Screening Of Exopolysaccharide Producing Bacterium Frateuria Aurentia From Elephant Dung

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**Abstract**

Exopolysaccharide producing strain was isolated from elephant dung in Srivilliputhur, Tamilnadu, India. It was identified as Frateuria aurentia by cultural, biochemical and 16S rDNA genetic factor sequencing. The maximum production of EPS recovered from Frateuria aurentia in basal medium 90.66 ± 16.8mg/100ml of dry weight.

**Keywords**: EPS, Frateuria aurentia, 16S rDNA Sequences

**Introduction**

In the natural environment, Exopolysaccharide (EPSs) is generally heteropolymeric (made of different monomeric units), non-sugar components like uronic acid, methyl esters, sulphates, pyruvates, proteins, nucleic acids and lipids. EPS also contain divalent metal cations that act as ionic bridges linking adjacent polysaccharide chains. Many microorganisms like bacteria, fungi and actinomycetes are produce high molecular weight, hydrated polymeric compounds called exopolysaccharides (EPS) during their lifecycle. Many bacterial cultures produce different types of EPS during its lifecycle. For example, most bacteria produce capsular form of EPS during the exponential growth phase and slime type EPS during the stationary growth phase (Wingender et al., 1999).

Only a few of the huge number of new bacterial EPSs reported over recent decades have emerged as industrially important biopolymers with significant commercial value, particularly with regard to their use as biomaterials (e.g., bacterial cellulose) (Chawla et al., 2009; Rehm, 2009) or as rheology modifiers of aqueous systems (e.g. xanthan gum). Some bacterial EPS can directly replace polysaccharides extracted from plants (e.g. guar gum or pectin) or algae (e.g. carrageenan or alginate) in traditional applications, because of their improved physical properties (e.g. xanthan gum or gellan gum) (Fialho et al., 2008; Rehm, 2009). Conversely, other bacterial EPS possess unique properties that can launch a range of new commercial opportunities (e.g. bacterial cellulose or levan) (Kumar et al., 2007; Ulrich, 2009). Two newly reported bacterial EPS with great potential include GalactoPol, synthesized by Pseudomonas oleovorans, which is composed mainly of galactose and FucPol, a fucose-containing EPS that is synthesized by Enterobacter A47 (Freitas et al., 2009; Freitas et al., 2011). The secretion of EPS is a challenging process for the bacterium, in which hydrophilic, high molecular weight polymers assembled in the cytoplasm must traverse the cell envelope, without compromising the critical barrier properties. In contrast with the wide diversity of the molecular structures found in EPSs, the pathways for their biosynthesis and export in most Gram-negative bacteria have been reported to follow one of two mechanisms: the Wzx-Wzy-dependent pathway, in which the polymer repeat unit is assembled at the inner face of the cytoplasmic membrane and polymerized at the periplasm, and the ABC transporter-dependent pathway, in which polymerization occurs at the cytoplasmic face of the inner membrane (Cuthbertson et al., 2009).

The global market for hydrocolloids, which includes many polysaccharides, is still dominated by plant and algal polysaccharides (e.g. Starch, Galactomannans, Pectin, Carrageenan and Alginate). This market valued at >4 million US$ in 2008, with xanthan gum being the only significant bacterial EPS, which accounted for 6% of the total market value. Bacterial EPSs are widely used in the food industry as viscofying, stabilizing and emulsifying agents (Liu et al., 2010). Moreover, EPS can be used as bioflocculants, bioabsorbants, encapsulating materials, heavy metal removing agents, drug delivery agents, ion exchange resins and hosts for hydrophobic molecules (Ismail and Nampoothiri, 2010). The polysaccharides are believed to protect bacterial cells from desiccation, penetration of toxic metals, antibiotic, phagocytosis, phage attack and to produce biofilms. In recent years, bacterial polysaccharides have become an alternative of interest as immunostimulatory, immunomodulatory, antitumor, antiviral, anti-inflammatory and antioxidant agents in various medical and pharmaceutical industries. Microbial EPSs such as dextrans, xanthan, gellan, pullulan, yeast glucans and bacterial alginates are potentially used in many industries as food additive and microorganisms are more suited for...
EPS production than microalgae and plant ((Wang et al., 2008). In this background we isolate and identify the EPS producing particularly bacterium Frateuria aurentia from elephant dung and observed the maximum production of EPS.

