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Production, purification and application of polysaccharide-based bioflocculant by *Paenibacillus mucilaginosus*

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ABSTRACT

The production and purification of polysaccharide-based bioflocculants (PSBs) by *Paenibacillus mucilaginosus* GIM1.16 in metal ion-supplemented medium and basal medium were evaluated. Three purified PSB1-1, PSB2-1 and PSB3-1 possessed different monosaccharide composition and their molecular weights were 2.53×10^6, 7.77×10^6 and 13.2×10^6 Da, respectively. FT-IR spectrometry indicated the presence of hydroxyl, carboxyl and phosphate groups in the three samples. Scanning electron microscopy showed that they had linear structure. The potential of these PSBs on wastewater treatment was evaluated. Among them, PSB1-1 exhibited the best performance, as it had high flocculating activities (above 94%) at 0.5–4 mg/L and could achieve high flocculating activities (above 97%) in the kaolin suspensions of pH 3–9. PSB1-1 was the key factor that might explain the enhanced flocculating activity of the supernatant from metal ion-supplemented medium. The performance of PSB1-1 on industrial wastewater was also satisfactory. PSB1-1 might be a good candidate as bioflocculant.

1. Introduction

Industrial wastewater treatments have been a focus of research in the worldwide, and flocculation was thought to be an excellent approach for removing pollutants from wastewater. Though many inorganic and organic synthetic flocculants performed well during the flocculation process, the environmental and health problems could not be neglected (Li et al., 2013). In this case, bioflocculant could be an alternative due to its innocuousness and biodegradability (Liu et al., 2013). Generally speaking, bioflocculants are the metabolites of microorganisms, which are composed of polysaccharide, protein, nucleic acid and lipid, etc. (Shih, Van, Yeh, Lin, & Chang, 2001). Among these biomacromolecules, the polysaccharide-based bioflocculants (PSBs) received considerable attention in recent years due to their high efficiency in disposing of different kinds of pollutants, including dyeing pigment, heavy metal ion, and other suspended pollutants (Deng, Yu, & Ting, 2005; Li et al., 2013; Ye et al., 2014). However, low flocculating activity and narrow application scope were the main barriers for the large-scale application of PSBs (Li et al., 2013). For example, the application of many reported bioflocculants was constrained by the pH range (Aljuboori, Idris, Abdullah, & Mohamad, 2013; Elkady, Farag, Zaki, Abu-Elreesh, & Abd-Ei-Haleem, 2011; Li, Zhou, Zhang, Wang, & Zhu, 2008). Although many studies have been carried out to improve the flocculating activity of PSBs, or develop new-type bioflocculants (Aljuboori et al., 2013; Li et al., 2013; Xia et al., 2008), results were not good enough for commercial applications due to high production cost. A bioflocculant candidate with high flocculating activity and wide application range of pH would be of significance.

Up to now, several reported PSBs were produced by different microorganisms including bacterium and fungi, such as *Bacillus mojavensis*, *Pseudoalteromonas* sp. and *Aspergillus flavus* (Aljuboori et al., 2013; Elkady et al., 2011; Li et al., 2008). Most of these microorganisms were isolated from sludge, wastewater or seawater (Xia et al., 2008; Yim, Kim, Ahn, & Li, 2007). *Paenibacillus mucilaginosus* is one kind of soil bacteria that has been widely studied in the aspect of silicate mineral weathering and agriculture (Basak & Biswas, 2009; Hu, Chen, & Guo, 2006). In recent years, *P. mucilaginosus* and its metabolites (mainly PSBs) have been used in heavy ions absorption (Mo & Lian, 2010; Yi & Lian, 2012). However, the studies of *P. mucilaginosus* in PSBs production are rare. In our previous work, we found that by adding small amount of metal

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ions complex (Mg^{2+}, Ca^{2+} and Fe^{3+}) to the culture medium for *P. mucilaginosus* GIM1.16 could improve the flocculating activity of the culture supernatant significantly. Further studies are needed to find out the key factor that might explain the phenomenon. In this study, *P. mucilaginosus* GIM1.16 was cultured in two different culture medium (metal ion-supplemented medium and the metal ion-free basal medium) to produce PSBs. The purification, characterization, flocculating activity and the application of the PSBs in wastewater treatment were also investigated.

2. Material and methods

2.1. Bacterial strain and culture conditions

P. mucilaginosus strain GIM1.16 was isolated from a soil sample and provided by the Microbial Culture Collection Center of Guangdong Institute of Microbiology, Guangzhou, China. The strain was cultured in nitrogen-free slant (sucrose 10 g/L, Na_2HPO_4 1 g/L, $MgSO_4$ 0.2 g/L, $CaCO_3$ 0.1 g/L, $FeCl_3$ 5 mg/L, agar 20 g/L, pH 7.5) at 30 °C for 3 d, and then stored at 4 °C.

