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Increasing viscosity and yields of bacterial exopolysaccharides by repeatedly exposing strains to ampicillin

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ABSTRACT

A universal method to enhance productivity and viscosity of bacterial exopolysaccharides was developed. The technique was based on the principle that ampicillin can inhibit the biosynthesis of peptidoglycan, which shares a common synthetic pathway with that of bacterial exopolysaccharides. Serial passages of three typical representatives of bacterial EPS-producing strains, namely *Sphingomonas elodea*, *Xanthomonas campestris*, and *Paenibacillus elgii*, were subjected to ampicillin, which was used as a stressor and a mutagen. These mutant strains are advantageous over other strains because of two major factors. First, all of the resulting strains were almost mutants with increase in EPS productivity and viscosity. Second, isolated serial strains showed different levels of increase in EPS production and viscosity to satisfy the different requirements of practical applications. No differences were observed in the monosaccharide composition produced by the mutant and parent strains; however, high-viscosity mutant strains exhibited higher molecular weights. The results confirmed that the developed method is a controlled universal one that can improve exopolysaccharides productivity and viscosity.

1. Introduction

Bacterial exopolysaccharides (EPSs) have been widely reported in recent decades. Given their unique structures and excellent physicochemical properties, EPSs have a wide range of applications such as biomaterials [e.g., cellulose from *Acetobacter* (Jung, Jeong, et al., 2010; Jung, Lee, et al., 2010)], rheology modifiers of aqueous systems [e.g., xanthan and gellan gums (Banik, Santhiagu, & Upadhyay, 2007)], and bioflocculants [e.g., EPSs from *Paenibacillus elgii* B69 (Li et al., 2013) and *Paenibacillus polymyxa* SQR-21 (Raza, Makeen, Wang, Xu, & Qirong, 2011; Raza, Yang, Wu, Huang, Xu, & Shen, 2010)] in food, pharmaceutical, chemical, oil field, environmental pollutant treatment and other industries. Among various EPSs with diverse molecular structures and properties, xanthan and gellan gums are two of the most industrially and commercially developed EPS. Xanthan gum is produced by *Xanthomonas campestris* and consists of repeated pentasaccharide units formed by β-1,4-linked D-glucose backbone and trisaccharide side chains. These trisaccharide side chains are composed of glucuronic acid, mannose, pyruvate, and acetyl residues (Becker, Katzen, Pühler, & Ielpi, 1998; Kamal, Mehrgan, Assadi, & Mortazavi, 2003; Palaniraj & Jayaraman, 2011). Gellan gum is produced by *Sphingomonas elodea* and consists of tetrasaccharide-repeating units with D-glucose, D-glucuronic acid, and L-rhamnose in the backbone with *O*-acetate and glycerate linked to the same glucose residue (Banik & Santhiagu, 2006; Banik, Santhiagu, & Upadhyay, 2007; Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982). In our previous study, a new EPS PE-69 that is composed of glucose, mannose, xylose, and glucuronic acid has been produced by the *P. elgii* B69 strain (Li et al., 2013).

Based on molecular structures and biosynthetic mechanisms, numerous studies have focused on increasing EPS productivity and viscosity by remodeling the biosynthetic pathway. For instance, Vartak, Lin, Cleary, Fagan, and Saier (1995) interrupted the *zwf* gene, which encodes the glucose-6-phosphate dehydrogenase to divert carbon flow toward gellan synthesis. However, single gene mutation strain does not significantly improve the EPS production. Coleman, Patel, and Harding (2008) observed that EPS production is slightly increased and viscosity is significantly increased by a recombinant *Sphingomonas* ATCC 53159 harboring a plasmid with EPS biosynthetic genes. Furthermore, Galván et al. (2013) found