Material And Methods
Collection and processing of the sample
Elephant dung sample was collected from Shenbagathoopu, Srivilliputhur, Tamilnadu North Latitude, 11° 00’ and 12° 00 N, East Longitude, 77° 28’ and 78° 50’. Isolates were obtained by serial dilution plating on nutrient agar medium. A total of 10 different colonies were isolated and the exopolysaccharide producing bacteria were screened based on their morphological characters, mucous and ropy appearances.

Biochemical Characterization
Collected samples were transported immediately to the laboratory for analysis.
They were manipulated according to conventional microbiological methods and Frateuria sp. are identified based on biochemical characteristics

Identification of bacteria by 16S rRNA gene sequencing analysis
Genomic DNA isolation from isolates
The isolated bacterial strain was grown in 25ml LB broth overnight at 35°C. The culture was spin at 5000rpm for 5min. The pellet was resuspended in 400µl of sucrose TE buffer (Tris EDTA). Lysozyme was added to a final concentration of 8mg/ml and incubated for 1 hour at 35°C. To this tube, 100µl of 0.5M EDTA (pH 8.0), 60µl of SDS and 3µl of protease K (20 mg/ml) were added and incubated at 55°C. After incubation, they were centrifuged at 7000 rpm for 3min and then the supernatant were extracted twice with phenol: chloroform (1: 10 and again with chloroform: isoamylalcohol (24:1). It was precipitated with ethanol. The DNA pellet was resuspended in sterile buffer.

Amplification of 16S rDNA gene sequence
Bacterial 16S rDNA was amplified from the extracted genomic DNA using the following universal eubacterial 16S rRNA primers: forward primer 5’ AGAGTTTGATCCTGGCTCAG 3’ and reverse primer 5’ ACGGCTACCTTGTTACGACT 3’. Polymerase chain reaction was performed in a typical reaction mixture was 2µl of template DNA and 1.5µl of forward primer, 1.5µl of reverse primer, 10µl of 2X PCR master mixes and 5µl of nuclease free water for 20µl reaction. The reaction was performed with an initial denaturation at 94°C for 2 minutes. 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56° for 1 minute, extension at 72°C for 1 minute 30 seconds followed by final extension at 72°C for 5 minutes and hold at 4°C. The amplification of 16S rRNA gene was confirmed by running the amplification product in 1% agarose gel electrophoresis.

16S rRNA gene sequence
Full length partial sequencing of the rRNA gene for the isolated bacteria was carried out by Macrogen, Korea.

Basal Medium for exopolysaccharide (EPS) production from isolated bacteria
Frateuria aurentia culture was maintained on nutrient agar plates. It was sub cultured and slants were inoculated and maintained at 28°C for 24 hours. Experiments were done using 250ml flask each contained 100ml of basal medium contains Dextrose -10gm; yeast extract – 3gm; malt extract – 3gm; peptone – 5gm; MgSo4, 7H2O – 1gm; KH2PO4 – 0.3gm and Vitamin B1– 10mg incorporated at 28°C on an orbital shaker incubator at 110 rpm for 72 hours.

Bacterial EPS quantification
After 72 hours of incubation, basal medium were centrifuged at 5000rpm for 20 min. The EPS was then precipitated from the supernatant by addition of equal amount of carbinol. The mixture were agitated with addition of methanol to prevent local high concentration of the precipitate and left night at 4°C and centrifuged at 7000 rpm for 20 mins. After centrifugation the precipitate was collected in a petri plate and dried at 60°C (Wang et al.2008).

Results And Discussion
Elephant dung samples were taken from Shenbagathoopu, Srivilliputhur area for this study. The samples were serially diluted and plated for screening of efficient EPS producing microorganism. After 24 hrs of incubation, the plates were kept at 37°C. Selected strain which exhibited EPS production on basal medium after further confirmation was used for further studies (Fig. 1.) Arena et al. (2009) stated that the B3 -72 strain of geobacillus thermodenitrificans, isolated from a shallow, marine vent of Vulcano Island (Eolian Islands, Italy). Fritas et al. (2010) screened EPS producing bacteria from French spoiled ciders allowed us to isolate a ropy bacterium belonging to the B. Licheniformis species. Strains of B. Licheniformis are also common in foods including natural agricultural cereals. Likewise we isolated the exopolysaccharide producing microorganisms from elephant dung, collected from shenbagathoopu, Srivilliputhur Tamilnadu, India. In the present study, we recovered maximum production of EPS observed from Frateuria aurentia in basal medium 90.66 ± 16.8mg/100ml of dry weight. The bacterial EPS recovered from optimized medium was 231 ± 0.8 mg/100ml of dry weight.
respectively. Frateuria aurentia exopolysaccharide content under the optimised conditions was 2.5 times higher than the basic culture medium of initial conditions.