2.2. Production of PSBs by P. mucilaginosus GIM1.16

The preculture of *P. mucilaginosus* GIM1.16 was prepared from nitrogen-free medium (sucrose 10 g/L, Na_2HPO_4 1 g/L, $MgSO_4$ 0.2 g/L, $CaCO_3$ 0.1 g/L, $FeCl_3$ 5 mg/L, pH 7.5) for 36 h at 30 °C with a shaking speed of 200 rpm. Then, the seed culture was inoculated into basal medium (sucrose 20 g/L, yeast extract 0.5 g/L, Na_2HPO_4 2 g/L, pH 7.5) or metal ion-supplemented medium (sucrose 20 g/L, yeast extract 0.5 g/L, Na_2HPO_4 2 g/L, $MgSO_4$ 0.144 g/L, $CaCl_2$ 0.028 g/L, $FeCl_3$ 1.6 mg/L, pH 7.5), respectively. The PSBs were produced by shaking the inoculated flasks at 30 °C and 200 rpm for 72 h. After 72 h incubation, cell-free supernatant was collected by centrifugation at 10,000 × *g* for 20 min and used for the purification of PSBs. Two kinds of supernatant were collected, i.e., culture supernatant 1 (from the metal ion-supplemented medium) and culture supernatant 2 (from the basal medium). The concentrations of PSBs were determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3. Purification of the PSBs

The PSBs were purified from the culture supernatant 1 or the culture supernatant 2, respectively. The supernatant was mixed with anhydrous ethanol (1:3, v/v), stirred vigorously and left overnight at 4 °C for PSB precipitation. The precipitate was collected by centrifugation at 10,000 rpm for 15 min and redissolved in distilled water. Equal volume of Sevag reagent (chloroform: *n*-butanol = 5:1, v/v) was mixed thoroughly with the above-mentioned solution for seven times to remove the residual protein. The deproteinized solution was then dialyzed against distill water and lyophilized to provide the crude PSB for further purification. As a result, two kinds of crude PSB, namely, crude P1 (from culture supernatant 1) and crude P2 (from culture supernatant 2) were obtained. Crude P1 and crude P2 were purified sequentially by chromatography of DEAE-52 and Sephadex G-200. Crude P1 or crude P2 (10 mg/mL) was added to a column of DEAE-52 (2.6 × 30 cm) and the column was stepwise eluted with distill water, 0.1, 0.3, 0.5, 0.7 and 1 M NaCl at a flow rate of 0.6 mL/min. Eluate (6 mL/tube) was collected automatically and carbohydrates were determined by the phenol-sulfuric acid method. Three fractions (PSB1, PSB2 and PSB3) were obtained, concentrated and followed by further purification through Sephadex G-200 column (2.6 × 50 cm) to afford PSB1-1, PSB2-1 and PSB3-1, respectively. The PSB1-1, PSB2-1 and PSB3-1 were then concentrated, dialyzed and lyophilized for purity analysis.

2.4. Physical and chemical analysis of the purified PSB1-1, PSB2-1 and PSB3-1

2.4.1. Molecular weight analysis

The molecular weight of the purified PSB1-1, PSB2-1 and PSB3-1 were measured by Gel Permeation Chromatography (GPC). In brief, two TSK columns (G5000PWXL and G3000 PWXL) in series were equipped on HPLC (Waters 2965, USA) and eluted with 0.02 M KH_2PO_4 at a flow rate of 0.6 mL/min. The eluent was monitored with a refractive index detector (RID). The molecular weight analysis was based on the standard curve established by dextrans with different molecular weight.

2.4.2. Monosaccharide composition analysis

Monosaccharide composition analysis of the purified PSB1-1, PSB2-1 and PSB3-1 was carried out as follows: five microgram of PSB1-1, PSB2-1 or PSB3-1 was hydrolyzed with 4 mL of 2 M trifluoroacetic acid (TFA) at 105 °C for 4 h. The residual TFA were removed by methanol. The trimethylsilyl (TMS) derivative of hydrolyzate was prepared by adding 1 mL pyridine, 0.4 mL hexamethyldisilazane and 0.2 mL trimethychlorosilane and heating at 80 °C for 30 min, then followed by gas chromatography (GC) analysis (Agilent 7890A, USA) equipped with a flame ionization detector (FID) and a HP-5 fused silica capillary column (30 cm × 0.32 mm × 0.25 mm). The chromatographic conditions were as follows: nitrogen gas (carrier gas) was at a flow rate of 1 mL/min, temperature of injector and detector were 250 °C and 300 °C, respectively. The initial column temperature was held at 110 °C for 1 min, then ascended at a rate of 5 °C/min to 180 °C and held for 3 min, subsequently at a rate of 10 °C to 240 °C and held for 3 min.

2.4.3. Functional group analysis

The functional group analysis of the purified PSB1-1, PSB2-1 and PSB3-1 was carried out in a Fourier transform infrared (FT-IR) spectrophotometer (Bio-Red FTIR Model FTS 135, USA) over a wave number range of 4000–500 cm^{-1}.

2.4.4. Ultra-structure characterization

The ultra-structure of the purified PSB1-1, PSB2-1 and PSB3-1 was characterized by scanning electron microscopy (SEM) (EVO-LS10, ZEISS, Germany).

2.4.5. Carbohydrate content analysis

The contents of carbohydrate in the purified PSBs (PSB1-1, PSB2-1 and PSB3-1) were analyzed by phenol-sulfuric acid method (Dubois et al., 1956).

