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# GROWTH AND SURVIVAL OF LACTIC ACID BACTERIA DURING THE FERMENTATION AND STORAGE OF SEAWEED OLIGOSACCHARIDES SOLUTION

Shao-Chi Wu\*, Fu-Jin Wang\*\*, and Chorng-Liang Pan\*

Key words: agarases, lactic acid bacteria, seaweed oligosaccharides solutions, fermentation.

## ABSTRACT

The current study is to evaluate the performance of two lactic acid bacteria (LAB) that could grow in seaweed oligosaccharides solutions (SOsS) for future development on algal health food. During the storage of fermented SOsS at 4°C, the changes in pH values, titratable acidity (TA), and LAB count were monitored. The seaweed polysaccharide extract solutions (SPsES) were prepared from *Gelidium* sp., *Gracilaria* sp., *Mon. nitidum*, and *Por. dentate*, and these SPsES were digested by agarases derived from *Aeromonas salmonicida* MAEF108 and *Pseudomonas vesicularis* MA103. The results of four seaweed polysaccharide extract solutions (SPsES) or four SOsS were inoculated with *Enterococcus faecalis* BCRC13076 and/or *Lactobacillus rhamnosus* BCRC14068 indicated that their TA increased, pH decreased, optical density at 600 nm increased, and their LAB counts increased during the fermentation period (0-24 h). During storage at 4°C, four SPsES or four SOsS fermented by *E. faecalis* BCRC13076 and/or *L. rhamnosus* BCRC14068 exhibited pH decreasing, TA increasing, viability decreasing, and decreasing in sugar contents from day 7 to day 14. These results show that four SOsS allowed better growth for *E. faecalis* BCRC13076 and/or *L. rhamnosus* BCRC14068 than SPsES did.

## INTRODUCTION

Nondigestible oligosaccharides (NDO) including fructo-oligosaccharides (FOS), lactulose and lactitol, galacto-oligosaccharides (GOS), and soybean oligosaccharides, are resistance to digestive enzymes in the small intestines due to their (chemical) structures [29]. NDO are associated with physiological actions such as reducing cholesterol levels and attenuating blood glucose, maintaining gastrointestinal health, and posi-

tively affecting calcium bio-availability and immune function [32]. NDO also have physicochemical properties including water dispersibility and solubility, viscosity effects, bulk, absorption and fermentability, and binding abilities to other compounds. Based on these properties, NDO can help improve the organoleptic properties and nutritional value of food [32].

Recently, NDO derived from different substances has been shown to have potential prebiotics properties. For example, FOS from yacon roots can be fermented by *Lactobacillus acidophilus* NRRL-1910, *L. plantarum* NRRL B-4496, and *Bifidobacterium bifidum* ATCC15696 [23]. Sanz *et al.* [27] obtained honey oligosaccharides by utilizing nanofiltration, yeast treatment, and adsorption onto activated charcoal to remove monosaccharides in honey. These honey oligosaccharides could increase the populations of bifidobacteria and lactobacilli. Olano-Martin *et al.* [22] observed that pectic oligosaccharides, POS I and POS II, were better candidate prebiotics than the pectins they derived from.

Agarose is composed of alternating of D-galactose and 3,6-anhydro-L-galactose linked by alternating  $\beta$ -(1,4) and  $\alpha$ -(1,3) bonds. The enzyme  $\beta$ -agarases cleaves the  $\beta$ -(1,4) linkage between D-galactopyranose and 3,6-anhydro-L-galactose to give a series of neoagarooligosaccharides (NAOs) [14]. The NAOs are water soluble, moisture absorbing, colorless, sweet tasting (sweetness 20% of sucrose), and can avoid starch aging. On the other hand, NAOs have the physiological property of lowering calories, that is making them difficult for intestinal flora of rat to ferment retaining 95% NAOs after 24 h administration [30]. Among these results, NAOs exhibits some properties so could be regarded as NDO.

Seaweed polysaccharides, xylan/mannan, prophyllan, and floridean starch extracted from *Porphyra yezoensis* were evaluated on the changes of intestinal flora of rat cecum. The cecum microflora of rat were changed by the administration of xylan/mannan or

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porphyran added to the diet, and the number of *Bifidobacterium* counts were increased by xylan/mannan [16]. Gudiel-Urbano and Goñi [13] discovered that the adaptation by rats to diets containing *Undaria pinnatifida* or *P. ternera* was associated with changes in microbial activity such as  $\beta$ -D-glucuronidase, azoreductase, nitroreductase, nitratedreductase, and  $\beta$ -D-glucosidase involving a decrease of the reductive and hydrolytic enzymatic activities in the conversion of procarcinogens into carcinogens.

To the best of our knowledge, lactic acid fermentation of seaweed oligosaccharides, produced by seaweed polysaccharide extracts digested by agarase has not been reported. This work deals with two strains selected from 35 lactic acid bacteria (LAB) strains. Both *Enterococcus faecalis* BCRC13076 and *L. rhamnosus* BCRC14068 utilize seaweed oligosaccharides that are derived from the seaweed polysaccharide extract of *Gelidium* sp., *Gracilaria* sp., *Monostroma nitidum*, and *P. dentate* by agarases digestion.

## MATERIALS AND METHODS

### 1. Crude enzyme preparation

The preparation of two crude enzyme solutions from the strains *Aeromonas salmonicida* MAEF108 and *Pseudomonas vesicularis* MA103 were obtained according to the method used by Wu and Pan [37], and the assay of agarase activity was carried out according to the same reference.

### 2. Types of seaweed polysaccharide extract (SPsE)

The seaweed polysaccharide extract (SPsE) of four seaweeds, *Gelidium* sp., *Gracilaria* sp., *M. nitidum*, and *P. dentate* were prepared according to the protocol described by Wu and Pan [37]. The four SPsEs from *Gelidium* sp., *Gracilaria* sp., *M. nitidum*, and *P. dentate* were abbreviated as Gel, Gra, Mon, and Por, respectively. The seaweed extracts are composed of ash (4.4%-11.33%), protein (0.31%-4.13%), and mostly carbohydrates (28.72%-49.25%) in the form as polysaccharide (data not shown).