The selected strain was identified by various physical, biochemical and molecular characters. This strain exhibited Gram negative, rod shaped and motile bacteria. According to Bergey’s manual of Determinative Bacteriology, the selected microbe are identified Fraturia sp. All the carbon utilizing and biochemical studies were performed and results were presented (Table.1).

The 16S rDNA gene of the Fraturia sp. was amplified using Polymerase Chain Reaction (PCR) with the help of 16S rDNA Universal primers. The sequences were compared against 16S rDNA sequences available in the RDP database (http://rdp.meb.ncsu.edu/). The sequence analysis revealed that the strains were phylogenetically closely related to the genus Fraturia. Blast analysis of the 16S rDNA sequence of isolate revealed that the selected isolates showed maximum similarity of 98% with Fracteria aurentia. The Phylogenetic relationship was obtained using neighbor joining by pair wise comparison among the 16S rDNA gene sequence of selected isolates with species. The dendrogram was constructed for their Phylogenetic relationship and it revealed that the isolate Fraturia aurentia was distinctly placed under separate clusters. The 16S rRNA gene sequences of the isolates had been submitted to the NCBI Genbank (Fig.2 and 3.) The bacterial EPS recovered from basal medium was 90.66 ± 16.8mg/100ml of dry weight. Similarly, Kannmani et al. (2011) find out the EPS production by S. phocae PI80, EPS (g/L) production (7.8 ± 0.29, 7.9 ± 0.34 and 8.1 ± 0.27) and also Ismail and Nampoothiri (2010) reported the maximum EPS production by Lactobacillus plantarum MTCC 9510 at 35°C. Wang et al. (2008) stated that the S. phocae was found to be best for EPS production. EPS production was also studied at various concentration of lactose and it is found that maximum EPS production (11.75 ± 0.20 g L⁻¹) occurred at 20 g L⁻¹ of lactose. The amount of EPS production and properties are greatly dependent on the microorganisms and their culture condition such as temperature, pH and media composition by the amount of EPS production and properties are greatly dependent on the microorganisms and their culture condition such as temperature, pH and media composition. Ismail and Nampoothiri (2010) reported that maximum EPS production by L. plantarum MTCC 9510 was observed in presence of lactose (40 g L⁻¹). Growth and EPS production by lactic acid bacteria was also enhanced by nitrogen sources. Liu et al. (2009) stated that the effect of nitrogen sources on EPS production by S. phocae showed that yeast extract was most effective than other tested nitrogen sources. This may be due to the presence of larger quantities of free amino acids, short peptides and more growth factors in yeast extract. Among the various concentration, yeast extract at 20 g L⁻¹ showed maximum EPS (12.14 ± 0.31 g L⁻¹) production. Yeast extract was reported to be the most efficient nitrogen source. Yeast extract was reported to be the most efficient nitrogen source, which greatly enhanced the EPS production by Lactobacillus plantarum MTCC 9510 observed maximum EPS production was high in the presence of yeast extract by Paenibacillus polymyxa EJS-3. Chowdhury et al. (2011) reported that Bacillus pumilus UW-02, an isolate from agricultural soil irrigated with waste water was found to produce a carbohydrate polymer in the form of extracellular polysaccharide (EPS) in glucose mineral salts medium (GMSM).

**Conclusion**

A pure bacterial strain capable of producing EPS bacterial strain was isolated and identified as Frateuria aurentia by carbohydrate fermentation profile and sequence analysis of 16S rRNA gene sequences.

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**Figure 1.** Exopolysaccharide production by Frateuria aurentia on basal medium

GGGGAGGCGTATCGGATACGGGCGTAAAACCGGTAGGGGCGTTTGTAAAA
GTCTGCTGTAACATCCCCGGGCTCAAACCTGGGAATGGCAGTGGATACCTGGG

107
Figure 2. Partial 16SrDNA sequences for Fraturia sp. (1737bp)

Figure 2. Phylogenetic analysis for Fraturia sp. (1737bp)
Table 1. Morphological and biochemical characteristics of Fraturia sp.

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<th>Observation</th>
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<td>Colony morphology</td>
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<tr>
<td>Gram staining</td>
<td>Gram negative, rod</td>
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<td>Motility</td>
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<td>Biochemical Characters</td>
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<td>Indole production test</td>
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<td>Hydrogen sulphide production</td>
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References