2.5. Flocculating activity of the PSBs

Flocculating activities of the culture supernatant 1, culture supernatant 2, crude P1, crude P2, purified PSB1-1, PSB2-1 and PSB3-1 samples were studied. In general, a 1 L beaker containing 5 g/L kaolin suspension was placed in a six league of electric mixer for agitation (150 rpm for 4 min, then 50 rpm for 10 min, and a final standing still for 10 min). Appropriate amount of sample and 2 mM $CaCl_2$ were added to the kaolin suspension at pH 8. In the case of studying the effect of sample concentration on flocculating activity, the concentration of samples were prepared in the range of 0.5–4 mg/L, while in the case of studying the effect of pH on flocculating activity, the pH of kaolin suspension was adjusted from

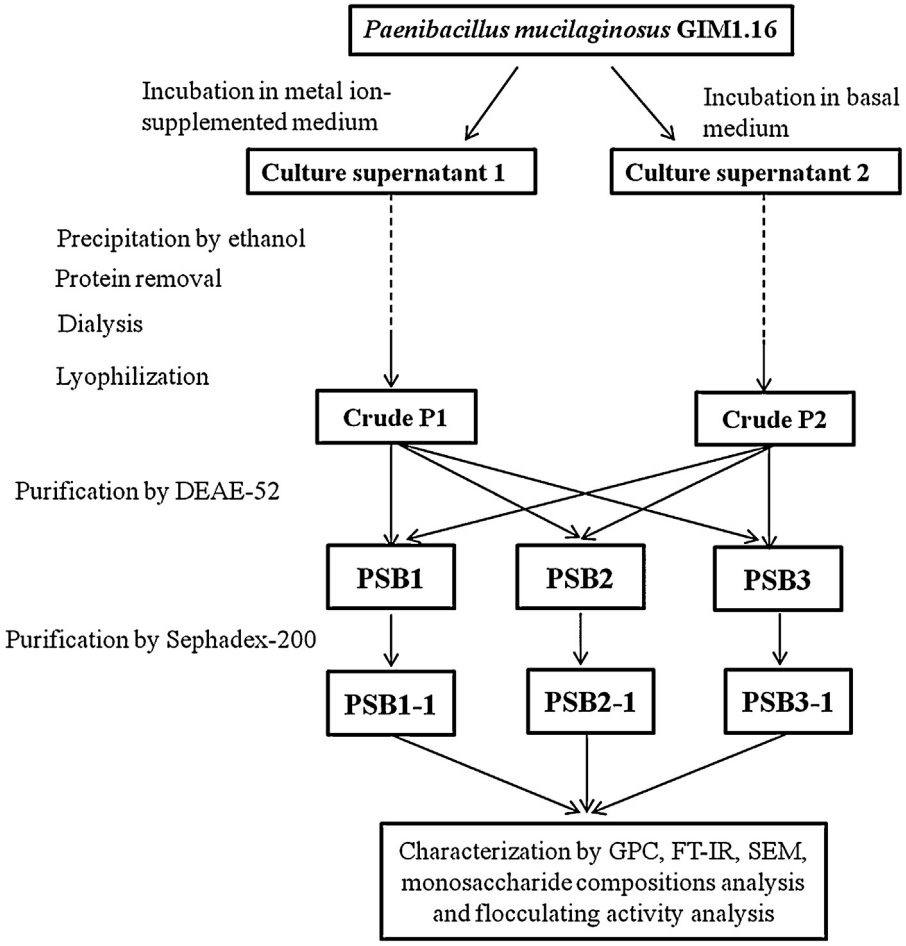

Fig. 1. The flow chart of the production and purification of the PSBs.

3 to 9. The absorbance of the sample (OD_{sample}) and the blank (OD_{blank}) were measured at 550 nm with a spectrophotometer (APL-752N, Shanghai, China). All experiments were performed in triplicates. The flocculating activity was measured by the following equation:

$$\text{Flocculating activity}\ (\%) = \frac{(OD_{blank} - OD_{sample})}{OD_{sample}} \times 100\% \quad (1)$$

2.6. Wastewater treatment assay

To further evaluate the capacities of wastewater treatment of crude P1 and PSB1-1, three different types of industrial wastewater with different pH were collected from a paper mill (pH 8.57), a biological product factory (pH 7.11) and a garbage incineration plant (pH 6.08), respectively. Flocculating activities were determined as described in 2.5, pH adjustment excluded. The chemical oxygen demand (COD) and solid suspension (SS) were detected according to National Standard of People's Republic of China. The removal rates of COD and SS were calculated as follows:

$$\text{Removal rate} = \frac{(C_0 - C_1)}{C_0} \times 100\% \quad (2)$$

where C_0 is the initial concentration and C_1 is the residual concentration after treatment.

3. Results and discussion

3.1. Production and purification of the PSBs by P. mucilaginosus GIM1.16

In our previous work, we observed that by adding small amount of metal ions complex (Mg^{2+}, Ca^{2+} and Fe^{3+}) to the culture medium for *P. mucilaginosus* GIM1.16 could improve the flocculating activity of the culture supernatant by 33%, compared to that of basal medium. However, the underlying reason was unclear. Therefore, in this study, the production and purification of the PSBs by *P. mucilaginosus* GIM1.16 in metal ion-supplemented medium and basal medium were studied, aiming to find out the key factor that might explain the phenomenon. The flow chart of the production and purification of the PSBs was shown in Fig. 1.

3.1.1. Production of PSBs in metal ion-supplemented medium and basal medium

As shown in Fig. 1, culture supernatant 1 and culture supernatant 2 were collected after *P. mucilaginosus* GIM1.16 was cultured in metal ion-supplemented medium (0.144 g/L $MgSO_4$, 0.028 g/L $CaCl_2$, 1.6 mg/L $FeCl_3$) and basal medium for 72 h, respectively. The concentration of PSBs from culture supernatant 1 (about 1.335 g/L) was 3.3 times as much as which from the culture supernatant 2 (about 0.410 g/L). Besides, the flocculating activity of culture supernatant 1 (about 89.7%) was increased by 33% when compared with culture supernatant 2 (about 67.4%) (Table 1). It appeared that the flocculating activity increasement of culture supernatant 1 compared with culture supernatant 2 was due to the improved

Table 1
Effect of metal ions complex on the PSBs concentration and the flocculating activity of culture supernatant.

