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Original Article

ISOLATION, OPTIMIZATION AND QUANTIFICATION OF EXOPOLYSACCHARIDE PRODUCING BACTERIUM FROM WASTE WATER

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ABSTRACT

Exopolysaccharides (EPS) are high molecular weight polymers which are long chain composed of sugar residues and secreted by microorganisms into the surrounding environment. Bacterial EPS as a complex mixture of macro molecular poly electrolytes including polysaccharides, proteins and nucleic acids, each comprising variable molecular mass and structural properties. Many bacteria possess an ability to synthesize and excrete exopolysaccharides. Attempt has been made to isolate and identify the EPS producing bacteria using basal and malt medium which was maintained in 72hrs at 37°C, the dry weight of the fractionated products was found to be 1.98 ± 0.13 mg/100ml and 0.89 ± 0.10 mg/100ml in basal and malt medium, respectively. From the results, it was concluded that EPS extract was higher in EPS basal medium than malt medium. Bacteria were identified by their morphological, biochemical characterization. Out of twenty five isolates, isolate no-3,5,7,9,11,15 and 21 was found to be potential isolates for EPS production. EPS production of potential isolate was optimized in nutrient broth containing 2 percent sucrose for different environmental and nutritional conditions viz. incubation periods, pH, temperature, carbon source and different sodium chloride (NaCl) conc. Sucrose was found to be the suitable carbon source to produce EPS. Three days of incubation period was found to be an optimum for production of maximum yield of EPS in nutrient broth containing 4% sucrose. The optimum pH, temperature and NaCl for EPS production were found to be 7.5, 30^{0}C and 80 mM, respectively.

Keywords: .

INTRODUCTION

Exopolysaccharides (EPS) are high-molecular-weight polymers that compose sugar residues. Many bacteria, yeasts, fungi and algal cells possess an ability to synthesize and excrete exopolysaccharide [1, 6, and 9]. Also referred an extracellular polysaccharides or EPS material, these complex carbohydrates are widely varied in structure and function. The synthesis of exocellular investments of a polysaccharide nature by bacterial cells is generally considered as directly related to environmental constraints on the producing microorganisms. Exopolysaccharides (EPS) produced by Lactic acid bacteria possess the possibility of replacing stabilizer and thickeners, currentlyproduced commercially by non-food grade bacteria [4, 14]. Microbial exopolysaccharides have found a wide range of applications in the food, the pharmaceutical and other industries, due to their unique structure and physical properties. Some of these applications include their use as emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening and suspending agents [15]. Exopolysaccharides are generally composed of monosaccharide and some non carbohydrate substituent, like acetate, succinate. Capsular exopolysaccharides can protect pathogenic bacteria and contribute to their pathogenicity. Attachment of nitrogen-fixing bacteria to plant roots and soil particles are important for colonization of rhizosphere and roots and for infection of the plant, can be mediated by exopolysaccharides [16]. Cyanobacterial and bacterial Exopolysaccharides (EPS) have been reported to play a significant role in providing protection to the cell as a boundary layer [3], contributing to soil aggregation due to its gluing properties [10] and binding heavy metals due to the presence of several active functional groups onto it [7, 12].Therefore, it is important to isolate and characterize EPS producing microorganisms from waste water. It might be helpful for reclamation of such habitat.

MATERIALS AND METHODOLOGY

Screening and isolation of Exopolysaccharide producing bacteria

Serially diluted waste water sample were used for microbial isolation. 0.1ml waste water suspensions were spread on nutrient agar plates. Plates were incubated at 48 hrs at room temperature. Isolates were maintained on nutrient agar plates. Mucoid colonies were screened and re-streaked on another nutrient agar plate to obtain pure culture. Isolated cultures were identified on the basis of morphological, biochemical and microscopic observations. The identification work was done according to the methods described in Bergeys Manual of determinative bacteriology 9th edition.

EPS Production

Isolated organisms were used for production of exoplysaccharides. Bacterial isolates were maintained on Nutrient agar slant and stored in refrigerator. Production was carried out in 250 ml flasks containing 50 ml of medium .The medium consisting of the following components (g/l): peptone 10 gm, meat extracts 3 gm, sodium chloride 5gm and sucrose 2%. Media were sterilized at 1210C for 20 min. The pH was adjusted to 6.5. The flasks were incubated on a rotary shaker at room temperature for 72 hrs.

