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Isolation and Screening of Extracellular Polymeric Substances Producing Bacteria from the Sewage Water in Nagercoil

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ABSTRACT

Extracellular polymeric substances or extracellular polysaccharides are very high molecular weight polymers mainly consist of carbohydrate, protein and nucleic acid. In this study, more than 50 bacterial strains were isolated from the sewage water and subjected for the secretion of extracellular polysaccharides. Among them ten bacterial cultures showed slime or mucoid appearance on nutrient agar plates. These bacterial isolates were identified based on morphological and biochemical characters. Among all bacterial strains, Pseudomonas aeruginosa produced more extracellular polysaccharides (1.125 ± 0.007 g/l). The total protein content of extracellular polysaccharide from P. aeruginosa was 1.9 ± 0.08 mg/g. The extracellular polysaccharides from P. aeruginosa may have various industrial applications.

Keywords: *Pseudomonas aeruginosa; polysaccharides; screening*

1. Introduction

Microorganisms secrete very high molecular weight compounds known as the extracellular polymeric substances (EPS). The EPS comprises of mainly polysaccharides but also contain non-sugar components such as nucleic acids and proteins. The bacterial EPSs are synthesized in two forms, *i.e.*, capsular EPS and slime EPS [1]. Capsular EPSs are attached to the bacterial cell whereas the slime is either not bound to the cell or is free from it. Capsular EPSs play an important role in the flocculation of sludge. In capsular EPS, the slime is not involved in this process because it is totally free from the cell [2]. Based on their sugar compositions, the EPSs can be classified into homopolysaccharides and heteropolysaccharides. Homopolysaccharides are composed of a single type of monosaccharide, and heteropolysaccharides are composed of different types of monosaccharides [3]. EPSs are products of bacterial metabolism, accumulating on the bacterial cell surface. They form a protective layer for the cells against the harsh external environment such as salinity, temperature, pH, and also serve as carbon and energy reserves during starvation. This EPS helps the organism to survive at extreme environmental conditions and impart protection against stress such as UV radiation, biocides, desiccation, and heavy metals [1].

EPSs are secreted by various microorganisms such as bacteria, fungi, blue-green algae and macro algae [4]. It consists of lipids, proteins, polysaccharides, extracellular DNA and form 'houses' of biofilm cells. Recently, there has been an increased attention in exploring EPS from various sources because of their applications in various industries. The wide structural, physical diversity and other properties of EPS produced by the biofilm-forming bacteria make it biotechnologically and industrially significant [5]. Various bacterial species including *Vibrio harveyi* [6], *V. Alginolyticus* [7], *Lactobacillus* sp. G77 [8], *Lactobacillus* sp. [9], *Lactococcus* sp. [10], *Bifidobacterium* [11] and *Bacillus* sp. [12] were reported to produce EPSs.

EPS producing bacteria is found in a variety of ecological niches. So the physiological role of exopolysaccharides is diverse and may be dependent on the specific nature of the environment of the particular organism. EPSs are useful in textiles, adhesives, paint, paper, beverage, food industries, heavy metal polluted soils, oil recovery [13], petroleum and mining industries [14]. In spite of the various types of extracellular polysaccharides which are commercially available researchers are continuing to search novel polysaccharides for various biotechnological applications.

2.0. Materials and Methods

2.1. Isolation and screening of EPS producing bacterial isolate

The wastewater samples were collected in and around Nagercoil area from 2011 to 2012 Kanniyakumari district, Tamilnadu. The samples were serially diluted and plated on nutrient agar plates [(in g/l), (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract, 1.5; sodium chloride, 5.0; and agar, 15)]. All plates were incubated at 37 °C for 96 h. The potent EPS producing isolate was retained by observing for good mucoid colony morphology [18].

2.2. Morphological and biochemical identification of EPS producing bacteria

Isolated bacterial cultures were identified on the basis of biochemical, morphological and microscopic observations. Bacterial identification was carried out according to the methods provided in Bergey's Manual of systematic bacteriology.

2.3. Production of EPS in submerged fermentation

EPS production was carried out in cultures performed in nutrient broth medium (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract, 1.5; and sodium chloride, 5.0). Twenty five EPS producing bacterial isolates were incubated individually at 37 °C for 7 days after which the EPS was extracted by standard methods.

2.4. Extraction of EPS

The EPS was precipitated from the cell free extract by adding double volumes of ice cold ethanol. The mixture was kept in ice for 2 h and the precipitate was collected by centrifugation (10,000 rpm, 15 min, 4°C). The white precipitate obtained was washed with double distilled water. About 2.0 ml of double distilled water was added and filtered through Whatman filter paper. The filtrate was collected and dried at room temperature. The carbohydrate content of the dried EPS was further analyzed.

2.5. Determination of EPS by phenol-sulfuric acid method

The extracted EPS was quantified using phenol-sulphuric method [15] using glucose as standard. Total EPS was expressed as mg/l of glucose in each sample.

