Food Sci Biotechnol (2018) 27(6):1735–1746
https://doi.org/10.1007/s10068-018-0409-y

Exopolysaccharide from *Pantoea* sp. BCCS 001 GH isolated from nectarine fruit: production in submerged culture and preliminary physicochemical characterizations

Seyyed Vahid Niknezhad[1,2] · Mohammad Hossein Morowvat[2,3] · Ghasem Najafpour Darzi[1] · Aida Iraji[4] · Younes Ghasemi[2,3]

Received: 21 February 2018 / Revised: 17 May 2018 / Accepted: 25 May 2018 / Published online: 12 June 2018

Abstract Exopolysaccharide (EPS), as potential microbial base polysaccharide source, has plenty of applications due to its unique physicochemical structure. A *Pantoea* sp. BCCS 001 GH bacterium with the ability to produce a high amount of EPS was identified by 16S rRNA gene sequencing and biochemical tests. The synthesis of EPS by *Pantoea* sp. BCCS 001 GH was 13.50 g/L in 48 h when sucrose was used as substrate. The proposed protocol was desirably rapid for massive prodcution of EPS and showed the remarkable impact of sucrose and disodium hydrogen phosphate, peptone, Triton x-100 and 2% (v/v) inoculum size on the yields of EPS production. The EPS was mainly composed of glucose and galactose in a relative molar ration (glucose/galactose) of 85.18:14.82, respectively. The preliminary characterization showed the average molecular-weight of EPS is about 2.522×10^6 Da. The microscopics morphology of polymer was formed irregularly shaped structures.

Keywords *Pantoea* sp. BCCS 001 GH · Exopolysaccharide · Physicochemical structure

Introduction

Exopolysaccharides (EPSs) are high molecular weight polymers with specific functions and rheological properties. They can change the flow characteristics of fluids, flocculate particles, and produce emulsions (Freitas et al., 2011; Silvi et al., 2013). These polymers not only secret extracellularly into the environment, but also closely attach to the cell wall of bacteria and release to the broth as capsules or loosely bound onto cell surface as slime. Bacterial EPS can protect bacteria from various external stresses (Amellal et al., 1998; Chawla et al., 2009). The carbohydrate structure of these biopolymers is mainly composed of various monomers, such as glucose, galactose, and mannose as the most common monomers (Kumar et al., 2007). EPS is eco-friendly due to its biodegradability, biocompatibility, nontoxicity and the feasibility of large-scale production. Therefore, it has sought due attention in the food industry, as well as pharmaceutical and medical applications (Matsumoto and Kuroyanaqi, 2010; Takeuchi et al., 2009). The ability of EPS that are engineered to nanomaterials, hydrogel or scaffold is recently highlighted in biomedicine and tissue regeneration (Bueno et al., 2013; Zanchetta et al., 2003). Only a few species of bacteria are recognized with the high capability of EPS production, driving scientists in recent years towards the discovery of new bacteria for EPS biosynthesis and testing the composition, structure, and characterization of the new EPSs (Trabelsi et al., 2015; Wang et al., 2007; Wang et al., 2015). Finding new bacteria with high potential of EPS production is of paramount importance for industries (Silvi

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10068-018-0409-y) contains supplementary material, which is available to authorized users.

✉ Younes Ghasemi
ghasemiy@sums.ac.ir

[1] Department of Chemical Engineering, Faculty of Engineering, Noshirvani University of Technology, Babol, Iran

[2] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran

[3] Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran

[4] Central Research Laboratory, Shiraz University of Medical Science, Shiraz, Iran

et al., 2013). Based on previous attempts, numerous microorganisms from different parts of the environment, including deep-sea, salt lakes, hot springs, soil, milk, wine and plants (roots, leaves, and fruits) are isolated to screen their ability in EPS production (Ahmed et al., 2013; Freitas et al., 2011; Li et al., 2015; Nicolaus et al., 2010; Silvi et al., 2013; Wang et al., 2015). In most of the bacteria, EPS is obtained under aerobic conditions (limited oxygen and nitrogen) and in the presence of excess carbon source (substrate) (Chawla et al., 2009; Kumar et al., 2007). Alternative and cheap substrate like agriculture waste, a by-product from value-added products, are nowadays of interest to produce several types of EPSs (Freitas et al., 2009; Niknezhad et al., 2015; Premjet et al., 2007; Sirajunnisa et al., 2016). Although organic and inorganic nitrogen sources could be utilized by bacteria to produce an exopolysaccharide (Sirajunnisa et al., 2016), it was found that organic nitrogen sources were not a suitable option due to unidentified composition and high price. Phosphorus source has a key role in the biosynthesis of monomers and their release out of the membrane of bacterial cells (Niknezhad et al., 2015). Micronutrients, mineral salts, vitamins, amino acids, and phosphate ions are vital components of bacterial metabolism that may be very influential parameters on biopolymer production (Li et al., 2010; Premjet et al., 2007; Sirajunnisa et al., 2016). A plethora of methods is reported to optimize and investigate the effect of pH on EPS yield (Li et al., 2010). The pH range is a significant factor for EPS production, depending on bacterial strain (Li et al., 2010). *Pantoea* sp. BM39, as a new marine bacteria, a strain known for remarkable hemoexopolysaccharide (21 g/L) production is suggested as a new microorganism for industrial applications (Silvi et al., 2013). Generally, *Pantoea* sp. strains produce bacterial EPSs in high yield with various compositions and structures (Amellal et al., 1998; Silvi et al., 2013).

The object of this study was to evaluate the detailed phylogenic a new plant pathogen bacterium and producing high levels of new EPS. The strain was identified as *Pantoea* sp. BCCS 001 GH by 16S rRNA gene sequencing and biochemical tests. In the fermentation process, the impact of the substrate, nitrogen source, nutrient and culture conditions were also investigated to enhance the yield of polymers synthesis by *Pantoea* sp. BCCS 001 GH under aerobic conditions. In addition, the physicochemical properties of new EPS has been investigated by determination of molecular weight, and monosaccharide profile. To the best of our knowledge, this is the first study that reports on the effect of different conditions of culter media on the synthesis of EPS from *Pantoea* strains.

Materials and methods

Culture conditions

A biopolymer-producing bacterium, nominated as *Pantoea*, was isolated from the nectarine fruits around Ghadir garden in the south of Yasuj city, Iran. Medium composition of 2 g/L KH_2PO_4, 0.2 g/L $MgSO_4$, 2 g/L NH_4NO_3, 2 g/L citric acid, 0.006 g/L H_3BO_3, 0.006 g/L $ZnCl_2$, 0.0024 g/L $FeCl_3$, and 0.02 g/L $CaCl_2$ were supplemented with 20 g/L glucose and 1.5% agar. pH of the solutions was adjusted to 7 before autoclaving at 121 °C for 15 min (Niknezhad et al., 2016). Production of the EPS by the bacteria was carried out at 30 °C and pH 7 for 2 days with an agitation rate of 200 rpm in the same medium without agar using different carbon source (glucose, maltose, sucrose, mannose, xylose, arabinose, galactose, and lactose) as described above. After selection of the best carbon source with high production of the biopolymer in large scale, different concentration of chosen carbon source was investigated. The best nitrogen source (3 g/L) was selected among several sources tested (peptone, yeast extract, ammonium nitrate, ammonium sulfate, ammonium chloride and urea). The sterile solutions were inoculated by adding 1–5% (v/v) of inoculum for cell culture and cultivations were performed in cotton plugged Erlenmeyer flasks to find out the best percentage of initial inoculum. The impact of pH on culturing was examined by adjusting the pH in the range of 3–9 using 1 M HCl and 1 M NaOH. Finally effects of 0.5 and 3 g/L phosphate ions (K_2HPO_4, KH_2PO_4, NaH_2PO_4, Na_2HPO_4), 0.1 and 0.5 g/L detergents (Tween 20, Tween 80 and Triton X-100), 2 and 10 mg/L vitamins (cobalamin, riboflavin, thiamine, pyridoxine, B complex, Ascorbic acid), 0.05 and 0.3 g/L amino acids (tyrosine, glutamine, lysine, glycine, arginine, leucine) and 0.06 and 0.2 g/L inorganic salts ($MgSO_4$, $CuSO_4$, $FeSO_4$, KCl, NaCl, $MgCl_2$, $ZnCl_2$, $CaCl_2$, $FeCl_3$) on EPS production were studied. For the preparation of chromosomal DNA, the bacteria were cultured at 30 °C with an agitation rate of 200 rpm overnight in nutrient broth (NB) liquid media.