### 3. Preparation of seaweed oligosaccharides solutions (SOsS)

#### (1) Degradation of SPsE by agarases

The degradation of the four SPsEs (Gel, Gra, Mon, and Por) by agarases was done according by the method of Wu *et al.* [38] with the highest sugar reducing result. 100 mL of the four raw seaweed oligosaccharides solu-

tions (RSOsS), obtained from Gel, Gra, Mon, or Por was chosen to be fermented by LAB strains. Take *Gracilaria* oligosaccharides hydrolysate as example, when extracted by 100°C boiling water for 4 h followed by cellulase and macerozyme treatment, it is composed of monosaccharides (0.47%), neoagarobiose (3.35%), neoagarotetraose (2.64%), and neoagarohexaose (4.18%) (data not shown).

#### (2) Preparation of seaweed oligosaccharides solutions (SOsS)

In the preliminary experiment of the four RSOsS, fermented by the cultivated LAB strains, the results showed that the LAB strains did not grow well after 96 h.

Preliminary experiments showed that the four RSOsS did not support growth of LAB. Therefore, 1.0% beef extract (Difco Laboratories, Detroit, MI, U.S.A.), 1.0% proteose peptone (Difco), and/or 0.5% yeast extract (Difco) were used as a nitrogen source based on the nitrogen ingredients in the MRS broth. The preliminary data showed that 1.0% beef extract and 0.5% yeast extract added to the four RSOsS would significantly increase LAB growth. Therefore, 100 mL of four SPsE or four RSOsS were placed in a 125 mL glass bottle with a screw cap, and 5 mL of a nitrogen source solution was added, containing 1.0 g of beef extract and 0.5 g of yeast extract. The RSOsS containing a nitrogen source of fermented LAB is called a seaweed oligosaccharides solution (SOsS). The SOsS from Gel, Gra, Mon, and Por are named SOsS-Gel, SOsS-Gra, SOsS-Mon, and SOsS-Por, respectively. The SPsE containing a nitrogen source of fermented of LAB, is called SPsE solution (SPsES) and set as a control. The SPsES from Gel, Gra, Mon, and Por are named SPsES-Gel, SPsES-Gra, SPsES-Mon, and SPsES-Por, respectively.

### 4. Organisms and the preparation of inocula

In the preliminary experiment, 35 strains of LAB, which were obtained from the Bioresources Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan were tested for their capability of utilizing the four SOsS. The results showed that the LAB strains which were capable of fermenting the four SOsS including the mentioned nitrogen sources were *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068. After two successive transfers of the test organisms in MRS agar (Difco) incubation at 37°C for 24 h, each activated culture was inoculated into a 250 mL Erlenmeyer flask containing 100 mL of MRS broth at 37°C. The optical density was measured at 600 nm ( $OD_{600nm}$ ) using a spectrophotometer (Coleman® model 35, Beckman Products Inc., Boise, ID, U.S.A.), the organisms were about 8 log

CFU/mL while the OD<sub>600nm</sub> value reaches approximately 0.8, then the suspension was served as the inoculum.

### 5. Starter combinations

The 100 mL of four SPsES or four SOsS were inoculated with three LAB groups, (1) 5 mL *E. faecalis* BCRC13076, (2) 5 mL *L. rhamnosus* BCRC14068, and (3) 2.5 mL *E. faecalis* BCRC13076 and 2.5 mL *L. rhamnosus* BCRC14068. In these experiments, the initial population of each LAB was about 6 log CFU/mL. The duration of the fermentation time was 24 h, and the pH values, titratable acidity (TA), and LAB counts were determined every six hours.

### 6. Microbiological and chemical analyses

#### (1) Determination of the acidity

The pH of the fermented samples were measured at room temperature (23 to 25°C) using a pH meter (MP220, Mettler Toledo, Schwerzenbach, Switzerland) (triplicately) after calibrating with fresh pH 4.0 and 7.0 standard buffers (Panreac) [36].

#### (2) Determination of titratable acidity (TA)

TA was determined by the AOAC method and expressed as % lactic acid [1].

#### (3) Determination of the lactic acid bacteria count

MRS agar (Difco) was used for the enumeration of *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068. 1 mL of each sample was diluted with 9 mL of sterilized 0.85% (w/v) NaCl solution, and vortex thoroughly. Subsequent serial dilutions were prepared, and viable numbers enumerated using the spread-plated technique onto the MRS agar. After 48 h of incubation at 37°C, the colonies appeared on the plates were counted and the CFU/mL was calculated [35].

#### (4) Determination of bacterial growth by optical density

The optical density (OD) of the samples were measured at 600 nm in a Coleman® model 35 spectrophotometer (Beckman) [17].

### 7. Refrigerated storage: Determination the percentage of pH decreasing, TA increasing, and viable LAB counts change in fermented products

When the pH of the LAB fermented four SPsES or four SOsS decreased to below 4.4 ~ 4.6 and they were

stored in a refrigerator (GR-B500A, LG, Seoul, Korea) at 4°C for two weeks. During the storage period, viable counts of lactic acid bacteria were determined once a week. SPsES or SOsS containing no LAB were used as controls. Percentage of pH decrease, TA increase, and viabilities of each culture in the presence of different LAB were calculated as follows:

$$\% \text{ pH decrease} = \left[ \frac{\text{pH each week of storage}}{\text{initial pH}} - 1 \right] \times 100$$

$$\% \text{ TA increase} = \left[ \frac{\text{TA each week of storage}}{\text{initial TA}} - 1 \right] \times 100$$

$$\% \text{ viability} = \left[ \frac{\log(\text{CFU/mL}) \text{ each week of storage}}{\text{initial log(CFU/mL)}} \right] \times 100 \text{ [4]}$$

### 8. Statistical analysis

All pH values, TA, and lactic acid bacteria counts resulting from the lactic acid fermentation solutions are expressed as mean  $\pm$  SD (n = 3). Data were analyzed by one-way analysis of variance (ANOVA). When the ANOVA identified differences among groups, multiple comparisons among means were made, using Duncan's new multiple range test. Statistical significance was determined by setting the aggregate type I error at 5% ( $p < 0.05$ ) for each set of comparisons, using the Statistical Analysis System software package [29].