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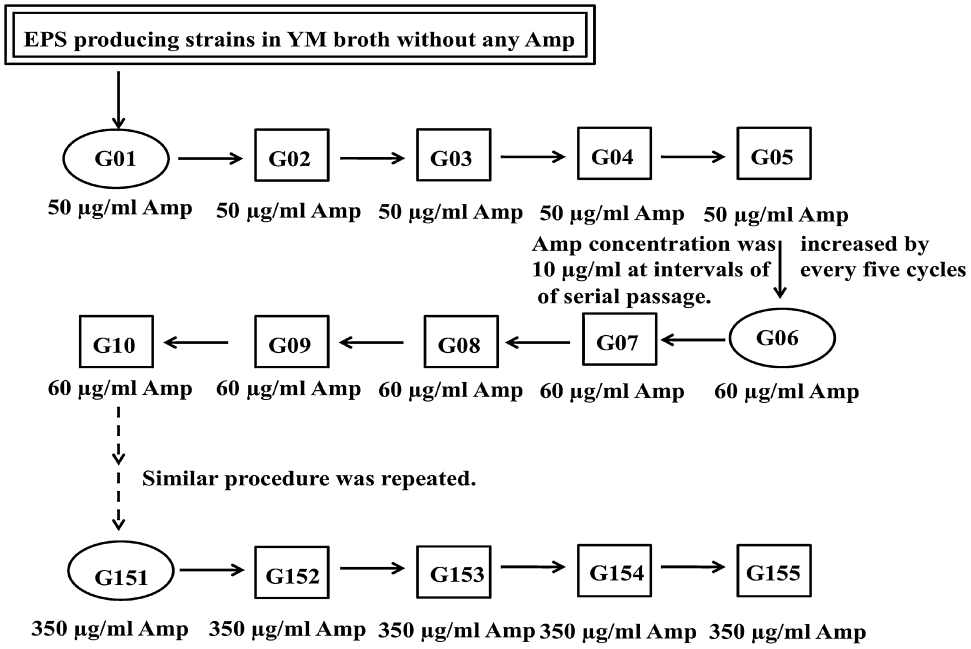

Fig. 1. Process of Amp exposure treatment during serial passages. G01–G155 represent the serial passage times and the arrows represent that the cultures were inoculated to another YM broth at a ratio of 1:10.

that xanthan viscosity increased by up-regulating the expression a polysaccharide co-polymerase protein.

Gene knockout or overexpression can be manipulated to obtain positive effects. However, genetically modified products especially as food additives are not acceptable in many countries. Some genetic modifications cannot be implemented without the knowledge about the relative gene or gene cluster. To solve this problem, a universal method that can be applied to the majority of bacterial EPS-producing strains should be developed to improve productivity and solution viscosity. In this study, the strains that produce xanthan and gellan gums as well as PE-69 from three different genera were repeatedly exposed to ampicillin (Amp) at gradually increasing concentrations and subjected to serial passages. Results indicated that Amp-resistant mutant strains can produce more EPS with higher viscosity.

2. Materials and methods

2.1. Bacterial strain and growth conditions

Gellan gum-producing strain *S. elodea* ATCC 31461 and xanthan gum-producing strain *X. campestris* ATCC 13951 were purchased from the American Type Culture Collection. The *P. elgii* B69 was a laboratory strain (Li et al., 2013). These three strains were maintained in YM agar slants [containing glucose (20 g/l), yeast extract (3 g/l), peptone (5 g/l), malt extract (3 g/l), and agar (20 g/l) at pH 7.0].

2.2. Amp sensitivity test

Each exponentially growing culture in YM broth was diluted at a ratio of 1:100 and dispensed into 96-well plates containing various concentrations of Amp. After incubated for 24 h at 30 °C, the optical density at 595 nm (OD_{595}) was determined using a Thermo Multiscan MK3 microplate reader at 0 h (initial time) and 24 h (final time). The minimum inhibitory concentration (MIC) was the lowest concentration of Amp in wells that presented no bacterial growth.

2.3. Amp exposure treatment during serial passages

The procedure for Amp treatment is illustrated in Fig. 1. Each exponentially growing culture was inoculated in YM broth containing an initial concentration of 50 μg/ml of Amp at a ratio of 1:10. The cultures were incubated at 30 °C, 200 rpm for 24 h and then inoculated in another YM broth containing the same concentration of Amp. The same procedure was repeated for another four cycles before Amp concentration was subsequently increased by 10 μg/ml. Amp concentration was increased by 10 μg/ml at intervals of every five cycles of serial passage. Growth rates were significantly reduced when the strains were treated by 300 μg/ml of Amp, and this procedure almost failed to yield enough strains when strains were exposed to higher Amp concentration (>350 μg/ml). Thus, the final Amp concentration used was 350 μg/ml, and the final serial passage was 155 times. Each of the three cultures, after being transferred for 55, 105, and 155 times, was plated on a YM agar medium containing the corresponding antibiotic and incubated at 30 °C for 72 h. Twenty random single colonies from each sample were isolated.

