INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–828/2017/19–3–437–444
DOI: 10.17957/IJAB/15.0298
http://www.fspublishers.org

*Full Length Article*

# Orange Peel Fiber and *Tremella flava* Fermented Powder Effectively Induce Exopolysaccharide Production by *Lactobacillus plantarum* SLC 13

**Jing-Yao Huang[1†], Min-Lang Huang[1†], Cheng-Yen Kao[2], Tony J. Fang[1, 3*]**
[1]*Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan*
[2]*Department of Biotechnology and Laboratory Science in Medicine, School of Biomedical Science and Engineering, National Yang Ming University, Taipei, Taiwan*
[3]*Food industry Research and Development Institute, Hsinchu, Taiwan*
[*]For correspondence: tjfang@nchu.edu.tw
[†]These authors contributed equally to this work

## Abstract

The aim of this study was to evaluate the effect of orange peel fiber powder (OPFP) in comparison to that of *Tremella flava* Chen fermented powder (TFP) on *Lactobacillus plantarum* SLC 13 exopolysaccharide (EPS) production. Growth of and EPS production by SLC 13 in MRS medium supplemented with different concentrations of glucose, OPFP, and TFP were investigated. The antioxidant activity of EPS and antibacterial property of the culture medium were investigated by adding 6% glucose, 2% OPFP, and 2% TFP to the SLC 13 culture medium. Supplementation with 6% glucose, 2% OPFP, and 2% TFP significantly promoted EPS production and bacterial viability. Maximum EPS production (2.6647 ± 0.0380 g/L) of SLC 13 was observed when 2% OPFP pretreated with 3 N HCl was added to the culture medium. Supplementation of 2% OPFP treated with 20 U xylanase, 20 U cellulase, and 10 U cellulase/10 U xylanase increased the EPS production by strain SLC 13. The highest reducing power and DPPH and ABTS radical scavenging activities of EPS were observed when the culture medium was supplemented with 6% glucose. Supplementation with 6% glucose and 2% OPFP enhanced the antibacterial property of the SLC 13 medium against *Staphylococcus aureus* and *Salmonella typhimurium*, respectively. Our findings that OPFP promotes growth, EPS production and antibacterial property of SLC 13 suggest that it has potential for industrial application as an economic prebiotic. 

**Keywords:** Exopolysaccharide; Lactic acid bacteria; Orange peel fiber; Agro-industrial waste; Prebiotics

## Introduction

Citrus fruits are among the important economic crops, with a worldwide production of more than 88 million tons per year, and one-third of citrus fruits are used for the production of fruit juice (Izquierdo and Sendra, 2003). Agricultural wastes from citrus fruit processing account for 50% of the total fruit weight (Cohn and Cohn, 1997). Most of these agricultural wastes are non-reusable, except a small portion that is used for the extraction of essential oils and the preparation of pectin and animal feeds (Malla *et al*., 2015; Liu *et al*., 2016; Masson *et al*., 2016). Hence, the effective use of citrus processing wastes is a research topic worthy of investigation. A previous study indicated that citrus fibers interact with foods and alter their nutritional value, tissues, and flavors during food processing (Sendra *et al*., 2008). The fibers enhanced the viability of lactic acid bacteria (LAB) in yogurts under cold storage, and it was speculated that they contain oligosaccharides that promote the growth of lactic acid bacteria (Sendra *et al*., 2008).

*Tremella fuciformis* is an edible mushroom that is used in traditional Chinese medicinal mushrooms. Since the concepts of health maintenance and disease prevention are gradually becoming popular, the development of potential health supplements has become more important. A previous study on *T. flava* Chen suggested its multiple physiological activities; mice that continually drank *T. flava* Chen fermented soymilk (TFS) exhibited increased anti-inflammatory activity and resistance against Salmonellosis (Chen *et al*., 2014). However, research on *T. flava* Chen as a supplementary food remains scarce.

Animal- and plant-derived LAB are food-grade microorganisms, and their exopolysaccharides (EPS) are being employed as additives with GRAS (Generally Recognized as Safe) status (Lin *et al*., 2007). This type of additives can improve the rheological properties (Roller and Dea, 1992), texture, and stability of products (Lin *et al*., 2007). Hassan *et al*. (1996) have pointed out that the use of EPS-producing LAB in yogurt production can improve the water retention capacity of the product and prevent

**To cite this paper:** Huang, J.Y., M.L. Huang, C.Y. Kao, T.J. Fang, 2017. Orange peel fiber and *Tremella flava* fermented powder effectively induce exopolysaccharide production by *Lactobacillus plantarum* SLC 13. *Int. J. Agric. Biol.*, 19: 437–4

syneresis. In addition, EPS-producing LAB exhibit health-promoting effects in humans, and the EPS have prebiotic effects that are indirectly associated with human health and exhibit antioxidative, antitumor, immunomodulatory, and antibacterial activities, and blood cholesterol-reducing effects (Li *et al*., 2014; Deepak *et al*., 2016). Therefore, the use of LAB-derived EPS in the development of functional polysaccharide products with health benefits is worthy of study.

In this study, we aimed to evaluate the effects of orange peel fiber powder (OPFP) and *T. flava* Chen fermented powder (TFP) on *Lactobacillus plantarum* SLC 13 (SLC 13) EPS production for future development of EPS-rich fermented drinks.