## RESULTS

### 1. Changes of pH and TA in SPsES or SOsS during the growth of the LAB

Changes in pH and TA during the fermentation of the four SPsES or four SOsS inoculated with BCRC13076 and/or BCRC14068 are shown in Figures 1 and 2. In general, the TA increased and the pH decreased with the fermentation time from 0 to 24 h for four SPsES or four SOsS inoculated with BCRC13076 and/or BCRC14068. The initial pH of four SPsES or four SOsS were from  $6.11 \pm 0.02$  to  $6.37 \pm 0.02$ . In this study, the pH of four SPsES fermented with BCRC13076 and/or BCRC14068 after 24 h ranged from  $4.32 \pm 0.00$  to  $4.62 \pm 0.01$ , and their pH lowers to 4.6 after 24 h fermentation. The pH of four SOsS fermented with BCRC13076 and/or BCRC14068 after 24 h ranged from  $4.16 \pm 0.04$  to  $4.44 \pm 0.03$ , and their pH value lowers to 4.6 after 18 h fermentation. The results showed that the pH of all four SPsES or four SOsS fermented with BCRC13076 and/or BCRC14068 were below 4.6 after 24 h. In four SPsES fermented with BCRC13076 and/or BCRC14068 after 24 h, the highest TA was found to be  $0.64 \pm 0.03\%$ , the lowest TA was found to be  $0.36 \pm 0.01\%$ . In four SOsS fermented with BCRC13076 and/

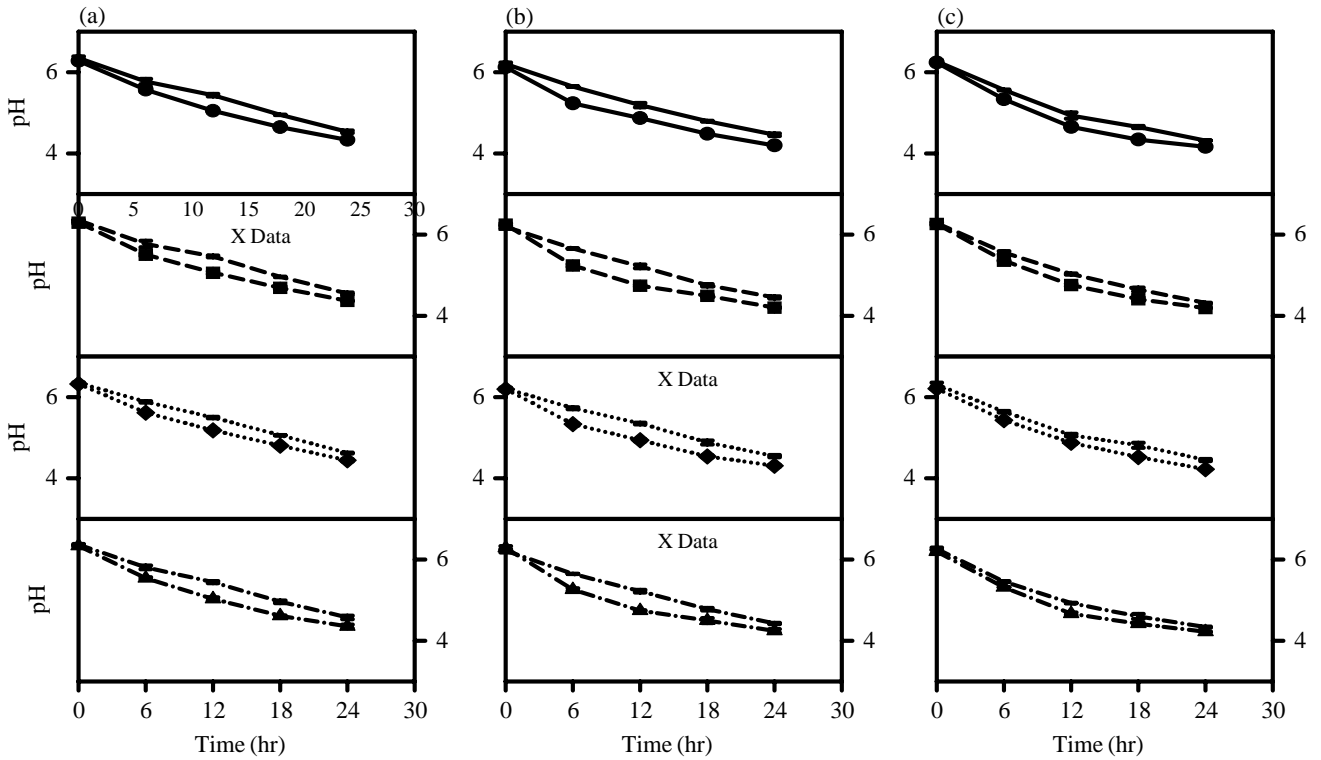


Fig. 1. pH values of the four SPsES or four SOsS during fermentation with (a) LAB BCRC13076; (b) LAB BCRC14068, and (c) LAB Com (LAB BCRC13076 and BCRC14068). (—) SPsES-Gel, (---) SPsES-Gra, (···) SPsES-Mon, (- · -) SPsES-Por, (●) SOsS-Gel, (■) SOsS-Gra, (◆) SOsS-Mon, (▲) SOsS-Por.