2.4. EPS fermentation

The strains were inoculated in the YM broth medium at 200 rpm for 24 h at 30 °C. The preculture was inoculated at a volume ratio of 1:10 in each of the production media. To produce gellan and xanthan, the flasks were maintained at 200 rpm for 48 h at 30 °C. The same conditions were used to produce PE-69 except for the longer duration of 96 h. The production media of the three different strains contained the following: (1) gellan: 1.5 g/l of K_2HPO_4, 1 g/l of KH_2PO_4, 0.6 g/l of $MgSO_4$, 0.2 g/l of yeast extract, 2 g/l of soy protein, and 30 g/l of sucrose; (2) xanthan: 30 g/l of glucose, 3 g/l of yeast extract, 2 g/l K_2HPO_4, 0.1 g/l of $MgSO_4{\cdot}7H_2O$, and 2 g/l of KH_2PO_4 (Rodriguez, Aguilar, & LaO, 1997); and PE-69, 1.5 g/l of K_2HPO_4, 1 g/l of KH_2PO_4, 0.6 g/l of $MgSO_4$, 0.5 g/l of yeast extract, 5 g/l of peptone, and 30 g/l of sucrose.

2.5. EPS productivity and viscosity measurement

To determine to the crude EPS productivity, the broths were diluted 10 times and centrifuged at 15,000 × *g* for 30 min at 4 °C to remove the cells. A double volume of ice-cold ethanol was added and the solution was maintained at 4 °C overnight. EPS were then separated by centrifugation at 10,000 × *g* for 15 min, dried at 80 °C, and weighed.

The crude EPSs was re-dissolved in ddH_2O (1.0 g/100 ml), and then the viscosity was measured using a Brookfield viscometer (Model RVDV-II Pro; Brookfield Engineering Laboratories, Stoughton, MA) with spindle no. 6 at 60 rpm and 25 °C.

2.6. Purification, monosaccharide composition, and molecular weight measurement

The obtained crude EPSs (Section 2.5) were re-dissolved in appropriate ddH_2O and deproteinization was performed using papain and Sevag reagent (Yan, Li, Wang, & Wu, 2010). Papain (1 U/mg EPS) was added and mixed at pH 6.4 for 3 h at 60 °C. The resulting mixture was centrifuged, and then the supernatant was mixed with Sevag reagent (chloroform/1-butanol at 5:1 (v/v)), constantly stirred for 2 h, and centrifuged again. The EPSs were lyophilized after the dialyzation (with a 9 kDa membrane) in deionized water for 48 h.

To analyze the chemical compositions, 2 mg sample was suspended in 1 ml of 2 M trifluoroacetic acid and heated for 8 h at 105 °C. Neutral sugars were determined by gas chromatography after the hydrolyzate has been converted into alditol acetates (Jones & Albersheim, 1972). Glycerate, acetate, pyruvate content, and standard were analyzed by Agilent 1200 HPLC system equipped with a Bio-Rad carbohydrate analysis column Aminex HPX-87H (300 mm × 1.8 mm internal diameter) coupled with a refractive index detector at 40 °C. H_2SO_4 (5 mM) was used as an eluent at a flow rate of 0.5 ml/min. Glucuronic acid was quantified according to the improved carbazole-sulfuric acid method (Li, Kisara, Danielsson, Lindström, & Gellerstedt, 2007).

The molecular weight was measured by gel permeation chromatography in an Agilent 1200 HPLC system equipped with two TSK columns, namely, G4000 PWXL and G6000 PWXL in series. The sample (20 μl) was injected and eluted with 0.05 M $NaNO_3$ solution at a flow rate of 0.5 ml/min. Dextrans with a standard molecular weight (10 kDa to 5700 kDa) were used to establish the standard curve.

2.7. Stability of mutant strains

The genetic stability of mutant strain was investigated after being transferred for 30 consecutive generations in a shake flask. The separated colonies were examined for EPSs production, viscosity, and Amp resistance.