## Materials and Methods

### LAB Strain

The EPS-overproducing LAB strain used in this study was isolated from Meinong District, Kaohsiung, Taiwan by Jing-Yao Huang (Department of Food Science and Biotechnology, National Chung Hsing University, Taiwan), and was identified as *L. plantarum* via API 50 CHL and 16S rDNA sequencing (Roy *et al*., 2001). The strain was named *L. plantarum* SLC 13.

### Preparation of TFP

*T. flava* Chen (ATCC 201807) is a fungal strain identified by Dr. Chee-Jen Chen (Department of Biotechnology, Southern Taiwan University, Taiwan; Chen 1998). Liquid *T. flava* Chen fermentation broth was lyophilized and stored until used in our experiments.

### Preparation of OPFP

Orange peel wastes were collected from orange juice vendors of the local market. The oranges were *Citrus sinensis*, originating from the Gukeng town ship, Yunlin County, Taiwan. The orange peels were washed and immersed in reverse osmosis water for 1 h until the peel fibers swelled. Then, the remaining flesh was removed and the white peel fibers were isolated and dried in an oven at 80°C for 4 h, followed by pulverization using a pulverizer. The pulverized peel fibers were filtered through an 80-mesh sieve to obtain OPFP that has good water solubility. The OPFP was stored in a moisture-proof cabinet until use. The preparation procedure of OPFP is shown in Fig. 1.

### Chemically Treated OPFP

The OPFP was dissolved in 1 N and 3 N HCl or NaOH solutions at a concentration of 10% (w/v). The solution was hydrolyzed by stirring for 1 h, after which the pH was adjusted to 7.0 with NaOH or HCl, respectively. Then, the solutions were freeze-dried and stored in a moisture-proof cabinet until used.

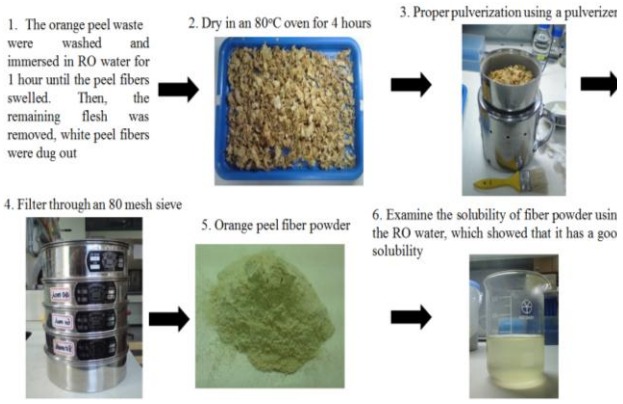


**Fig. 1:** Procedure for preparing OPFP

### Enzyme-Treated OPFP

The OPFP was treated with 20 U xylanase (EC 3. 2. 1. 8), 20 U cellulase (EC 3. 2. 1. 4), and 10 U xylanase + 10 U cellulase dissolved in in reverse osmosis water. The solutions were adjusted to a concentration of 2% (w/v) and hydrolyzed by stirring for 1 h. Then, the solutions were freeze-dried and stored in a moisture-proof cabinet until used.

### Effects of MRS Broth Supplemented with Different Substrates on the Growth of SLC 13

MRS broth was supplemented with different substrates at various concentrations as follows: 0, 2, 4, 6 and 8% glucose; 0, 0.5, 1.0, 1.5 and 2.0% OPFP; 0, 0.5, 1.0, 1.5 and 2.0% TFP; 2% OPFP pretreated with different chemicals; and 2% OPFP pretreated with different enzymes. After sterilization, the cooled media were inoculated with SLC 13 and incubated for 48 h to measure the EPS yield, bacterial growth, residual sugar and pH.

### Analysis of EPS Production

EPS production by strain SLC 13 was evaluated according to a previous study with a modification (Kim *et al*., 2005). In brief, the liquid fermented by LAB for 48 h was centrifuged at 6,000 × *g* for 15 min and the supernatant was carefully collected. A 1-mL aliquot of supernatant was mixed with 4 mL of 95% ethanol, and the mixture was incubated at 4°C for 24 h. The precipitated EPS was centrifuged at 9,000 × *g* for 15 min and the supernatant was discarded. The pure EPS precipitate was oven-dried at 60°C for 24 h. EPS production was analyzed by the phenol-sulfuric method using glucose as a reference standard (Torino *et al*., 2001).

**Determination of Bacterial Growth**

A ten-fold serial dilution of SLC 13 culture in PBS was prepared. Each dilution was homogenized by vortexing, and 1 mL was transferred into a petri dish. Molten MRS agar was poured into the petri dishes, mixed with the bacteria, and left to cool before incubation at 37°C for 48 h to determine the viable counts by colony counting.

**Determination of the Amount of Residual Sugars**

Residual sugars were determined according to the method described by Miller (1959). Fermentation broth that had been cultured for 48 h was centrifuged. One milliliter of diluted (1:10) supernatant was transferred into 3 mL of 3, 5-dinitrosalicylic acid reagent. After thorough mixing, the solution was incubated in boiling water for 5 min followed by incubation in cold water for 3 min. Then, 5 mL of reverse osmosis water was added to the solution, the mixture was homogenized by vortex prior, and the absorbance at 540 nm was measured. The absorbance values of glucose solutions of different concentrations were plotted as a calibration curve to determine the amount of residual sugars.