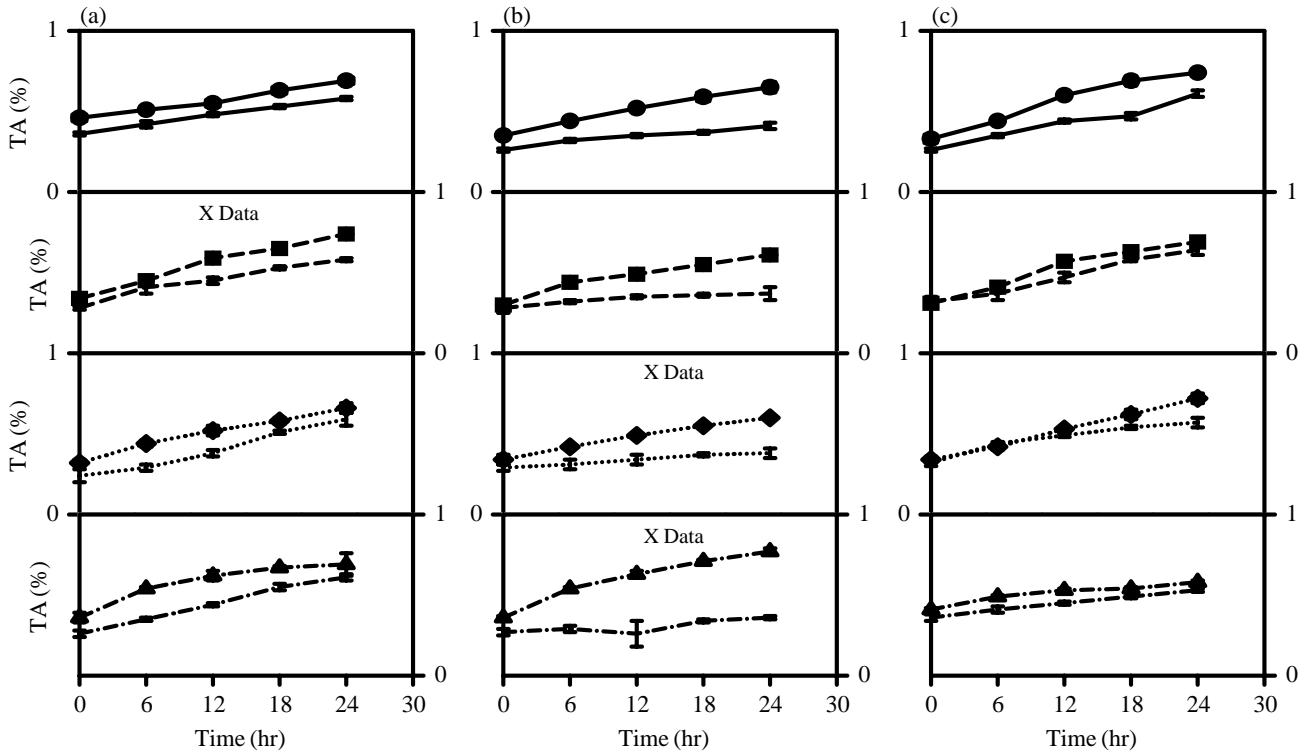


Fig. 2. Titratable acidities of the four SPsES or four SOsS during fermentation with (a) LAB BCRC13076, (b) LAB BCRC14068, and (c) LAB Com (LAB BCRC13076 and BCRC14068). (—) SPsES-Gel, (---) SPsES-Gra, (···) SPsES-Mon, (- · -) SPsES-Por, (●) SOsS-Gel, (■) SOsS-Gra, (◆) SOsS-Mon, (▲) SOsS-Por.

or BCRC14068 after 24 h, the highest TA was  $0.77 \pm 0.02\%$ , and the lowest TA was  $0.58 \pm 0.01\%$ . In the preliminary experiments of the four RSOsS without nitrogen source and directly fermented by 35 LAB strains, the results of pH values showed that the 35 LAB strains could not lower the pH below 4.6 after 96 h. After adding nitrogen ingredients in four RSOsS according to the MRS broth, which were 1.0% beef extract, 1.0% proteose peptone, and/or 0.5% yeast extract, only BCRC13076 and BCRC14068 showed a pH value below 4.6 after 48 h. When the nitrogen source was mixture of 1.0% beef extract and 0.5% yeast extract in four RSOsS, the pH was lowered to 4.6 after 18 h fermentation.

## 2. Growth of LAB during SPsES or SOsS fermentation

Figures 3 and 4 were results of changes in the  $OD_{600nm}$  and log CFU/mL during the fermentation of four SPsES or four SOsS inoculated with BCRC13076 and/or BCRC14068. In general, log CFU/mL increased with the  $OD_{600nm}$  as the fermentation time goes through 0 to 24 h for four SPsES or four SOsS when inoculated with BCRC13076 and/or BCRC14068. In the  $OD_{600nm}$  of the fermented solution of four SPsES that were

fermented with BCRC13076 and/or BCRC14068, except for SPsES-Gel and SPsES-Gra which were fermented with BCRC13076 or BCRC14068 and were higher than 1.0, the rest were below 1.0. In the  $OD_{600nm}$  of the fermented solution of four SOsS fermented with BCRC13076 or/and BCRC14068, except for SOsS-Mon which were fermented with BCRC13076 or a combination of BCRC13076 and BCRC14068 which were lower than 1.0, the rest were higher than 1.0.

The viable cell count of the four SPsES fermented with BCRC13076 after 24 h were below 8.0 log CFU/mL, and the four SPsES fermented with BCRC14068, and BCRC13076/14068 were above 8.0 log CFU/mL after 24 h. The fermentation of the four SOsS fermented with BCRC13076 after 24 h were above 8.0 log CFU/mL, and the four SOsS fermented with BCRC13076/14068 were above 8.0 log CFU/mL after 24 h, except for SOsS-Mon. When the four SPsES or four SOsS were inoculated with BCRC13076 or BCRC14068, the results show that BCRC14068 grew better than BCRC13076. The four SPsES or four SOsS inoculated with BCRC13076/BCRC14068 were better than the BCRC13076 or BCRC14068 alone. Otherwise, the LAB counts increased with the reducing sugar contents. These results indicated that four SOsS fermented with

