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Exopolysaccharide production by an indigenous isolate *Pseudomonas stutzeri* XP1 and its application potential in enhanced oil recovery

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ABSTRACT

In this study, a rapid and efficient method for screening biopolymer producers was established using 96-well plates. An indigenous biopolymer producer *Pseudomonas stutzeri* XP1 was isolated from Xinjiang oil reservoirs, China. Strain XP1 can grow and produce 16 g/l biopolymer using corn starch and nitrate. Produced biopolymer increased culture viscosity up to 2384 mPa s. Biopolymer showed rheological properties and pseudo-plastic behavior. The viscosity of 8 g/l biopolymer solution kept higher than 25 mPa s at 20–50 °C and pH values (5–9) and increased to 7600 mPa s with NaCl concentrations increasing to 2%. Gel permeation chromatography data showed that the biopolymer average molecular mass was 1.65×10^6 Da. Gas chromatography revealed that the monosaccharide composition in biopolymer was glucose. Core flooding experiments revealed that extra 13.56% of oil was recovered by in situ biopolymer production of strain XP1. Properties of strain XP1 and the biopolymer produced make them promising for enhanced oil recovery.

1. Introduction

Improving sweep efficiency is one of key factors to effectively enhance residue oil recovery in oil reservoirs (Chen, Feng, Liu, & Sepehrnoori, 2017; Dhanarajan et al., 2017; Olajire, 2014). Polymers can increase the viscosity of displacement fluid, thus reducing the fluidity ratio and improving the sweep efficiency (Guo et al., 2014; Zhang, Wang, Yang, Chen, & Bai, 2015). Although chemical polymers flooding effectively improved oil recovery, the use of chemical polymers caused environmental pollution (Fakhru'l-Razi et al., 2009; Olajire, 2014). The sewage produced after chemical polymers flooding is not up to the reinjection standard (Zhang et al., 2010). The sewage treatment is high-cost and difficult (Dong, Lu, Huang, & Xu, 2011; Zhang et al., 2010). Therefore, eco-friendly oil recovery technologies are urgently needed. Microbial enhanced oil recovery (MEOR) is considered to be an eco-friendly enhanced oil recovery technology (Brown, 2010; Lazar, Petrisor, & Yen, 2007). Many MEOR studies were focused on biosurfactants flooding by improving the oil displacement efficiency (Al-Wahaibi et al., 2014; Brown, 2010; Zhao, Li, Guo, Shi, & Zhang, 2018). Relatively few MEOR studies were involved using biopolymers to enhance oil recovery (Fernandes et al., 2016; Lazar et al., 2007; Pfiffner, McInerney, Jenneman, & Knapp, 1986)

Some microorganisms can produce biopolymers to enhance oil recovery (Pfiffner et al., 1986; Soudmand-asli, Ayatollahi, Mohabatkar, Zareie, & Shariatpanahi, 2007). Produced biopolymers can improve the sweep efficiency by increasing the viscosity of displacement fluid (Pfiffner et al., 1986; Soudmand-asli et al., 2007; Sun et al., 2011). Moreover, microbial produced biopolymers are salt tolerant and thermotolerant, which makes them become promising oil displacement agents under the environmental conditions that exist in the reservoir (Li et al., 2017; Pfiffner et al., 1986).

Currently, microbial produced biopolymers, such as exopolysaccharide, are mostly used in biopharmaceutical, biomedical and food industries (Freitas, Alves, & Reis, 2011; Habibi & Khosravi-Darani, 2017). Only a few biopolymers were used for enhanced oil recovery, such as xanthan gum and welan gum (Jang, Zhang, Chon, & Choi, 2015; Xu, Xu, Liu, Chen, & Gong, 2013). Discovering appropriate biopolymers for targeted oil reservoirs will be significant to use biopolymers to enhance oil recovery. Screening of biopolymer producing bacteria as much as possible will help to discover biopolymers suitable for

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enhanced oil recovery. Oil reservoirs harbored abundant microbial resource (Gao, Tian, Li, Sun, & Ma, 2015; Gevertz, Telang, Voordouw, & Jenneman, 2000). The functional microorganisms isolated from oil reservoirs can well adapt the complex oil reservoirs conditions (Magot, Ollivier, & Patel, 2000; Zhao et al., 2015).

In the present study, a rapid and efficient method for screening biopolymer producers was established using 96-well plates. An indigenous biopolymers producer was isolated from the production water of Xinjiang oilfield, China. The low-cost substrate for biopolymer production was also studied. The biopolymer product was extracted and characterized. Its rheological properties were also evaluated. The enhanced oil recovery potential was also investigated by core flooding experiments.

2. Materials and methods

2.1. Water samples, chemicals and cultivation conditions

Water samples were collected from production wells in Xinjiang oilfield, China. The oil reservoirs depth, temperature and salinity are 1088 m, 39 °C and 15,000 mg/L, respectively. Chemicals of analytical grade were used. The mineral salts (MS) medium used in this study contained 40.0 g/l corn starch, 2.0 g/l $NaNO_3$, 0.50 g/l $MgSO_4{\cdot}7H_2O$, 1.5 g/l $K_2HPO_4{\cdot}3H_2O$ and 1.0 g/l KH_2PO_4. The LB medium contained 10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl. LB medium with 2% agar was also used in this study. Bacteria screening and biopolymer production experiments were both conducted at 39 °C that simulated the zone temperature in Xinjiang oil reservoirs.