**Determination of Antioxidant Capacity**

Freeze-dried EPS was obtained from MRS broth, MRS broth supplemented with 6% glucose, MRS broth supplemented with 2% OPFP, and MRS broth supplemented with 2% TFP after SLC 13 cultivation for 48 h. The EPS was dissolved at 0.625, 1.25, 2.5, 5, 10 and 20 mg/mL to determine the antioxidant capacity as follows:

**Reducing power:** The reducing power was determined according to the method described by Oyaizu *et al.* (1986) with modifications. First, 125 µL of EPS solutions of different concentrations were evenly mixed with 125 µL of 0.2 M potassium phosphate buffer solution (pH, 6.62) and 125 µL of 1% potassium ferricyanide, followed by incubation in a water bath at 50°C for 20 min. After snap-cooling, 125 µL of 10% trichloroacetic acid was added and the mixtures were centrifuged at 2,300 × *g* for 10 min. One hundred microliters of the supernatant was reacted in the dark for 10 min with 100 µL of de-ionized water and 20 µL of 0.1% $FeCl_3$. The absorbance at 700 nm wavelength was measured. Using vitamin C (0.625 mg/mL) as a positive control, the reducing power (%) was calculated with the following formula:

Reducing power (%) = ($OD_{700}$ value of sample/$OD_{700}$ value of positive control) × 100

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging capacity:** This assay was performed according to the method described by Shimada and others (1992). EPS solutions of different concentrations (160 µL) were mixed 40 µL of freshly prepared 1 mM DPPH solution in ethanol. After 45 min of reaction in the dark, the absorbance at 540 nm was measured. Using ethanol as a positive control, DPPH radical scavenging capacity (%) was calculated with the following formula:

DPPH radical scavenging capacity (%) = [1 – ($OD_{540}$ value of sample/$OD_{540}$ value of control group)] × 100

**ABTS free-radical scavenging capacity:** The assay was performed according to the method described by Roberta *et al.* (1999) with modifications. Twenty microliters of ABTS, 20 µL peroxidase, and 20 µL $H_2O_2$ were mixed with 120 µL deionized water and allowed to react in the dark for 60 min. Then, 20 µL of EPS solutions of different concentrations were added and allowed to react in the dark for 10 min, after which the absorbance at 734 nm was measured. Using deionized water as control, the ABTS free-radical scavenging capacity was calculated with the following formula:

ABTS radical scavenging capacity (%) = [1 – ($OD_{734}$ value of sample/$OD_{734}$ value of control group)] × 100

**Antibacterial-activity Assay**

The agar well diffusion method as described previously was applied to detect and determine the antibacterial activities of the SLC 13 strain (Touré *et al*., 2003). *Staphylococcus aureus* (BCRC 10908), *Bacillus cereus* (BCRC 12813), and *Listeria monocytogenes* Scott A (Briers and others, 2011) were used as gram-positive indicator strains, and *Escherichia coli* (BCRC 11778) and *Salmonella typhimurium* (BCRC 10747) served as gram-negative indicator strains. All indicator strains were incubated in Luria-Bertani (LB) broth overnight and then diluted to 7 $\log_{10}$ CFU/mL with LB broth. LB agar plates were prepared with five wells of 6 mm in diameter. One hundred microliters of the indicator strains in LB broth was spread on the surface of an LB agar plate, and 50 µL of an overnight LAB culture medium supplemented with 6% glucose, 2% OPFP, or 2% TFP was placed into the wells. The LB agar plates were incubated at 37°C for 24 h. The antibacterial activities of LAB strains were assessed by the diameter of the inhibition zones. The interpretation of antibacterial activities of these LAB strains was as follows: $<$10 mm, (–) no antibacterial activity; 10–15 mm, (+) weak antibacterial activity; $>$ 15, (++) strong antibacterial activity. The antibacterial activity of each strain was evaluated in three independent experiments.

**Statistical Analysis**

Data are expressed as the mean ± standard deviation (SD) of triplicate experiments. Significant differences ($p$ $<$0.05) between means were identified using Duncan's multiple-range test (DMR-Test).

## Results

**Effects of Supplementation of SLC 13 Culture Medium with Glucose, OPFP and TFP**

Previous studies have indicated that glucose is the best

carbon source for EPS production in LAB (Cerning *et al*., 1994) and that EPS yield in LAB is proportional to the glucose concentration (Walling *et al*., 2005). Another study suggested that MRS broth supplemented with different polysaccharides of mushroom wastes enhances the viability of LAB during cold storage (Chou *et al*., 2013). Therefore, this study further analyzed and compared the culture of SLC 13 in MRS broth supplemented with different concentrations of glucose, OPFP, and TFP to assess their EPS production- and viability-enhancing effects. We first established a procedure for OPFP production for potential further commercialization (Fig. 1). As shown in Table 1, the tested additives did not significantly affect the pH of the culture medium and the supplementation of 8% glucose, 2% OPFP and 2% TFP, maximized the EPS yield up to 2.2581 ± 0.3881, 2.4691 ± 0.0268 and 2.5582 ± 0.0873 g/L, respectively, while the viable counts were 8.9487 ± 0.0345, 7.6696 ± 0.0656, and 7.9558 ± 0.0746 $\log_{10}$ CFU/mL, respectively. Based on the amount of residual sugars, excessive glucose enabled a higher EPS yield and viable count in SLC 13, but the bacteria were unable to fully utilize the excessive glucose during the late stage of fermentation (Table 1). Supplementation with 6% or 8% glucose showed no significant difference; therefore, 6% glucose was used in further experiments. In contrast, OPFP not only was utilized by SLC 13, it also promoted glucose utilization. In addition, SLC 13 was capable of utilizing TFP as a raw material for EPS production and bacterial growth, but retained more glucose than when the broth was supplemented with OPFP (Table 1).

