AsPac J. Mol. Biol. Biotechnol. 2023
Vol. 31 (1) : 14-25

Production and functional characteristics of exopolysaccharide by *Lactobacillus plantarum* co-cultivation with *Saccharomyces cerevisiae*

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Received 21st June 2022 / Accepted 17th December 2022

Abstract. Exopolysaccharides (EPS) are well-known biopolymers secreted by several lactic acid bacteria with combination of various strains. The aim of this study is to increase EPS production by co-culturing *Lactobacillus plantarum* ATCC 8014 with *Saccharomyces cerevisiae* ICA-Y01 and study the changes in the functional characteristics of the EPS from both cultivations. In this study, the production and functional characteristics of EPS from co-cultivation culture of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 were evaluated. The co-cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01was markedly increased EPS production up to 55.84% with 6.8 g/l yield after 20 hours cultivation. The pH of the co-cultivation culture was remained constantly at 5.2 until the end of cultivation. Furthermore, co-cultivation under pH 6 in the 16L bioreactor showed a higher growth rate of 0.214 h^{-1} and EPS production increased up to 104.44% when compared with single cultivation of *L. plantarum* ATCC 8014. This result clearly indicates the importance of growing the cells in the controlled pH condition when cultivated with *S. cerevisiae* ICA-Y01 to enhance EPS production. The functional characteristics of EPS secreted from both cultivation strategies were also evaluated. FT-IR spectroscopy confirmed EPS presence from both cultivations, indicating functional group of the polysaccharide with D-glucose units bound by α-(1→6). The EPS production from single cultivation showed a higher DPPH radical scavenging activity (88.21%) and IC_{50} (19.57%) as compared to EPS produced from co-cultivation with DPPH scavenging exhibited 32.45% with no IC_{50} value detected. Furthermore, solubility and water uptake of EPS from single cultivation are higher in comparison to co-cultivation. In conclusion, higher efficiency in the bioactivity of EPS from the single cultivation of *L. plantarum* ATCC 8014 was confirmed even though the EPS yield is low as compared to EPS synthesis through inter-kingdom cultivation.

Keywords: *Lactobacillus plantarum, Saccharomyces cerevisiae,* exopolysaccharide, functional characteristics, co-cultivation, stirred tank bioreactor

INTRODUCTION

The human microbiota, especially in our gastrointestinal system has a variety of enzymes, which can convert various food sources into compounds that are beneficial to human health. Exopolysaccharides (EPS) are well-known biopolymers secreted by several lactic acid bacteria (LAB) into a culture medium (Dilna *et al.,* 2015; Panthavee *et al.,* 2017). EPS was valuable in the food industry as its water-soluble characteristics and high viscosity properties enables it to be a good emulsion agent and thickener (Patel *et al.,* 2010). Co-cultivation of

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different bacterial species was known to influence the productivity of metabolite production including the secretion of EPS (Dailin *et al.,* 2016; Bertsch *et al.,* 2019). *Saccharomyces cerevisiae* is a well-known strain that can coexist with *Lactobacillus* sp. in the same ecosystem (Maligoy *et al.,* 2008; Prado *et al.,* 2015; Jin *et al.,* 2019) and has been reported to increase the growth factors in the cultivation medium such as organic acids, amino acids, vitamins, terpenes, and pyruvate which will improve the growth performance of the cultured strain or even enhance the flavour profile including the texture of fermented foods (Gadaga *et al.,* 2001; Jin *et al.,* 2019). Since the secretion of lactic acid will affect the growth of LAB during fermentation, it often experiences growth deterioration that will directly affect the production of other metabolites such as EPS (Abedi & Hashemi, 2020). Accumulation and increase of organic acid including lactic acid in the cultivation broth as a by-product of LAB itself will affect the cell growth when lactic acid absorbs into the cell cytoplasmic region through unbroken membrane cells resulting in cytoplasmic acidification and proton motility failure (Othman *et al.,* 2017). A good bioprocess strategy should be designed for high cell mass cultivation of *Lactobacillus* sp. concomitantly with the secretion of EPS in order to maintain the transmembrane pH gradient by the secretion of lactic acid to control the cellular function of *Lactobacillus* sp. (Othman *et al.,* 2018). The differences of functional characteristics of EPS from co-cultivation culture solely depend on for EPS-producing microorganisms (Trabelsi *et al.,* 2015; Rejiniemon *et al.,* 2015; Wang *et al.,* 2017). This was also supported by studies by Cerning *et al.* (1994) in which the composition of carbohydrate monomer in EPS was influenced by the types of carbon source and the growth stage of *Lactobacillus* sp. cultivation. However, the production of EPS under different types of carbon source was differed for different strains when referring to *Lactobacillus plantarum* 70810 where it was a homopolysaccharide of galactose when the cultivation broth was consisting of lactose (20 g/l) and mannose (10 g/l) (Wang *et al.,* 2014).

Furthermore, it was known that *Lactobacillus plantarum* ATCC 8014 RJF4 produced EPS composed of mannose and glucose residues when lactose was supplemented as the only carbon source in the cultivation medium (Dilna *et al.,* 2015). The aim of this study is to increase the EPS production by co-culturing *L. plantarum* ATCC 8014 with *S. cerevisiae*. Different growth conditions of batch cultivation and bioreactor cultivation were applied to investigate on the cell growth and production of EPS. Furthermore, the changes of the functional characteristics of the EPS from both cultivations were evaluated with the view of utilizing them as a promising biomaterial candidate in the development of enriched health-promoting foods.