3. Results and discussion

3.1. Determination of Amp MIC

Based on OD_{595}, the strains *S. elodea* and *X. campestris* showed similar sensitivities at the MIC of 85 μg/ml, and *P. elgii* B69 showed the lowest sensitivity at the MIC of 97.5 μg/ml. Considering that the Amp sensitivities of the three tested strains were similar, the subsequent Amp exposure experiment was conducted at the same Amp concentration (50 μg/ml) to obtain a moderate initial stress condition.

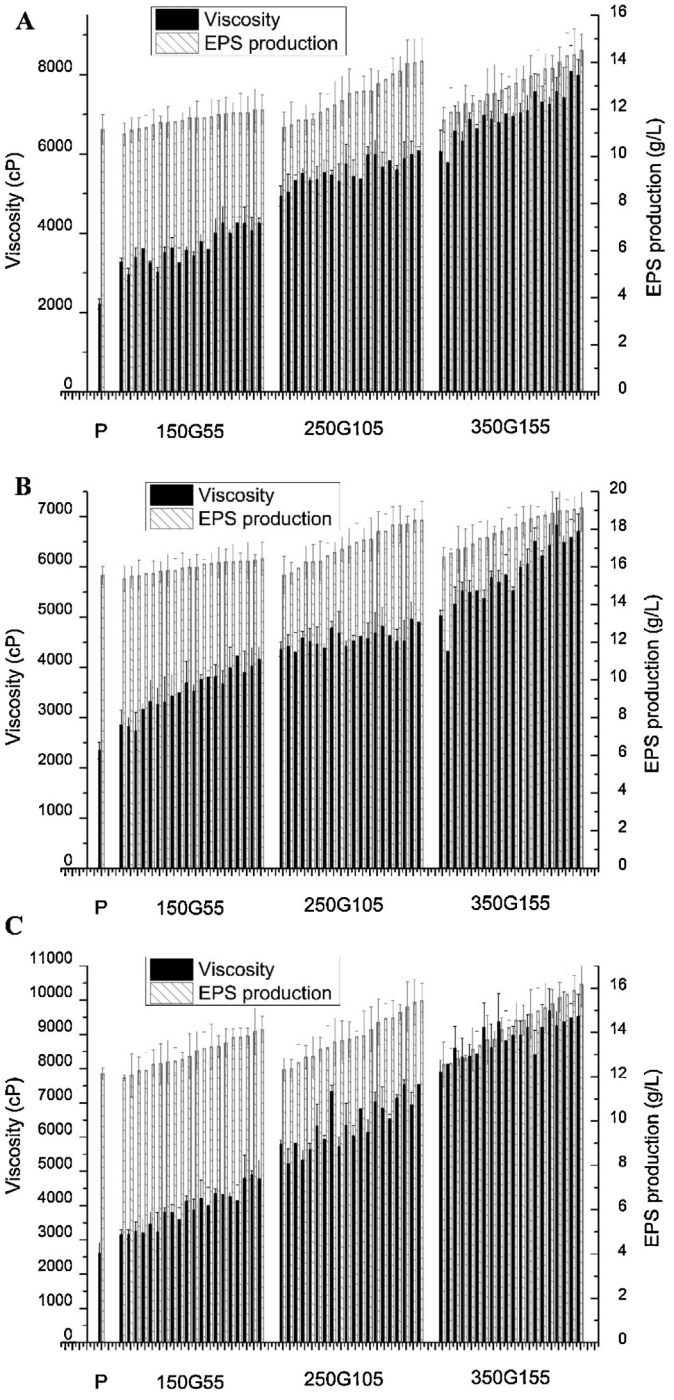

Fig. 2. EPS production, viscosity of Amp exposure mutants of *S. elodea* ATCC 31461 (A), *X. campestris* ATCC 13951 (B) and *P. elgii* B69 (C). The data are represented as the mean value of three experiments ± SD. Numbers 150, 250, and 350 represent Amp concentrations of 150, 250, and 350 μg/ml, respectively. G55, G105, and G155 represent the serial passage times. 150G55, 250G105, and 350G155 represent 20 single colonies of different Amp concentrations and different serial passage times of the three strains arranged in the order of elevated EPS production.