**Effects of SLC 13 Culture Medium Supplemented with OPFP Pretreated with Enzymes or Chemicals**

Chemical treatment using acid and alkali as well as enzymatic treatment can be applied for the pretreatment of fibers of agriculture waste, of which acid treatment is the most widely used (Kumar *et al*., 2009). This study compared the effects of 2% OPFP pretreated with acid, alkali, and enzymes on EPS production by and viability of SLC 13, to enhance the applicability of the fibers. The results of chemical treatments showed that pretreatment with 3 N HCl exhibited the greatest effect with an EPS yield of 2.6647 ± 0.0380 g/L and a bacterial count of 7.9098 ± 0.0491 $\log_{10}$ CFU/mL (Table 2). Notably, proper chemical treatment may digest OPFP to produce abundant oligosaccharides and monosaccharides for SLC 13. The amount of residual sugars revealed that acid treatment had the greatest effect, with 3 N HCl yielding the maximum amount of residual sugars of 9.6928 ± 0.0183 g/L. However, the overproduction of salts produced by neutralization possibly led to salt stress, thus inhibiting the growth of SLC 13, rendering the bacteria unable to utilize mono- and oligosaccharides at the late stage of fermentation (Table 2).

The results of enzymatic treatments of OPFP showed that treatment with 20 U xylanase, 20 U cellulase, and 10 U cellulase + 10 U xylanase all significantly improved the EPS yield (Table 3). This might be due to the production of large amounts of monosaccharides and oligosaccharides from the enzymatic hydrolysis of OPFP, which become available to SLC 13 for EPS production. Moreover, based on the amount of residual sugars, enzymatic treatment, especially with xylanase, promoted a more complete utilization of monosaccharides in the medium by SLC 13 to produce abundant EPS. Interestingly, the viable counts were not significantly improved in comparison with that of the untreated group.

**Antioxidant Capacity of EPS Produced by SLC 13 in Culture Medium Supplemented with glucose, OPFP and TFP**

The effects of the different substrates on the antioxidant capacity of EPS produced by SLC 13 were further analyzed. The results indicated that 6% glucose had the greatest positive effect on the reducing power of EPS at a concentration of 20 mg/mL, followed by no supplementation, 2% OPFP, and 2% TFP, with reducing powers of 121.1548 ± 2.1861%, 109.1203 ± 6.1522%, 101.1332 ± 4.1096%, and 98.4888 ± 2.2972%, respectively (Fig. 2A).

The greatest enhancing effect on the DPPH free-radical scavenging capacity of EPS at a concentration of 20 mg/mL was noted for 6% glucose, followed by 2% TFP, 2% OPFP, and no supplementation, with DPPH scavenging capacities (%) of 73.7676 ± 3.1502, 67.1828 ± 3.6326, 58.2132 ± 3.2622% and 52.2335 ± 1.8811, respectively (Fig. 2B). MRS broth without supplementation of any substrate showed a greater effect than MRS broth supplemented with 2% OPFP on the DPPH free radical scavenging capacity (%) of 10 mg/mL EPS.

In terms of the ABTS free-radical scavenging capacity (%) of EPS, 6% glucose exhibited the greatest enhancing effect at high concentration of EPS (20 mg/mL), followed by 2% OPFP, no supplementation, and 2% TFP, with ABTS scavenging capacities (%) of 85.1297 ± 0.7075, 82.4351 ± 2.4061, 76.2974 ± 6.2307 and 69.8104 ± 5.4724, respectively (as shown in Fig. 2C). Interestingly, MRS broth supplemented with 2% OPFP exhibited the greatest effect on EPS at low concentration (5 mg/mL).

**Antibacterial Activity of SLC 13 Culture Medium Supplemented with Glucose, OPFP and TFP**

In this study, we analyzed whether supplementation of the culture medium with 6% glucose, 2% OPFP and 2% TFP affects the antibacterial activity of SLC 13. Agar well diffusion testing showed that regardless of whether the MRS broth was supplemented or not with 6% glucose or 2% OPFP, the inhibition zone diameter was greater than 10 mm for all indicator strains (Table 4). Culture medium supplemented with 6% glucose and 2% OPFP exhibited the greatest antibacterial effect against *S. aureus* and *S.*

**Table 1:** EPS production, bacterial growth, residual sugar, and pH of *L. plantarum* SLC 13 culture medium with different supplements