MATERIALS AND METHODS

Microorganisms

Lactobacillus plantarum ATCC 8014, was cultivated in Man Rogosa Sharpe's (MRS) medium (Merck, Darmstadt, Germany) containing of (g/l): peptone, 10.0; meat extract, 5.0; yeast extract, 5.0; D(+)-glucose, 20; K_2HPO_4, 2; $C_6H_{14}N_2O_7$, 2; $C_2H_3NaO_2$, 5; $MgSO_4$, 0.1; $MnSO_4$, 0.05; agar, 12 and incubated at 30°C for 48 hours. While *S. cerevisiae* ICA-Y01 was incubated for 3-5 days at 26°C on potato dextrose agar, PDA (Merck, Darmstadt, Germany) with the following compositions (g/l); dextrose 20; potatoes infusion 4 and agar 20. For both strains, the cells were harvested at the early stationary phase cells and stored in glycerol solution (50% w/v) at -80°C prior to further use.

Single and co-cultivation of L. plantarum ATCC 8014 in the shake flask and bioreactor

Single cultivation of *L. plantarum* ATCC 8014 was carried out in a 250 ml Erlenmeyer flask containing 50 ml mixtures of 40 g/l molasses, 16.8 g/l yeast extract, 2.72 g/l KH_2PO_4 and 3.98 g/l $C_2H_3NaO_2$ (at pH 6.0) and incubated at 150 rpm, 30°C for 24 hours (Othman *et al.,* 2018). All cultivations were inoculated with *L. plantarum* ATCC 8014 as an active inoculum at the size of 10% (v/v). Similar cultivation medium and growth conditions were used for the co-cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01, with each strain at the size of 10% (v/v). Both strains were also cultivated in stirred tank bioreactor 16 L (Bio Engineering, Wald, Switzerland) with the following conditions;

a 24 hours-grown starter culture was inoculated into the 16 L bioreactor containing *in-situ* sterilized 8 L of medium containing (g/l) of molasses,40; yeast extract, 16.8; KH_2PO_4, 2.72 and NaOAc, 3.98 at pH 6.0 (adjusted by 1M HCl or 1M NaOH) and incubated for 72 hours at 30°C and 200 rpm at the beginning of cultivation.

Aeration was supplied at a rate of 1 v/v/min throughout cultivation to control the dissolved oxygen (DO) concentration at 30% of saturation by controlling agitation speed and air flow rate through in situ monitoring system by the polarographic electrode (Ingold, Mettler-Toledo, Urdorf, Switzerland). Antifoam-A concentrate (Sigma-Aldrich), as the antifoaming agent was used to control the development of foaming. At regular time intervals, the total cell mass analysis was carried out by transferring a small volume of samples into a 50 ml falcon tube followed by centrifugation at 6339 × g for 15 minutes. The cell pellets were washed using small volumes of sterilized deionized water prior to drying at 65°C until constant weight. The resulting supernatant was used for subsequent EPS extraction as follows; supernatant was mixed with cold absolute ethanol (95%) in the ratio of 1:3. Then, the sample was left for 24 hours at 4°C followed by centrifugation at 6339 × g for 15 minutes. The crude EPS sediment was dried to a constant weight in a vacuum oven at 35–40°C. The EPS yield was measured. After weighing, the dried EPS was evaluated for functional characterization (Seesuriyachan *et al.,* 2012; Imran *et al.,* 2016). All experiments were performed in triplicates.

Physical and biochemical characterization of EPS

Monosaccharide composition analysis

The monosaccharide composition was analyzed using High-Performance Liquid Chromatography, HPLC (Agilent, USA) equipped with Hi-Plex H column (8µm, 7.7 x 300 mm, Agilent, USA) with evaporative light scattered detector (ELSD) used to identify monosaccharide composition in EPS. Dried EPS (20 mg) was hydrolyzed using 20 ml of 1M H_2SO_4 at 100°C for 2 hours in a thermostat bath followed by neutralization with 1N NaOH. Elevated temperature pure water acted as an isocratic mobile phase in the column at a flow rate of 0.6 ml/min for 15 minutes. The column temperature was set and controlled at 70°C. The monosaccharide composition was determined against various standards namely glucose, galactose, ribose, and mannose (HPLC grade, purity > 99%).

Fourier transform infrared spectroscopy analysis (FTIR)

The EPS sample was also determined for major functional groups using a Fourier Transform Infrared Spectrophotometer, FT-IR (Nicolet™ iS50 FTIR, US). The dried EPS sample was prepared in the form of a pellet on a disc using a hydraulic press at a pressure of 1 MPa. The IR spectrum of a sample was processed by passing a beam of infrared light in the frequency range of 4000–400 cm^{-1} for detailed structural analysis.

Water solubility index (WSI) and water-holding capacity (WHC) of EPS

The water solubility index (WSI) and water-holding capacity (WHC) of EPS were determined by dissolving approximately 0.2 g of dried EPS in 5 ml of deionized water and agitating for 30 minutes in a water bath at 40°C (Saravanan & Shetty, 2016). The solution was then centrifuged at 5000 × g for 10 minutes and dried at 105°C for 4 hours to obtain a soluble solid weight for evaluation of WSI. The WSI was determined using the following correlation (1);

WSI (%) = (weight of dry soluble solids in supernatant)/ weight of dry sample × 100 (*1*)

A similar procedure was repeated for the determination of WHC where cell suspension of dried EPS was centrifuged at 16,000 × g for 30 min and filtered through pre-weighed filter paper. The WHC was determined as follows (Saravanan & Shetty, 2016);

WHC (%) = total sample weight after water absorption/ total dry sample weight × 100 (*2*)

Scanning electron microscopy analysis of EPS

The microstructure and surface morphology of the dried EPS were analyzed using scanning electron microscopy (SEM: Quanta 250 FEG (FEI Company, Hillsboro, OR, EUA). The dried

EPS sample was mounted on an aluminum stub and gold-sputtered. Then, SEM analysis of the samples by maintaining an accelerated voltage of 10 keV. Micrographs were recorded at suitable magnification to ensure clear images.

