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Exopolysaccharide production and purification from polluted soil of *Bacillus cereus* GR9

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ABSTRACT

Exopolysaccharide (EPS) biopolymers produced by microorganisms play a vital role in the environment, such as in the health and bio-nanotechnology sectors, gelling agents in the food and cosmetic industries and bio-flocculants in the environments sector, because they are biodegradable and non-toxic. This study focuses on enhancing EPS production 2.98 ± 0.045 g/L at 28 ºC for incubation. Bacillus cereus GR9 was the most potent bacterial isolate in contaminated soil. Following production, EPS was extracted and purified using DEAE-cellulose and confirmed by Fourier transform infrared (FT-IR), Uv-Visible and SEM. The results showed inter-linkage with glucose, mannose and galactose

Keywords: Polluted soil, Exopolysaccharides, *Bacillus cereus*, Production, Purification

INTRODUCTION

The most crucial group of biopolymers are microbial exopolysaccharides (Ates, (2015). EPS can be found in either a capsular or slimy layer, depending on its location (Freitas et al., 2014). Microbial exopolysaccharides are mainly composed of sugar polymers synthesized by a wide range of microorganisms, including bacteria, fungi, and yeasts (Freitas et al., 2010). In comparison to harmful synthetic polymers, natural biopolymers (bacterial polysaccharides) are biodegradable and biocompatible (Wang et al., 2014). The number of known exopolysaccharides (EPS) produced by microbial fermentation has gradually increased during the last few decades. Microbial biopolymers have a wide range of biotechnology applications, including pharmaceuticals, tissue engineering, cosmetics, food, textiles, oil recovery, metal mining, and metal recovery (Alsharabasy et al., 2016; Khopade et al., 2012).

The nutritional and environmental circumstances significantly impact the production of microbial EPS (Staudt et al., 2012; Suresh Kumar et al., 2007).

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The new age of industrial-scale microbial biopolymer production focuses on developing low-cost sources such as agro-industrial wastes (Pacwa-Pociniczak et al., 2011). Molasses is the ultimate effluent produced through repeated crystallization during the sugar production process (Razack et al., 2013). Sugarcane molasses may be a better carbon source due to its more significant overall sugar concentration of 48.3%. Wide-scale synthesis of microbial exopolysaccharides necessitates many research activities in order to put novel concepts into action on a large scale. This study aims to increase EPS production from the most potent bacterial strain by utilizing a sophisticated statistical experimental design as one of the first steps toward industrial production.

MATERIALS AND METHODS

Isolation, Identification and purification of producing bacterial exopolysaccharide (EPS):

Soil samples were collected from polluted soil from the Bodhan Sugarcane industries (Nizamabad, Telangana). Each sample was serially diluted from 10–1 to 10–7 in phosphate-buffered saline, pH 7.2 ± 0.2.

Isolated pure colonies of bacterial culture were identified using 16S rDNA gene sequencing, as reported by Ruimy et al. (1994). Eurofins Genomics, India Pvt Ltd carried out the genome sequencing (Bangalore, India). The obtained sequences were compared to GenBank sequences using the BLAST tool (Altschul et al., 1997), submitted to NCBI Genbank, and the accession number was obtained.

Exopolysaccharide production was done in 500 ml Erlenmeyer flasks containing 250 ml of medium broth. The basal medium contains (gm/Lit): Basal extract 10gm - Dextrose, 3.0gm – Yeast extract, 5.0gm – Peptone, 1.0gm – $MgSO_4$.7H2O, 0.3gm - KH_2PO_4,10mg – Vitamin B1(Atlas and Parks, 1997) 1000 ml- Distilled H_2O, sucrose 20g used as a source of carbohydrate. The medium was sterilized at 121°C for 15 minutes. All the test flask was inoculated with fresh growing 18 hours old 10% v/v culture, having a cell count of $3X10^6$ cells/ml. The flasks were incubated in a rotary shaker (Thermo scientific maxQ 6000) at 28 ± 2 °C for 4 days. Viscosity and EPS production was checked every 24 hrs.

UV-Vis

The sample was prepared by EPS (5 mg) was dissolved in 2.5 mL of distilled water. These solutions range between 200 and 600 nm peak showing noted using a spectrophotometer EPS followed this method. THERMO Scientific Evolution 600 UV–Vis NIR spectrophotometer used for absorption spectra (UV–Vis) was recorded.

FT-IR Analysis

PerkinElmer Spectrum 100 FT-IR spectrophotometer was used by the KBr pellet technique Sample were scanned ranging from 400–4000 cm−1.

EPS analysis by Scanning Electron Microscopic (SEM)

The EPS surface morphological behavior was studied through scanning electron microscopy (SEM) EPS surfaces. In addition, aluminum stubs and the air was dried with the EPS solution (1 mg/ml). The sample was analyzed through an SC7620 sputter coater. This study was performed at the National Institute of Technology-Warangal, Telangana, India.