| Characteristics | Supplements | Concentration (w/v) | | | | |
|---|---|---|---|---|---|---|
| EPS (g/L) | | 0% | 2.0% | 4.0% | 6.0% | 8.0% |
| | Glucose | $0.4322 \pm 0.0145^{cA}$ | $0.6922 \pm 0.0191^{cB}$ | $1.2569 \pm 0.2567^{bA}$ | $2.0302 \pm 0.1572^{aA}$ | $2.2581 \pm 0.3881^{aA}$ |
| | | 0% | 0.5% | 1.0% | 1.5% | 2.0% |
| | OPFP | $0.4340 \pm 0.0142^{cA}$ | $0.7262 \pm 0.0157^{dAB}$ | $1.5061 \pm 0.0820^{cA}$ | $2.0941 \pm 0.0172^{bA}$ | $2.4691 \pm 0.0268^{aA}$ |
| | TFP | $0.4372 \pm 0.0395^{cA}$ | $0.9651 \pm 0.1298^{dA}$ | $1.6100 \pm 0.1260^{cA}$ | $2.1184 \pm 0.0977^{bA}$ | $2.5582 \pm 0.0873^{aA}$ |
| Bacterial count | | 0% | 2.0% | 4.0% | 6.0% | 8.0% |
| ($\log_{10}$CFU/mL) | Glucose | $7.0570 \pm 0.0806^{cA}$ | $8.5177 \pm 0.0373^{bA}$ | $8.5267 \pm 0.0910^{bA}$ | $8.8821 \pm 0.0523^{aA}$ | $8.9487 \pm 0.0345^{aA}$ |
| | | 0% | 0.5% | 1.0% | 1.5% | 2.0% |
| | OPFP | $7.0211 \pm 0.0117^{dA}$ | $7.1364 \pm 0.0224^{cB}$ | $7.1577 \pm 0.0342^{cB}$ | $7.2516 \pm 0.0120^{bC}$ | $7.6696 \pm 0.0656^{aC}$ |
| | TFP | $6.9856 \pm 0.0444^{dA}$ | $7.1536 \pm 0.0194^{cB}$ | $7.2509 \pm 0.0585^{cB}$ | $7.6712 \pm 0.0393^{bB}$ | $7.9558 \pm 0.0746^{aB}$ |
| Residual sugars | | 0% | 2.0% | 4.0% | 6.0% | 8.0% |
| (g/L) | Glucose | $0.0450 \pm 0.0016^{cA}$ | $0.1567 \pm 0.0016^{dA}$ | $0.3412 \pm 0.0006^{cA}$ | $0.5285 \pm 0.0051^{bAB}$ | $0.7150 \pm 0.0062^{aB}$ |
| | | 0% | 0.5% | 1.0% | 1.5% | 2.0% |
| | OPFP | $0.0452 \pm 0.0091^{bA}$ | $0.1484 \pm 0.0274^{aA}$ | $0.1291 \pm 0.0365^{aA}$ | $0.1033 \pm 0.0183^{abB}$ | $0.0968 \pm 0.0274^{abC}$ |
| | TFP | $0.0426 \pm 0.0420^{cA}$ | $0.2065 \pm 0.2373^{bcA}$ | $0.4388 \pm 0.3833^{bcA}$ | $0.8841 \pm 0.3742^{abA}$ | $1.1551 \pm 0.0821^{aA}$ |
| pH | | 0% | 2.0% | 4.0% | 6.0% | 8.0% |
| | Glucose | $3.7250 \pm 0.0212^{cA}$ | $3.5750 \pm 0.0071^{cC}$ | $3.5550 \pm 0.0071^{bcC}$ | $3.5350 \pm 0.0071^{bC}$ | $3.5350 \pm 0.0071^{aC}$ |
| | | 0% | 0.5% | 1.0% | 1.5% | 2.0% |
| | OPFP | $3.7400 \pm 0.0141^{cA}$ | $3.7850 \pm 0.0071^{dB}$ | $3.7650 \pm 0.0071^{cB}$ | $3.7450 \pm 0.0071^{bB}$ | $3.7300 \pm 0.0000^{aB}$ |
| | TFP | $3.7450 \pm 0.0212^{cA}$ | $3.8300 \pm 0.0141^{aA}$ | $3.8550 \pm 0.0071^{abA}$ | $3.9200 \pm 0.0141^{bcA}$ | $3.9400 \pm 0.0141^{cA}$ |

Data are the mean of three replicates ± SD. Different letters indicating a column or a row are statistically significant differences at $p<0.05$; OPFP, orange peel fiber powder; TFP *T. flava* Chen fermented powder

**Table 2:** EPS production, bacterial growth, residual sugar, and pH of *L. plantarum* SLC 13 medium after incubation with OPFP pretreated with chemical reagents in MRS broth

| Characteristics | Chemically treated OPFP* | | | | |
|---|---|---|---|---|---|
| | Control | Acid treatment | | Alkali treatment | |
| | | 1 N HCl | 3 N HCl | 1 N NaOH | 3 N NaOH |
| EPS (g/L) | $2.4691 \pm 0.0268^{b}$ | $1.2794 \pm 0.1193^{d}$ | $2.6647 \pm 0.0380^{a}$ | $1.6596 \pm 0.0007^{c}$ | $2.3151 \pm 0.0910^{b}$ |
| Bacterial count ($\log_{10}$CFU/mL) | $7.6696 \pm 0.0656^{b}$ | $6.9494 \pm 0.0069^{e}$ | $7.9098 \pm 0.0491^{a}$ | $7.1072 \pm 0.0096^{d}$ | $7.2731 \pm 0.0425^{c}$ |
| Residual sugars (g/L) | $0.0968 \pm 0.0274^{cd}$ | $0.2259 \pm 0.0639^{b}$ | $9.6928 \pm 0.0183^{a}$ | $0.0516 \pm 0.0548^{d}$ | $0.1871 \pm 0.0456^{bc}$ |
| pH | $3.7300 \pm 0.0000^{a}$ | $3.5950 \pm 0.0071^{d}$ | $3.6800 \pm 0.0141^{b}$ | $3.6350 \pm 0.0071^{c}$ | $3.5900 \pm 0.0000^{d}$ |

*The MRS broth was supplemented with 2% chemically treated OPFP; *Data are the mean of three replicates ± SD. Different letters indicate statistically significant differences at $p < 0.05$; OPFP, orange peel fiber powder.

*typhimurium*, respectively. Surprisingly, supplementation of the MRS broth with 2% TFP reduced the inhibition zones of *L. monocytogenes* and *B. cereus* below 10 mm.