Identification of strain and bioinformatic analysis of the amplified sequence

The bacterium was identified using the phylogenetic analysis based on the 16S rRNA gene sequence and testing biochemical properties. Genomic DNA of the pure isolate was extracted using Cinna Pure-DNA Kit (for Gram-negative bacteria) (Sina Clon, Tehran, Iran) according to the manufacturer's instructions. The amplification of partial 16SrDNA gene was performed with a universal bacterial primer set; 63F (5′-CAGGCCTAACACATGCAAGTC -3′)

and 1378R (5′-CGGTGTGTACAAGGCCCGGGAACG -3′), which amplifies an about 1200 bp region of the 16S rDNA gene. PCR reactions were performed in a total volume of 40 µl containing 20 µl PCR Master mix, 2 µl template DNA, 1 µl of each primer (10 pmol), and 16 µl distilled water. Amplifications were performed using a DNA thermocycler (BIO-RAD, Hercules, CA, USA) with the following temperature profiles: initial template denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 5 min. The final extension step was 5 min at 72 °C. Successful amplifications were confirmed by electrophoresis of 5 µl PCR products on a 1% agarose gel. Unidirectional PCR product sequencing was performed. The nucleotide sequences of certainly known microorganisms (was compared to the found 16S rDNA gene sequence and was aligned) using Basic Local Alignment Tool (BLAST) in Gene Bank database of the National Center for Biotechnology Information (NCBI). The phylogenetic study was conducted using MEGA software version 7 (Zare et al., 2015). For annotating multiple sequence alignments, shading and alignment editing, a bioinformatic tool called the CLC sequence viewer software, version 6.8.1., was used to compare the retrieved sequences. Also, the conserved domains among these sequences were identified. The biochemical characteristics of the nectarine isolated bacteria were concluded by *Enterobacteriaceae* identification kit (KB003 Hi25).

Extraction and preparation EPS for characterization

After the cultivation of the bacteria at different conditions, 1.5 ml of the culture media was used for high-speed centrifugation at 11,000 rpm for 20 min at 4 °C. Cell bacteria were washed with Milli-Q water for two times, and then, the final precipitants were dried at 50 °C for 48 h and weighed. EPS crude solutions, i.e. cell-free supernatant were blended with 4.5 ml solution of 0.1% $CaCl_2$ in isopropanol (Niknezhad et al., 2015). The biopolymer was extracted by centrifugation (20 min, 10.000 rpm). The final precipitants were dried at 50 °C for 48 h and weighed. For characterization of EPS, it was very important to have samples without any $CaCl_2$ and enzyme that cause degraded EPS. Thus, the supernatant underwent high-speed centrifugation for several times to completely separate bacteria cells. The supernatant was kept in autoclave at 100 °C for 30 min to deactivate enzymes, and then, it was rapidly cooled down (Ahmed et al., 2013; Trabelsi et al., 2015). The biopolymer solution cell-free supernatant was mixed with isopropanol for 3 times without any salt and extracted according to the mentioned method. The polymer was dissolved in water and again extracted by isopropanol. The biopolymer precipitated with isopropanol and the supernatant was removed. High concentration of biopolymer was dissolved in Milli- Q water, and then the EPS solution was lyophilized and used for characterization.

Monosaccharide analysis

The polysaccharide sample (20 mg) was hydrolyzed with Trifluoroacetic acid (TFA) at 121 °C for 4 h. After hydrolysis, methylation was performed and the sample was finally analyzed by GC–MS according to the method developed by Li et al. (2010) and Wang et al. (2015). The hydrolyzed sample was dried with the addition of methanol at 90 °C. Therefore, methylation was performed at room temperature for 30 min with about 60 µl of iodomethane in presence of 120 mg of NaOH. In addition, DMSO (50 µl) was utilized as the solvent for reagents and reactants. Afterward, the methylated sample was precipitated with milli-Q water and completely blended with the same volume of chloroform. Based on the result, the chloroform layer was separated by low-speed centrifuge from water layer and then injected to a GC–MS (Agilent 7890A, USA) equipped with an HP-5 capillary column and using He as the carrier gas. The initial temperature of the oven was adjusted at 100 °C, which was then constantly increased by 6 °C/min to 250 °C, where it was held for 5 min.

UV–vis and FTIR spectra analysis

Ultraviolet–visible (UV–vis) spectroscopy analysis of the EPS was conducted using UV–vis spectrophotometer (Cary Series UV/VIS Spectrometer, Agilent Technologies, USA) to highlighting the presence of nucleic acid and protein in the EPS sample. The EPS solution was prepared by suspending 1 g/L biopolymer in milli-Q water for UV–vis measurement in the wavelength range of 200–700 nm. To investigate the fractional groups of the EPS was used a Fourier transform infrared spectrophotometer (Vertex 70/70v FT-IR, Bruker, USA). The dry sample powder was mixed with spectroscopic grade KBr powder (Sigma-Aldrich) and then pressed into a 1 mm pellets under reduced pressure for FT-IR measurement by scanning between 4000 and 400 cm^{-1}.

Molecular mass determination (M_w)

The gel permeation chromatography (GPC) was used to measure molecular mass of EPS. The GPC system was consisted of a Waters Ultra hydrogel linear column. The EPS was detected using a refractive index detector (IR) (Shimadzu LC-20A, Japan), at an internal temperature of 35 °C. The column was eluted with 0.1 M $NaNO_3$ solution

at a flow rate of 1 mL/min. The polysaccharides were then dissolved in 0.1 M $NaNO_3$ (60 °C, 1 h), cooled and filtered through a 0.45 μm nylon filter prior to injection into the column.

Nuclear magnetic resonance (NMR) analysis of EPS

NMR spectra of the lyophilized EPS were recorded using a Bruker 300 MHz Spectrometer (Bruker, Germany) at 25 °C with 5 mm inverse probe to showing the monomers configuration in the polysaccharide. The sample was dissolved in D_2O (Sigma) for two times at a concentration of 5 mg/mL (for ^{1}H NMR) and 20 mg/mL (for ^{13}C NMR) (Wang et al., 2015). Chemical shifts (δ) were described in parts per million (ppm).

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy was used for characterization of biopolymer and bacteria. EPS was fixed on the aluminum stub and then SEM image was captured (Tescanvega 3, Czech Republic). To prepare SEM samples of bacteria, the suspension of bacterial cells was washed with Ringer's solution for three times, and a thin layer was prepared on a glass slide. In order to heat-fix the bacterial stain, the air-dried slides were passed through the flame of a Bunsen burner three times. The bacterial cells were fixed in 2.5% glutaraldehyde in Ringer's solution for 45 min. For rinsing, the slides were kept in Ringer's solution for 15 min. Cell dehydration of the samples were conducted by frequent immersion of the slides in different solutions of ethanol (30, 50, 70, 80 and 90%) for 10 min. Consequently, prepared samples were left in 100% ethanol for 1 min. The slides were stored at room temperature for overnight to completely evaporate the ethanol. SEM was used to observe the three-dimensional structure and surface morphology of EPS macromolecules.

X-ray diffraction (XRD)

For finding the nature of EPS resembling amorphous or crystalline was used X-ray diffraction (XRD). XRD studies were performed on the Bruker D8 Advance model XRD with Ni-filtered Cu Kα radiation generated at conditions of 40 kV, 40 mA, the data gathered in the 2Ø range 5–40 with step size of 0.05 and step time 1 s.

Statistical analysis

The statistical evaluations of all data were carried out with Sigma Plot (version 12.0, Systat Software Inc., USA) using one-way ANOVA. The means comparisons were determined by Tukey's test ($P < 0.05$).