3.2. Increased productivity and viscosity after serial passages with Amp exposure

Despite the diversity in monosaccharide compositions and structures of various bacterial EPSs, their biosynthetic pathway closely resembles the biosynthesis of bacterial cell wall peptidoglycan. Most bacterial EPSs, including xanthan gum, gellan gum, and PE-69, are synthesized in the cell membrane and then exported

out of the cell. EPS biosynthesis begins by adding a sugar phosphate from nucleoside diphosphate sugars to an undecaprenyl phosphate lipid carrier to form an undecaprenyl phosphate-linked sugar. This procedure results in a complete repeating unit after the stepwise addition of other sugar phosphates (Guo, Yi, Song, & Wang, 2008).

The initial step in the biosynthesis of bacterial EPS is the transferring of nucleotide sugar to a lipid carrier. EPS shares a common synthetic pathway with that of bacterial peptidoglycan that can be inhibited by Amp and bacitracin. This inhibition results in the synthesis of more lipid carriers and nucleotide sugars, which can also be used to synthesize EPS, thereby increasing EPS production and viscosity. Thus, we aimed to use Amp or bacitracin to isolate the mutant bacterial EPS-producing strains which increases EPS viscosity and production. Our preliminary experiment showed that the result of using Amp was better than that of bacitracin (data not shown). Previous studies have also found that bacitracin-resistant mutants of *X. campestris* lost the ability to secrete xanthan gum (Marquet, Mikolajczak, Thorne, & Pollock, 1989; Pollock, Thorne, Yamazaki, Mikolajczak, & Armentrout, 1994). Therefore, Amp was chosen to function as the stressor in the present study. Increased Amp concentration and serial passages were used to treat three typical representatives of bacterial EPS-producing strains, namely, *S. elodea* ATCC 31461, *X. campestris* ATCC 13951, and *P. elgii* B69. The experimental results would determine whether the method could be used to establish a universal method to improve the productivity and viscosity of various bacterial EPSs.

Fig. 2 shows the effect of Amp exposure and serial passages on EPS productivity and viscosity of *S. elodea*, *X. campestris* and *P. elgii*. When strain *S. elodea* ATCC 31461 was exposed to Amp with gradually increasing concentration from 50 to 150 μg/ml during the initial 55 successive passages, no significant change in EPS production was detected. However, an increase in EPS viscosity was observed compared with the change in EPS production (Fig. 2A). With the increased repetitions serial passage and Amp concentration, the increasing trends in EPS production and viscosity in *S. elodea* became more significant. The assayed colonies from the strains after 105 serial passages (Amp concentration increased to 250 μg/ml) and after 155 serial passages (Amp concentration increased to 350 μg/ml) were all mutant strains with elevated EPS productivity, except for those from the strains after 155 serial passages, which exhibited a more significant improvement in EPS viscosity and productivity. To determine whether the other bacterial EPS-producing strains show a similar increase in trend after Amp exposure and serial passages, xanthan-producing strain *X. campestris* ATCC 13951 and PE-69-producing strain *P. elgii* B69 were subjected to the same treatment. The same general increasing trends in productivity and viscosity were observed on the different strains that belong to various genera with different characteristics (Fig. 2B and C). A slight difference in the rate of increase of in the EPS production and viscosity among the three strains was also found. Among the three strains, *P. elgii* B69 showed the highest significant increase. Furthermore, the highest mutant strain from *P. elgii* B69 was 9%, 27%, and 33% higher in terms of EPS production than its parent strain for the three corresponding Amp concentrations. The difference may be attributed to the different EPS compositions and Amp sensitivities. Similarly, West (2002) isolated an Amp-resistant strain of *S. elodea* with an increasing production of gellan after the strains were treated with ethylmethane sulfonate (EMS). In the present study, we found that Amp can effectively improve EPS productivity and viscosity for the strain of *S. elodea*, which is similar to the result of West, as well as for the other strains of different genera. Consequently, we can expect that this Amp exposure procedure may be used as a universal method for the improvement of other bacterial EPS produced by various strains given that the initial biosynthetic steps are similar to that of peptidoglycan.

