and Jemal, A. 2017. Colorectal Cancer Statistics. Cancer Journal for Clinicians. 67:177-193.

Roberfroid, MB. 1998. Prebiotics and synbiotics: concept and nutrition properties. British Journal of Nutrition. 80:S197-S202.

Rowland, L.R., Rumney, C.J., Coutts, J. T and Lievense, L. C. 1998. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis. 19: 281-285.

Saulnier, D.M.A., Gibson, G.R. and Kolida, S. 2008. In vitro effects of selected synbiotics on the human faecal microbiota composition. FEMS Microbiology Ecology. 66(3): 1-12.

Sunha, D. and Geetha, N. 2017. Evaluation of acute toxicity of the methanolic extract of *Tanacetum parthenium* L. in albino wistar rats. Journal of Scientific & Innovative Research.

6(3): 113-115

Tripathi, M.K. and Giri, S.K. 2014. Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods. 9: 225-241.

Verma, A. and Shukla, G. 2012. Probiotics *Lactobacillus rhamnosus GG, Lactobacillus acidophilus* Suppresses DMH-Induced Procarcinogenic Fecal Enzymes and Preneoplastic Aberrant Crypt Foci in Early Colon Carcinogenesis in Sprague Dawley Rats. Journal Nutrition and Cancer. 65: 84-91.

Virani, S., Bilheem, S., Chansaard, W., Chitapanarux, I., Daoprasert, K., Khuanchana, S., Leklob, A., Pongnikorn, D., Rozek, L.S., Siriarechakul, S., Suwanrungruang, K., Tassanasunthornwong, S., Vatanasapt, P. and Sriplung, H. 2017. National and subnational population-based incidence of cancer in Thailand: Assessing cancers with the highest burdens. Cancers. 9(108): 1-27.

Wichienchot, S., Jatupornpipat, M. and Rastall, R.A. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. Food Chemistry. 120(3): 850-857.