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Production and characterization of exopolysacharides (EPS) from the bacteria isolated from Pharma lab sinks

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Abstract: Exopolysacharides (EPS) are polymeric substances of microorganisms of high molecular weight and long chain composed of sugar residues secreted by them into the surrounding environment. Bacterial EPS are also complex mixture of macro molecular poly electrolytes including polysaccharides, proteins and nucleic acids, each comprising of variable molecular mass and structural properties. Three bacteria viz., *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* were isolated from the Micro. Laboratory sinks of Pharmaceutical company. They were inoculated in EPS yeast mannitol glucose broth, as this medium promotes the production of exo-cellular polysaccharide. The pH of the medium was adjusted at 6.9+/- 0.2, later sterilized at 121degree for 15 min/15lbs. The dry weight of the fractionated products were found at 0.10g ± 0.02g/500ml, 0.41g ± 0.03g/500ml and 0.13g ± 0.05g/500ml of media produced from *S. aureus*, *B. subtilis* and *P. aeruginosa* in the YMGB broth respectively. During the study period, it was found that EPS extract was higher in *Bacillus subtilis* in comparison with other two. The physiochemical characterization of EPS was studied and the structure was confirmed by the FTIR and HPLC analysis for the *B. subtilis* only. **From the Clinical Editor**: In this study, bacteria isolated from adverse medical environment, particularly washing sink area, were subjected to EPS production and their (EPS) physiochemical characterization by FTIR and HPLC in order to get the structural confirmation.
Key words: Exopolysacharides (EPS), *B. subtilis,* FTIR, HPLC, Pharma lab sinks.

Introduction

Extracellular polysacharides or exopolysacharides (EPS) are often found in the surroundings of the outer structures of prokaryotic as well as eukaryotic microbial cells. They either closely associated with the cell in the form of discrete capsules or else excreted as slime unattached to the cellular surface. They exist in a wide variety of unique and complex chemical structures and are believed to provide self protection against anti microbial substances growing nearby[1]. Exopolysacharides have major roles in different processes viz., formation of biofilm[1,2,3], protection of bacterial cell from desiccation[4], for maintaining primary cellular functions and antibacterial activity against predators, gelling ability, pollutant degradation kinetics[5], bioremediation activity[6] and plasma substituting capacity[4]. It was found that bacterial EPS are not consumed as an energy source by the producing bacteria, but are released to protect the producer organisms under starvation conditions and also at extreme pH and temperature conditions. They (polysaccharides) play vital roles in many biological processes and they can work as the virulence determinants in the pathogens[7].

The present study was focused to find a comparative study of EPS production from different bacteria isolated from an adverse environment like Micro. Laboratory sinks of a Pharmaceutical Company, Hosur using

single medium to understand the influence of nutrients. The physiochemical characterization of EPS produced by *Bacillus subtilis* was carried out to determine its structural configuration by HPLC and FTIR analysis.

Materials and Methods

Isolation of bacteria from adverse environment

The sample for the present study was collected from a pharmaceutical industry, MICROLABS Ltd. Microlabs is a leading Pharma industry located in SIPCOT phase I, Hosur, Tamilnadu, India. They manufacture almost all pharmaceutical preparation like Injection, Ointments, Syrups and Tablets etc. The Company has a well established Microbiology Department and they perform different tests pertaining to adulteration of medicine before moving to the market. The sample was collected by swab method from the washing sink of the microbiology laboratory. Three bacterial strains viz., *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were isolated based on the routine biochemical tests[8] in the Microbiology laboratory, M.G.R. College, Hosur, Tamilnadu, India and it was followed by subculture and storage at 4°C in nutrient agar slants as stock cultures. Further the strains were grown in nutrient broth for maintenance in order to experiment for the production of EPS.

Production of Exopolysacharides

Three isolated bacteria were inoculated in a standardized medium "Yeast mannitol glucose broth" as this medium support the growth rate of bacteria & also good production rate EPS. Three flasks containing 100 ml of the above said medium (YMGB) were inoculated with each bacterium separately and the flasks were incubated at 37^0 c for overnight. After overnight incubation 100 µl was transferred to 500 ml of a fresh media in a conical flask. Above step was carried out in an aseptic manner, the flask was incubated for 5 days at 37^0 c.