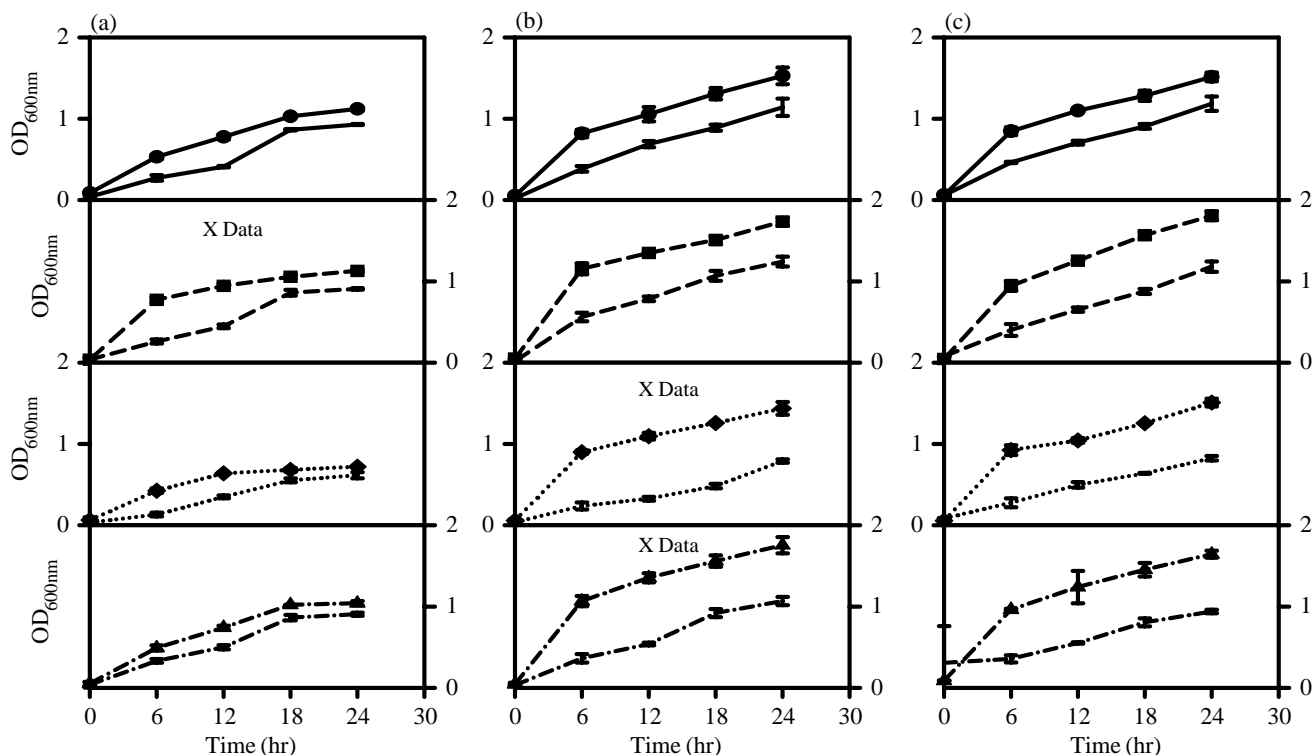


Fig. 3. The  $OD_{600nm}$  of the four SPsES or four SOsS during fermentation with (a) LAB BCRC13076, (b) LAB BCRC14068, and (c) LAB Com (LAB BCRC13076 and BCRC14068). (—) SPsES-Gel, (---) SPsES-Gra, (···) SPsES-Mon, (-·-) SPsES-Por, (●) SOsS-Gel, (■) SOsS-Gra, (◆) SOsS-Mon, (▲) SOsS-Por.

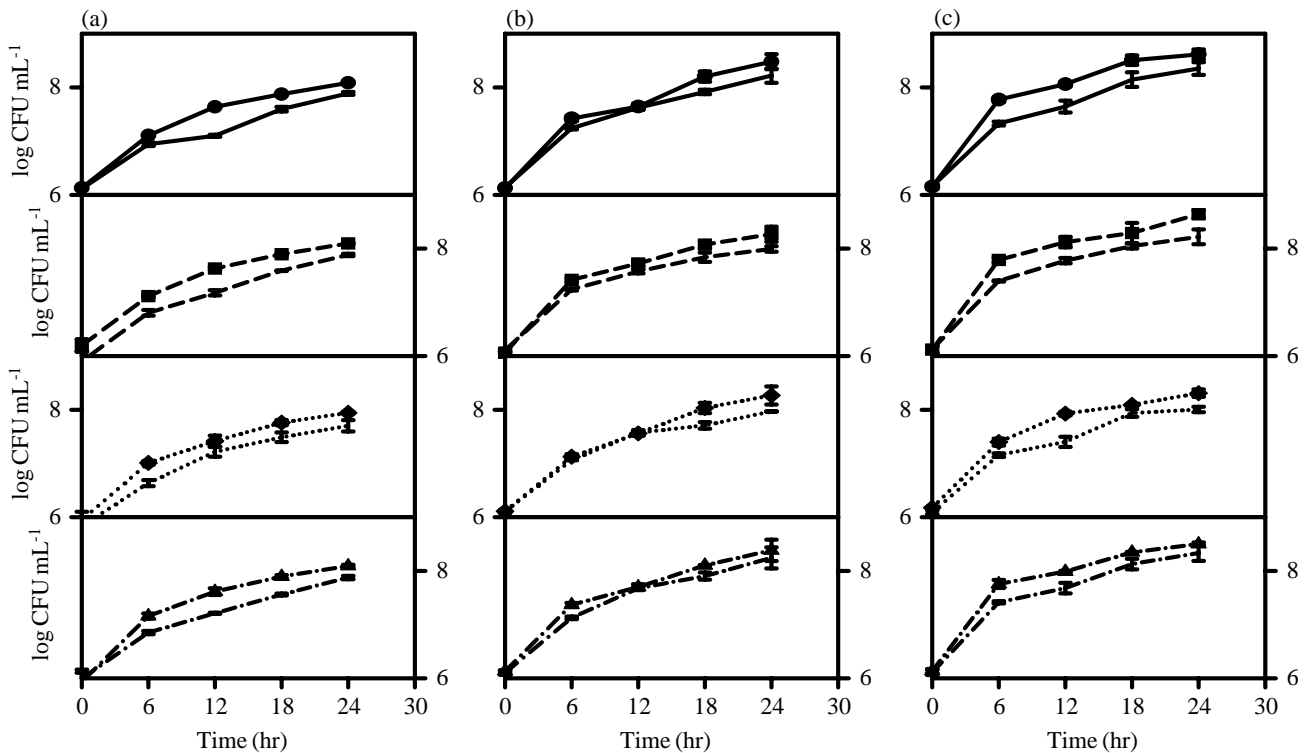


Fig. 4. Lactic acid bacterial count (Log CFU/mL) of the four SPsES or four SOsS during fermentation with (a) LAB BCRC13076, (b) LAB BCRC14068, and (c) LAB Com (LAB BCRC13076 and BCRC14068). (—) SPsES-Gel, (- - -) SPsES-Gra, (···) SPsES-Mon, (- · -) SPsES-Por, (●) SOsS-Gel, (■) SOsS-Gra, (◆) SOsS-Mon, (▲) SOsS-Por.

BCRC13076 and/or BCRC14068 can lower the pH quicker with a nitrogen source addition than those without added nitrogen source.

### 3. Survival of LAB in the refrigerated storage of cultured SPsES or SOsS

Table 1 shows the % pH decrease of the four SPsES or four SOsS fermented with BCRC13076 and/or BCRC14068 stored at 4°C after 1 or 2 weeks. In general, the results of the % pH decrease related to the storage time from 1 to 2 weeks, when four SPsES or four SOsS fermented by BCRC13076 and/or BCRC14068 stored at 4°C. Meanwhile, the pH decreases as the reducing sugar content rises. The % pH decrease of the four SPsES fermented with BCRC13076 and/or BCRC14068 stored at 4°C ranged from  $0.7 \pm 0.1$  to  $1.7 \pm 0.0\%$  after 1 week, and  $1.5 \pm 0.2$  to  $3.1 \pm 0.1\%$  after 2 weeks. The SPsES-Mon in four SPsES fermented by BCRC13076 and/or BCRC14068 had the lowest % pH decrease among the four SPsES. Otherwise, the % pH decrease of the four SOsS fermented with BCRC13076 and/or BCRC14068 that were stored at 4°C ranged from  $1.1 \pm 0.0$  to  $2.2 \pm 0.4\%$  after 1 week, and  $2.4 \pm 0.1$  to  $4.8 \pm 0.2\%$  after 2 weeks. The SOsS-Mon in four SOsS

fermented by BCRC13076 and/or BCRC14068 had the lowest % pH decrease among the four SOsS.