Results and discussion

Identification of bacteria

The low-quality bases from the ends of raw sequence data (ca. 1195 bp) were removed and then sequences obtained (ca. 849 bp). The evolutionary history was inferred using the Maximum Likelihood method. The tree with the highest log likelihood is shown in Fig. 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search was automatically obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 849 positions in the final dataset. Evolutionary analyses were directed in MEGA7. The isolate, bacteria from nectarine fruit closely affiliated to the genus *Pantoea* sp. The 16S rDNA sequence was published in the NCBI databases under the specific accession numbers "SUB3753631 *Pantoea* MH026116". However, the new name (BCCS 001 GH) was selected for *Pantoea* strain. The phylogenetic analysis showed that BCCS 001 GH constituted an external cluster quite far from the most similar species, *P. agglomerans*. The multiple sequence alignments, the similarity between the studied sequences and the conserved domains among the studied sequences were shown with a color scale (Supp Fig. 1). The red residues are the most conserved. Figure 1 shows the epidermis of nectarine that was wound by *Pantoea* sp. BCCS 001 GH and EPS on it, growing bacteria in the YPD (1% Yeast extract, 2% Pepton, and 2% D-glucose) medium culture and finally SEM image of the bacteria indicated that it has a rod shape (0.81 ± 0.15–2.4 ± 0.4 μm). The strain *Pantoea* sp. BCCS 001 GH, generally termed as *Pantoea,* is a mobile and gram-negative bacteria (Silvi et al., 2013). Preliminary biochemical tests were demonstrated a positive reaction for reduction of nitrate to dinitrogen, and negative reaction for β-galactosidase, urease, line utilization, and ornithine utilization. In addition, the *Pantoea* sp. BCCS 001 GH can be used arabinose, rhamnose, cellobiose, melibiose, saccharose, trehalose, glucose as sole carbon source. The isolated bacteria strain was stored in bacteria culture collection of Shiraz University of Medical Sciences (BCCS) for preservation and future cultivation.

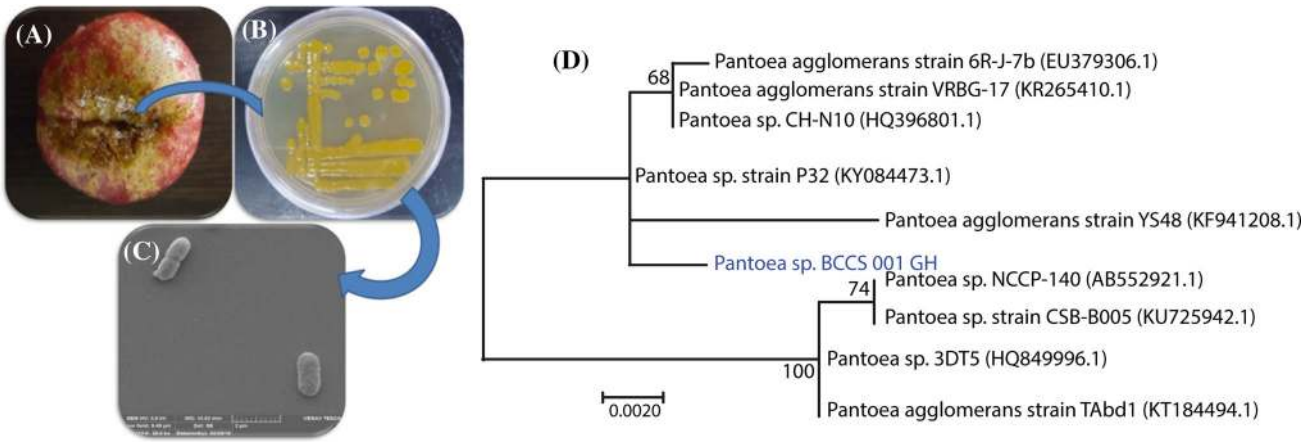

Fig. 1 Isolation and identification of *Pantoea* sp. BCCS 001 GH. (**A**) Nectarine fruit diseases by *Pantoea* sp. BCCS 001 GH bacteria. (**B**) Growth bacteria in YPD culture after 48 h. (**C**) SEM result of *Pantoea* sp. BCCS 001 GH at 10000×. (**D**) Molecular phylogenetic analysis of an isolated *Pantoea* strain with some related strains

Effect of different culture condition (carbon and nitrogen source, time harvesting, inoculum concentration and the pH of culture media) on EPS production

The carbon source concentration as the driving force for sugar transport to cells is an important factor in microbial EPS fermentation (Mao et al., 2014; Niknezhad et al., 2014; Xu et al., 2014). After 48 h of incubation, the results showed that *Pantoea* sp. BCCS 001 GH was able to utilize the eight carbon source. The concentration of EPS was studied under nine conditions, which is presented in Fig. 2(A), varied between 12.06 to 4.74 g/L. The highest EPS titers (12.06 g/L) were obtained using galactose as sole carbon source. Using inexpensive and more abundant carbon sources for EPS manufacturing can reduce the production cost and, therefore, enhance the economy of the process. Galactose has a high price, hence it is not economical to use them for EPSs production. Sucrose strongly induced EPS (9.57 g/L) production when comparing the other carbon sources. In order to produce EPS, the basic carbon source for experiments using sucrose and glucose as abundant sugar in nature were investigated. halophilic marine species *Pantoea.*BM 39 was growing up to 100% of salinity and produce a high level (21 g/L) a homopolymeric glucose EPS with 80 g/L glucose as a carbon source (Silvi et al., 2013). In this way, glucose and sucrose derived metabolic intermediates can be used for the biosynthesis of EPSs (Silvi et al., 2013; Wang et al., 2007). The maximum yield of 67.5% was achieved in this work, a value that has never reported before for the production of EPS from other *Pantoea* bacteria (Silvi et al., 2013; Wang et al., 2007). In this case, the biomass and EPS synthesis were controlled by the change of available nitrogen sources [Fig. 2(B)]. Effect of organic and inorganic nitrogen source was assessed and mentioned that, peptone as the organic matter had the great effect on EPS production (9.63 g/L) and urea had the most effective impact to produce biomass (2.73 g/L). As matter of fact, organic compounds, such as peptone and yeast extract are unknown, so they can use as another source such as carbon, phosphorus, and nutrients for microorganisms and their metabolism. Effect of carbon source concentration (2–10%) was not significant due to inhibition of EPS production and only bacteria try to grow more [Fig. 2(C)]. The carbon source concentration as the driving force for sugar transport to cells is an important factor in microbial EPS fermentation, but high concentration may inhibit growth and reduce production (Niknezhad et al., 2015). Conceiving that high concentrations of carbon only make growth and try to reproduce more bacteria will not be distracting. In addition, interaction between all additive (carbon, nitrogen, phosphorus and nutrient) in the culture media are significant to produce EPS (Niknezhad et al., 2014; Niknezhad et al., 2015). The results were indicated that the best concentration of sucrose for EPS production from *Pantoea* sp. BCCS 001 GH is 2% (w/v). Afterwards, EPS, biomass and optical density against time as the main effect in the biological process were obtained [Fig. 2(D)]. Accordingly, this strain exhibited a relatively short lag phase of less than 2 h because of the same component in inoculums culture and EPS production culture and start to utilize the carbon source. The *Pantoea* sp. BCCS 001 GH started to produce the EPS in the beginning of the exponential growth phase same as *Pantoea* sp BM39 (Silvi et al., 2013). After short logarithmic growth about 16 h, stationary phase was started and 9 g/L of the product was obtained till 72 h. Also, maximum specific growth rate (μ_m), the slope of the semi-logarithmic growth curve in a logarithmic growth phase, was calculated about 0.3 h^{-1}. Record the optical density (OD) shown the same pattern

