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Biopolymer from marine *Athelia* and its application on heavy oil recovery in heterogeneous reservoir

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ABSTRACT

Biopolymer produced from marine *Athelia* strain presented unique Pseudoplastic behaviors under extremely-high temperature and salinity conditions. Characteristic analysis with FT-IR spectroscopy, high performance liquid chromatography, 1H and 13C NMR and two-dimensional COSY and HMQC spectra showed the structure of β-(1–6) glucans. Single-factor and orthogonal experiment design were used to optimize the yield, the maximum yield of the biopolymer was 28.32 g/L with 56.64% carbon conversion rate under optimized conditions. Economic investigation demonstrated that this novel biopolymer has great potential of commercialization with the competitive cost of $2896.04-5228.94 per ton for powder. Resistance factor and residual resistance factor were evaluated with core flooding experiments showed that this biopolymer had excellent performance of plugging capacity and profile modification, and indicating the great potential of application on heavy oil recovery.

1. Introduction

Petroleum-based polymers have the disadvantage of their limited resources and the substantial environmental impact. In this context, biopolymer from microorganisms, representing a valuable alternative with the benefit of the compatibility of environment and ecology, minimization of carbon release, consumption reduction of water and energy (Angelina & Vijayendra, 2015; Ahmad, Mustafa, & Man, 2015), have recently attracted worldwide attentions with their unique physi-chemical and biochemical properties on numerous applications.

Over the past decades of achievements in the field of microbial biopolymer biosynthesis have developed many biopolymers suitable for abundant applications such as the medicine, food, cosmetics, construction, drilling, oil recovery and so on (Angelina & Vijayendra, 2015; Lapasin & Pricl, 1995; Laurienzo, 2010; Safdel, Anbaz, Daryasafar, & Jamialahmadi, 2017). As investigation, the global industry of biopolymer grew by 37 million tonnes to 194 million tonnes, corresponding to an average annual consumption growth of around 3%, with that the related market is projected to witness a CAGR of 12.0% from 2016 to reach a market size of USD 5.08 Billion by 2021, mainly driven by implementation of strict environment regulations to reduce carbon content and fluctuation of fossil-fuel prices (Bernardo, Simões, & Pinto, 2016; Chaabouni, Gassara, & Brar, 2014; Morris & Harding, 2009; Rehm, 2010). Based on the microbial carbohydrate structure database, four hundred different biopolymers with various chemical structures have been documented, and many reports describe microbes to be capable of producing biopolymer but without giving structural information (Rühmann, Schmid, & Sieber, 2015; Toukach, Joshi, Ranzinger, Knirel, & Von Der Lieth, 2007). Furthermore, the better understanding of the molecular mechanisms and regulatory processes underlying the biopolymer synthesis has provided the powerful tools to engineer microbes that are capable of not only efficient production but also the production of modified and even unnatural polymers exhibiting unique material properties for specific applications, all at a viable economic cost (Rehm, 2010). These all impressively demonstrate the tremendous diversity of available biopolymer and the capacity for new variants to be of technical and commercial interest.

Microbes can efficiently convert different carbon sources into a diverse range of polymers with varying chemical and material properties (Gronau, Krishnaji, Kinahan, Giesa, & Wong, 2012; Rühmann et al., 2015). The exploring of microbial strains for their competence in biopolymer production has therefore been a major issue, especially in the

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search for new biopolymer variants among natural strain isolates. Concerning the fact that most of all microbes carry the genetic codes for the synthesis of polysaccharides under specific conditions, the naturally provided biopolymer portfolio seems to be still massively under explored. Although microbes synthesize only a few intracellular polymers, the range of extracellular polymers that they can synthesize is vast (Angelina & Vijayendra, 2015; Busuioc, Mackiewicz, Buttaro, & Piggot, 2009; Manivasagan & Kim, 2014; Sukan, Roy, & Keshavarz, 2015). Some of these polymers serve the same function in a wide range of prokaryotes, whereas other polymers can be specific for certain microbial taxa and serve distinct biological functions (Angelina & Vijayendra, 2015; Sukan et al., 2015; Sutherland, 2005). Therefore, the discovery of newly biopolymer is not limited to be proceeded in the unexplored microorganism, but also can be focused on the existing identified microorganisms.

Several microbial polymers have been already produced commercially through various scale fermentations, with annual world production volumes of around 2000 t and 100,000 t for the polysaccharides xanthan, welan, gellan, and polyesters (Ahmad et al., 2015; Angelina & Vijayendra, 2015; Manivasagan & Kim, 2014; Sutherland, 2005). The market value depends on the material properties that impact on the field of application. Besides the property, microbial polymers are required to compete with petroleum-based and non-renewable polymers, the cost of production is a crucial parameter. Low-value applications of biopolymers become economically viable when they can be produced at low costs, like for petroleum industry. Therefore, it is essential to focus the efforts on the reduction of the production cost. Among the factors that fluctuate the cost, the nutrients, process control, and purification are generally crucial.

This study reports a marine *Athelia* strain can produce a novel polysaccharide. Structural analysis of this biopolymer is preliminarily conducted. Compared with the reported or marketed biopolymer, it shows significantly different pseudoplastic characteristics and can keep stability even under extremely high temperature and salinity. The estimation of the production cost under the optimal production conditions is made. And its potential application of enhancing heavy oil recovery for high temperature and salinity reservoir is evaluated in stimulated porous medium.