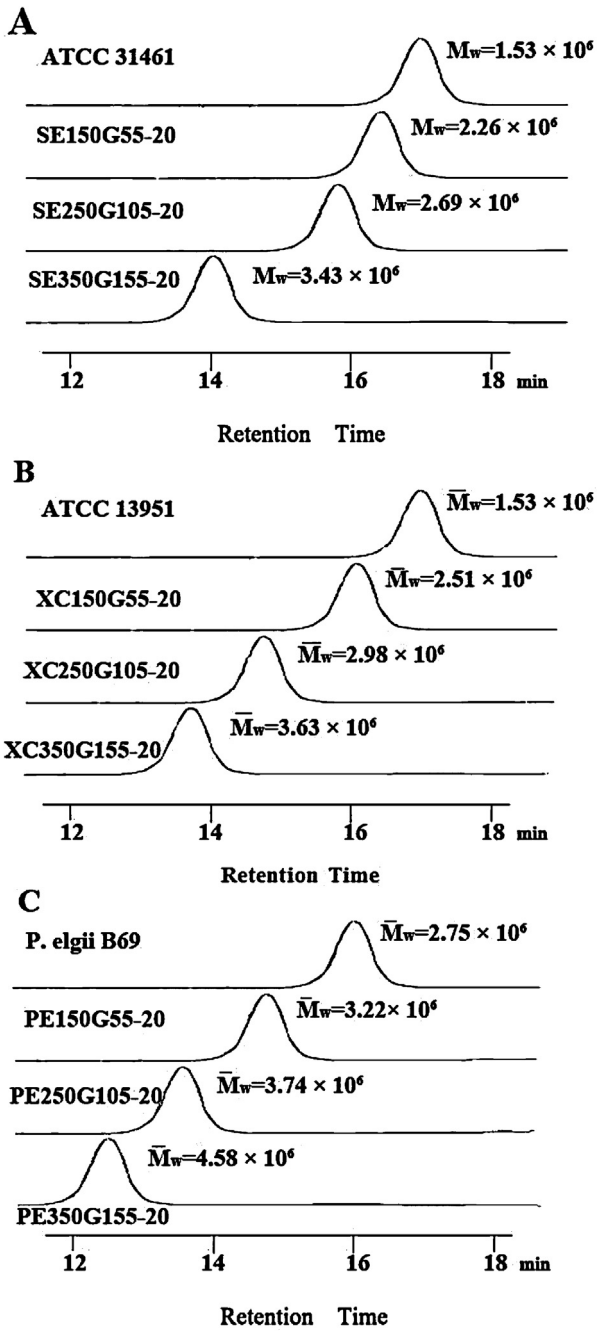

Fig. 3. Gel permeation chromatography profiles of EPS produced by wild type and derived mutant strains of *S. elodea* (A), *X. campestris* (B) and *P. elgii* B69 (C). SE, XC, and PE represent the derived strains from *S. elodea* ATCC 31461, *X. campestris* ATCC 13951, and *P. elgii* B69, respectively. Numbers 150, 250, and 350 represent Amp concentrations of 150, 250, and 350 μg/ml, respectively. G55, G105, and G155 represent the serial passage times. Tested sample in Fig. 2 are the strains which produced the most of the 20 colonies picked after 55, 105, and 155 serial passage times.

Another important feature of this study is that the strains isolated from the three treated strains were almost the mutant strains with high EPS productivity, and few of the isolated strains had low productivity or viscosity (Fig. 2). On the contrary, other studies also isolated many mutants with lower EPS production or no capability of producing EPS (West, 2002). The difference may be explained by the following. First, this study used a gradually increasing concentration of Amp as a sustained stress during the serial passages, whereas West used Amp as a temporary and short-term

Table 1
EPS production, viscosity, monosaccharide compositions, and molecular weight of Amp exposure mutants and their parental strains.[a]