Table 2 shows the % TA increase of the four SPsES or four SOsS fermented with BCRC13076 and/or BCRC14068 stored at 4°C after 1 or 2 weeks. In general, the results of the % TA increase rose with the storage time from 1 to 2 weeks, when four SPsES or four SOsS which were fermented by BCRC13076 and/or BCRC14068 stored at 4°C. Percentage of TA increase also match to the rising reducing sugar content. The % TA increase of the four SPsES fermented with BCRC13076 and/or BCRC14068 stored at 4°C ranged from  $2.6 \pm 0.2$  to  $10.6 \pm 0.8\%$  after 1 week, and  $6.1 \pm 0.9$  to  $22.9 \pm 1.4\%$  after 2 weeks. The SPsES-Mon in four SPsES fermented by BCRC13076 and/or BCRC14068 had the lowest % TA increase among the four SPsES. Otherwise, the % TA increase of the four SOsS fermented with BCRC13076 and/or BCRC14068 stored at 4°C ranged from  $4.7 \pm 1.2$  to  $14.2 \pm 1.3\%$  after 1 week, and  $10.1 \pm 2.0$  to  $30.3 \pm 2.6\%$  after 2 weeks. The SOsS-Mon in four SOsS fermented by BCRC13076 and/or BCRC14068 had the lowest % TA increase among the four SOsS.

Table 3 showed the % viability of the four SPsES or four SOsS fermented with BCRC13076 and/or



**Table 1. pH percent decrease of SPsES or SOsS fermented with *E. faecalis* BCRC13076 and/or *L. rhamnosus* BCRC14068 after 1 or 2 weeks of refrigerated storage at 4°C**

Treatments	Seaweeds	% pH decrease					
		13076*		14068		Com	
		1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
SPsES**	Gel	1.0 ± 0.1 <sup>bc</sup>	2.1 ± 0.2 <sup>c</sup>	1.3 ± 0.1 <sup>b</sup>	2.9 ± 0.2 <sup>b</sup>	1.5 ± 0.1 <sup>ab</sup>	2.8 ± 0.2 <sup>b</sup>
	Gra	1.0 ± 0.1 <sup>bc</sup>	2.2 ± 0.1 <sup>c</sup>	1.2 ± 0.1 <sup>bc</sup>	2.7 ± 0.1 <sup>bc</sup>	1.5 ± 0.1 <sup>b</sup>	3.1 ± 0.1 <sup>b</sup>
	Mon	0.7 ± 0.1 <sup>c</sup>	1.5 ± 0.2 <sup>d</sup>	0.9 ± 0.2 <sup>c</sup>	1.9 ± 0.3 <sup>b</sup>	1.1 ± 0.0 <sup>c</sup>	2.4 ± 0.0 <sup>c</sup>
	Por	1.1 ± 0.3 <sup>bc</sup>	2.3 ± 0.4 <sup>c</sup>	1.7 ± 0.0 <sup>a</sup>	2.9 ± 0.2 <sup>c</sup>	1.5 ± 0.3 <sup>b</sup>	3.0 ± 0.3 <sup>b</sup>
SOsS	Gel	2.2 ± 0.2 <sup>a</sup>	3.9 ± 0.3 <sup>a</sup>	1.7 ± 0.1 <sup>a</sup>	3.4 ± 0.2 <sup>a</sup>	1.7 ± 0.1 <sup>ab</sup>	3.7 ± 0.2 <sup>a</sup>
	Gra	1.9 ± 0.3 <sup>a</sup>	3.6 ± 0.2 <sup>a</sup>	1.7 ± 0.3 <sup>a</sup>	3.1 ± 0.4 <sup>ab</sup>	1.7 ± 0.1 <sup>ab</sup>	3.7 ± 0.2 <sup>a</sup>
	Mon	1.2 ± 0.1 <sup>b</sup>	2.8 ± 0.3 <sup>b</sup>	1.1 ± 0.0 <sup>bc</sup>	2.4 ± 0.1 <sup>c</sup>	1.4 ± 0.1 <sup>b</sup>	2.9 ± 0.0 <sup>b</sup>
	Por	2.2 ± 0.4 <sup>a</sup>	3.9 ± 0.3 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>	3.5 ± 0.3 <sup>a</sup>	1.8 ± 0.2 <sup>a</sup>	4.8 ± 0.2 <sup>a</sup>

\*: "13076" or "14068" represent the inoculated amounts of *E. faecalis* BCRC13076 or *L. rhamnosus* BCRC14068 at 5 mL, and "Com" represents the inoculated amounts of *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068 at 2.5 mL, respectively.

\*\* : SPsES express SPsE solution, and SOsS express seaweed oligosaccharides solution.

\*\*\*: Data are mean values of triplicate determinations ± standard deviation. Different superscript letters in the same column (vertical comparison) indicate significantly different values ( $p < 0.05$ ).

**Table 2. TA percent increase of SPsES or SOsS fermented with *E. faecalis* BCRC13076 and/or *L. rhamnosus* BCRC14068 after 1 or 2 weeks of refrigerated storage at 4°C**