	Concentration of PSBs (g/L)	Flocculating activity (%)
Culture supernatant 1	1.335 ± 0.052	89.7 ± 3.4
Culture supernatant 2	0.410 ± 0.038	67.4 ± 2.8

production of PSBs. With regard to the PSBs production, similar result was observed in the production of antifungal compound in *Paenibacillus polymyxa* SQR-21, where the production of antifungal compound was increased by 2.2 times through adding multiple metal ions (Ca^{2+}, Ni^{2+}, Mn^{2+} and Cu^{2+}) to the medium (Raza, Wu, & Shen, 2010a). Effects of individual metal ion (Mn^{2+}, Mg^{2+} or Ca^{2+}) on the flocculating activity of PSBs from the culture supernatant were reported, with only 3–6% increasement in the flocculating activity (Liu, Wang, Li, Yuan, & Yang, 2010; Aljuboori et al., 2013). In this study, the effects of metal ions complex (Mg^{2+}, Ca^{2+} and Fe^{3+}) on the production of PSBs by *P. mucilaginosus* GIM1.16 were investigated for the first time. Results suggested that the metal ions complex might exert important effects on the production and flocculating activity of metabolites in *P. mucilaginosus* GIM1.16. Besides, adding metal ions complex to medium might also be a cost-effective way for PSBs production. As comparing with another common way to enhance PSBs production and activities by increasing carbon and nitrogen sources which are known to be expensive ingredients for industrial production (Li et al., 2013; Aljuboori et al., 2013), metal ions complex are relatively cheaper but with better performance in improving PSBs production and activities.

3.1.2. Purification of PSBs

After ethanol precipitation, protein removal, dialysis and lyophilization, crude P1 was prepared from culture supernatant 1 and crude P2 was prepared from culture supernatant 2, respectively. Then, after separation through an anion-exchange chromatography of DEAE-52, both crude P1 and crude P2 were separated into three independent elution fractions, designated as PSB1, PSB2 and PSB3, respectively (Fig. 2). The recovery rates of the purification from crude P1 and crude P2 were 82.9% and 75.6%, respectively. The amount (mg) of PSB1, PSB2 and PSB3 in the crude P1 and crude P2 was demonstrated in Table 2. Results revealed that PSB2 was the major component in crude P1, whereas PSB2 and PSB3 were the major components in crude P2. When comparing with the different amount of PSB1, PSB2 and PSB3 from crude P1

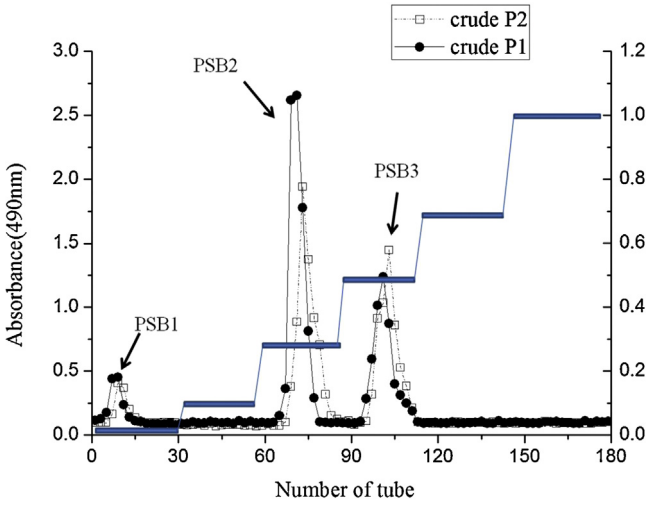

Fig. 2. Stepwise elution curves of crude P1 and crude P2 on anion-exchange chromatography column DEAE-52

Table 2
The amount of PSB1, PSB2 and PSB3 in crude P1 and crude P2.

sample	PSB1 (mg)	PSB2 (mg)	PSB3 (mg)
*Crude P1	77.4	882.5	147.1
*Crude P2	20.9	178.0	111.0

* The crude P1 was prepared from 1 L culture supernatant 1 and the crude P2 was prepared from 1 L culture supernatant 2.

and crude P2, the amount of PSB1 and PSB2 from crude P1 was 3.7 times (77.4 mg vs. 20.9 mg) and 5.0 times (882.5 mg vs. 178.0 mg) as much as which from crude P2, while the amount of PSB3 from crude P1 was slightly higher than that from crude P2 (147.1 mg vs. 111.0 mg). Therefore, based on the result, PSB1 and PSB2 might be the main components contribute to the enhanced flocculating activity of culture supernatant 1.

In addition, the result implied that the metal ions complex (Mg^{2+}, Ca^{2+} and Fe^{3+}) in the culture medium not only increased the PSBs productivity, but also changed the constitution of PSBs. Similar result was observed in brown algae, in which the production of cell wall polysaccharides was significantly increased, and the monosaccharide composition of polysaccharide was changed under high level of Zn^{2+} and Cd^{2+} (Andrade et al., 2010). In fact, different metal ions as well as their oxide minerals are important to the types and the quantity of polysaccharides, proteins and enzymes during the growth process of bacteria (Raza et al., 2010b). However, the underlying mechanisms of how the metal ions complex exerted their influences on the production and the constitution of bacterial metabolites were not clear. It is presumed that the metal ions might activate certain enzymes responsible for activating the regulatory function of the cells in favor of PSB biosynthesis.