Extraction of EPS

Samples from flasks were separated and concentrated to small volumes[1]. The EPS was then precipitated from the supernatant by addition of equal volume of alcohol. The mixture were agitated during addition of alcohol to prevent local high concentration of the precipitate and left over night at 4°C before centrifuged at 7000 rpm for 20 min. After centrifugation, the precipitate was collected in petriplates and dried at 60°C. EPS was extracted according to the method followed by Ohno *et al*[2].

The total carbohydrate content was estimated by phenol sulphuric acid method proposed by Dubois *et al* [8]. The amount of protein present in *B. subtilis* extract for YMGB medium was estimated by the Lowry's method[10].

FTIR analysis

The bacterial exopolysacharides from *B. subtilis* were characterized using a Fourier transform infrared spectrophotometer. IR spectroscopes of bacterial EPS along with a standard, dextran sulfate (DS) were tested using Perkin-Elmer FTIR instrument, which helped to analyze different sulfate, carboxyl and hydroxyl groups of these sample molecules[11]. One part of extract was mixed with ninety nine parts of dried potassium bromide (KBr) separately and then compressed to prepare a salt disc of 3mm diameter. These discs were subjected to IR spectra measurement in the frequency range of 400 and 4000 cm^{-1}[12].

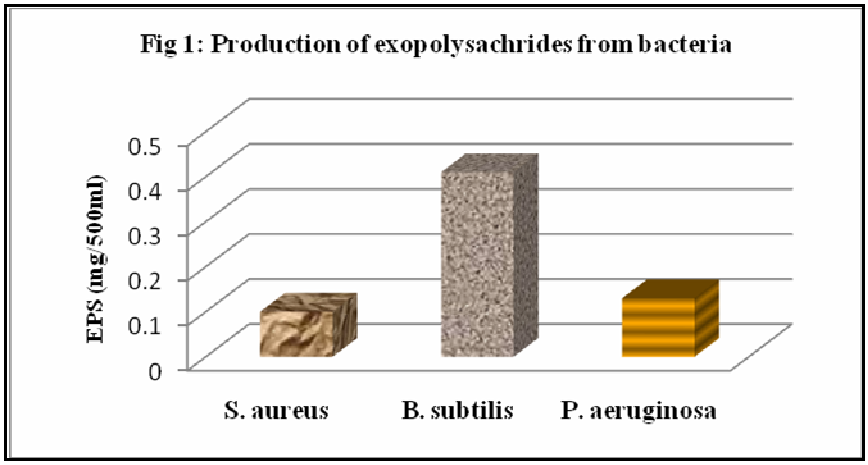
HPLC analysis

The crude EPS of *B. subtilis* were analyzed by high performance liquid chromatography (HPLC) system (Agilent 1100) equipped with Aqueous GPC start up Kit column and eluted with distilled water at a flow rate of 1.0ml/min at 20°c. The separated components were monitored by a refractive index detector. The EPS after being hydrolyzed and dissolved with methanol was analyzed for its sugar composition by HPLC. The column was calibrated with different molecular mass standard and a standard curve was then established.

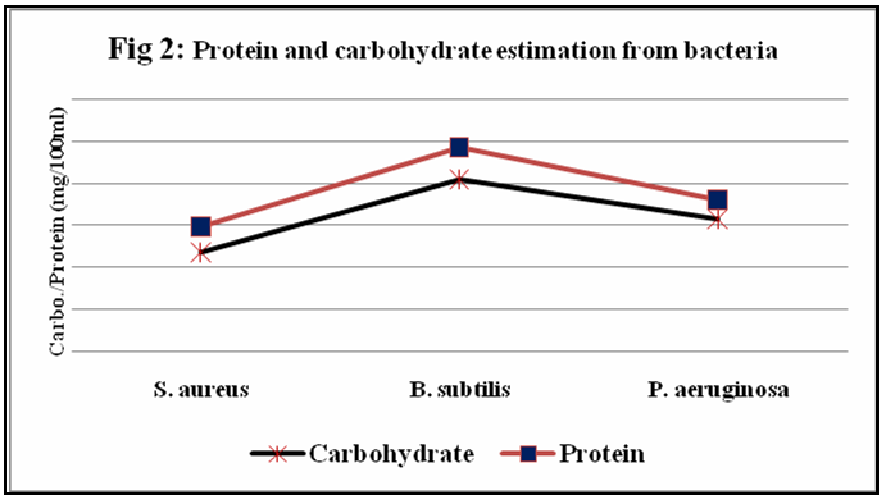
Results and Discussion

Isolation of three bacteria viz., *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* were made by swab culture from Pharma Lab. Sink, Pharmaceutical Co. Ltd, Hosur, India. Exopolysaccharides was produced in the complex was separated by dissociation of high ionic Yeast Mannitol Glucose Broth. The