2.2. Isolation and characterization of biopolymer-producing bacterial strain

2.2.1. Strain isolation

Firstly, 5 ml of reservoirs production water mixture was added to the 250 ml flasks containing 100 ml of MS medium. Flasks were incubated at 39 °C, 180 rpm for 3 days. Then, 0.2 ml of enrichment culture was sampled. Enrichment culture was serially diluted and dispersed on LB medium plates. Then LB plates were incubated at 39 °C for 24 h.

Each distinct colony was inoculated into wells of 96-wells plates. Each well contained 0.1 ml of MS medium. Sterile breathable membrane was covered on 96-well plates. Then the 96-well plates were incubated at 39 °C for 3 days. The culture in each well of 96-well plate was picked up by sterilizing toothpicks to observe whether visible silk was pulled out from the culture liquid. The colonies whose culture liquid can be pulled out visible silk were the candidate biopolymer producers.

To verify the biopolymer production, candidate strains were inoculated into 250 ml flasks containing 100 ml of MS medium. After incubated at 39 °C, 180 rpm for 4 days, the viscosity of culture liquid was determined using DV-IIviscometer (Brookfield Company, Massachusetts, USA) at 39 °C and 50 rpm. The strain whose culture liquid had maximum viscosity was selected for further study. Here the selected strain was referred to as strain XP1.

2.2.2. Characterization and identification of strain XP1

Using scanning electron microscope (ESEM) Quanta™250 (FEI Company, American), the photomicrograph of strain XP1 was taken for morphological characterization. Genomic DNA of strain XP1 was extracted using TIANamp Bacteria DNA Kit (TIANGEN BIOTECH CO., LTD, Beijing, China). 16S rDNA of strain XP1 was amplified using PCR primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR product was sequenced by Beijing Genomics Institution (Beijing, China). Using the online BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the obtained sequence was aligned with those sequences available in the GenBank database. MEGA 5.0 software was used to construct the phylogenetic tree by the neighbor-joining method.

2.3. Selection of carbon source and nitrogen source for biopolymer production

In order to select the optimum and low-cost carbon source, strain XP1 was cultivated in the MS media containing equal amount of diff ;erent substrates, respectively. Nitrate ($NaNO_3$ of 2 g/l) was used as nitrogen source. The concentrations of all the tested substrates were 40 g/l. The tested substrates included glucose, sucrose, corn starch, glycerol, corn steep powder, molasses.

To select the optimum nitrogen source, strain XP1 was cultivated in the MS media containing equal amount of diff ;erent nitrogen source. Corn starch (40 g/l) was used as carbon source. The concentrations of all the nitrogen sources were 2 g/l. The tested nitrogen source included $NaNO_3$, NH_4Cl, yeast extract, peptone. Strain XP1 was inoculated into 250 ml flasks containing 100 ml of MS medium. After incubated at 39 °C, 180 rpm for 5 days, viscosity of culture fluid was measured at 39 °C and 50 rpm.

2.4. Biopolymer extraction

In order to remove cells and insoluble substances, diatomite was added into fermentation liquid to a final concentration of 2% (m/v). Then the mixture was shocked at 40 °C, 100 rpm for 6 h. The precipitation substance was removed by centrifugation (10,000 *g*, 10 min). Then 6-fold volumes of ethanol was added into the supernatant, mixed and kept at 4 °C overnight to precipitate biopolymer product (Shih, Van, Yeh, Lin, & Chang, 2001). The crude product was obtained by centrifugation (10,000 *g*, 10 min). The crude biopolymer product was dissolved by 200 ml distilled water. Proteins were removed by sevag method (Chen, Xie, Nie, Li, & Wang, 2008). Then the liquid was treated by the ethanol precipitation process again. After vacuum freezing and drying, the biopolymer product was used for further study.

2.5. Effect of biopolymer concentration on the solution viscosity

Using the extracted biopolymer product, biopolymer solution with concentrations of 2 g/l, 4 g/l, 6 g/l, 8 g/l and 10 g/l was prepared. The viscosity of biopolymer solution with different concentrations was determined using DV-IIviscometer (Brookfield Company, Massachusetts, USA) at 39 °C and 50 rpm. The zone temperature in Xinjiang oil reservoirs is 39 °C. The suitable concentration was chosen for further experiments according to the polymer viscosity value used for oil displacement in oilfield.

2.6. Biopolymer stability under different temperatures, pH values and salinities

Biopolymer solution of 8 g/l was prepared using the extracted biopolymer product. The viscosity was determined at 39 °C and 50 rpm. Then biopolymer solution was divided into 20 parts. Each part was 100 ml. The viscosity of 8 parts of biopolymer solution was measured by viscometer at different temperatures (i.e., 20, 30, 40, 50, 60, 70, 80 and 90 °C) with rotate speed of 50 rpm. Other 6 parts of biopolymer solution was adjusted to different pH (i.e., 5, 6, 7, 8, 9 and 10) with 1 mol/l HCl and 1 mol/l NaOH. Then the viscosity was measured by viscometer at 39 °C and 50 rpm. The last 6 parts of biopolymer solution was added NaCl to different salinity (i.e., NaCl concentration of 0, 2 g/l, 5 g/l, 10 g/l, 20 g/l and 25 g/l). The viscosity was measured by viscometer at 39 °C and 50 rpm.

2.7. Rheological property analysis

Rheological properties of biopolymer produced by strain XP1 were analyzed as follows. Biopolymer solution with concentrations of 4 g/l,