Antioxidant activity

The biochemical properties of the dried EPS were assessed for its antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Approximately, 500 mg of dried EPS was grounded to fine powder form and dissolved in 20 ml of deionized water at 26°C for 20 min. It was then filtered prior to use. The DPPH reagent was prepared by dissolving approximately 8 mg of DPPH reagent in 100 ml of MeOH (80 % v/v) to obtain a solution concentration of 80 µl/ml. The samples (100 µl) were pipetted into a 96-well microplate followed by the addition of 100 µl of DPPH reagent. The mixtures were then mixed well and incubated at 26°C for 30 min. Absorbance at 517 nm was recorded using 96 well plate reader (Brand Molecular Device) and methanol was used as the control sample (Farinazzo *et al.,* 2019; Sagar & Singh, 2011). The DPPH radical scavenging activity was determined using the following formula (3):

$$\text{Radical scavenging (\%)} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test compound}}{\text{[Absorbance of control]}}\right] \text{x} 100 \quad (3)$$

The IC_{50} DPPH values were achieving through linear regression of dose-response curve plotting between different concentrations of EPS against DPPH free radical scavenging activity and then was determined based on the IC_{50} value. The stronger the antioxidant activity showed by lower IC_{50} value.

RESULTS AND DISCUSSION

Shake flask of single cultivation of L. plantarum ATCC 8014 and co-cultivation with S. cerevisiae ICA-Y01

The growth curve of the single cultivation of *L. plantarum* ATCC 8014 and co-cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 is shown in Figure 1. The cultivation of *L. plantarum* ATCC 8014 only gave a yield of cell mass with 4.44 g/l and 4.47 ± 0.25 g/l of EPS for 44 hours of cultivation (Othman et al., 2018). When co-cultured with *S. cerevisiae* ICA-Y01, the total cell mass starts to increase during the first 4 hours of cultivation. After 4 hours of cultivation, total cell mass was observed to increase proportionally with the production of EPS in the cultivation medium (Figure 1) with a specific growth rate recorded of 0.645 h^{-1} which gave the total cell mass about 12.78 ± 2.11 g/l at the growth rate of 0.63 ± 0.23 g/l h.

At this growth rate, the maximal volumetric EPS production increased up to 55.84% with 6.81 ± 0.71 g/l yield after 20 hours of cultivation. The specific EPS production of 0.526 ± 0.01 g EPS /cell was obtained in the co-cultivation as compared to the more production secreted by single cultivation with 1.19 ± 0.01 EPS g/cell. After 10 hours of cultivation, the pH of the cultivation broth drastically dropped from pH 6.0 to 5.2 and maintained pH throughout the end of cultivation.

The cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 can regulate the pH of the cultivation medium to not lower than 5.2 until the end of cultivation (Figure 1). These observations prove that the production of high volumetric EPS production is mainly due to the performance of *L.plantarum* ATCC 8014 to produce EPS under acceptable pH conditions. *L. plantarum* ATCC 8014 was reported to produce low yields of EPS due to the secretion of lactic acid during its growth. In this study, it is clearly observed single and co-cultivation of *L. plantarum* ATCC 8014 showed pH reduction from 6.0 to 4.2 (Othman *et al.,* 2018) and 5.2, respectively. For co-cultivation, cell biomass and EPS production were increased up to 190.04% and 55.00% respectively, which was mainly due to a higher specific growth rate (0.645 h^{-1}) as compared to single culture cultivation (0.132 h^{-1}). Another plausible reason for the low production of EPS by single culture of *L. plantarum* ATCC 8014, was the drop of growth pH from 6.0 to 4.2, which is not within the pH range for the production of EPS by *Lactobacillus* (Aslim *et al.,* 2005) because the secretion of an organic acid such as lactic acid during growth of *Lactobacillus* sp. may result in a low cell mass and EPS yield. Therefore, the cultivation of lactic acid bacteria with *S. cerevesiae*

ICA-Y01 is essential to prevent the accumulation and side effects of lactic acid on the growth performance of lactic acid bacteria when producing EPS under controlled pH conditions (Bertsch *et al.,* 2019).