Treatments	Seaweeds	% TA increase					
		13076*		14068		Com	
		1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
SPsES**	Gel	8.6 ± 1.9 <sup>cd</sup>	17.8 ± 2.8 <sup>b</sup>	10.6 ± 0.8 <sup>c</sup>	22.9 ± 1.4 <sup>b</sup>	9.9 ± 1.4 <sup>bc</sup>	18.7 ± 2.0 <sup>cd</sup>
	Gra	9.8 ± 0.7 <sup>bcd</sup>	20.1 ± 0.5 <sup>b</sup>	5.4 ± 0.7 <sup>d</sup>	10.8 ± 2.7 <sup>cd</sup>	8.9 ± 2.4 <sup>bc</sup>	18.8 ± 2.5 <sup>cd</sup>
	Mon	5.2 ± 2.2 <sup>e</sup>	12.6 ± 3.8 <sup>c</sup>	2.6 ± 0.2 <sup>e</sup>	6.1 ± 0.9 <sup>d</sup>	6.5 ± 1.3 <sup>c</sup>	10.6 ± 2.4 <sup>e</sup>
	Por	9.2 ± 0.8 <sup>bcd</sup>	17.9 ± 1.1 <sup>b</sup>	6.5 ± 1.8 <sup>d</sup>	13.0 ± 6.2 <sup>c</sup>	8.8 ± 0.8 <sup>bc</sup>	20.7 ± 1.1 <sup>bc</sup>
SOsS	Gel	12.2 ± 1.5 <sup>ab</sup>	24.8 ± 1.5 <sup>a</sup>	14.2 ± 1.3 <sup>a</sup>	30.3 ± 2.6 <sup>a</sup>	14.9 ± 0.9 <sup>a</sup>	27.5 ± 1.5 <sup>a</sup>
	Gra	13.9 ± 3.0 <sup>a</sup>	25.1 ± 1.4 <sup>a</sup>	12.8 ± 0.4 <sup>ab</sup>	26.8 ± 0.4 <sup>ab</sup>	11.7 ± 2.9 <sup>ab</sup>	24.5 ± 5.0 <sup>ab</sup>
	Mon	7.4 ± 2.2 <sup>de</sup>	16.4 ± 1.7 <sup>bc</sup>	4.7 ± 1.2 <sup>d</sup>	10.1 ± 2.0 <sup>cd</sup>	7.5 ± 0.5 <sup>c</sup>	14.4 ± 0.9 <sup>de</sup>
	Por	11.6 ± 0.8 <sup>abc</sup>	24.5 ± 4.0 <sup>a</sup>	11.3 ± 1.3 <sup>bc</sup>	23.1 ± 2.0 <sup>b</sup>	12.3 ± 3.0 <sup>ab</sup>	27.0 ± 3.3 <sup>a</sup>

\*: "13076" or "14068" represent the inoculated amounts of *E. faecalis* BCRC13076 or *L. rhamnosus* BCRC14068 at 5 mL, and "Com" represents the inoculated amounts of *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068 at 2.5 mL, respectively.

\*\* : SPsES express SPsE solution, and SOsS express seaweed oligosaccharides solution.

\*\*\*: Data are mean values of triplicate determinations ± standard deviation. Different superscript letters in the same column (vertical comparison) indicate significantly different values ( $p < 0.05$ ).

BCRC14068 stored at 4°C after 1 or 2 weeks. In general, the results of the % viability decreased with the storage time from 1 to 2 weeks, when four SPsES or four SOsS fermented by BCRC13076 and/or BCRC14068 were stored at 4°C. Meanwhile the percentage viability ranges decreased with the reducing sugar contents. The % viability of the four SPsES fermented with BCRC13076 and/or BCRC14068 stored at 4°C ranged

from 61.7 ± 0.8 to 89.2 ± 2.4% after 1 week, and 48.9 ± 1.1 to 78.9 ± 0.2% after 2 weeks. The SPsES-Mon in the four SPsES which were fermented by BCRC13076 and/or BCRC14068 had the highest % viability among the four SPsES. Furthermore, the % viability of the four SOsS fermented with BCRC13076 and/or BCRC14068 stored at 4°C ranged from 62.2 ± 0.1 to 93.9 ± 3.4% after 1 week, and 49.6 ± 0.2 to 79.7 ± 1.2% after 2 weeks. The

**Table 3. Viability percent of SPsES or SOsS fermented with *E. faecalis* BCRC13076 and/or *L. rhamnosus* BCRC14068 after 1 or 2 weeks of refrigerated storage at 4°C**

Treatments	Seaweeds	% viability					
		13076*		14068		Com	
		1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
SPsES**	Gel	87.6 ± 0.1 <sup>b</sup>	75.1 ± 0.1 <sup>b</sup>	88.5 ± 0.4 <sup>b</sup>	76.8 ± 0.9 <sup>ab</sup>	89.0 ± 1.8 <sup>a</sup>	78.7 ± 1.9 <sup>a</sup>
	Gra	75.4 ± 0.5 <sup>c</sup>	63.1 ± 0.7 <sup>c</sup>	88.5 ± 3.3 <sup>b</sup>	77.8 ± 0.2 <sup>ab</sup>	89.2 ± 2.4 <sup>a</sup>	77.3 ± 1.8 <sup>a</sup>
	Mon	61.7 ± 0.8 <sup>d</sup>	48.9 ± 1.1 <sup>d</sup>	74.5 ± 1.6 <sup>c</sup>	63.5 ± 2.0 <sup>c</sup>	75.1 ± 2.1 <sup>c</sup>	67.9 ± 2.1 <sup>b</sup>
	Por	75.1 ± 0.1 <sup>c</sup>	62.6 ± 0.1 <sup>c</sup>	88.4 ± 1.7 <sup>b</sup>	76.4 ± 3.4 <sup>ab</sup>	89.0 ± 2.0 <sup>a</sup>	78.9 ± 0.2 <sup>a</sup>
SOsS	Gel	88.3 ± 0.1 <sup>a</sup>	76.6 ± 0.2 <sup>a</sup>	89.9 ± 0.2 <sup>ab</sup>	78.8 ± 2.4 <sup>ab</sup>	87.9 ± 1.3 <sup>a</sup>	79.2 ± 1.3 <sup>a</sup>
	Gra	75.5 ± 0.5 <sup>c</sup>	63.2 ± 0.7 <sup>c</sup>	93.3 ± 3.4 <sup>a</sup>	79.7 ± 1.2 <sup>a</sup>	87.2 ± 2.4 <sup>a</sup>	77.5 ± 3.5 <sup>a</sup>
	Mon	62.2 ± 0.1 <sup>d</sup>	49.6 ± 0.2 <sup>d</sup>	77.4 ± 2.9 <sup>c</sup>	67.7 ± 1.7 <sup>c</sup>	79.5 ± 0.5 <sup>b</sup>	68.5 ± 0.2 <sup>b</sup>
	Por	75.2 ± 0.2 <sup>c</sup>	62.8 ± 0.2 <sup>c</sup>	90.3 ± 0.8 <sup>ab</sup>	75.8 ± 1.9 <sup>b</sup>	86.5 ± 0.1 <sup>a</sup>	79.2 ± 0.4 <sup>a</sup>

\*: “13076” or “14068” represent the inoculated amounts of *E. faecalis* BCRC13076 or *L. rhamnosus* BCRC14068 at 5 mL, and “Com” represents the inoculated amounts of *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068 at 2.5 mL, respectively.

\*\* : SPsES express SPsE solution, and SOsS express seaweed oligosaccharides solution.