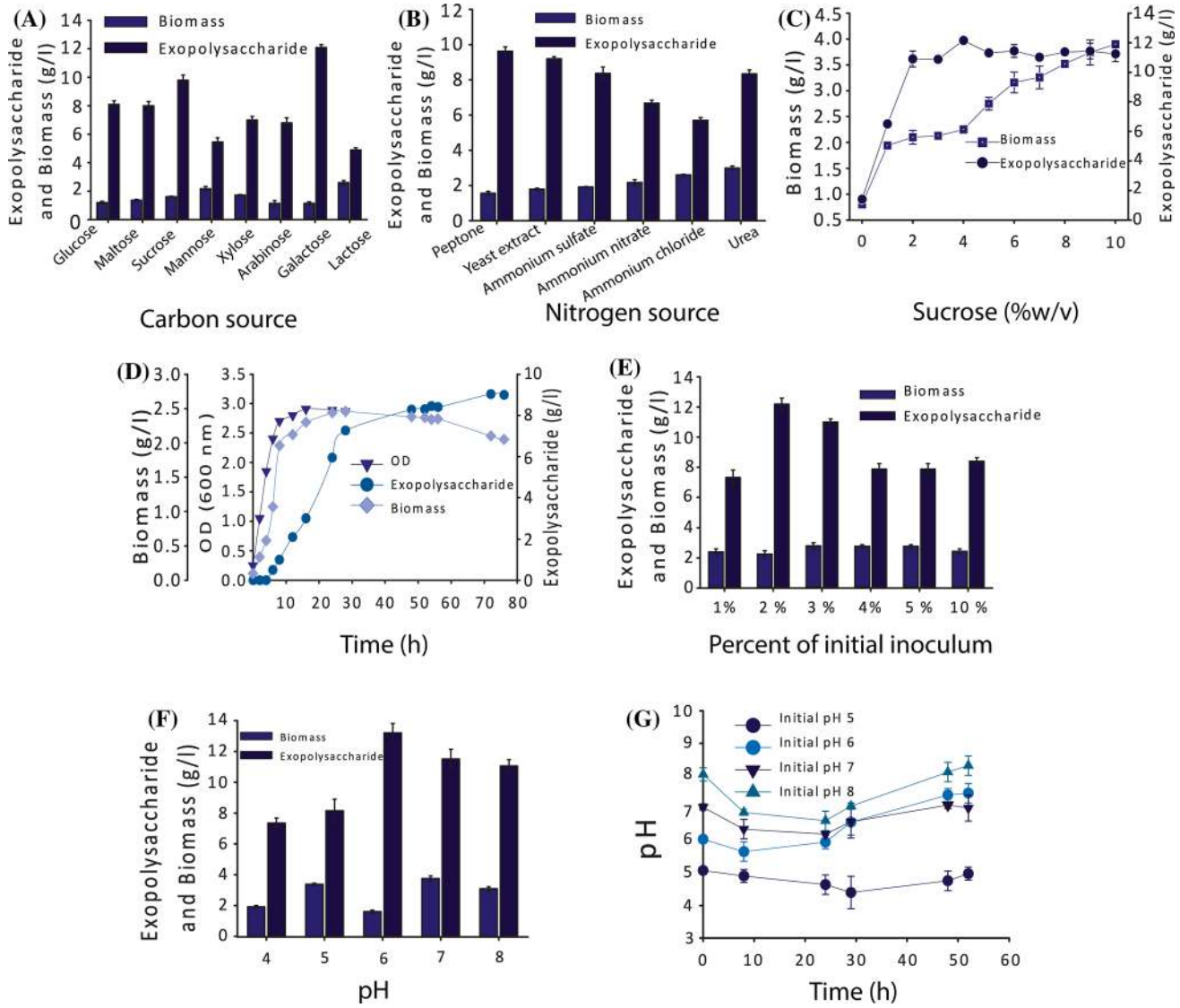

Fig. 2 Effect of different condition culture on EPS and biomass production. (**A**) *Pantoea* sp. BCCS 001 GH utilize the nine carbon source and produce EPS and biomass after 48 h. (**B**) Effect of organic and inorganic nitrogen source assess on EPS and biomass. (**C**) Show the effect of a percent of sucrose as selected carbon source on biomass and EPS production. (**D**) Growth in a different time of fermentation. (**E**) Changes in percent of initial inoculums and the effect on EPS and biomass production. (**F**) Effect of initial pH in batch fermentation. (**G**) Effect of pH against time. All results represent standard deviations ± S.D.; n = 3

like biomass and useful for replacing biomass as the first factor in seed culture. At the respective optimum inoculum concentration, the maximum yield of polysaccharide (12.18 ± 0.4 g/L) was found at the lowest level of inoculum concentration (2% v/v) as shown in Fig. 2(E). Although the amounts of EPSs from different inoculum size completely depend on bacterial strains, many results have been shown by other researchers that there was a tendency of negative influence at the high inoculum size on the EPSs production (Rawal et al., 2016; Ruffing and Chen, 2006). Overall, a large inoculum size (3–5%) seemed to highly lower the amount of EPS produced, due to the reduced availability of bacteria to the substrate and an increase in the concentration of bacteria to the rich culture sources (Niknezhad et al., 2015; Rawal et al., 2016; Sivakumar et al., 2012). Effect of initial pH in batch fermentation was studied by varying the pH of the culture medium from 3 to 9 for an incubation period of 56 h. In the first and last pH range (3 and 9), there was no significant growth of bacteria. The production of EPS has increased to (13.20 ± 0.61 g/L) at pH 6 and then decrease with additional increase in pH as shown in Fig. 2(F). The initial pH of culture medium was indicated as an important factor for cell growth, EPS production and uptake of different nutrients by *Daedalea dickinsii* (Mao et al., 2014). EPSs synthesis needs the biosynthesis of activated precursors

(e.g. monosaccharides or amino acids) during fermentation process (Freitas et al., 2011; Ruffing and Chen, 2006). Thus, it was evident that the pH at the beginning of fermentation decrease by the production of amino acids, several metabolite products, and precursors. In the late stage once amino acids were utilized by the bacteria, then pH increased [Fig. 2(G)].

Effect of different phosphate ions, detergent, amino acids, inorganic salts and vitamins on EPS production

As shown in Fig. 3(A), among the several phosphate ions salts (Na_2HPO_4 (DSHP), NaH_2PO_4 (SHP), KH_2PO_4 (PHP), and K_2HPO_4 (DPHP)) tested, maximum EPS production was obtained in Na_2HPO_4 and K_2HPO_4 cultures with 13.28 ± 0.28 and 11.11 ± 0.11 g/L, respectively. Also, low concentrations of phosphate ions (0.5 g/L) were not effective in EPS production. The same results reported that Na_2HPO_4 is a significant factor to produce gellan gum from *Sphingomonas paucimobilis* ATCC-31461 (Banik et al., 2007). The results illustrated a huge difference in EPS production between two concentrations of phosphate ions (0.5 and 3.0 g/L). However, phosphate as a factor for cell growth and a buffering agent to prevent pH fluctuations for EPS was investigated (Niknezhad et al., 2015).

When detergents were added to the culture medium, final EPS concentration was improved compared to the control, which received no detergent [Fig. 3(B)]. 0.5 g/L Tween 80 and Triton X-100 in culture broth had the most important effect, producing 1.40 and 1.42 times more EPS compared to the absence of detergent (control). Simultaneously, the limitations of polymer production from 8 to 6 g/L were observed with both concentrations of Tween 20. Another study used Triton X-100, Tween 40 and Tween 80 for the production of xanthan gum and wellan gum (Galindo and Salcedo, 1996; Li et al., 2010). They showed that using detergents led to higher oxygen concentrations during fermentation compared to the culture with no detergent. It was found that detergents had affected on the bacterial cells size since cells size were smaller than those of observed in the absence of the detergent (Galindo and Salcedo, 1996).

Amino acids as an essential factor in microbial metabolism were applied to EPS production. The amino acids, such as glutamine was essential for the growth of some *Lactobacillus* bacteria also the omission of glycine affected growth only slightly (Grobben et al., 1998). Although, it appeared that most amino acids were essential for the EPS production, due to produced amino acids as activated precursors at the beginning of fermentation process by bacteria, all amino acids with two concentrations (0.05 and 0.3 g/L) added to the culture did not have significant effect on EPS production [Fig. 4(A)]. It means organisms do not require an excess amount of amino acids supply. A similar result was indicated that amino acids were not a crucial component for cellulose production by *Acetobacter xylinum* ATCC 10245 (Premjet et al., 2007).