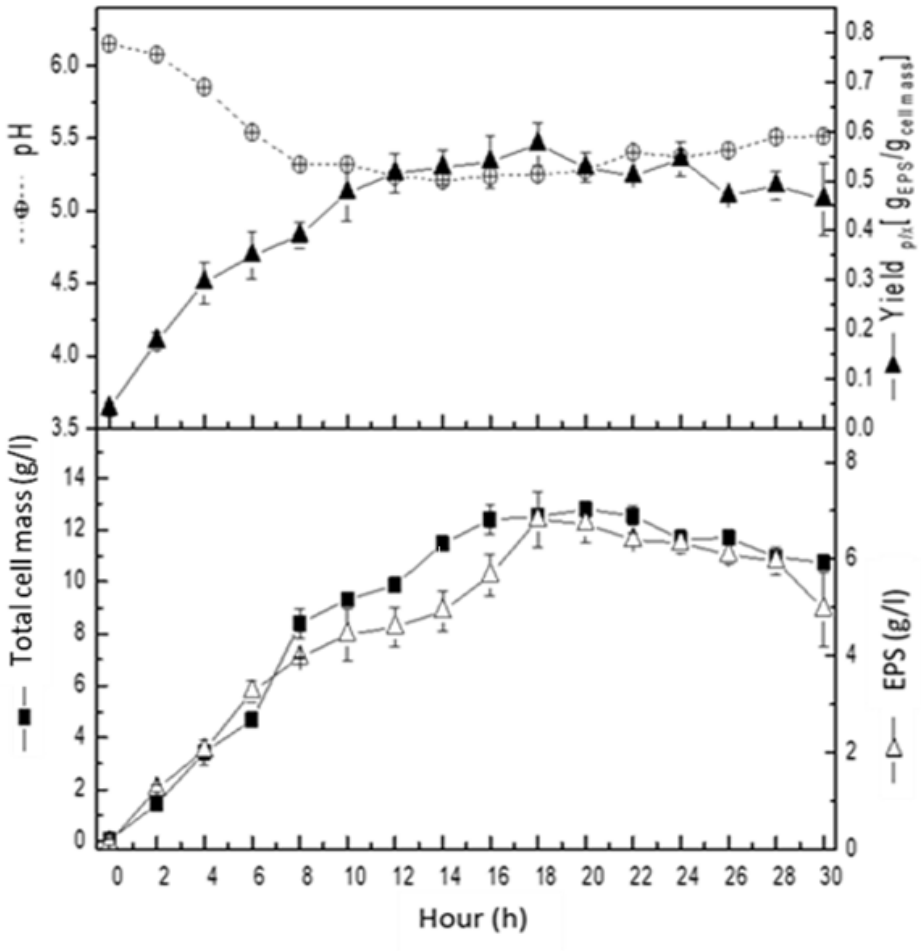

Figure 1. Total cell mass, EPS production and changes of pH for co-culture cultivation of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01 in shake flask level at initial pH of 6.0

Single cultivation of L. plantarum ATCC 8014 and co-cultivation with S. cerevisiae ICA-Y01 under bioreactor condition

Figure 2 showed the growth curves for cell growth, pH, EPS production, and specific EPS production during the batch cultivation of *L. plantarum* ATCC 8014 at pH 6.0, and the oxygen saturation level in the bioreactor was controlled at 30%. The cells showed exponential growth at growth rates of 0.142 ± 0.01 g/l/h after an initial 5 hours of lag phase. After 36 hours of cultivation, a maximal cell mass of 5.13 ± 0.11 g/l was reached with EPS production of 6.73 ± 0.27 g/l and specific EPS production, $Y_{p/x}$ of 1.35 ± 0.01 g EPS/cell. When grown together with *S. cerevisiae* ICA-Y01 using similar cultivation condition under uncontrolled pH was observed to gradually decrease to pH 5.5 which directly indicate the production of lactic acid (Figure 3A). A slightly higher total cell mass of 5.52 ± 0.33 g/l was achieved after 30 hours of incubation (oxygen saturation at 30%) at a growth rate of about 0.198 ± 0.01 g/l/h. In this condition, the EPS production was at the rate of 0.121 ±0.01 g/l/h and extended its maximal value up to 6.75 ± 0.312 g/l after 28 hours. Finally, *L. plantarum* ATCC 8014 stopped producing EPS after 50 hours of cultivation with a specific EPS production of only 1.465 ± 0.01 g EPS/cell.

Figure 3B showed results obtained from the co-cultivation of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01 under controlled pH of 6.0. A

much higher total cell mass of about 14.38 ± 0.23 g/l at a growth rate of 0.211 g/l/h was obtained. This value is around 150% higher compared to the total cell mass obtained when grown in the uncontrolled pH environment. From Figure 3B, a linear correlation between the production of EPS and cells' exponential growth can be observed. Maximal EPS production in the controlled pH cultivation reached its maximal value of 13.8 ± 1.91 g/l (around 104% higher than when grown in uncontrolled pH after 60 hours at an EPS production rate of 0.23 ± 0.01 g/l/h.

A scalability study carried out for single cultivation in the bioreactor 16L under controlled pH showed exponential cell growth concomitantly with the EPS production with a low specific growth rate of 0.140 h^{-1} and low EPS yield of only 6.73 ± 0.303 g/l. However, under similar growth conditions for co-cultivation showing a higher growth rate of 0.214 h^{-1}, total cell mass (151.61 %) and EPS production (104.44%) were obtained. This clearly indicates the importance of growing the cells in the controlled pH conditions rather than uncontrolled pH, as growth rates were reduced to approximately 0.158 h^{-1}. Production of organic acid in the uncontrolled pH condition was primarily responsible for the cell growth reduction and EPS production. Several studies mentioned that the importance of optimization in the bioprocessing strategies to maximize the secretion of essential metabolites including EPS although favorable condition was granted under co-cultivation of *Lactobacillus sp*. with yeast (Saleski *et al.,* 2017; Khan *et al.,* 2020).