Fraction PSB1, PSB2 and PSB3 were further purified through a column of Sephadex-200. As a result, each fraction generated one single elution peak (Fig. 3), namely, PSB1-1, PSB2-1 and PSB3-1, respectively. The recovery rates of the purification from crude P1 and crude P2 were 74.2% and 69.4%, respectively. The carbohydrate contents of PSB1-1, PSB2-1 and PSB3-1 were 96.8%, 95.7% and 97.8%, respectively, which could confirm that the major components of the purified PSB1-1, PSB2-1 and PSB3-1were polysaccharides.

3.2. Characterization of the purified PSB1-1, PSB2-1 and PSB3-1

3.2.1. Molecular weight of PSB1-1, PSB2-1 and PSB3-1

For the determination of molecular weight, the purified PSB1-1, PSB2-1 and PSB3-1 were measured by Gel Permeation Chromatography (GPC), respectively. GPC results showed there was only one main peak for each purified sample (see Supplementary information Fig. S1), indicating that the PSB1-1, PSB2-1 and PSB3-1 were quite homogeneous. Referring to a calibration curve by dextrans, the average molecular weight of PSB1-1, PSB2-1 and PSB3-1 were estimated to be 2.53×10^6, 7.77×10^6 and 13.2×10^6 Da, respectively. The molecular weight of the purified PSB2-1 and PSB3-1 were higher than other bioflocculants produced by a variety of microorganisms, such as *Bacillus licheniformis*, *Arthrobacter* and *Paenibacillus elgii* (Li et al., 2009, 2013; Ye et al., 2014).

3.2.2. Monosaccharide compositions of PSB1-1, PSB2-1 and PSB3-1

The purified PSB1-1, PSB2-1 and PSB3-1 were hydrolyzed with TFA to determine the sugar composition. The results revealed that PSB1-1 was composed of fructose, galactose, sorbose, glucose and rhamnose at a ratio of 1:1.08:2.45:1.66:2.78, whereas PSB2-1 and PSB3-1 were composed of mannose, fructose, galactose, sorbose, glucose and xylose at a ratio of 1:1.16:1.12:2.4:1.72:2.88 and 1:1.23:1.07:3.3:1.7:3.8, respectively. The monosaccharide compositions of PSB1-1, PSB2-1 and PSB3-1 were different from each

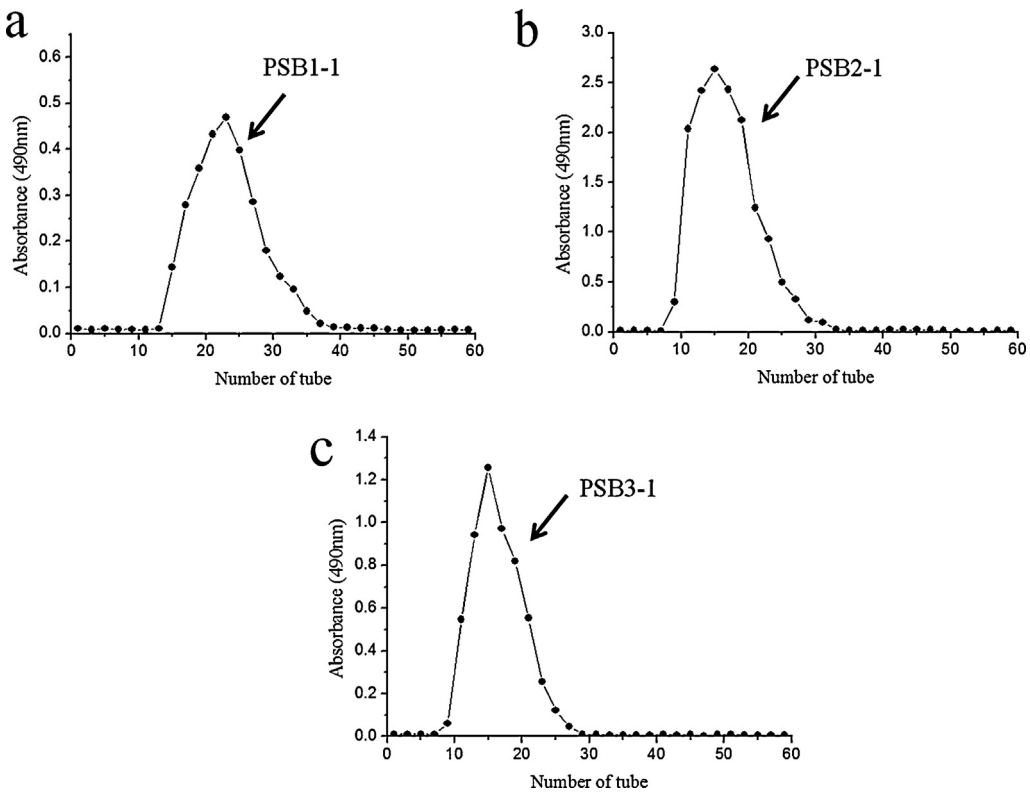

Fig. 3. Elution curve of fractions (PSB1, PSB2 and PSB3) on Sephadex G-200.

other. And the polysaccharides were different from those produced by other *Paenibacillus* strains (Li et al., 2013; Liu et al., 2009; Raza, Makeen, Wang, Xu, & Qirong, 2011). These dissimilarities reflect the species specific production and biotechnological potential of polysaccharides (Raza et al., 2011).