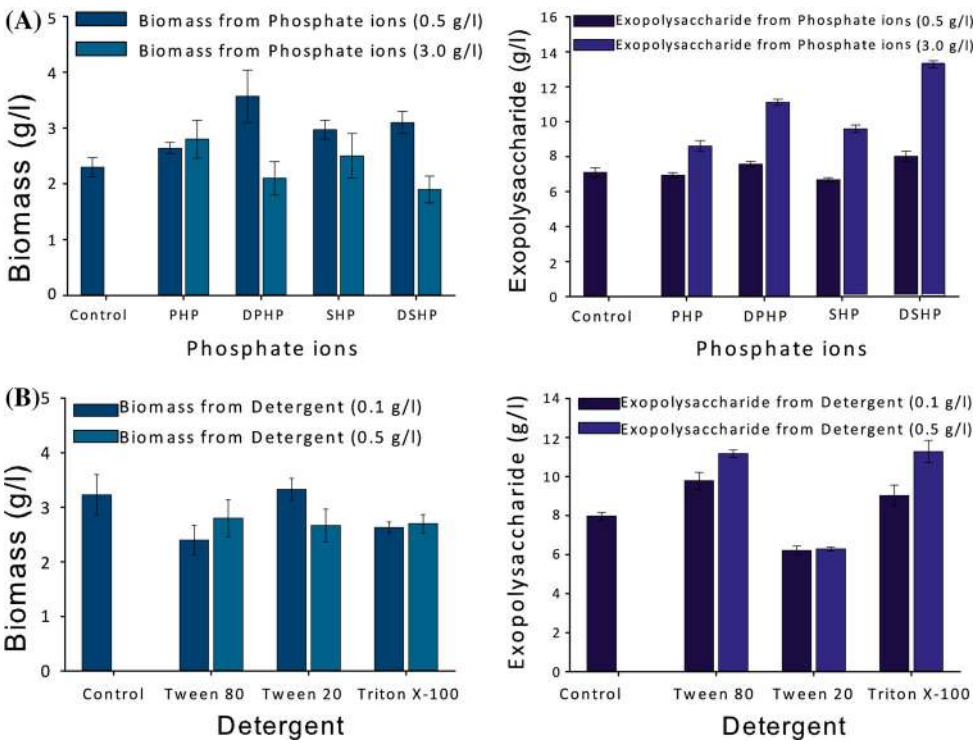

Fig. 3 Effect of different phosphate ions and detergent on EPS and biomass production. (**A**) Effect of several phosphate ions salts (Na_2HPO_4 (DSHP), NaH_2PO_4 (SHP), KH_2PO_4 (PHP), and K_2HPO_4 (DPHP)) on EPS and biomass production (**B**) Improve EPS concentration with adding detergent to media culture. All results represent standard deviations ± S.D.; n = 3

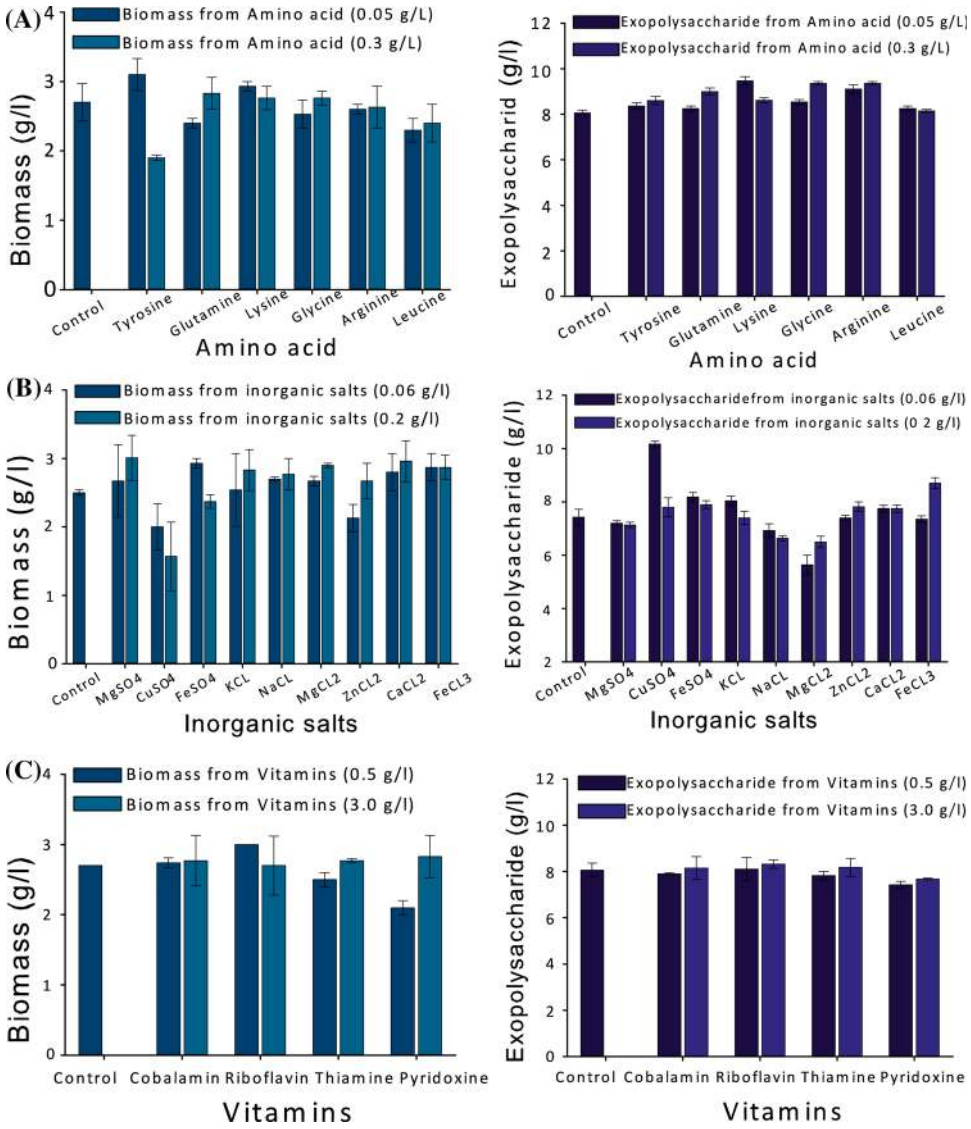

Fig. 4 Effect of different nutrient on EPS and biomass production, (**A**) amino acids, (**B**) inorganic salts and (**C**) vitamin. All results represent standard deviations ± S.D.; n = 3

The inorganic salts had no significant effect on EPS production except for $CuSO_4$ and $FeCl_3$ [Fig. 4(B)]. The two most effective inorganic salts for EPS production in the medium were $CuSO_4$ (10.18 ± 0.11 g/L) and $FeCl_3$ (8.71 ± 0.2 g/L). Only one inorganic salt ($CuSO_4$) had a positive effect on EPS production. It was evident that this bacteria does not require inorganic salts as a growth factor. The growth and EPS production by *Pantoea* sp. BCCS 001 GH in the different media that is rich in vitamins are depicted in the Fig. 4(C). Although many researchers reported that vitamins were essential materials in culture medium for EPS production (Lin, 2011; Prasertsan et al., 2006), results showed that vitamin in two concentrations (2 and 10 mg/L) compare to control did not have a synergetic effect on EPS production [Fig. 4(C)].