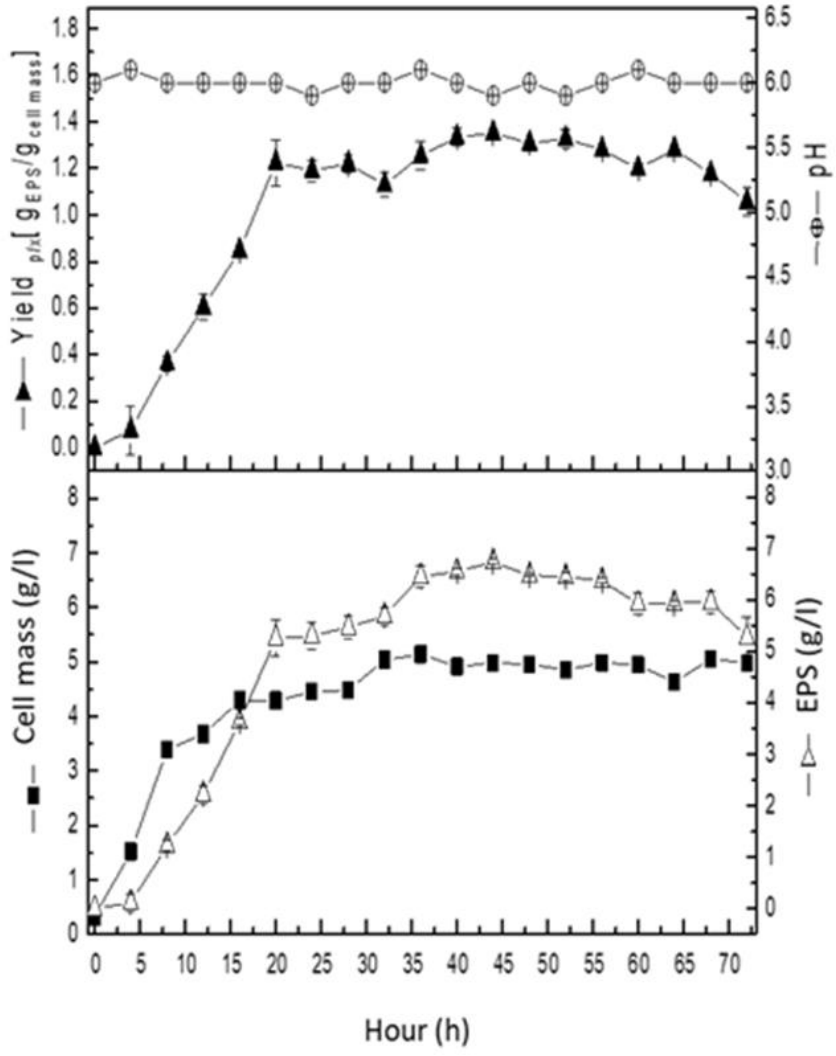

Figure 2. Total cell mass and EPS production by single culture of *L. plantarum* ATCC 8014 cultivation in stirred tank bioreactor 16-L under controlled pH of 6.0.

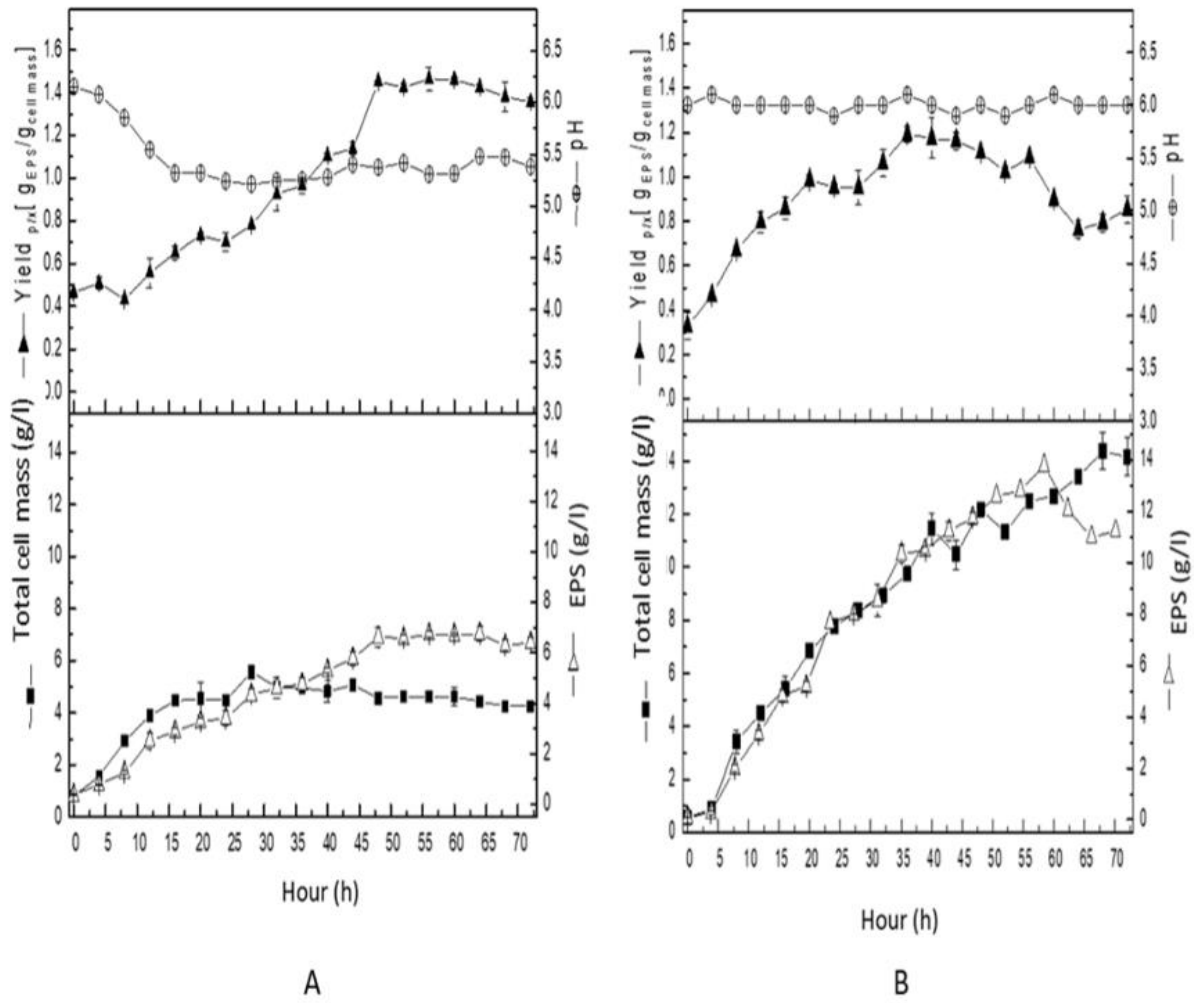

Figure 3. Total cell mass, EPS production and changes of pH for co-culture cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 in stirred tank bioreactor 16-L under un-controlled (A) and controlled pH condition (B).