Isolation and Extraction of EPS

Cells were harvested by centrifugation for 20 min at 10,000 rpm. After centrifugation, two volumes of ice cold Isopropanol were added into it and stored overnight at 40^{0}C. Precipitated material was collected by centrifugation (20min at 10,000 rpm) and the pellets were dried at 1000^{0}C. After drying weigh the pellet to know which organism were showed high production of exoplysaccharides and the best strain which showed higher production was used for the optimization of exopolysaccharide production at different environmental conditions

Optimization of exopolysaccharide production

To study the effect of different parameters, 1% inoculum containing 5×106 cells/ml were inoculated in 100 ml of production medium. EPS production was optimized under different environmental and nutritional conditions viz.incubation period (1 to 5 days), pH (6.5, 7, 7.5, 8), Temperatures (20, 30, 37, 450C), carbon sources (glucose, lactose, fructose, sucrose, mannitol), salt concentrations (80, 100, 120, 140, 160,

180 mM NaCl) and different sucrose concentrations (1, 2, 3, 4 and 5 %) also as per [14, 9].

Estimation of Carbohydrate and Protein content of crude EPS

The total carbohydrate content was estimated by phenol sulphuric acid method [5]. The amount of protein present in the EPS was estimated by the Lowry et al. [8].

Qualitative tests for carbohydrate

Monosaccharide, oligosaccharide ands polysaccharide were detected by Molish test, Fehling's test, Benedicts test and Barfoed test respectively [13].

RESULT AND DISCUSSION

The waste water sample was collected from nearby area of Madurai Kamaraj university. From this sample 25 single colonies were isolated and out of that 7 EPS producing microorganisms were screened and further optimisation parameters are analysed for the potential production. Identification of isolates was done on the basis of their morphological, microscopic and biochemical characters with reference to the key of Bergeys manual of determinative bacteriology (9th edition). The isolates are 3. *Azotobacter spp. 5. Pseudomonas Spp. 7. Agrobacterium spp. 9. Alpha Proteobacterium group and 11.Xanthomonas spp. 15 Klebisella and 21 Pseudomonas Spp* .These isolates were further used for the screening of high amount of exopolysaccharide production.(table1-4)

Table 1: Plate morphology of the microorganism

Characters	3	5	7	9	11	15	21
Size	2 mm	2 mm	3 mm	2 mm	3 mm	2 mm	3 mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Color	Colorless	Greenish blue	Yellow	Pale yellow	Yellow	orange	blue
Margin	Convex	Plain	Plain	Convex	Convex	Convex	Convex
Elevation	Irregular	Regular	Entire	Entire	Entire	Entire	Entire
Opacity	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Mucoid	Moist	Mucoid	Mucoid	Mucoid	Moist	Mucoid

Table 2: Microscopic view of the microorganism

Characters	3	5	7	9	11	15	21
Motility	Motile	Motile	Motile	Motile	Motile	Motile	Motile
Gram Staining	Negative rods	Negative rods	Positive rods	Negative rods	Negative rods	Negative rods	Negative rods
Spore staining	Non spore forming	Non spore forming	Non spore forming	Non spore forming	Non spore forming	Non spore forming	Non spore forming
Capsule staining	capsulated	Non capsulated	capsulated	capsulated	Non capsulated	capsulated	capsulated

Table 3: Sugar utilization test by the microorganism

Sugar	3	5	7	9	11	15	21
Glucose	U, F	U, F	U, F	U, F	U, F	U, F	U, F
Sucrose	U, F	U, F	U, F	U, F	U, F	U, F	U, F
Mannitol	U, F	U, F	U, F	U, F	U, F	U, F	U, F
Fructose	U, F	U, F	U, F	U, F	U, F	U, F	U, F
Lactose	U, F	U, F	U, F	U, F	U, F	U, F	U, F

Table 4: Other biochemical test for the microoganim

Characters	3	5	7	9	11	15	21
Oxidase	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+

The purpose of this experiment was to obtain the efficient strain isolated from waste water which produce high amount of EPS. Seven different isolates from waste water were screened for EPS producing activity. Various factors such as pH, temperature , NaCl concentration , sucrose concentration, incubation period and carbon source were analysed. After optimizing these parameters for the selected 7 isolates the EPS production was increased in all the isolated strains.