Characterization of EPS

The EPS that is produced with sucrose was hydrolyzed with 4 M TFA and finally converted to methylated, and analyzed by GC–MS. Two monosaccharides were revealed that the composition of the EPS was glucose and galactose in an approximate ratio of 5.75:1. Glucose had the highest part of the polymer as depicted in result shows the types and percentage of glycosidic linkages of the EPS, as determined by GC–MS. The GC–MS analysis exhibited the presence of D-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl and D-galactofuranoside, methyl 2,3,5,6-tetra-O-methyl (Data not shown, base on a library of GC–MS). Among the EPS derivatives, the D-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl- was the most profuse component (5.75), indicating that the backbone of the EPS was mainly composed of a (1 → 6) glycosidic linkage. In

addition, the presence of D-galactofuranoside, methyl 2,3,5,6-tetra-O-methyl inferred the occurrence of some branches connected to the backbone in the ratios of 1. The absolute configuration of the monosaccharides were investigated, and it was found that glucose and galactose were present in the D configuration. Wang et al. (2007) isolated 9 *Pantoea agglomerans* and showed that EPS consists of a protein-bound polysaccharide as well as arabinose, glucose, galactose and glucuronic acid in the molar ratio of 1.0:2.2:2.8:0.9. Likewise, a glucose homopolysaccharide was produced with *Pantoea* sp. (BM39) (Silvi et al., 2013). The multiple combinations of monomeric units that were produce with a variety of bacteria lead to very complex chemical structures with different and highly branched heteropolysaccharides. Undoubtedly, *Pantoea* genes can produce a variety of polysaccharide with different structure and components.

The UV–vis absorption spectra method was robust, allowing to successfully extract EPS without any other material, such as protein. UV–vis absorption spectra of the EPS from strain *Pantoea* sp. BCCS 001 GH showed absorption at (256 nm) and no distinctive absorption at 280 and 260 nm [Fig. 5(A)], implying the absence of both nucleic acid and protein present in the EPS sample. To investigate the fractional group of the EPS, the FTIR spectrum was analyzed as shown in Fig. 5(B). It is indicated that the polysaccharide possesses a significant number of hydroxyl groups as it displayed a broad band around 3379 cm^{-1}. The bound water which overlaps in part with C–H vibration peak appearing at 2927 cm^{-1} (Trabelsi et al., 2015). The absorption at 1730 cm^{-1} corresponded to the stretch vibration of the amide C–O and carboxyl groups. Wang et al. (2007), produced EPS from *Pantoea agglomerans* KFS-9 against this research showed absorption at 1650 and 1534 cm^{-1}, which were the special absorption peaks of the amide. The presence of a band at 1606 cm^{-1} can not be attributed to protein because EPS does not have any protein. So, the carboxylate group was denoted by the vibrational stretching band at 1608–1418 cm^{-1} (Torres et al., 2014). The intense peak at 1256 and 1079 cm^{-1} is identified for the main properties of the polysaccharide. The extensive absorption bands at 1079 cm^{-1} in the range of 1200–1000 cm^{-1} in the FTIR spectrums was due to the C–O–C stretching vibration of ether in pyranose-ring which mentioned that the presence of carbohydrates and monosaccharide in EPS had a pyranose ring (Trabelsi et al., 2015). The band around 892 cm^{-1} described the P–O–C bond. Eventually, the weak absorption band at 777 cm^{-1} and strong adsorption band at 544 cm^{-1} were indicative of glycosidic binding peak for polysaccharide.

For the evaluation of polysaccharide absolute molecular weight, the GPC was considered to be the method to determine the molecular weight [Fig. 5(C)]. The average molecular weight of EPS from *Pantoea* sp. BCCS 001 GH that produced with sucrose as carbon source was about (2.522×10^6 Da). This molecular weight was 3.04 times higher than the glucose homopolymer form *Pantoea* sp.

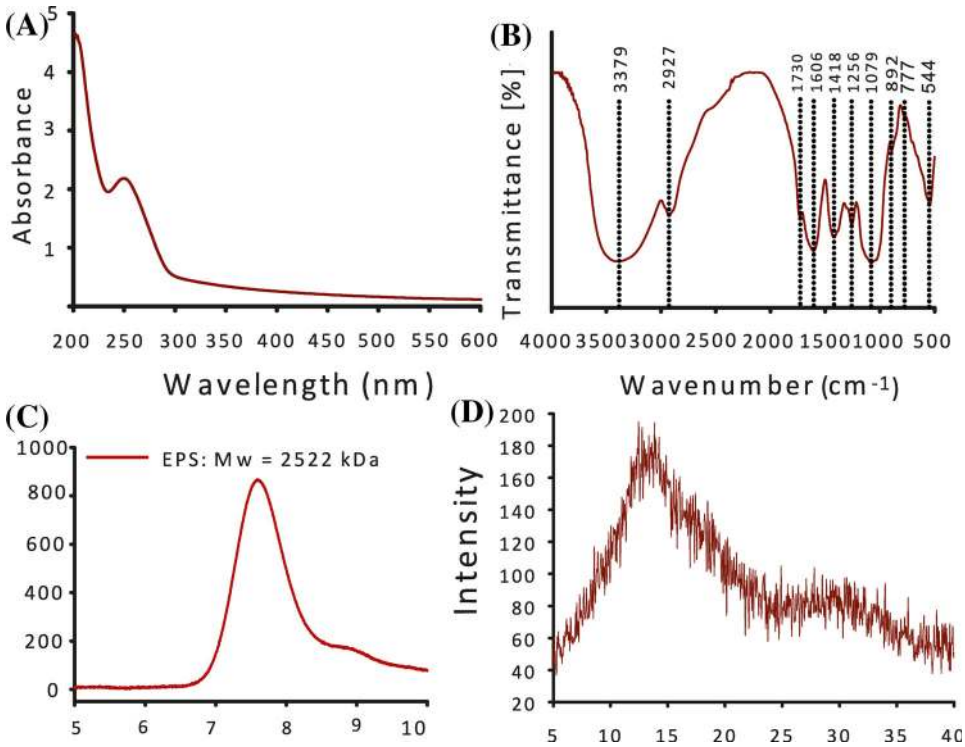

Fig. 5 Physicochemical characterization of EPS. (**A**) UV–vis spectrum of purified EPS. (**B**) FTIR in the region 4000–400 cm^{-1}. (**C**) Gel permeation chromatography (GPC), and (**D**) XRD of EPS

BM39 (*Enterobacteriaceae*) (Silvi et al., 2013). The EPS produced by *Enterobacter* A47 with the different initial nitrogen concentrations had molecular weight values of 2.73×10^6–6.65×10^6. On the contrary, there was a reduction in the molecular weight (7.20×10^5) of the polymers obtained with different nitrogen/glycerol feeding rates, which may be related to the higher volumetric productivities and different carbon/nitrogen ratio of the fermentation broth (Abdhul et al., 2014). High productivity and high molecular weight are special properties of this EPS that make it more useful for the new application. High molecular weight EPSs have the ability to be exploited as hydrogel in medical applications, such as tissue engineering and drug delivery because of great swelling behavior and having great capacity to hold water (Bueno et al., 2013).

