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Characterization of an exopolysaccharide synthesized by *Bacillus subtilis*

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SUMMARY

Plant leaves were examined for bacteria capable of producing polysaccharide extra-cellularly. A bacterium identified as belonging to genus *Bacillus* efficiently produced polysaccharide. Polysaccharide productivity was optimal in medium which contained peptone, yeast extract and NaCl. The physiological and gelling properties of partially purified exopolysaccharide were similar to those of levan gum. The sugar constituent of this exopolysaccharide was fructose.

Key words: Sonication, CPC(acetyl pyridinium chloride), EPS(extra cellular polysaccharide), and Levan

Many bacteria are able to produce various kinds of polysaccharide outside the cells as Capsules or slime layers. These extra-cellular polysaccharide secreted from bacteria are biodegradable and much safer than chemically synthesized polymers. For example, Dextron (i) is used as a blood plasma substitute, Xanthan gum (2) Alginate (3) are added to food as thickening and suspending agents and Gellan gum (4) can be used in the agricultural industry as a plant tissue culture medium. Furthermore the affinity of ionic polysaccharide to metal ion is of potential importance for water clarification and metal recovery. As only a small number of bacterial strains have been adopted to industrial application. It is therefore, further important to isolate new polysaccharide producing agents.

A bacterium that produces copious amounts of extra-cellular polysaccharide in a liquid medium was isolated, during the course of screening for polysaccharide producing bacteria from plant leaf surface. This paper describes identification of the bacterium isolated, the culture condition for stable polysaccharide production, and the chemical as well as physiological properties of the polysaccharide produced.

MATERIALS AND METHOD

Screening of a polysaccharide-producing micro-organism- A polysaccharide producing micro-organism was screened from plant leaves (Neem plant). Leaves were soaked in distilled water followed by few minutes (3-4 mins) of sonication. The water thus obtained was streaked on agar plates consisting of 1% peptone, 0.5% yeast extract, 0.5%NaCl, and 2% Agar. The plates were incubated at 30°C for 4-5 days. Plates were observed for the appearance of mucoid colonies after 3-5 days.

Production of Microbial EPS- Mucoid and rough colonies were selected and incubated at 30°C on a rotatory shaker at 2000 rpm for 4-5 days in a liquid medium composed of 1% sucrose, 0.08% peptone, 0.04% yeast extract and 0.14% Na_2HPO_4. After 4-5 days of incubation, the culture with the rich mucous was subjected to further investigation.

Identification and culture of the polysaccharide producing micro-organism- Taxonomical study of the isolated polysaccharide producing micro-organism was performed according to Bergey's Manual of systematic bacteriology based on its morphological and biochemical characteristics.

The micro-organism was inoculated into 50 ml of a polysaccharide producing medium containing1% sucrose, 0.2% peptone, 0.05% yeast extract and 0.02% $MgSO_4$. $7H_2O$ in a 300 ml flask and centrifuged at 10,000 rpm for 5-10 mins. Polysaccharide synthesized was precipitated by the addition of three times the volume of acetone to the cell free culture broth followed by washing with acetone. All the three mediums were compared for maximal production of polysaccharide.

Partial purification of the polysaccharide- Acetone precipitated polysaccharide was then dissolved in distilled water followed by re-precipitation with acetone. This procedure was repeated twice. The polysaccharide obtained, were dialyzed against water for 24h. After dialysis, the dialyzate was diluted with water to bring the polysaccharide concentration to around 0.3%. The dialyzed polysaccharide solution was treated with 0.3% trifluro-acetic acid. Finally, three times the volume of acetone was added to the dialyzate. The precipitate, partially purified exopolysaccharide, was then dried under reduced pressure and stored in a dessicator for further analysis.

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