Strain[b]	Monosaccharide compositions[c]	Molecular weight (kDa)
ATCC 31461	GlcA:Glc:Rha:Gly:Ace = 1:1.81:1.29:0.48:0.26	1.53×10^6
SE150G55-20	GlcA:Glc:Rha:Gly:Ace = 1:1.72:1.31:0.46:0.28	2.26×10^6
SE250G105-20	GlcA:Glc:Rha:Gly:Ace = 1:1.85:1.35:0.40:0.38	2.69×10^6
SE350G155-20	GlcA:Glc:Rha:Gly:Ace = 1:1.88:1.25:0.57:0.19	3.43×10^6
ATCC 13951	GlcA:Glc:Man:Pyr:Ace = 1:2.19:1.93:1.09:1.41	1.53×10^6
XC150G55-20	GlcA:Glc:Man:Pyr:Ace = 1:2.27:1.86:1.14:1.25	2.51×10^6
XC250G105-20	GlcA:Glc:Man:Pyr:Ace = 1:2.10:1.83:1.05:1.51	2.98×10^6
XC350G155-20	GlcA:Glc:Man:Pyr:Ace = 1:2.25:1.76:1.16:1.31	3.63×10^6
P. elgii B69	GlcA:Glc:Man:Xyl = 1:1.89:2.17:0.87	2.75×10^6
PE150G55-20	GlcA:Glc:Man:Xyl = 1:1.95:2.27:0.93	3.22×10^6
PE250G105-20	GlcA:Glc:Man:Xyl = 1:1.83:2.15:0.89	3.74×10^6
PE350G155-20	GlcA:Glc:Man:Xyl = 1:1.94:2.09:0.79	4.58×10^6

[a] The data of viscosity and EPS are mean value of three experiments ± SD.
[b] SE, XC, and PE represent the derived strains from *S. elodea* ATCC 31461, *X. campestris* ATCC 13951, and *P. elgii* B69, respectively. Numbers 150, 250, and 350 represent Amp concentrations of 150, 250, and 350 μg/ml, respectively. G55, G105, and G155 represent the serial passage times. Mutant strains in Table 2 are the ones which produced the most EPS of the 20 colonies picked after 55, 105, and 155 serial passage times (Fig. 2).
[c] Abbreviation: GlcA, Glucuronic acid; Rha, Rhamnose; Gly, Glycerate; Ace, Acetate; Man, Mannose; Pyr, Pyruvate; Xyl, Xylose.

screening method. The strains with low productivity in this study might be gradually phased out by serial passages and increase in Amp concentration because high EPS production had higher resistance to antibiotics, which is confirmed by other researchers (Drayson, Leggat, & Wood, 2011; Ito, Taniuchi, May, Kawata, & Okabe, 2009; Lopez-Torres & Stout, 1996). Second, the strains were treated with EMS as a mutagen in the West's research, and the corresponding DNA damage may be very strong. By contrast, studies have indicated that Amp can stimulate bacteria to produce reactive oxygen species that can directly damage DNA as an active, reactive mutagen (Dwyer, Kohanski, Hayete, & Collins, 2007; Kohanski, Dwyer, Hayete, Lawrence, & Collins, 2007). Thus, Amp was used in our study as a stressor and as a moderate mutagen that could cause sufficient mutation without excessive damage to the cells.

3.3. Monosaccharide composition and molecular weight measurement

To characterize the possible alterations in EPS that caused an increase in viscosity, we compared the monosaccharide composition, ratios, and molecular weight, which are closely related to viscosity, of EPS from the mutant strains with their parent strains. Purifying EPS from more than 60 strains and measuring their monosaccharide composition, ratios, and molecular weights constitute a huge workload. Thus, nine representative mutant strains were selected to compare with their parent strains. These strains had the highest EPS production among the 20 colonies picked after 55, 105, and 155 serial passage times, which reflected the general trend to some extent. No significant difference was found in the monosaccharide composition and ratios of the EPSs. By contrast, the retention time is gradually reduced in the GPC profile in Fig. 3. This result indicates that the molecular weights of the EPSs gradually increased (Table 1) with the increasing passage numbers and Amp concentrations. At the end of the Amp exposure treatment, the highest EPS molecular weights for strains SE350G155-20, XC350G155-20, and PE350G155-20 were 2.24, 2.37, and 1.67 times higher than those of the corresponding parent strains. However, the increase in molecular weight was slightly lower than that of viscosity.