## Discussion

In this study, we evaluated the effect of orange peel fiber powder (OPFP) in comparison to that of *T. flava* Chen fermented powder (TFP) on *L. plantarum* SLC 13 exopolysaccharide (EPS) production. The results showed that the supplementation of 8% glucose, 2% OPFP and 2% TFP, maximized the EPS of SLC 13 (Table 1). These results suggested that OPFP and TFP are potential prebiotics that can replace glucose to increase the EPS yield and bacterial growth of SLC 13. However, as an agricultural waste that can be added into the culture medium after a simple processing procedure, OPFP is the more economical candidate for industrial application.

The stress tolerance and antioxidant activity of LAB isolated from tropically grown fruits and leaves are highly variable (Fessard *et al*., 2016). Our results showed that, although chemical treatments are relatively more simple and economic than enzymatic treatments, in addition to monosaccharides, large amounts of salts are produced in the neutralization after chemical treatments, which might inhibit bacterial growth (Table 2 and 3). A previous study showed that *L. casei* Z3-1, especially with the addition of cellulase, could improve the fermentation quality of wheat straw silage as compared to cellulase-only-treated silages (Ni *et al*., 2014). Hence, enzymatic treatment would be the method of choice for OPFP treatment, to obtain fermented beverages of SLC 13 with higher viscosity.

The antioxidant activity of a polysaccharide depends on its combined structural characteristics, including the configuration of the glycosidic bond, molecular weight, and monosaccharide content (Zheng *et al*., 2014). In this study, 6% glucose exhibited the greatest enhancing effect on the antioxidant capacity of EPS. In addition, 2% OPFP increased the DPPH and ABTS scavenging capacities of EPS produced by SLC 13 (Fig. 2). Whether these additives affect EPS synthesis/structure in the bacterial cells, thereby influencing its antioxidant capacity, remains to be further clarified.

Previous studies have shown that LAB inhibit

**Table 3:** EPS production, bacterial growth, residual sugar, and pH value of *L. plantarum* SLC 13 incubated with OPFP pretreated with different enzymes in MRS broth

| Characteristics | Enzyme-treated OPFP* | | | |
|---|---|---|---|---|
| | Control | Xylanase (20 U) | Cellulase (20 U) | Xylanase (10 U) + cellulase (10 U) |
| EPS (g/L) | 2.4691 ± 0.0268[b] | 3.1488 ± 0.0410[a] | 3.1467 ± 0.0574[a] | 3.2169 ± 0.0895[a] |
| Bacterial count ($log_{10}$CFU/mL) | 7.6696 ± 0.0656[a] | 7.5341 ± 0.0806[ab] | 7.4265 ± 0.0917[b] | 7.6433 ± 0.0140[a] |
| Residual sugars (g/L) | 0.0968 ± 0.0274[a] | 0.0129 ± 0.0183[a] | 0.0839 ± 0.0821[a] | 0.0129 ± 0.0183[a] |
| pH | 3.7300 ± 0.0000[a] | 3.7900 ± 0.1980[a] | 3.7150 ± 0.1626[a] | 3.6900 ± 0.1273[a] |

*The MRS broth was supplemented with 2% OPFP pretreated with different enzymes.
Data are the mean of three replicates ± SD. Different letters indicate statistically significant differences at $p < 0.05$.
OPFP, orange peel fiber powder

**Table 4:** Antibacterial activity of *L. plantarum* SLC 13 incubated in culture medium supplemented with different substrates

| Bacterial strains characteristics | MRS broth supplemented with | | | |
|---|---|---|---|---|
| | - | 6% glucose | 2% OPFP | 2% TFP |
| Pathogenic bacteria* | | | | |
| Gram-positive | | | | |
| *Staphylococcus aureus* (BCRC 11863) | + | ++ | + | + |
| *Listeria monocytogenes* (BCRC 14848) | + | + | + | - |
| *Bacillus cereus* (BCRC 12813) | + | + | + | - |
| Gram-negative | | | | |
| *Salmonella typhimurium* (BCRC 10746) | + | + | ++ | + |
| *Escherichia coli* (BCRC 11778) | + | + | + | + |

*Inhibition level was determined by the diameter of the inhibition zone: (-) <10 mm; (+) 10–15 mm; (++) > 15 mm
OPFP, orange peel fiber powder; TFP, *T. flava* Chen fermented powder