The ^{1}H NMR and ^{13}C NMR spectroscopy of the EPS from *Pantoea* sp. BCCS 001 GH is shown in (Supp Fig. 2). The signals in the^{1}H and^{13}C NMR spectra of the EPS were determined based on the component and methylation analyses and on values reported in the researchers (Aruna et al., 2017; Fathi et al., 2016; Ibarburu et al., 2015; Li et al., 2015; Li et al., 2016; Polak-Berecka et al., 2015; Wang et al., 2015). Two major chemical shift in the anomeric region (4–5.5) were found that (5.311 and 5.298) in ^{1}H NMR (Supp Fig. 2), suggesting the presence of α-anomers (Aruna et al., 2017). The peak at δ 5.311 and δ 5.298 ppm correspond to glucopyranosyl reducing end. Moreover, the intensity of signal around δ 2 ppm, characteristic of N-acetyl groups from glucosamine (Ibarburu et al., 2015). The peak δ 3.566 ppm indicated the presence of carbohydrate derivatives and alcohols, H–C–OH and δ 3.710, 3.889, 3.910, 3.930 and 3.942 ppm indicated that the presence of esters RCOO–C–H (Aruna et al., 2017). The ^{13}C NMR spectrum (Supp Fig. 2), of EPS included anomeric carbons peaks in the regions (δC 92–105 ppm) and ring carbons (δC 50–85 ppm) regions (Wang et al., 2015). Also, it was indicated that the sugar moieties in the polysaccharide possessed an α-configuration (δC 103.6). The signal at δ 103.610 corresponds to the anomeric carbon region of α-glucopyranosyl unit reducing end (Li et al., 2015; Wang et al., 2015). This result corresponds to previous results from monosaccharide profile. In addition, the signal at between range δC 60 and 70 ppm is related to C_6 and δC 70–81 ppm which is corresponded to C_2, C_3, and C_5 (Li et al., 2016). No signal at δC 90 ppm was suggesting that there was only pyranose ring configuration for all sugars (Liang et al., 2016), that it prove FTIR results. The amino sugars were not detected by monosaccharide analysis of the EPS (GC-M) as described above. Besides, no signals between δC 50 and 60 ppm were exhibited for amino sugars, only signals at δC 23.636 that was probably from C-2 and N-acetyl group. Based on our knowledge, the detailed chemical structure of the EPS from strain *Pantoea* sp. BCCS 001 GH needs to be further studied. Hence, on the basis of the methylation analysis, FTIR and NMR spectrum, the structure of the repeating glucose unit present in the EPS isolated from *Pantoea* sp. BCCS 001 GH strain was established in (Supp Fig. 3). XRD was carried out to understand the nature of EPS resembling amorphous or crystalline. Figure 5(D) depicts all diffraction peaks of EPS. From XRD pattern, it was found that the EPS has an amorphous structure without any characteristic diffraction peaks. Similar patterns without any crystalline nature were reported earlier for very important EPS (xanthan gum) that was produced by kitchen waste as the sole substrate (Li et al., 2016). In addition, characterization of galactan EPS produced by *Weissella confuse* KR780676 was shown two sharp narrow diffraction peaks that indicate the crystalline nature whereas broad peak indicates amorphous component, it is difficult to interpret broad amorphous peaks of several amorphous biopolymers (Kavitake et al., 2016).

Scanning electron microscopic of EPS

Scanning electron microscopy is a powerful tool to observe the three-dimensional structure and surface morphology of macromolecules and it can be helpful to clarify their common physical properties. The micrograph of the microstructures of EPS is presented by SEM, shown in Fig. 6. The EPS revealed the tine film, leaf-like with glittering surface structural units. At higher magnification, additional details of the microstructure of the EPS were visible. The EPS had smoother uniform particles rather than a feather-like structural units (Li et al., 2015), the different shape, structure and surface topography of the EPS were most likely caused by differences in the physicochemical properties of the EPS and by differences in sample extraction, purification, drying or method of sample preparation for analysis. Ahmed et al. (2013), reported EPSs similar to thin films with very smooth and glittering surface. In addition, EPS sheet-like compact morphology from *L. plantarum* KF5 was identified by Wang et al. (2010) that was very similar to *Pantoea* sp. BCCS 001 GH EPS. The highly branched and porous structure of the EPS from strain *Lactobacillus Plantarum* YW11 was observed and stressed about its application in foods to improve the physical properties including, viscosity, water-holding capacity of the product (Wang et al., 2015).

The EPS fermented by newly bacterium *Pantoea* sp. BCCS 001 GH strain isolated from nectarine fruit that showed different physicochemical structure. The highest potential in EPS production with the maximum yield of 67.5% (13.50 g/L) was investigated when sucrose was used in the medium as a carbon source. Monosaccharide

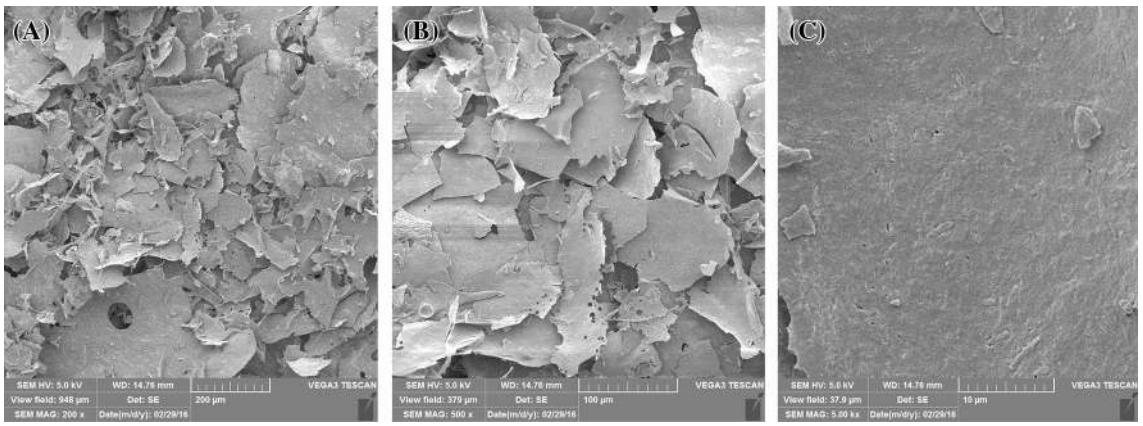

Fig. 6 Scanning electron microscopic of EPS, (**A**) at 200×, (**B**) at 500×, and (**C**) at 5000×

analysis showed that EPS are heteropolysaccharide mainly consisted of two monomers, including glucose and galactose in an approximate ratio of 5.75:1 with high average molecular weight (2.522×10^6 Da) that it has a high potential for using in the medical and food product in future works. The UV–vis absorption spectra of the EPS showed no absorption at 280 and 260 nm, implying the absence of both nucleic acid and protein present in the EPS sample. The FTIR spectrum indicated that the polysaccharide contained a significant number of hydroxyl and the amide C-O and carboxyl groups. The ^{1}H NMR and ^{13}C NMR spectroscopy of the EPS *Pantoea* sp. BCCS 001 GH were shown possessed monomer with α–configuration and corresponds to previous results from monosaccharide analysis as new EPS. High EPS production makes this opportunity for further studies to motivate the more detailed analysis of its physicochemical characterizations and on the application of fermentation EPS in different food products with the abundant substrate.

Acknowledgements Authors are sincerely thankful to Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences for supporting the joined project.

References

Abdhul K, Ganesh M, Shanmughapriya S, Kanagavel M, Anbarasu K, Natarajaseenivasan K. Antioxidant activity of exopolysaccharide from probiotic strain *Enterococcus faecium* (BDU7) from Ngari. Int. J. Biol. Macromol. 70: 450–454 (2014)

Ahmed Z, Wang Y, Anjum N, Ahmad A, Tariq Khan S. Characterization of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir Part II. Food. Hydrocoll. 30: 343–350 (2013)

Amellal N, Burtin G, Bartoli F, Heulin T. Colonization of wheat roots by an exopolysaccharide-producing *Pantoea agglomerans* strain and its effect on rhizosphere soil aggregation. Appl. Environ. Microbiol. 64: 3740–3747 (1998)

Aruna J, Selvakumar S, Sathishkumar R, Moovendhan M, Ananthan G, Maruthiah T, Palavesam A. In vitro antioxidant activities of an exopolysaccharide from a salt pan bacterium *Halolactibacillus miurensis*. Carbohydr. Polym. 155: 400–406 (2017)

Banik RM, Santhiagu A, Upadhyay SN. Optimization of nutrients for gellan gum production by *Sphingomonas paucimobilis* ATCC-31461 in molasses based medium using response surface methodology. Bioresour. Tech. 98: 792–797 (2007)

Bueno B, Bentini R, Catalani LH, Petri DF. Synthesis and swelling behavior of xanthan-based hydrogels. Carbohydr. Polym. 92: 1091–1099 (2013)

Chawla PR, Bajaj IB, Survase SA, Singhal RS. Microbial cellulose: fermentative production and applications. Food Tech. Biotech. 47: 107–124 (2009)

Fathi M, Mohebbi M, Koocheki A. Introducing *Prunus cerasus* gum exudates: chemical structure, molecular weight, and rheological properties. Food Hydrocoll. 61: 946–955 (2016)