RESULTS AND DISCUSSION

Isolation and identification of bacteria

The isolated bacteria were white and irregular in form, with positive gram staining outcomes. The presence of rod-shaped bacteria was clearly revealed by SEM analysis (Fig. 3B). The 16S rDNA gene sequence data indicated that the isolated strain was more similar to *Bacillus cereus*. The sequences were submitted to Genbank and obtained the accession number MH553075.

Production of EPS

On 72 h, EPS and biomass production in the culture medium were insignificant (exponential growth phase). It is important to note that total cell biomass and EPS production (2.98 ± 0.045 g/L EPS) would be significant. However, by 96 h (2.4±0.023), there was a reduction in biomass production, resulting in a decline phase. The results showed that EPS production was closely related to biomass growth over most of the culture period. Sun and colleagues recently reported that the marine fungus *Epicoccum nigrum* JJY-40 produced significantly fewer EPS (0.83 g/L) in a 7-day culture (Sun et al., 2011). After 72 h of fermentation, *Pestalotiopsis sp*. BC55 produced the most biomass and EPS (1.32 ± 0.045 gm/L EPS).

Purification and determination of EPS yield

Bacterial culture was combined with two volumes of absolute cold ethanol and centrifuged at 10,000 rpm for 10 minutes. This procedure was performed twice to eliminate low molecular weight contaminants. Ion exchange chromatography using the DEAE-Cellulose column to purify the EPS yielded in 61 fractions. Exopolysaccharide, the first recovered polysaccharide in the nine eluting solutions, was dissolved in deionized water and measured using the phenol-sulfuric acid test (Michel et al., 1956). The experiment was carried out three times to obtain the results mean value.

Chemical analysis of EPS

UV-Visible

It strongly absorbs electronic transitions used for chromophore groups of atoms characterization by analysis of UV-visible spectroscopy. The present study of the UV spectrum of the EPS was calculated and shown in (Fig. 1) UV absorption area indicated the results. The GR9 recorded the highest spectrum area from the 240~320 nm spectrum region. It has been found in many functional groups carboxyl, amine, ester and carbonyl. The EPS samples were used to record UV spectra (Fan et al., 2014). The spectrum range at 1,100 cm^{-1} could be credited to the existence of sulphate groups C−O−S and S = O (Stuart, (2004); Parikh et al., 2006). The elemental analysis of EPS in the presence of sulphur was confirmed, which presents many visible bands and peaks less than 1,000 cm^{-1} (Comte et al., 2006).

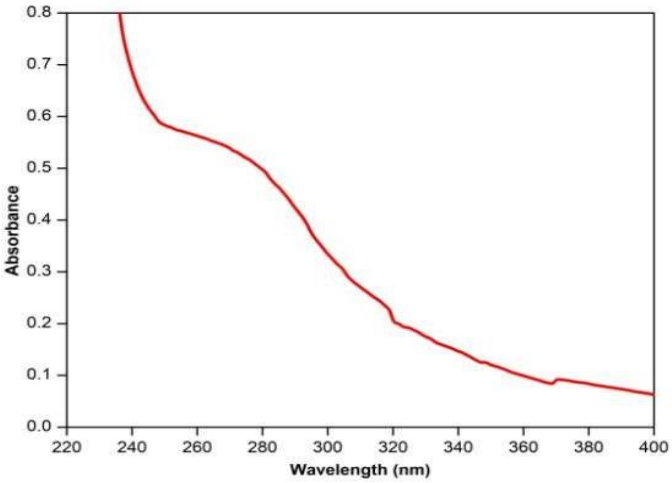

Figure-1. EPS UV-Visible

FT-IR analysis of EPS

The FTIR spectrum of the *Bacillus cereus* GR9 (Fig. 2) was clearly shown to signal peaks of carbonyl compounds. 2927 cm^{-1} (-O-H), 1446 cm^{-1} (CH_2 bind) and 1020 cm^{-1} (C-O) are related to the hydrolic function group, like glucose or Galactose, bending carbon functional group vibrations of alkanes was identified and ether groups were identified. Spectrum at 2926.30 cm^{-1} and 1032.30 cm^{-1} to be common polysaccharides has been reported.

