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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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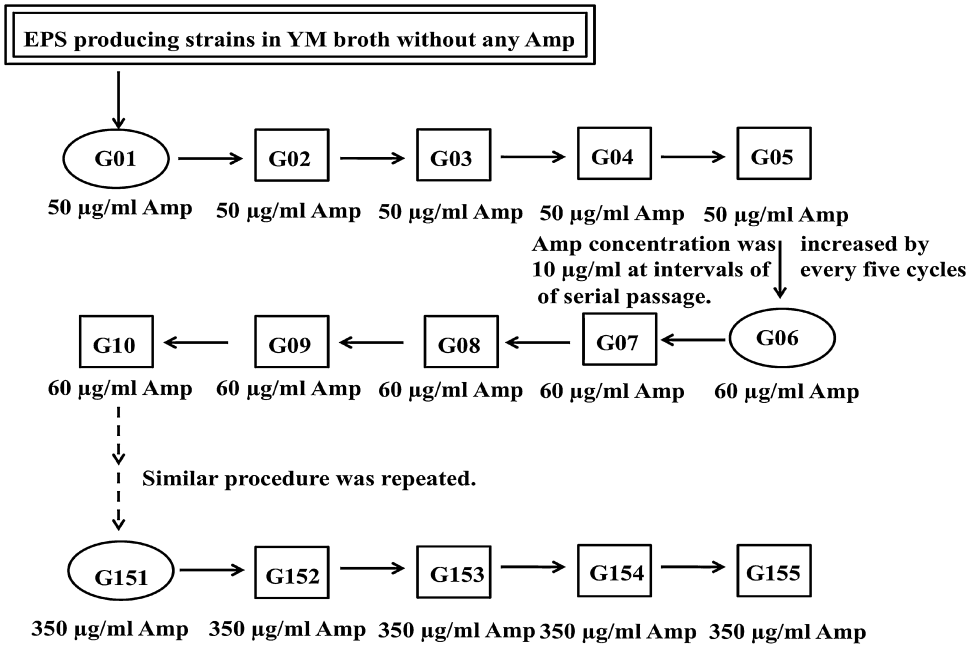

Fig. 1. Process of Amp exposure treatment during serial passages. G01–G155 represent the serial passage times and the arrows represent that the cultures were inoculated to another YM broth at a ratio of 1:10.

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.