Freitas F, Alves VD, Pais J, Costa N, Oliveira C, Mafra L, Hilliou L, Oliveira R, Reis MA. Characterization of an extracellular polysaccharide produced by a *Pseudomonas* strain grown on glycerol. Bioresour. Technol. 100: 859–865 (2009)

Freitas F, Alves VD, Reis MA. Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends Biotechnol. 29: 389–398 (2011)

Galindo E, Salcedo G. Detergents improve xanthan yield and polymer quality in cultures of *Xanthomonas campestris*. Enzyme Microb. Technol. 19: 145–149 (1996)

Grobben GJ, Chin-Joe I, Kitzen VA, Boels IC, Boer F, Sikkema J, Smith MR, De Bont JAM. Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii subsp. bulgaricus* NCFB 2772 with a simplified defined medium. Appl. Environ. Microbiol. 64: 1333–1337 (1998)

Ibarburu I, Puertas AI, Berregi I, Rodríguez-Carvajal MA, Prieto A, Dueñas MT. Production and partial characterization of exopolysaccharides produced by two *Lactobacillus suebicus* strains isolated from cider. Int. J. Food. Microbiol. 214: 54–62 (2015)

Kavitake D, Devi PB, Singh SP, Shetty HP. Characterization of a novel galactan produced by *Weissella confusa* KR780676 from an acidic fermented food. Int. J. Biol. Macromol. 86: 681–689 (2016)

Kumar AS, Mody K, Jha B. Bacterial exopolysaccharides–a perception. J. Basic Microbiol. 47: 103–117 (2007)

Li P, Li T, Zeng Y, Li X, Jiang X, Wang Y, Xie T, Zhang Y. Biosynthesis of xanthan gum by *Xanthomonas campestris* LRELP-1 using kitchen waste as the sole substrate. Carbohydr. Polym. 151: 684–691(2016)

Li W, Xia X, Tang W, Ji J, Rui X, Chen X, Jiang M, Zhou J., Zhang Q, Dong M. Structural characterization and anticancer activity of

cell-bound exopolysaccharide (c-EPS) from *Lactobacillus helveticus* MB2-1. J. Agric. Food Chem. 63: 3454–3463 (2015)

Li H, Xu H, Xu H, Li S, Ouyang PK. Biosynthetic pathway of sugar nucleotides essential for welan gum production in *Alcaligenes* sp. CGMCC2428. Appl. Microbiol. Biotechnol. 86: 295–303 (2010)

Liang TW, Tseng SC, Wang SL. Production and characterization of antioxidant properties of exopolysaccharide (s) from *Peanibacillus mucilaginosus* TKU032. Mar. Drugs 14: 40 (2016)

Lin ES. Production of exopolysaccharides by submerged mycelial culture of *Grifola frondosa* TFRI1073 and their antioxidant and antiproliferative activities. World J. Microbiol. Biotechnol. 27: 555–561 (2011)

Mao DB, Shi CW, Wu JY, Xu CP. Optimization of exopolysaccharide production in submerged culture of *Daedalea dickinsii* and its antioxidant activity. Bioprocess. Biosyst. Eng. 37: 1401–1409 (2014)

Matsumoto Y, Kuroyanaqi Y. Development of a wound dressing composed of hyaluronic acid sponge containing arginine and epidermal growth factor. J. Biomater. Sci. Polym. Ed. 21: 715–726 (2010)

Nicolaus B, Kambourova M, Oner ET. Exopolysaccharides from extremophiles: from fundamentals to biotechnology. Environ. Technol. 31: 1145–1158 (2010)

Niknezhad SV, Asadolahi MA, Zamani A, Biria D. Production of xanthan gum by *Xanthomonas* species using starch as carbon source. Minerva Biotechnologica. 26: 191–197 (2014)

Niknezhad SV, Asadolahi MA, Zamani A, Biria D. Production of xanthan gum by free and immobilized cells of *Xanthomonas campestris* and *Xanthomonas pelargonii*. Int. J. Biol. Macromol. 82: 851–856 (2016)

Niknezhad SV, Asadolahi MA, Zamani A, Biria D, Dostmohammadi M. Optimization of xanthan gum production using cheese whey and response surface methodology. Food Sci. Biotechnol. 24: 453–460 (2015)

Polak-Berecka M, Choma A, Waśkoa A, Górskad S, Gamian A, Cybulska J. Physicochemical characterization of exopolysaccharides produced by *Lactobacillus rhamnosus* on various carbon sources. Carbohydr. Polym. 117: 501–509 (2015)

Prasertsan P, Dermlim W, Doelle H, Kennedy JF. Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. Carbohydr. Polym. 66: 289–297 (2006)

Premjet S, Premjet D, Ohtani Y. The Effect of ingredients of sugar cane molasses on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245. Sen'i. Gakkaishi. 63: 193–199 (2007)

Rawal PM, Chauhan PB, Prajapati H, Gahlout M. Evaluation of cultivation condition for enhanced production of exopolysaccharide by bacterial isolate P 11 under submerged culture condition. Int J Adv. Res. Biol. Sci. 3: 183–190 (2016)

Ruffing A, Chen RR. Metabolic engineering of microbes for oligosaccharide and polysaccharide synthesis. Microb. Cell. Fact. 5: 25 (2006)

Silvi S, Barghini P, Aquilanti A, Jimenez BJ, Fenice M. Physiologic and metabolic characterization of a new marine isolate (BM39) of *Pantoea* sp. producing high levels of exopolysaccharide. Microb Cell. Fact. 12: 10 (2013)

Sirajunnisa AR, Vijayagopal V, Sivaprakash B, Viruthagiri T, Surendhiran D. Optimization, kinetics and antioxidant activity of exopolysaccharide produced from rhizosphere isolate, *Pseudomonas fluorescens* CrN6. Carbohydr. Polym. 135: 35–43 (2016)

Sivakumar T, Sivasankara Narayani S, Shankar T, Vijayabaskar P. Optimization of cultural conditions for exopolysaccharides production by *Frateuria aurentia*. Int. J. Appl. Biol. Pharm. Technol. 3: 133–143 (2012)

Takeuchi A, Kamiryou Y, Yamada H, Eto M, Shibata K, Haruna K, Naito S, Yoshikai Y. Oral administration of xanthan gum enhances antitumor activity through Toll-like receptor 4. Int. Immunopharmacol. 9: 1562–1567 (2009)

Torres CA, Marques R, Ferreira AR, Antunes S, Grandfils C, Freitas F, Reis MA. Impact of glycerol and nitrogen concentration on *Enterobacter* A47 growth and exopolysaccharide production. Int. J. Biol. Macromol. 71: 81–86 (2014)

Trabelsi I, Slima SB, Chaabane H, Riadh BS. Purification and characterization of a novel exopolysaccharides produced by *Lactobacillus* sp. Ca6. Int. J. Biol. Macromol. 74: 541–546 (2015)

Wang H, Jiang X, Mu H, Liang X, Guan H. Structure and protective effect of exopolysaccharide from *P. Agglomerans* strain KFS-9 against UV radiation. Microbiol. Res. 162: 124–129 (2007)

Wang Y, Li C, Liu P, Ahmed Z, Xiao P, Bai X. Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet Kefir. Carbohydr. Polym. 82: 895–903 (2010)

Wang J, Zhao X, Tian Z, Yang Y, Yang Z. Characterization of an exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibet Kefir. Carbohydr. Polym. 125: 16–25 (2015)

Xu X, Hu Y, Quan L. Production of bioactive polysaccharides by *Inonotus obliquus* under submerged fermentation supplemented with lignocellulosic biomass and their antioxidant activity, Bioprocess. Biosyst. Eng. 37: 2483–2492 (2014)

Zanchetta P, Lagarde N, Guezennec J. A new bone-healing material: a hyaluronic acid-like bacterial exopolysaccharide. Calcif. Tissue Int. 72: 74–79 (2003)

Zare MH, Mohkam M, Morowvat MH, Nezafat N, Ghasemi Y. Probiotic potential of five *Lactobacillus* strains isolated from traditional persian yoghurt in fars province, Iran: viewing through the window of phylogenetics. Biosc. Biotechnol. Res. Asia. 12: 1265–1272 (2015)