Chemicals characteristics of dried EPS by DPPH free radical scavenging activity

Figure 4 exhibited DPPH radical scavenging profiles by different concentrations of dried EPS obtained from the growth of a single cultivation of *L. plantarum* ATCC 8014 as well as a co-cultivation of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01. Maximum DPPH radical scavenging activity of 88.21 % was obtained for dried EPS from *L. plantarum* ATCC 8014 with IC_{50} value of 19.57 ± 0.53 mg/ml.

The scavenging activity increased proportionally with increasing concentration of EPS with a slightly higher scavenging activity, relative to ascorbic acid (acted as positive control), at EPS concentrations of 25 mg/l, 50 mg/l, and 100 mg/l. At this concentration, the scavenging activity of EPS was 54.27% (25 mg/l EPS), 79.23% (50 mg/l), and 89.15% (100 mg/l) compared to ascorbic acid at 25 mg/l (49.05%), 50 mg/l (75.76%) and 100 mg/l (82.19%). However, a much lower DPPH radical scavenging activity of 29.28% (25 mg/l), 36.82% (50 mg/l), and 34.96% (100 mg/l) was observed when dried EPS from co-culture were used with no IC_{50} value was detected.

From Figure 4, the EPS from single cultivation of *L. plantarum* ATCC 8014 exhibited strong antioxidative effects activity on the DPPH radical scavenging. The finding presented the potential scavenging capacity from the EPS of a single cultivation, which showed the largest hydroxyl radicals scavenging activities even though at a low concentration (100 mg/l) still has abilities for hydrogen donation when compared with EPS from a co-culture (Figure 4). This result

is much higher in comparison to the study conducted by Wang *et al.* (2017) and Adesulu-Dahunsi *et al.* (2018) in which they described the scavenging activity for EPS produced by *Lactobacillus plantarum* KX041 and *L. plantarum* YO175 was approximately 80% and 56.89%, respectively, at a concentration of 5 mg/ml. In conclusion, a higher efficiency in the bioactivity of EPS from the cultivation of single cultivation *L. plantarum* ATCC 8014 was observed when compared with the EPS biosynthesis through co-cultured with *S. cerevisiae* ICA-Y01.

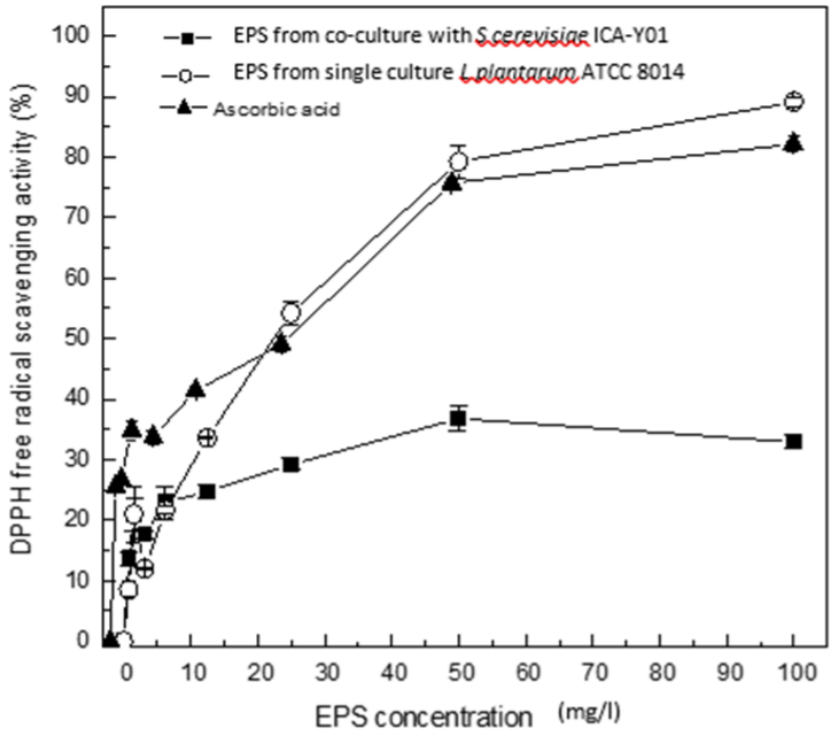

Figure 4. The DPPH free radical scavenging activity of EPS from cultivation single culture of *L. plantarum* ATCC 8014 and co-culture with *S. cerevisiae* ICA-Y01 and ascorbic acid as positive control.

Carbohydrate monomer analysis

Both HPLC chromatogram for EPS produced by single cultivation of *L. plantarum* ATCC 8014 and co-cultivation with *S. cerevisiae* ICA-Y01 showed a single peak at a retention time of 9.9 min (Figure 5). As glucose was the only sugar detected in the EPS, indicating that the possibility of glucan, a polysaccharide that made up from glucose monomer. In this study, molasses were used as the main carbon source in the cultivation medium in which presumably contain sucrose as the main component of carbohydrates where glycosidic bonds were responsible for linkage each glucose monomer (Van Hijum *et al.,* 2006). Further chemical characterizations of the synthesized EPS from both cultivations were classified as homopolysaccharides which consisted of glucose monomers as molasses were used as the main carbon source.

FTIR spectral analysis of the EPS

For functional groups characteristics using FTIR, EPS obtained from the growth of a single cultivation of *L. plantarum* ATCC 8014 showed a broad band at 3274.31 cm^{-1} indicating the existence of the stretching vibration of −OH group (Adesulu-Dahunsi *et al.,* 2018), strong band at 1647 cm^{-1} (C-O and C-N bond stretching) (Asgher *et al.,* 2019), 1458 cm^{-1} (CH_2 band), 1217.96 cm^{-1} (COOH group, indicating the presence of polysaccharides), a strong peak at 1037 cm^{-1} (pyranose sugar, Shi *et al.,* 2016) and the relative short peak at 780 cm^{-1} indicating the glycosidic linkage (C-O-C, You *et al.,* 2020).