Optimized parameters for efficient EPS production are

Incubation period (in days) **3**

Temperature **30^{0}C**

Carbon source **sucrose**

Sucrose concentration **4%**

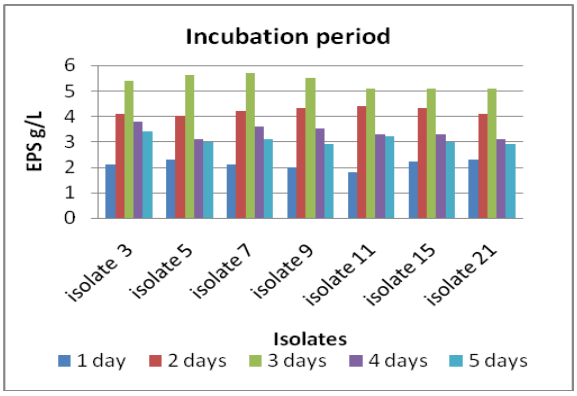
pH **7.5**

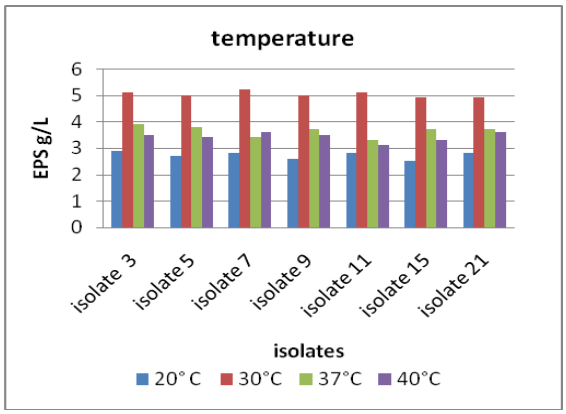
salt (NaCl) concentration in mM **80mM**

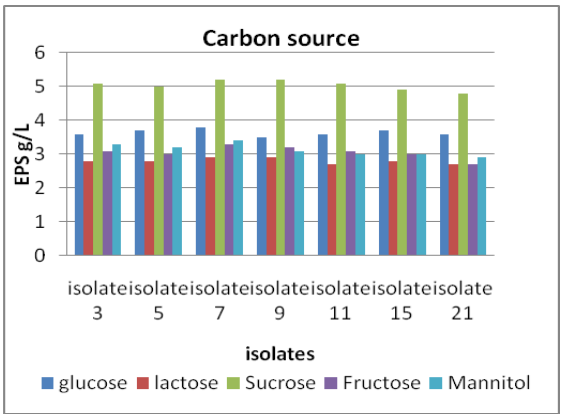
using these parameter the EPS production was done for further studies. The antibacterial studies were preformed against 2 positive and 2 negative strains using antibiotic ampicillin was used positive control and was as negative control.

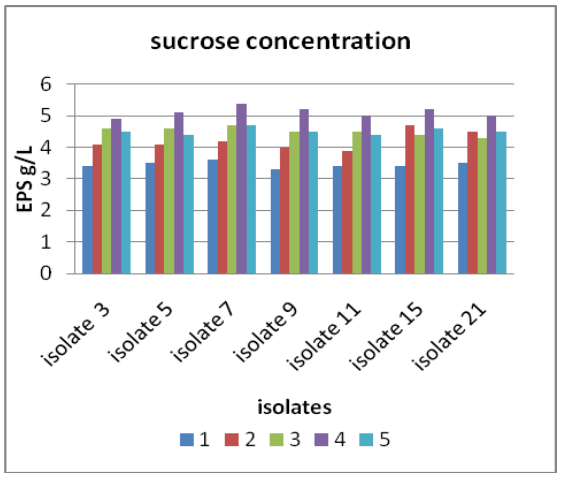
[9] Studied the different fungal strains for the production of exopolysaccharides. They screened different lactic acid bacterial strains for their exopolysaccharide production [2]. Out of seven isolates, isolate no-5, 11and 21 produced higher amount of exopolysaccharide as compared to other isolates. Identification of isolate no- 5,11and 21 was confirmed by their biochemical characterization. Therefore isolate no 3,5,7.9, 11,15 and 21 was used for the further work.as they are the Moist and Mucoid state.The exopolysaccharide production was optimized under different environmental conditions (figure1). EPS production was the maximum in Nutrient Broth, contains 2% sucrose. The EPS production from *Ganoderma* alppanatum were also reported [9]. EPS production was determined during different periods of incubation (1, 2, 3, 4and 5 Days). The EPS production was highest in 3 days incubation period (5.2 g/l). For *A. alternata*, [9] found that ninth day incubation period was optimum for EPS production. The pH and temperature of the culture medium is a vital factor that governs cell growth and EPS production [12, 14]. We found that, 7.5 pH and 300^{0}C temperature were optimum for EPS production. The production rate of EPS was decreased rapidly with increasing the pH and temperature. [12, 14] reported that pH 3 and 30^{0}C temperature would be suitable for EPS production.