dry weight of the fractionated products (EPS) were found at 0.10g ± 0.02g/500ml, 0.41g ± 0.13g/500ml and 0.13g ± 0.05g/500ml of media produced from *S. aureus, B. subtilis* and *P. aeruginosa* in the YMGB respectively, which has been given in Fig 1.



During the study period, the carbohydrate and protein estimation was done from all the three bacteria isolated from the washing basins of Pharma lab is given in Fig 2, which showed that the optical density for carbohydrate were 0.47±0.08mg/100ml, 0.82 ± 0.04 mg/100ml, 0.63 ± 0.05 mg/100ml for *S. aureus, B. subtilis and P. aeruginosa* respectively. Protein was also found higher in *B. subtilis* 0.15 ± 0.07 mg/100ml and it was followed by *S. aureus* (0.12 ± 0.04mg/100ml) and *P. aeruginosa* (0.09 ± 0.03mg/100ml) (Fig. 2).



FTIR analysis was used to identify the molecules, proteins and functional groups found in the exopolysacharides produced from *B. subtilis* (Table 1 & Fig 3). The FTIR analysis obtained for the EPS showed that the absorption peaks located at 3535.52/3250.05 cm^{-1} (N-H stretch of amines), 1631.78 cm^{-1}(C=O stretch of amides), 1402.99 cm^{-1} (O=H bend of esters), 1083.99cm^{-1} (C-O stretch of alcohols), 969.48cm^{-1} (C-H bend mono-substituted alkanes), 605.65cm^{-1} (Acetylenic C-H bend of alkynes). IR spectroscopy of the medium exopolysaccharides (EPS) showed the presence of hydrogen bonded compound, possible acid, amide, alkyne or amine salt. The bacterial EPS extracts revealed the characteristic of absorption bands of EPS as observed in the compound dextran sulphate. Thus, the FTIR spectrum revealed that the EPS is complex polysaccharide containing different functional group in addition to functional group reported earlier in polysaccharide structure[5].

Fig. 3: FTIR analysis of exopolysacharides

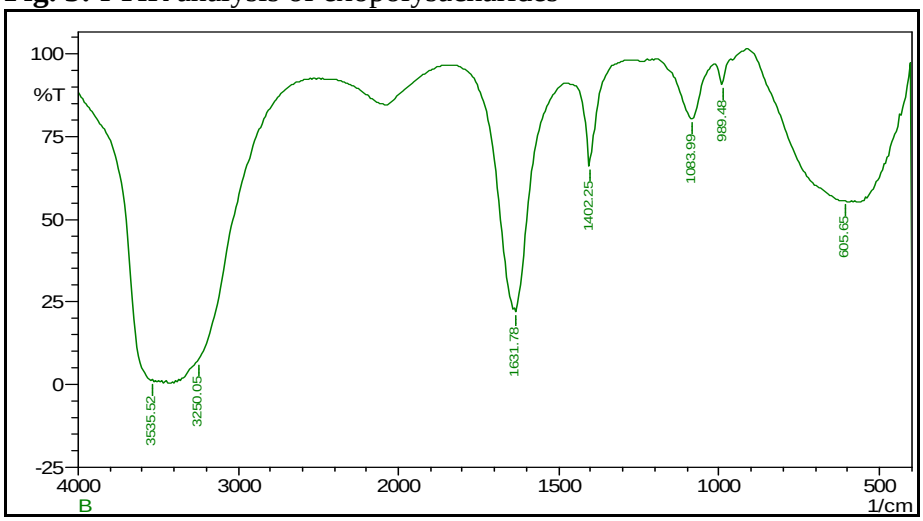
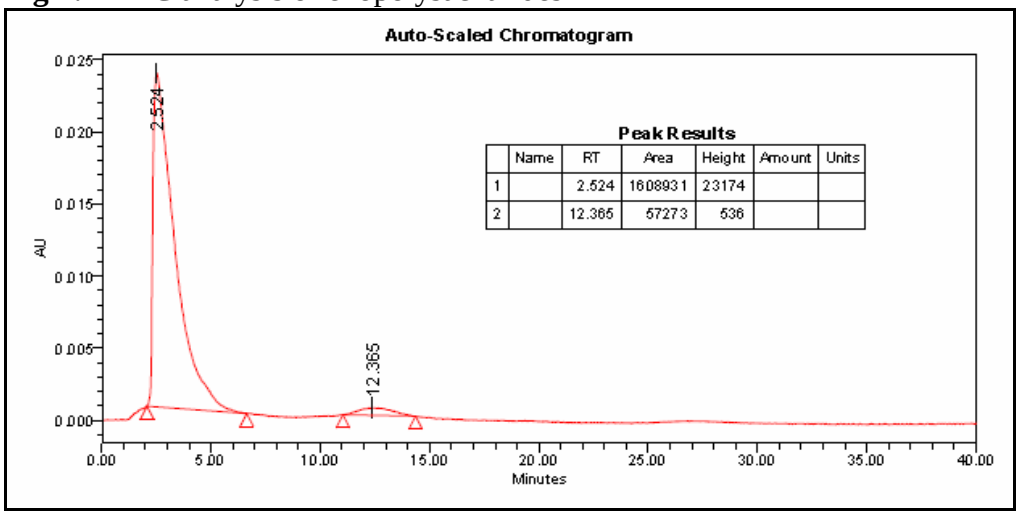