The concomitant small shifting and strengths of absorption bands for EPS were observed on the spectrum from the co-cultured of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01, with most of the band's strength being weak and had smaller shifts (Figure 6). Interestingly, only the short peak at 780 cm^{-1} was not shifted (Figure 6) the same as

EPS from single cultivation of *L. plantarum* ATCC 8014 which shows the presence of glycosidic linkage (C-O-C) in polysaccharides. Significantly, EPS from co-cultivation reduced the intensity of the −OH group and shifted from 3274.31 to 3210.8 cm^{-1}. Its elevated intensity was indicative of a larger hydrogen bond network that formed between the −OH groups of carbohydrate moiety with other −OH groups on EPS. Significant differences were observed in the peak at 2925.50 cm^{-1} of the single cultivation of *L. plantarum* ATCC 8014 which had shifted to a lower wavenumber and shifted to 2049.03 cm^{-1} resulting in co-culturing with *S. cerevisiae* ICA-Y01. This peak can be referred to an asymmetrical C-H stretching vibration of the aliphatic CH_2 group, which related to the presence of organic substances such as proteins or sugars in EPS (Wang *et al.,* 2015). FT-IR spectroscopy revealed an EPS from both cultivations displayed the configuration of D-glucose units bound by α-(1→6) to form polysaccharides. All samples presented almost identical feature peaks, indicating that they had the same functional group.

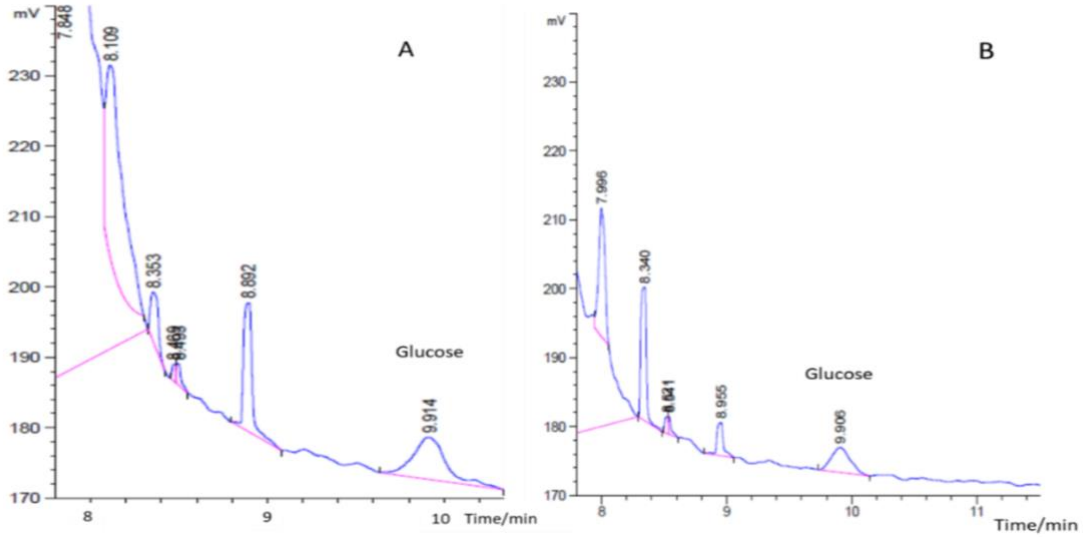

Figure 5. HPLC chromatogram showing the glucose monomer from cultivation single culture of *L. plantarum* ATCC 8014 (A) and co-culture of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01 (B).

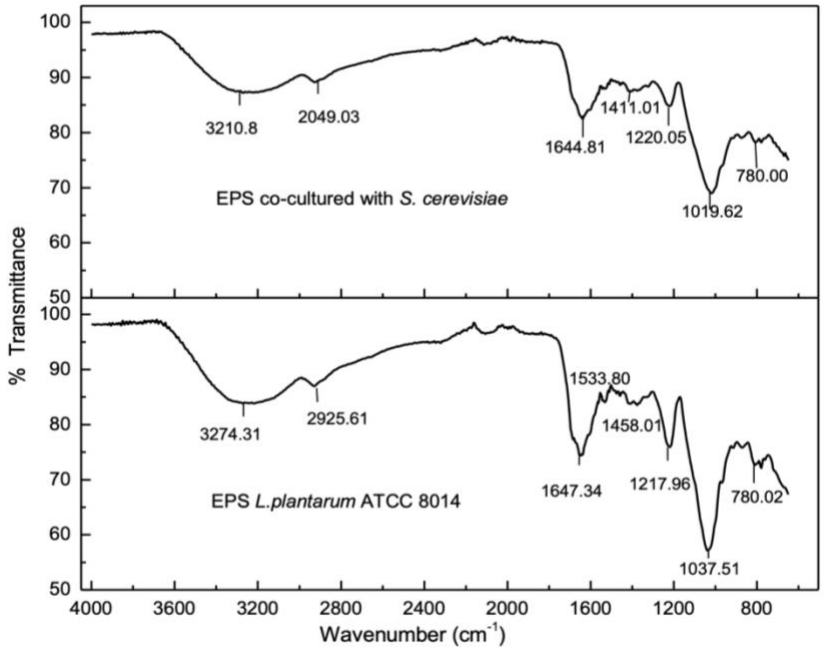

Figure 6. Fourier-transform infrared (FT-IR) spectra of exopolysaccharide yielded from single culture of *L. plantarum* ATCC 8014 and mixed culture of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01.