\*\*\*: Data are mean values of triplicate determinations ± standard deviation. Different superscript letters in the same column (vertical comparison) indicate significantly different values ( $p < 0.05$ ).

SOsS-Mon in the four SOsS which were fermented by BCRC13076 and/or BCRC14068 had the highest % viability among the four SPsES.

## DISCUSSION

Microorganism sustain their life cycles via a large number of interrelated/complex metabolic pathways covering both biosynthetic and energy-yielding functions. One such regulatory mechanism is derived from low molecular weight compounds which is present in the growth medium [24, 31]. *Streptococcus thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *Leuconostoc lactis* utilized lactose homofermentatively to produce lactic acid, whilst *Bifidobacterium* spp. ferments the same sugar heterofermentatively to produce lactic acid and acetic acid [31]. LAB have developed two different strategies to metabolize lactose or galactose depending on their mode of transport, which is either via the phosphoenolpyruvate-dependent phosphotransferase system (PEP-PTS) or via a permease system. In lactococci and some *L. casei* strains, the transport of lactose or galactose depends on a PEP-PTS system combined with phospho-β-galactosidase, resulting in phosphorylated galactose which is metabolised by the enzymes of the tagatose-6-P pathway including galactose-6-P isomerase, tagatose-6-P kinase and tagatose-1,6-diP. In *S. thermophilus*, *L. bulgaricus*, and *L. lactis*, usage of lactose permease system in which a β-galactosidase catalyzes sugar cleavage is not modified and the galactose is catabolised via the Leloir pathway. The

Leloir pathway includes three enzymes, galactokinase, galactose-1-uridylyltransferase and UDP-galactose 4-epimerase, involving in the conversion of galactose to glucose-1-P [8, 10-12, 19, 21, 26, 34].

Organic acids, such as acetic and lactic acid are a byproducts in lactic fermentation, inhibiting growth of food borne pathogen due to lower pH., causes fermentation, and resulting in toxins of food borne pathogens [6, 15]. The reducing sugar contents ranged from 0.4 to 0.7 mg galactose/mL in four SPsES, and were from 3.74 to 4.25 mg galactose/mL in four SOsS (data not shown). The oligo- and mono-saccharides of the four SOsS were richer than the four SPsES which were investigated by gel permeation chromatograms (data not shown). Narvhus *et al.* [20] indicated that lactose was the primary substrate for acid production in milk, affecting the production of organic acids. When lactose content in milk increasing, the TA increases with the organic acids produced. Thus, the pH decreases and the TA increases of the four SOsS fermented with BCRC13076 and/or BCRC14068 after 24 h were larger than the four SPsES, which is related to their oligo- and mono-saccharides contents, as mentioned in Section 3-A. of Material and Methods (data not shown).

In glucose-based oligosaccharide, oligodextrins, pectin-oligo-saccharides, and xylo-oligosaccharides had effective prebiotic performance potential, when compared to their original polysaccharides [22]. Dimitrijević-Branković and Baras [9] fermented soy milk with *Lactobacillus* sp. V3 or *Bifidobacterium* sp. A7, and chose beetroot juice or carrot juice as the additional

source of carbohydrates and brewer's yeast as an extra source of nitrogen. The results showed that the mixture with brewer's yeast had a better stimulating effect on the growth of *Lactobacillus* sp. V3 or *Bifidobacterium* sp. A7 compared to those with juices alone, and the numbers of LAB increased with the fermentation time to 8 h. Rycroft *et al.* [25] observed galacto-oligosaccharides (GOS) could effectively increase the numbers of bifidobacteria. Tzortzis *et al.* [33] observed that *L. mucosae*, *L. acidophilus*, or *L. reuteri* could grow in tested GOS, such as lactose, melibiose or raffinose. LeBlanc *et al.* [18] discovered soymilk fermented by *L. fermentum* CRL722 resulted in the reduction of  $\alpha$ -GOS concentrations in soymilk, due to *L. fermentum* CRL722 expressing  $\alpha$ -galactosidase, which is a promising solution for the degradation of  $\alpha$ -GOS in soymilk. On the other hand, *Lactococcus raffinolactis*, unlike most lactococci, is able to ferment  $\alpha$ -galactosides, such as melibiose and raffinose, while at the same time the  $\alpha$ -galactosidase activity was higher in lactose-, melibiose- and raffinose-grown cells than in galactose-grown cells, and no  $\alpha$ -galactosidase activity was detected in glucose-grown cells [3]. Therefore, the *E. faecalis* BCRC13076 or *L. rhamnosus* BCRC14068 which fermented four SPsES or four SOsS may produce  $\alpha$ -galactosidase to decompose oligosaccharides in the four SPsES or four SOsS used in this experiment, and the probable simple sugars produced is utilized by the two lactics.

During storage, Bruno *et al.* [4] explained that skimmed milk containing *Bifidobacterium* enables a decrease in the pH value, possibly due to post-fermentative production of acetic acid and lactic acid. Bonczar *et al.* [2] discovered that the pH values decreases and the TA increases in yoghurt, bio yoghurt, sour milk, and kefir with the storage time up to 14 days. Cais-Sokolińska *et al.* [5] determined that lactic acid and titratable acidity concentration of yogurt fermented by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were affected during the storage time of the first 3 weeks at 6°C. The pH values decreased during 3 weeks of storage time of the yoghurt. At the same time, the lactic acid and titratable acidity concentration of the yoghurt rose during 3 weeks of storage. Dave and Shah [7] observed that during storage of yoghurt containing other various ingredients, *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, and bifidobacteria showed alteration of bacterial counts. The counts of *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and bifidobacteria reduced during 35 days of storage. Bruno *et al.* [4] observed that for skim milk containing *Bifidobacterium*, the LAB count reduced while storage at 4°C, and the reduced LAB counts depended on the LAB types and the carbon source. From the four SPsES and four SOsS, the SPsES-

Por and SOsS-Por, on the basis of microbiological and chemical analyses (also flavor and aroma personally observed), have good potential as new lactic acid fermented drink products in the future.

In this study, *E. faecalis* BCRC13076 and *L. rhamnosus* BCRC14068, showed their ability to utilize seaweed oligosaccharides derived from seaweed polysaccharide extracts of *Gelidium* sp., *Gracilaria* sp., *Monostroma nitidum*, and *Porphyra dentate* digested by agarases. And these results could mean that these LAB had the potential being used in the future development of lactic fermentative seaweed oligosaccharide lysate health foods. Though, more research has to be done to promote the usage of marine seaweeds as bioresources.

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