Table 1: Functional groups found in the EPS produced from *B. subtilis*

Sl. No.	Wave number (cm^{-1})	Functional Group
1	3535.52, 3250.05	N-H stretch of amines
2	1631.78	C=O stretch of amides
3	1402.99	O-H bend of esters
4	1083.99	C-O stretch of alcohols
5	969.48	C-H bend (monosubstituted) alkenes
6	605.65	Acetylenic C-H bend of Alkynes

The obtained fractions were analyzed with a high performance liquid chromatography (HPLC) system (Agilent 1100). It was confirmed that the EPS production has been quantified through HPLC and independent peaks were identified with retention time (Fig. 4). Based on HPLC analysis, EPS was estimated to be a glucan. The HPLC chromatogram of EPS indicated that the EPS is a heteroploysaccharide consisting of rhamnose, raffinose and maltose.

Fig 4: HPLC analysis of exopolysacharides



High level of EPS production was achieved by the bacteria isolated from adverse environment in the YMG broth. In many habitats, bacteria form sessile communities known as biofilms[16], the main components of the biofilm matrix are the microbial cells products where together form a dynamic environment in which the microbial cells is organized to make use of all available nutrients. The main purpose of the study was to isolate bacteria from one community different from normal habitat in order to quantify the EPS from them (bacteria).

The novel properties of microbial exopolysacharides such as xanthan, alginate and curdlan may improve food viscosity, hydration of products and low calories food production[13]. The microbial EPS may be used for food edible coating production that effectively would protect products from spoilage[14]. The bacterial EPS extracts gave characteristics bands for EPS. The carbonyl (C=O) stretching peak and OH stretching peak was at broad and the maximum peak and the band at 1000-1500 showed the presence of polysaccharide. According to Sutherland[3], reduction of the cultivation temperature by 10°c below optimal level inhibits the EPS biosynthesis by microbial cells. However, under low temperature of the growth, environment profiles of the high productivity of extracellular polysaccharide occur by bacterial cells[1,15]. In the HPLC analysis, different independent peaks were identified and molecular mass was determined with retention time but previous authors have reported that the molecular mass of different fractions from exopolysacharides while in our study, fraction from *B. subtilis* from YMGB medium was considered[17].

Conclusion

During the study, extraction of EPS made from three bacteria isolated from Pharma lab sinks was found to be novel heteropolysaccharide with complex, dense and flakes like structure. The characterization of EPS was carried out by FTIR and HPLC analysis in order to detect the amine, amide, alkynes and carboxyl groups containing polysaccharides. The present result would be an initiatory step towards the utilization and modification of EPS in future research in the production of valuable drugs for antioxidant and anticancer properties.

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