The C – O carbon vibration stretching was allocated to the absorption peak at 1056.99 cm^{-1}. In addition, the

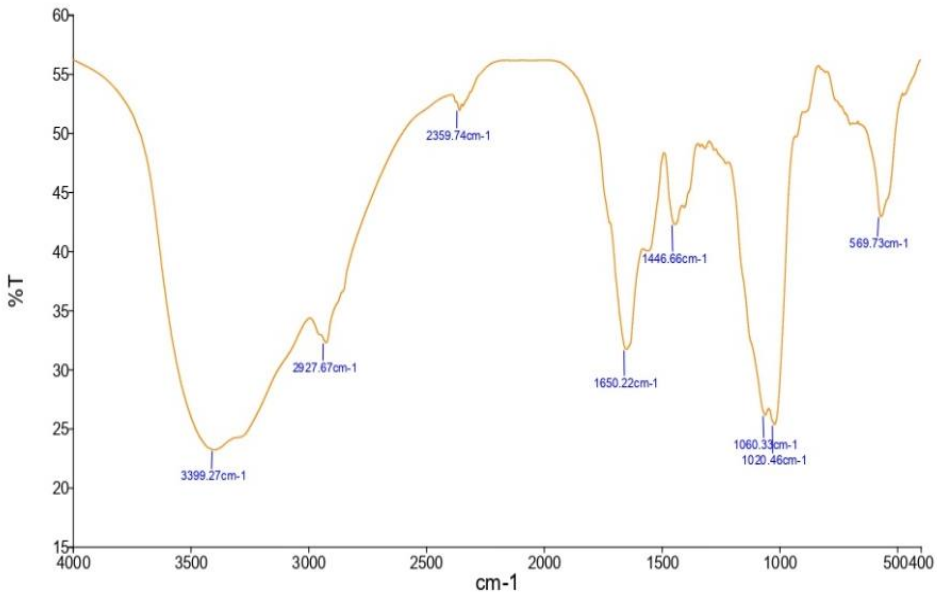

Figure-2. EPS FT-IR analysis

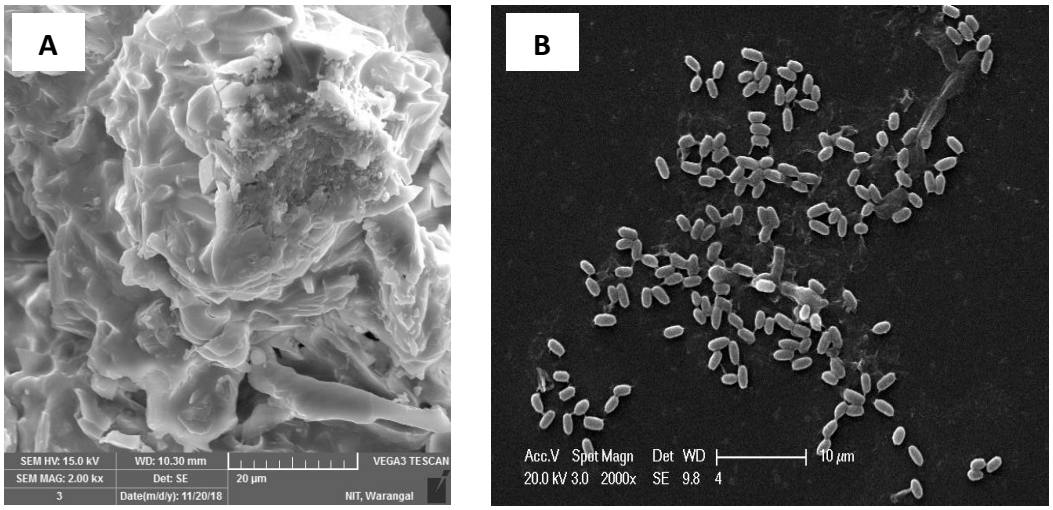

Figure-3. A) SEM images showing the shape and surface morphology of EPS and B) *Bacillus cereus* GR9

range of peaks between 1010.63 cm−1 and 690.47cm^{-1} was assigned for ethereal bonding and C-C-O bending and the O-C-O bending, both with mixed vibrational vibrations of C- C-H distortion (Miao et al. 2014). The spectrum peaks at 3401 cm^{-1} (O-H group), 2932 cm^{-1} (C- H & C-O groups) and 1094 cm^{-1} (C-O group). FT-IR spectrum analysis of major functional groups indicating mannose and galactose were revealed (Wang et al., 2017) and different functional groups of polysaccharide absorption peaks (Wang, et al., 2014).

SEM analysis of EPS

SEM was investigated in the composition of EPS from *Bacillus cereus* GR9. The internet's dense, porous and uneven design, such as the EPS SEM analysis, was shown (Fig. 3 (A)). The porous nature of EPS has also been reported in the surface morphology and the elemental composition of EPS made from *S. thermophiles* (Wang et al., 2015; Prasanna et al., 2012).

CONCLUSION

The novel strain *Bacillus cereus* was isolated strain was identified and the synthesis, optimization, and purification of EPS produced by *Bacillus cereus* GR9 were explored. Chemical characterized by Uv-Vis, FI-IR and SEM analysis done. Compared to the initial EPS production, the EPS production at optimal conditions increased by 2.98 ± 0.045 g/L. A cost-effective and optimum culture medium is required for industrial EPS production, mainly composed and inter-linkage of glucose, mannose, and galactose

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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