Many EPSs, such as xanthan and gellan gums, are primarily used as gels, thickening agents, or stabilizers. Thus, many applications would get more benefits from EPSs with higher viscosity

Table 2
Stability test of mutant strains[a]

Strains[b]	Amp mutant strain		Transferred strains[c]	
	Viscosity (cP)	EPS (g/l)	Viscosity (cP)	EPS (g/l)
SE150G55-19	4057 ± 350	11.98 ± 0.89	3989 ± 274	11.89 ± 0.59
SE150G55-20	4258 ± 125	11.98 ± 0.64	4178 ± 108	11.94 ± 0.54
SE250G105-19	5987 ± 338	13.98 ± 1.02	5887 ± 401	14.05 ± 0.83
SE250G105-20	6078 ± 114	14.06 ± 0.97	5938 ± 555	14.10 ± 0.62
SE350G155-19	8086 ± 654	14.33 ± 1.09	8213 ± 654	14.31 ± 0.80
SE350G155-20	7987 ± 397	14.51 ± 0.67	7971 ± 397	14.47 ± 0.62
XC150G55-19	4025 ± 367	16.35 ± 0.29	4095 ± 231	16.44 ± 0.59
XC150G55-20	4158 ± 248	16.44 ± 0.88	4043 ± 388	16.44 ± 0.61
XC250G105-19	4958 ± 357	18.46 ± 0.61	4713 ± 555	18.51 ± 0.49
XC250G105-20	4897 ± 246	18.47 ± 1.02	4785 ± 517	18.59 ± 0.64
XC350G155-19	6589 ± 358	19.07 ± 0.68	6803 ± 427	18.99 ± 0.75
XC350G155-20	6705 ± 354	19.13 ± 1.23	6613 ± 500	19.08 ± 0.48
PE150G55-19	4895 ± 129	14.05 ± 1.05	4910 ± 713	14.14 ± 0.88
PE150G55-20	4785 ± 541	14.12 ± 0.61	4595 ± 230	14.17 ± 1.01
PE250G105-19	6942 ± 364	15.35 ± 1.06	6999 ± 677	15.47 ± 0.53
PE250G105-20	7543 ± 465	15.43 ± 0.79	7301 ± 733	15.27 ± 0.41
PE350G155-19	9487 ± 410	15.91 ± 0.67	9103 ± 813	15.76 ± 0.27
PE350G155-20	9526 ± 648	16.17 ± 1.01	9666 ± 404	16.13 ± 0.53

[a] The data are represented as the mean value of three experiments ± SD.
[b] SE, XC, and PE represent the derived strains from *S. elodea* ATCC 31461, *X. campestris* ATCC 13951, and *P. elgii* B69, respectively. Numbers 150, 250, and 350 represent Amp concentrations of 150, 250, and 350 μg/ml, respectively. G55, G105, and G155 represent the serial passage times. -19 and -20 represent two highest EPS yield strains from each of the 20 single colonies (Fig. 2).
[c] Strains were transferred for 30 consecutive generations in a shake flask.

and higher molecular weight either to provide higher viscosity at an equivalent concentration or to reduce the concentration without changing its effectiveness. Furthermore, our study has advantages over other studies that randomly generated various mutant strains (Rodriguez, Aguilar, & Lao, 1997; West, 2002). Our study involved a series of mutant strains producing EPS with various orderly improvements in viscosity and molecular weight obtained from different stages of our procedure (such as at 55, 105, or 155 serial passages). The results of our study can meet all the requirements for practical application (Fig. 2 and Table 1).

3.4. Stability test of mutant strains

After 30 generations of growth in a non-antibiotic medium, all of the mutant strains remained Amp resistant. The levels of EPS production and broth viscosity of the mutant strains and their passage strains were unchanged. Table 2 shows some data of the high yield EPS-producing strains. The results of the stability test indicated that the mutant caused by Amp as a stressor and mutagen exhibited good stability and heritability.

4. Conclusions

Three bacterial EPS-producing strains were treated with serial passages of gradually increasing concentration of Amp, which was used as a stressor and mutagen. EPS productivity and viscosity of the mutant strains derived from the three species gradually increased as the number of serial passage and Amp concentration increased. Further analysis indicated that high viscosity is related to high molecular weight. Therefore, the serial passage with Amp exposure could be a controlled universal method to obtain bacterial EPS-producing strains with high EPS productivity and viscosity to meet the different requirements of various applications.

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