3.2.3. Functional group analysis of PSB1-1, PSB2-1 and PSB3-1

FT-IR spectroscopy was performed on the purified PSB1-1, PSB2-1 and PSB3-1. In general, the spectrum of PSB2-1 was similar with that of PSB3-1, but different from that of PSB1-1. The results agreed with their monosaccharide composition analysis which revealed that PSB2-1 and PSB3-1 were comprised of the same monosaccharides. The functional group characteristics of each sample were analyzed as follows (Fig. 4): a strong and wide absorption band of about 3200-3400 cm^{-1} was related to O—H stretching vibrations. The signal at 2800–3000 cm^{-1} was related to stretching vibrations of C—H bond, which indicated typical IR spectra of polysaccharide. The signal at 1720–1730 cm^{-1} was attributed to ester bonds. Two characteristic absorptions of carboxyl groups were a signal at 1600–1700 cm^{-1} (C=O asymmetric stretching vibrations) and a peak of about 1350–1450 cm^{-1} (C=O symmetric stretching vibrations). A peak of about 1250–1280 cm^{-1} indicated the presence of phosphate groups. The signal at 900–1150 cm^{-1} was related to C—O—C bond.

3.2.4. Ultra-structural characterization of purified PSB1-1, PSB2-1, PSB3-1

The surface ultra-structural images of the purified PSB1-1, PSB2-1 and PSB3-1 were observed by SEM. As shown in Fig. 5, all the purified PSBs had the linear structures, which indicated that the bridging might be the major function of the PSB1-1, PSB2-1 and PSB3-1 in the flocculation process (Xia et al., 2008). However, their linear structures were not the same. From the SEM images, the linear structure of PSB1-1 was rather regular, bulky and dendritic, while the linear structures of PSB2-1 and PSB3-1 were rather irregular and slender. The ultra-structure of a bioflocculant would influence its flocculating performance. Therefore, it is possible that individual PSB1-1, PSB2-1 and PSB3-1 may have different flocculating activity.

3.3. Application of PSBs in the wastewater treatment

3.3.1. Effects of PSBs concentrations on the flocculating activity

The effects of the PSBs concentrations on the flocculation activity of the kaolin suspension at pH 8 were studied. As shown in Fig. 6a, the five PSBs samples, i.e., crude P1, crude P2, PSB1-1, PSB2-1

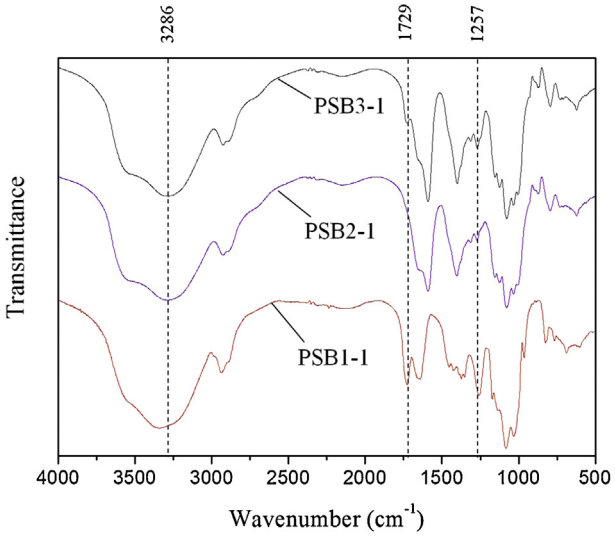

Fig. 4. FT-IR spectra of PSB1-1, PSB2-1 and PSB3-1.

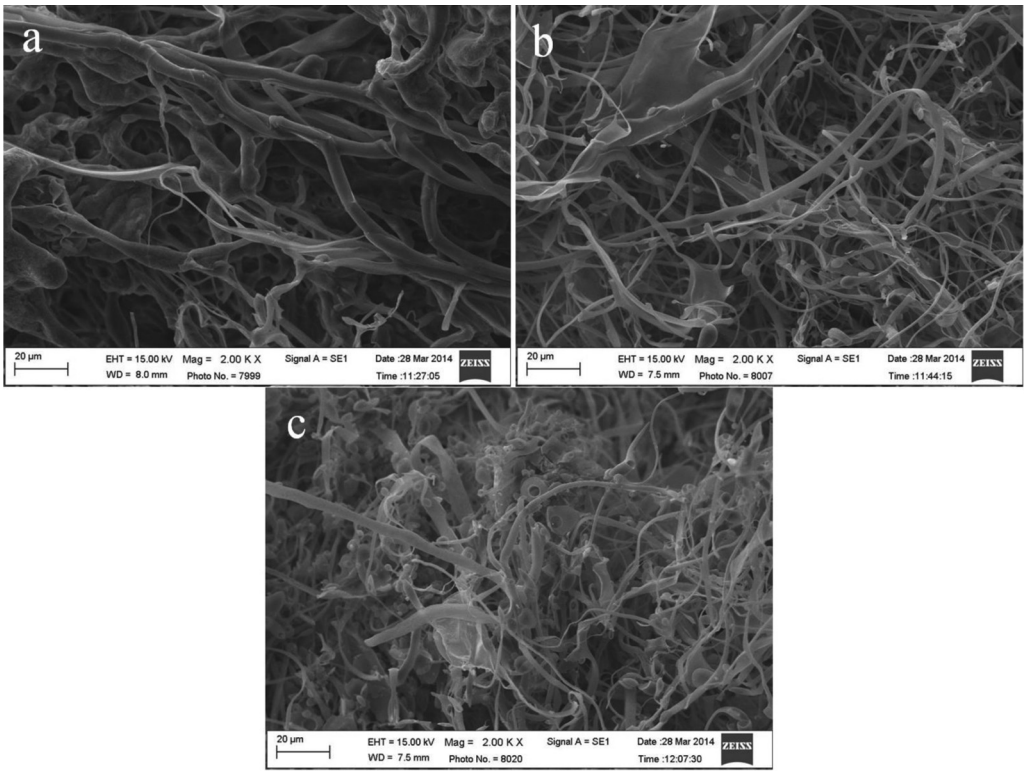

Fig. 5. SEM images of (a) PSB1-1, (b) PSB2-1, (c) PSB3-1.