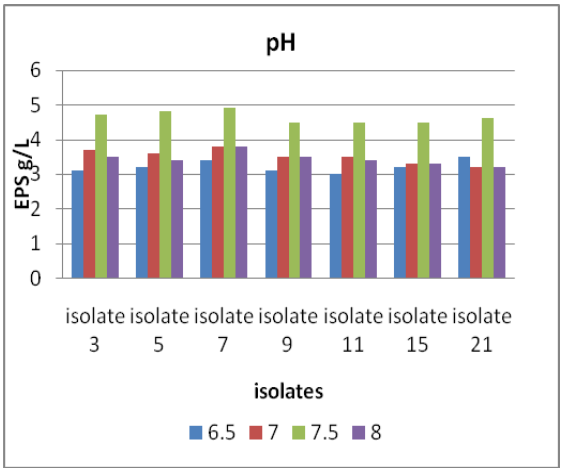
Fig. 1: Optimisation various parameters for exopolysacchaide production.

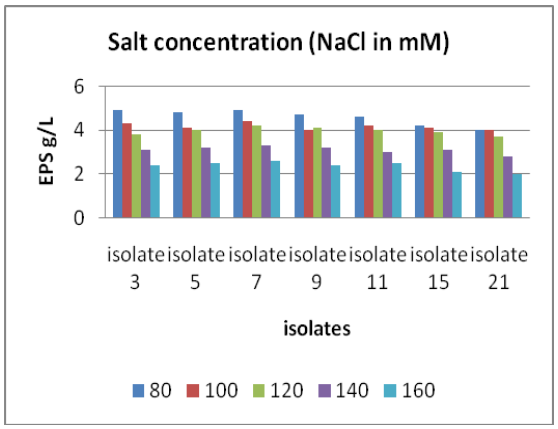






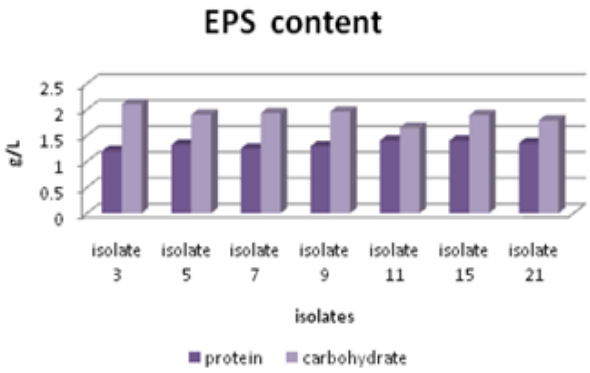






We have partially characterized the isolated polymer. A chemical analysis of the polymer reveals the presence of proteins and carbohydrates. The protein content in EPS was in the range of 9.4%. Whereas, carbohydrates accounted for more than 68%. A spot test for carbohydrates indicates the presence of reducing sugar (i.e. Glucose and Fructose).

Figure 2: Protein and Carbohydrate content in EPS.



The antibacterial activity was analysed for 2 gram positive and 2 gram negative strains were the zone of inhibition was more for gram negative than gram positive bacteria.

CONCLUSION

Out of twenty five isolates only seven isolates, were found to be moist and mucoid in nature and capable of producing exopolysaccharides and along them isolate no-5,11 and 21 produced higher amount of exopolysaccharide. The exopolysaccharide production was optimized under different Nutritional and environmental conditions. EPS production was found to be maximum in Nutrient broth in presence of 4% sucrose as a carbon source and 80mM salt concentration, at room temperature within three days of incubation. A chemical analysis of the polymer reveals the presence of proteins and carbohydrates. The EPS acts as potential antimicrobial agents.

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