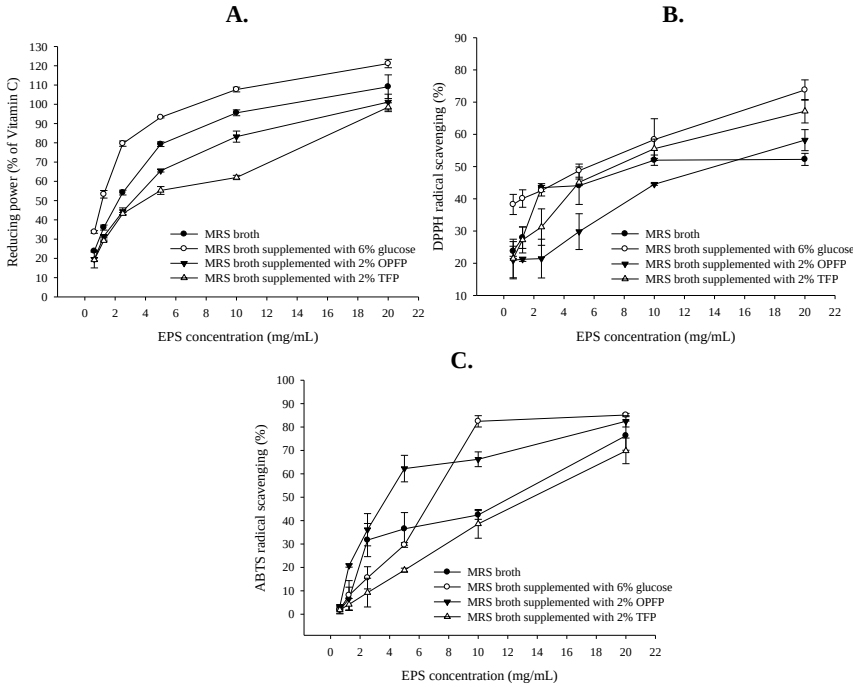


**Fig. 2:** Antioxidant capacity of EPS produced by SLC 13 incubated in MRS broth with different substrates. (A) Reducing power (% of vitamin C); (B) DPPH radical scavenging (%); (C) ABTS radical scavenging (%). OPFP, orange peel fiber powder; TFP *T. flava* Chen fermented powder. Each value is expressed as the mean ± SD (n = 3)

pathogens through several mechanisms. For instance, Zheng *et al*. (2016) reported that *L. pentosus* strain LPS16 produces lactic acid to inhibit multidrug-resistant *Helicobacter pylori*. In addition, the growth of pathogenic bacteria has been demonstrated to be inhibited by bacteriocins, such as plantaricins NC8, 35d, W, A, and C, and plantacin B, produced by *L. plantarum* (Hauge *et al*., 1999; Moll *et al*., 1999; Messi *et al*., 2001; Maldonado *et*

*al*., 2003). Maldonado *et al*. (2004) reported that the presence of specific bacteria could act as an environmental signal to switch on bacteriocin production in *L. plantarum* NC8 via a quorum-sensing mechanism mediated by PLNC8IF. However, the mechanism/molecules through which SLC 13 can inhibit the growth of pathogens needs to be clarified to develop alternative therapies in future. Bochmann *et al*. (2015) reported that the production of class I lantibiotics (subtilin-like lantibiotics) by *B. subtilis* is strongly influenced by glucose. Whether supplementation with different substrates affects the production of antibacterial molecules by SLC 13 is worth further investigation.

## Conclusion

The OPFP with potential as a prebiotic to improve the EPS yield and viability of *L. plantarum* SLC 13 and partially increased the EPS antioxidant capacity and antibacterial activities of the culture medium. Although the supplementation with TFP tended to have similar effects, OPFP has higher economic value for industrial application. The development of orange peel fibers and *T. flava* Chen as additives is expected to improve the functionality of food products such as yogurt and soy milk, thereby enhancing their health benefits.

## Acknowledgments

This study was supported by grant 99-AS-3.1.3-FD-Z1(2) from the Council of Agriculture, Executive Yuan, Taiwan.

## References

Bochmann, S.M., T. Spiess, P. Kotter and K.D. Entian, 2015. Synthesis and succinylation of subtilin-like lantibiotics are strongly influenced by glucose and transition state regulator AbrB. *Appl. Environ. Microbiol*. 81: 614–622

Cerning, J., C.M. Renard, J.F. Thibault, C. Bouillanne, M. Landon, M. Desmazeaud and L. Topisirovic, 1994. Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. *Appl. Environ. Microbiol*. 60: 3914–3919

Chen, C.J., 1998. Morphological and molecular studies in the genus *Tremella*. (Bibliotheca Mycologica, Band 174). Stuttgart: J. Cramer

Chen, T., I.C. Sheih, H.Y. Jeng and T.J. Fang, 2014. Anti-inflammation and anti-infection applicability of *Tremella flava* Chen fermented soymilk (TFS) in a BALB/c mice model. *2014 2nd International Conference on Food and Agricultural Sciences IPCBEE* Vol. 77. IACSIT Press, Singapore DOI: 10.7763/IPCBEE

Chou, W.T., I.C. Sheih and T.J. Fang, 2013. The applications of polysaccharides from various mushroom wastes as prebiotics in different systems. *J. Food. Sci*., 78: M1041–M1048

Cohn, R. and A.L. Cohn, 1997. Subproductos del procesado de las frutas. *In*: *Procesado De Frutas*, pp: 213–228. Arthey, D. and P.R. Ashurst (eds.). Acribia, Zaragoza

Deepak, V., S. Ramachandran, R.M. Balahmar, S.R. Pandian, S.D. Sivasubramaniam, H. Nellaiah and H. Sundar, 2016. *In vitro* evaluation of anticancer properties of exopolysaccharides from *Lactobacillus acidophilus* in colon cancer cell lines. *In Vitro Cell. Dev. Biol. Anim*. 52: 163–173

Fessard, A., E. Bourdon, B. Payet and F. Remize, 2016. Identification, stress tolerance, and antioxidant activity of lactic acid bacteria isolated from tropically grown fruits and leaves. *Can. J. Microbiol*. 62: 550–561

Hauge, H.H., D. Mantzilas, V.G. Eijsink and J. Nissen-Meyer, 1999. Membrane-mimicking entities induce structuring of the two-peptide bacteriocins plantaricin E/F and plantaricin J/K. *J. Bacteriol*., 181: 740-747

Hassan, A.N., J.F. Frank, K.A. Schmidt and S.I. Shalabi, 1996. Textural properties of yogurt made with encapsulated nonropy lactic cultures. *J. Dairy. Sci*. 79: 2098–2103