and PSB3-1 showed similar profiles on the effects of the concentrations. The optimal concentration to achieve the highest flocculating activity was 2 mg/L, higher or lower concentration would reduce the flocculating activity. It is speculated that at concentration lower than 2 mg/L, PSBs could not efficiently absorb kaolin particles, while at concentration higher than 2 mg/L, the excess negatively charged PSBs might cause the repulsion of negatively charged kaolin particles. Both cases would reduce the flocculating activities of PSBs. Similar results were observed from the PSBs produced by other microorganisms (Elkady et al., 2011; Gong et al., 2008; Zhao, Liu, & Zhou, 2013). Among the five samples, PSB3-1 showed the lowest flocculating activity in the range from 0.5 to 4 mg/L, followed by PSB2-1. On the contrary, PSB1-1 could achieve the highest flocculating activity in the same concentration range, with the flocculating activity as high as 98.4% at the concentration of 2 mg/L, while the flocculating activities of PSB3-1 and PSB2-1 were only 72.5% and 78.0% at the same concentration, respectively. During the process of PSB1-1, PSB2-1 and PSB3-1 purification, the column of DEAE-52 was stepwise eluted with distill water, 0.1, 0.3, 0.5, 0.7 and 1 M NaCl, thereby, the fraction PSB1-1 (eluted with distill water) should be a neutral polysaccharide and the fractions PSB2-1 (eluted with 0.3 M NaCl) and PSB3-1 (eluted with 0.5 M NaCl) should be acidic polysaccharides with negative charged groups. The negative charge sequence of PSB1-1, PSB2-1 and PSB3-1 was PSB3-1 > PSB2-1 > PSB1-1. In basic solution, electrostatic attractive was expected to be the dominant driving force for the suspension adsorption (Li, Du, Wu, & Zhan, 2004). At pH 8, due to its relatively high negative charge, PSB3-1 could cause the repulsion of negatively charged kaolin particle, which might explain the low flocculating activity of PSB3-1. The neutral PSB1-1 could manage to absorb the kaolin suspension by electrostatic attractive, resulting in high flocculating activity. To our knowledge, the flocculating activity of PSB1-1

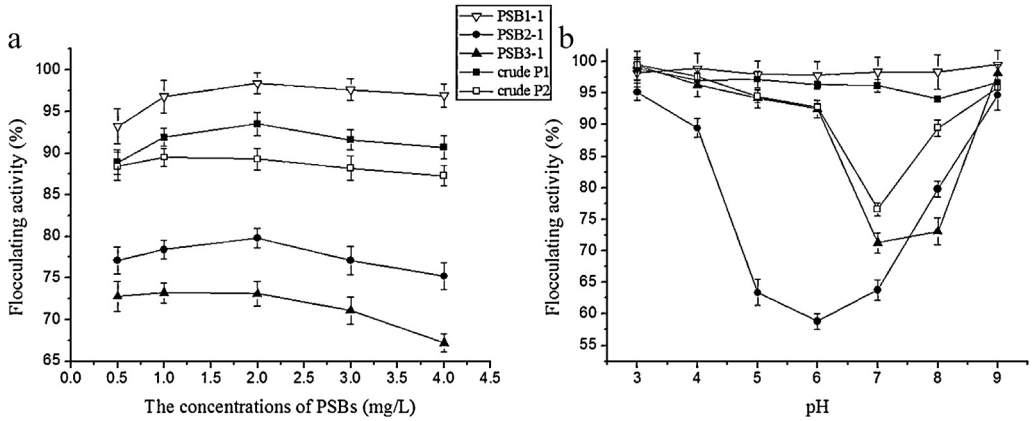

Fig. 6. (a) Effect of the PSBs concentrations on the flocculating activities in 5 g/L kaolin suspension at pH 8; (b) effect of pH on the flocculating activities in 5 g/L kaolin suspension.

was higher than any other bioflocculants produced by different microorganisms, such as *Bacillus subtilis*, *Halomonas* sp., *Gyrodinium impudicum* (He et al., 2010; Wu & Ye, 2007; Yim et al., 2007).

The flocculating activities of crude P1 and crude P2 were lower than that of PSB1-1, but higher than those of PSB2-1 and PSB3-1, which further confirmed that the PSB1-1 in the crude P1 and crude P2 contributed to the flocculating activities. Moreover, the flocculating activity of crude P1 was higher than crude P2, which was consistent with the results from Section 3.1.1 that the flocculating activity of culture supernatant 1 was higher than culture supernatant 2. And the results suggested that it was only the PSB1-1 contributed to the enhanced flocculating activity of culture supernatant 1 and crude P1, while PSB2-1 might exert weak influence during the flocculation process. Therefore, PSB1-1 was the key factor that might explain the enhanced flocculating activity of the culture supernatant from metal ion-supplemented medium.