Scanning electron microscopy of EPS

Results from the surface morphology analysis of EPS from the single cultivation of *L. plantarum* ATCC 8014 and co-cultivation with *S. cerevisiae* ICA-Y01 using SEM were shown in Figure 7. For a single cultivation of *L. plantarum* ATCC 8014 at 1000x magnification (Figure 7A), irregular lumps of small porous, sponge structures of different sizes were observed with a consistent polymeric matrix of a coarse surface can be referred to as the structural reliability needed for bio-based film formation (Kanamarlapudi & Muddada, 2017). SEM micrographs of EPS from co-cultivation of *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 showed a microstructure of the EPS when observed at 1000x magnification, revealing irregular big lumps structure of diverse size with a coarse surface and irregular pores (Figure 7B).

Water solubility index (WSI) and water-holding capacity (WHC) of EPS

The WSI and WHC of the EPS from the cultivation of single culture *L. plantarum* ATCC 8014 were 70.95 ± 1.49% and 95.0 ± 7.47%, respectively. However, the water solubility index and water holding capacity of EPS produced from co-cultivation of *L. plantarum* ATCC 8014 and *S. cerevisiae* ICA-Y01 were 61.0 ± 1.19% and 79.3 ± 5.40% respectively. Solubility and water uptake of EPS from single cultivation of *L. plantarum* ATCC 8014 was high when compared with EPS from co-cultivation. Furthermore, solubility and water uptake of EPS from the cultivation of single culture were higher when compared with the EPS from the co-cultivation. As mentioned by Asgher *et al.* (2019), EPS from the single ccultivation of *L. plantarum* ATCC 8014 was soluble in water (70.95%) with high efficiency for water retention capacity (95%) as measured by WSI and WHC attributable to the structure of the porous matrix of the polymer, as observed in SEM (Figure 7A and 7B). This structure was having an easing-interconnection with water molecules through the formation of hydrogen bonds as a potential binding and stabilizing agent (Farinazzo *et al.,* 2019). The highly porous structure of the EPS from single culture *L. plantarum* ATCC 8014 may aid its application in food industries to increase their physical properties, e.g., the viscosity and water-holding capacity of the product.

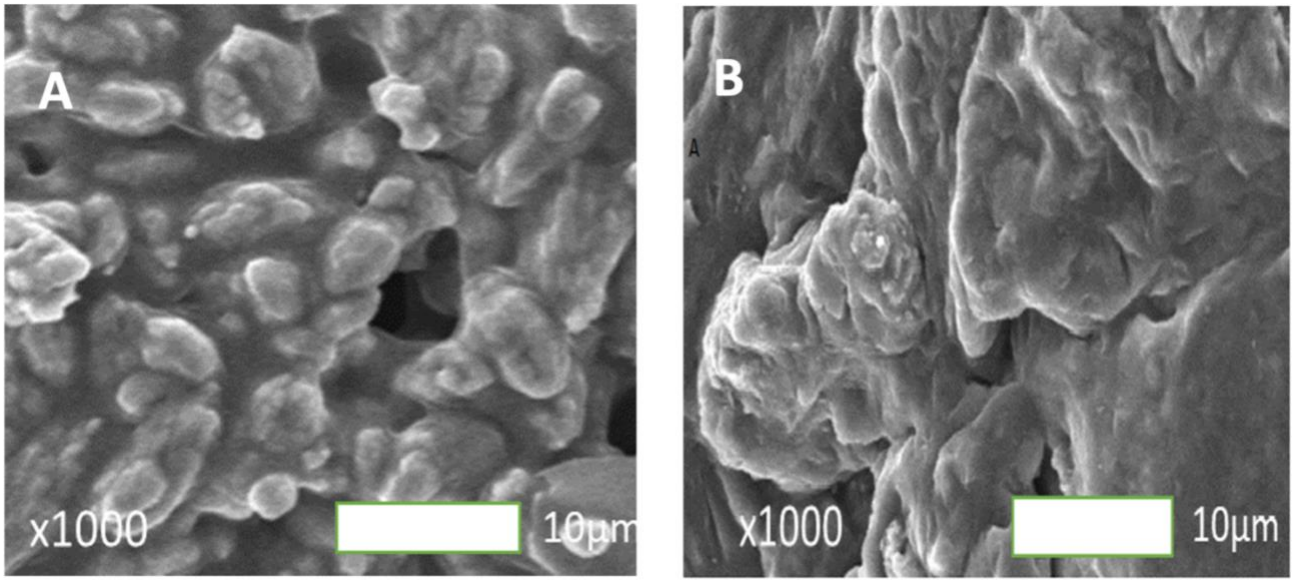

Figure 7. Scanning electron microscopy showing the surface morphology of the EPS at magnification of 1000X from single culture of *L. plantarum* ATCC 8014 (A) and co-cultured with *S. cerevisiae* ICA-Y01 (B).

CONCLUSION

In this research, the highest EPS production was achieved when *L. plantarum* ATCC 8014 with *S. cerevisiae* ICA-Y01 were co-cultured. Both EPS from the single cultivation of *L. plantarum* ATCC 8014 and the co-cultivation produced a similar homopolysaccharide of EPS with glucose as a monomer. FTIR confirmed the existence of OH/COOH functional groups and glycosidic linkages among monomeric subunits. However, EPS produced from single cultivation of *L. plantarum* ATCC 8014 gave a good antioxidant activity with high DPPH radical scavenging indicating its potential health benefits when compared to EPS from co-cultivation of *L. plantarum* ATCC 8014 with yeast. Furthermore, it demonstrated highly soluble material and water-binding capacity making it a potential compound for commercial application.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Universiti Teknologi Malaysia for the funding under UTM Fundamental Research Grant (UTMFR) (Q.J130000.3809.22H12).

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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