2. Materials and methods

2.1. Microorganism and culture condition

Among 178 strains isolated from water samples from yellow sea following the various methods (Manivasagan & Kim, 2014; Rühmann et al., 2015), fungi SCL was able to produce extracellular biopolymer when fed with sugar as carbon source. Mycelium was cultivated at 28 °C in basic medium (BM) for 5 days, containing (g/L): $NaNO_3$ 3.0, $K_2HPO_4{\cdot}3H_2O$ 1.3, Citric acid 0.7, $MgSO_4{\cdot}7H_2O$ 0.5, KCl 0.5, $FeSO_4{\cdot}7H_2O$ 0.05, yeast extract 1.0, and initial pH 4.5; sucrose 20 and glucose 18 as carbon source. Seed culture was prepared with the rest *Athelia* cell that washed triplicated with phosphate buffer solution and then incubated in 500 mL Erlenmeyer flasks with 100 mL BM medium at 250 rpm and 28 °C for 48 h. Fermentation optimization was carried out in 500 mL Erlenmeyer flasks with BM medium at 250 rpm, 28 °C for 72 h. It deposited at CCTCC (China Center for Type Culture Collection) with NO. M2014325 for further research.

2.2. Phylogenetic analysis

Chromosomal DNA was extracted using a E.Z.N.A.® Fungal DNA Mini Kit (OMEGA Bio-tek, USA). Amplification of 18S rRNA gene was performed by PCR using universal fungal specific primers TR1 [5′-GTTTCTAGGACCGCCGTA-3′] and TR2 [5′-CTCAAACTTCCATCGAC TTG-3′] (Cappa & Cocconcelli, 2001). PCR program was set as an initial denaturation step of 94◦C for 4 min, followed by 35 cycles of 94◦C for 30 s, annealing at 58◦C for 30 s, extension at 72◦C for 30 s and a final extension step at 72◦C for 7 min. PCR products were analyzed by 1.0% agarose gelelectrophoresis. Gel eluted PCR product was purified by QIAquick gel extraction kit (QIAGEN, US) as per manufacturer's protocol. The resulting sequences were determined for the orientation and then preliminarily compared with those available in the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov) using the BLAST service to determine their approximate phylogenetic affiliations. The phylogeny tree was constructed based on the neighbor-joining algorithm using MEGA 6.0 after multiple alignments of the data by CLUSTAL-X.

2.3. Purification and identification

Crude biopolymer was recovered by ethanol precipitation at 4◦C from culturing, then collected by filtration. Total sugar content of biopolymer was determined by the phenol-sulfuric acid assay (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

Further purification was carried out as following procedure for structure analysis. The crude biopolymer was dissolved in 1 M NaOH, then dialyzed and lyophilized. The soluble biopolymer was subjected to ion exchange chromatography (IEC) on a column of DEAE-Sephadex A-25 (18 × 1.8 cm, Sigma), pre-equilibrated using 0.01 M Tris-Cl (pH 7.8) (Mukhopadhyay, Chatterjee, Gauri, Das, & Mishra, 2014). The column was washed with equilibration buffer followed by successive elution with NaCl solution of increasing molarities in a stepwise manner. Fractions were collected and monitored spectrophotometrically by phenol-sulfuric acid assay. The major polysaccharide fractions eluted at 100 mM NaCl were pooled, dialyzed, and freeze-dried.

After IEC purification, the biopolymer was then purified by gel permeation chromatography (GPC) on Sepharose CL–6 B column (90 × 2.1 cm, GE Healthcare) with water as eluant (0.75 mL/min), and dextran molecular weight markers (range 40–2000 kDa, sigma-akdrich, USA) as standard (Ghosh, Chandra, Roy, Mondal, & Maiti, 2008; Mukhopadhyay et al., 2014). The fractions were collected and monitored spectrophotometrically at 490 nm with phenol-sulfuric acid reagent. GPC purified biopolymer was used for further chemical characterization.

FT-IR spectra of freeze-dried GPC purified biopolymer were obtained using a 5DX (Shimazdu Corporation, Tokyo, Japan) in transmittance mode in the range of 4000–450 cm-1. ^{13}C,^{1}H and HSQC NMR spectra analysis of biopolymer was carried out following the reported description (Dai, Wu, Chen, Zhu, & Yin, 2010; Gonzaga, Menezes, de Souza, Ricardo, & Soares, 2013). Monosaccharide composition was determined by high-performance liquid chromatography (HPLC) equipped with Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm) following the reported description with using pure monosaccharides (mannose, ribose, rhamnose, galacturonic acid, glucose, xylose, galactose, and arabinose, Sigma aldich, USA) as standard (Wu, Jia, Ying, & Wu, 2014). To obtain the spectra of 13C, 1H NMR and bi-dimensional COSY and HMQC, the samples were solubilized in DMSO. The spectra were obtained in Bruker equipment model Avance HD 400 M.

2.4. Production optimization, chemical analysis, and cost evaluation

Based on our previous data, Taguchi orthogonal array was implemented for the evaluation of selected four factors on the biopolymer yield, including temperature, initial pH, $K_2HPO_4{\cdot}3H_2O$, and $MgSO_4{\cdot}7H_2O$ in four levels (Table S1). All the experiments were carried out in 200 mL of BM medium where 10% (v/v) inoculum was added at 28 °C and 200 rpm for 72 h and samples were collected at 6 h interval. After obtaining the optimal medium, the scale-up production was performed in 20 L automatic stainless steel bioreactor (BLBIO-20SJ-S, Bailun, China).

Biomass and biopolymer were determined as follows. 10 mL of fermented broth was diluted 4 folds with distilled water, then heated at 80 °C for 30 min and vortexed for 1 min, and finally the pellet was