3.3.2. Effect of pH on the flocculating activity

The effects of pH on the flocculating activity were investigated on the PSBs samples at the optimal concentration (2 mg/L) (Fig. 6b). Results showed that PSB1-1 had the best flocculating activities (above 97%), followed by the crude P1 (94%–98%) at pH from 3 to 9. To our knowledge, this finding is exciting because hardly any reported bioflocculants produced by other microorganisms could achieve high flocculating activity (over 94%) in the pH range of 3 to 9. In fact, many reported bioflocculants were not suitable for the flocculation in neutral solution. For example, the purified MBF-5 produced by *Klebsiella pneumoniae* maintained higher flocculating activity (90–95%) under acidic condition (pH 2–5) and alkaline condition (pH 8–11), but obtained lower flocculating activity in neutral condition (pH 6–7) (Zhao et al., 2013). This phenomenon was similar to that of the crude P2. Crude P2 could maintain high flocculating activity (above 90%) in acidic suspensions (pH 3–6) and alkaline suspensions (pH 8–9). In addition, many kinds of bioflocculants performed well only in acidic condition, such as the bioflocculants of *Bacillus mojavesis*, *G. impudicum* and *Streptomyces griseus* (Elkady et al., 2011; Shimofuruya et al., 1996; Yim et al., 2007). Such observations were in accord with that of PSB3-1, which showed high flocculating activity (above 90%) in acidic solution. As for PSB2-1, its flocculating activities were relatively low in the pH range of 5–8. It is interesting to note that the relative amount of PSB1-1 was the lowest in the crude P1 and crude P2 (Table 2), however, it showed the highest flocculating activity, which indicated that PSB1-1 might exert significant effect during the flocculation process.

The results indicated the flocculation performance was in the order of PSB1-1 > PSB3-1 > PSB2-1. According to the results of monopolysaccharide composition (Section 3.2.2), compared with PSB2-1 and PSB3-1, the main difference of PSB1-1 was the existence of hydrophobic rhamnose. Better flocculation performance of PSB1-1 might be due to the hydrophobic groups which could enhance the interaction between anionic suspended particles and flocculants (Shang, Liu, Zheng, & Wang, 2009).

Several functional groups have been indicated as the preferred groups for enhanced flocculation activities, such as hydroxyl groups, phosphate groups and ester linkages (Salehizadeh & Shojaosadati, 2001; Shang et al., 2009). Based on the FT-IR spectrum of the purified PSBs (Fig. 4), compared with PSB2-1 and PSB3-1, the band related to O–H stretching vibration was shifted to a higher wave-number (from 3286 to 3342 cm^{-1}) in the spectrum of PSB1-1, indicating an increase in the content of hydroxyl groups due to the reduction of intermolecular hydrogen bonds (Nelson & O'Connor, 1964). Besides, the abundance of hydroxyl groups could build up strong attraction forces between polysaccharides molecules (Dermlim, Prasertsan, & Doelle, 1999). Thereby, PSB1-1 with regular linear structure (Fig. 5a) might cause better approachability between PSB1-1 molecules, resulted in higher flocculating activity of PSB1-1. Another worth noting feature of PSBs spectrum was that both the peak intensities at 1729 and 1257 cm^{-1} were in the order of PSB1-1 > PSB3-1 > PSB2-1, which meant that the contents of ester linkages and phosphate groups were also PSB1-1 > PSB3-1 > PSB2-1. These might further explain the flocculation performance was in the order of PSB1-1 > PSB3-1 > PSB2-1.

Table 3
The removal rates of COD and SS in wastewater from paper mill (pH = 8.57), biological product factory (pH = 7.11) and garbage incineration plant (pH = 6.08) by crude P1 and PSB1-1.

Wastewater types	Removal rate of COD (%)		Removal rate of SS (%)	
	Crude P1	PSB1-1	Crude P1	PSB1-1
Paper mill	70.2	75.2	81.5	88.0
Biological product factory	83.2	86.9	88.8	92.0
Garbage incineration plant	59.7	60.7	68.0	69.8

3.3.3. Wastewater treatment by crude P1 and purified PSB1-1

Based on the results of Sections 3.3.1 and 3.3.2, PSB1-1 and crude P1 had good flocculating activities on kaolin suspension, therefore, their performance on real wastewater was further evaluated. Three different types of industrial wastewater with different pH were used to evaluate the flocculating activities of crude P1 and PSB1-1. As shown in Table 3, both crude P1 and PSB1-1 had good performance on the wastewater with different pH. Similar COD and SS removal rates were detected for crude P1 and PSB1-1 in the treatment of three kinds of wastewater, with the best performance was in the treatment of biological product factory wastewater, followed by the paper mill wastewater and garbage incineration plant wastewater. The satisfactory flocculating performance of crude P1 and PSB1-1 in the treatment of industrial wastewater indicated the feasibility of their use for wastewater processing industries.

4. Conclusions

PSBs were produced from *P. mucilaginosus* GIM1.16 in two different medium with or without metal ions complex. Three PSBs (PSB1-1, PSB2-1 and PSB3-1) were obtained after purification. PSB1-1 not only had high flocculating activities in the concentration range from 0.5 to 4 mg/L but also it could achieve high flocculating activities in the pH range of 3 to 9 of kaolin suspensions. PSB1-1 was the key factor that might explain the enhanced flocculating activity of the culture supernatant from metal ion-supplemented medium. High removal rates of COD and SS were also obtained when PSB1-1 was applied in industrial wastewater treatment.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.07.045.

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