Izquierdo, L. and J.M. Sendra, 2003. Citrus fruits: composition and characterization. *In*: *Encyclopedia of Food Sciences and Nutrition*. Caballero, B., L. Trugo and P. Finglas, (eds.). Academic Press, Elsevier Science Ltd., Oxford

Kim, H.O., J.M. Lim, J.H. Joo, S.W. Kim, H.J. Hwang, J.W. Choi and J.W. Yun, 2005. Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybe cylindracea*. *Biores. Technol*., 96: 1175–1182

Kumar, P., D.M. Barrett, M.J. Delwiche and P. Stroeve, 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind. Eng. Chem. Res*., 48: 3713–3729

Li, S., R. Huang, N.P. Shah, X. Tao, Y. Xiong and H. Wei, 2014. Antioxidant and antibacterial activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315. *J. Dairy. Sci*., 97: 7334–7343

Lin, T.Y. Chang and M.F. Chien, 2007. Exopolysaccharides production as affected by lactic acid bacteria and fermentation time. *Food. Chem*. 100: 1419–1423

Liu, Y., M. Dong, Z. Yang and S. Pan, 2016. Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway. *Int. J. Biol. Macromol*. 89: 484–488

Maldonado, A., J.L. Ruiz-Barba and R. Jimenez-Diaz, 2003. Purification and genetic characterization of plantaricin NC8, a novel coculture-inducible two-peptide bacteriocin from *Lactobacillus plantarum* NC8. *Appl. Environ. Microbiol*., 69: 383–389

Maldonado, A., R. Jimenez-Diaz and J.L. Ruiz-Barba, 2004. Induction of plantaricin production in *Lactobacillus plantarum* NC8 after coculture with specific gram-positive bacteria is mediated by an autoinduction mechanism. *J. Bacteriol*., 186: 1556–1564

Malla, B.A., A. Rastogi, R.K. Sharma, A. Ishfaq and A.J. Farooq, 2015. Kinnow madarin (*Citrus nobilis* lour × *Citrus deliciosa* tenora) fruit waste silage as potential feed for small ruminants. *Vet. World,* 8: 19–23

Masson, J., E. Liberto, J.C. Beolor, H. Brevard, C. Bicchi and P. Rubiolo, 2016. Oxygenated heterocyclic compounds to differentiate Citrus spp. essential oils through metabolomic strategies. *Food. Chem*., 206: 223–233

Messi, P., M. Bondi, C. Sabia, R. Battini and G. Manicardi, 2001. Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a *Lactobacillus plantarum* strain. *Int. J. Food Microbiol*., 64: 193–198

Moll, G.N., W.N. Konings and A.J. Driessen, 1999. Bacteriocins: mechanism of membrane insertion and pore formation. Antonie. Van. *Leeuwenhoek,* 76: 185–198

Miller, G.L., 1959. Use of dinitrosalicyclic acid regent for determination of reducing sugar. *Anal. Chem*., 31: 426–428

Ni, K., Y. Wang, H. Pang and Y. Cai, 2014. Effect of cellulase and lactic acid bacteria on fermentation quality and chemical composition of wheat straw silage. *Am. J. Plant Sci*., 5: 1877–1884

Oyaizu, M., 1986. Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. *Jpn. J. Nutr. Diet*., 44: 307–315

Roberta, R.E., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and R.E. Catherine, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*., 26: 1231–1237

Roller, S. and I.C.M. Dea, 1992. Biotechnology in the production and modification of biopolymers for foods. *Crit. Rev. Biotechnol*., 121: 261–277

Roy, D., S. Sirois and D. Vincent, 2001. Molecular discrimination of lactobacilli used as starter and probiotic cultures by amplified ribosomal DNA restriction analysis. *Curr. Microbiol*., 42: 282–289

Sendra, E., P. Fayos, Y. Lario, J. Fernández-López, E. Sayas-Barberá and J.A. Pérez-Alvarez, 2008. Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiol*., 25: 13–21

Shimada, K., K. Fujikwa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.,* 40: 945–948.

Torino, M.I., M.P. Taranto, F. Sesma and G.F. de Valdez, 2001. Heterofermentative pattern and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in response to environmental pH. *J. Appl. Microbiol*., 91: 846–852

Touré, R., E. Kheadr, C. Lacroix, O. Moroni and I. Fliss, 2003. Production of antibacterial substances by bifidobacterial isolates from infant stool active against *Listeria monocytogenes*. *J. Appl. Microbiol*., 95: 1058–1069

Walling, E., M. Dols-Lafargue and A. Lonvaud-Funel, 2005. Glucose fermentation kinetics and exopolysaccharide production by ropy *Pediococcus damnosu*s IOEB8801. *Food Microbiol*., 22: 71–78

Zheng, J.Q., J.Z. Wang, C.W. Shi, D.B. Mao, P.X. He and C.P. Xu, 2014. Characterization and antioxidant activity for exopolysaccharide from submerged culture of *Boletus aereus*. *Process Biochem*., 49: 1047–1053

Zheng, P.X., H.Y. Fang, H.B. Yang, N.Y. Tien, M.C. Wang and J.J. Wu, 2016. *Lactobacillus pentosus* strain LPS16 produces lactic acid, inhibiting multidrug-resistant *Helicobacter pylori*. *J. Microbiol. Immunol. Infect*., 49: 168–174

**(Received 09 december 2016; Accepted 18 February 2017)**