2.6. Total protein estimation

Total protein content of the sample was estimated as described by Lowry *et al* [16]. This protein assay is mainly based on the biuret reaction of proteins with copper sulphate at alkaline conditions and the Folin-Ciocalteau phosphomolybdotungstate reduction to heteropolymolybdenum blue which is measured at 660 nm.

3.0. Results and Discussion

In the present study, the EPS producing bacterial isolates were screened from the municipal waste water. Different types of bacteria persisted in the sewage water. A range of bacteria are reported to produce EPS. The morphological and biochemical characters of potent EPS producing bacterial isolates are described in Table 1.

Table 1. Morphological and biochemical characters of EPS producing bacterial isolates

Sl. No	Gram's staining	Shape	MR	VP	H_2S	Indole	Citrate	Urease	Starch	Catalase	Nitrate	Oxidase	TSI
sw1	+	Rod	-	+	-	-	-	-	+	+	-	+	+
sw2	-	Rod	+	-	-	+	-	-	+	+	+	-	+
sw3	-	Rod	-	+	-	+	+	+	+	+	-	-	+
sw4	-	Rod	-	+	-	-	+	+	+	+	-	-	-
sw5	-	Rod	-	-	-	-	-	-	+	+	-	+	-
sw6	+	Rod	+	+	-	-	+	-	-	+	+	+	+
sw7	+	Cocci	+	+	-	-	+	+	+	+	+	-	-
sw8	+	Rod	-	+	-	-	-	-	+	+	+	+	+
sw9	-	Rod	-	+	-	-	-	-	+	+	+	+	+
Sw10	+	Rod	-	+	-	-	-	-	+	+	+	+	+

The creamy colonies were generally regarded as the secretion of EPS and in the present study the creamy colonies were observed when the organism was cultured in nutrient agar medium. The selection of suitable EPS producing bacterial strains was mainly based on the development of mucoid colonies. The development of mucoid colonies by the secretion of EPS was reported by various researchers in various bacterial species [17]. Fusconi and Godinho [18] observed mucoid colonies by the secretion of *Pseudomonas aeruginosa* and *Escherichia coli*. In the present study, based on mucoid colonies, the organisms were selected for further studies. In the present study, *Escherichia coli, Proteus* sp., *Klebsiella* sp., *Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus* and *B. subtilis* were identifiedas bacterial isolates. Among all the bacterial isolates, the sample no. sw5 (*P. aeruginosa*) showed potent EPS production. The EPS producing bacterial isolates from the genus *Pseudomonas* was selected based on mucoid secretion [19, 24].

In the present investigation, the EPS producing bacterial isolates were screened from the municipal waste water. EPS producing bacteria including *P. aeruginosa* and *Azotobacter vinelandii* were reported to produce EPS [5]. The selected bacterial strains (sw1 to sw10) were cultured in nutrient broth medium and produced potential quantity of EPS. Among the

bacterial isolates, *P. aeruginosa* produced more EPS (1.125±0.007 g/l) than other bacterial isolates (Table 2).

Table 2. Production of EPS by the bacterial isolates isolated from the sewage water

Sl. No	EPS (g/l)
Bacillus sp.	32±0.008
Escherichia coli	0.099±0.001
Proteus sp.	0.84±0.017
Klebsiella sp.	0.63±0.003
P. aeruginosa	1.125±0.007
S. typhimurium	0.11±0.005
S. aureus	0.93±0.018
B. cereus	0.59±0.004
B. subtilis	0.981±0.010
Bacillus sp.	0.78±0.005

Screening of EPS producing organisms are in increasing demand because EPS has many biological activities [20]. The sewage water contains rich of microorganisms and these can survive under extreme environmental conditions and impart protection against stress such as UV radiation, biocides, desiccation, and heavy metals [1]. Screening of microorganisms from the subsurface environment showed that the ground water microorganisms are potent to produce many pesticides [21].

The crude, ethanol precipitated EPS was subjected for total protein estimation. The protein content of EPS from *P. aeruginosa* was found to be 1.9 ± 0.08 mg/g EPS (Table 3).

Table 3. Total protein content of EPS produced by the bacterial isolates

Sl. No	Total protein (mg/g EPS))
Bacillus sp.	0.15±0.002
Escherichia coli	0.041±0.003
Proteus sp.	0.32±0.016
Klebsella sp.	0.06±0.008
P. aeruginosa	1.9 ± 0.08
S. typhimurium	0.10±0.007
S. aureus	0.53±0.008
B. cereus	0.49±0.009
B. subtilis	0.87±0.017
Bacillus sp.	0.92±0.059

This result was in accordance the observations made with other bacterial species [5, 7]. Carbohydrate and protein were the important components of the EPS [22]. In soil and water, *Pseudomonas aeruginosa* is ubiquitous in nature and also isolated from biofilms of effluent

treating systems [23]. Because of its biofilm forming ability, *P. aeruginosa* is a good model bacterium for membrane biofouling studies [5].

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