PRODUCTION OF EXOPOLYSACCHARIDE BY AN INDIGENOUS SOIL ISOLATE

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SUMMARY: An indigenous soil isolate identified as Pseudomonas aeruginosa produces exopolysaccharide. The organism is multiple antibiotic and multiple metal resistant. It shows resistance to ampicillin, chloramphenicol, kanamycin, copper, zinc, lead and nickel. Polysaccharide consists of glucose and unidentified pentose and its production is increased by the addition of sodium chloride. The isolate accumulates nickel from the medium.

Key Words: Pseudomonas aeruginosa, polysaccharide, indigenous soil isolates.

INTRODUCTION

Many bacteria produce extra cellular polysaccharide (ESP) and in genera such as Klebsiella, Rhizobium and Streptococcus they have been well characterized for a number of species and strains (12). In addition to this many Pseudomonas species such as *Pseudomonas atlantica* form extra-cellular polymers (8). Some other bacterial strains have been also reported to produce slime for example *Acetobacter xylinum*, a gram negative bacterium characterized by its capability to synthesize cellulose and other complex exopolysaccharide named as acetan (3). Propionibacteria is also reported to produce polysaccharide during lactose fermentation. This polysaccharide mainly contains methyl pentose, glucose and galactose (4). Microbial polysaccharide with important mechanical properties have significant impact in commercial applications (11) for example xanthan gum is an anion polysaccharide produced by the bacterium *Xanthomonas campestris*. Because of its high viscosity, pseudoplasticity and the rheological properties over wide range of temperature and pH, it has a wide variety of applications (13). It is commonly used as a thickening agent in food product, for mobility control in secondary and tertiary oil recovery, in petroleum drilling fluids and in the paint, pharmaceutical and cosmetic industries *Aureobasidium pullulans* is cultivated industrially for the production of useful polysaccharide, Pullulan. (10).

Polysaccharide production in few bacteria has been shown to be controlled by plasmid (Muc+plasmid), (15,1). Bacterial exopolysaccharide has been reported to help in biosorption of metal salt. *Arthrobacter viscosus* has found to accumulate more metal as compared to other strain that does not produce exopolysaccharide (14) in the same way Enterobacter specie was found to accumulate metals from culture medium (16).

In the present study, we report the isolation and characterization of a soil bacterium capable of polysaccharide production. The polysaccharide contains mainly glucose and unidentified pentose. In addition to this the bacterium also accumulate nickel.

MATERIALS AND METHODS

Isolation and purification of bacterial culture

Soil sample was collected from Botany Department, University of Karachi. Approximately 1 gm of soil sample was suspended in 100 ml of sterilized nutrient broth. This sample was incubated at

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37°C for 48 hours. After incubation, swabbing was done on nutrient agar plate and further incubated for 24 hours. Mucoid colonies were picked and re-streaked on another nutrient agar plate to obtain pure culture.

Identification of strain

Strain was identified on the basis of Gram's reaction, morphological and biochemical tests.

Antibiotic sensitivity test

Sensitivity to four antibiotics i.e. Streptomycin (100 ug/ml), Chloramphenicol (100 ug/ml), Ampicillin (200 ug/ml) and Kanamycin (200 ug/ml) was tested on nutrient agar plate with varying concentration of respective antibiotics. Stock solution was prepared as described in Maniatis (9).

Resistance to heavy metals

Resistance to six different heavy metals like nickel, copper, cadmium, cobalt, zinc and lead were checked by incorporating different concentrations of metal salts to nutrient agar. Stock solutions were prepared by dissolving 1 gm of metal salt in 100 ml of distilled water. Salt solutions were added after sterilization of media.

Composition of minimal media

The minimal media described by Miller (Miller, 1972) contained (g l^{-1}) K_2HPO_4, (10.5); KH_2PO_4, (4.5); $(NH_4)_2SO_4$, (1); Na citrate. $2H_2O$ (0.5); $MgSO_4.7H_2O$ 1 ml from stock solution after autoclaving; B1 (Thiamine Hydrochloride) 0.5 ml from sterilized stock solution; Methionine 10 ml from 4 mg/ml stock solution; Glucose was used as a carbon source for this 10 ml from 20% stock solution was added to media.

Assessment of nickel accumulation

8 ml of bacterial culture grown in minimal media was centrifuged at 3.000 rpm for 15 minutes. Supernatant was decanted and pellet was washed three times with deionized distilled water and digested by adding 3 ml of concentrated HNO_3 at 100°C for 16-18 hours. Acid digested samples were cooled and made up to 30 ml with sterilized distilled water. The content of Ni was determined by atomic absorption spectrophotometry.

ANALYTICAL METHODS

Maximal polysaccharide production

Production of polysaccharide was increased by adding 0.25 M NaCl to MacConkey's agar and incubated at 25°C for 3 to 4 days.

Isolation of polysaccharide

Polysaccharide was isolated by the modified method of Evans and Linker (5). The bacterial culture on agar plate was scraped into 25 ml of sterilized water and stirred to get a uniform mixture. The suspension was centrifuged at 10.000g for 30 minutes, the precipitate was discarded and supernatant was re-centrifuged for half an hour. The precipitate was again discarded. To the supernatant three volumes of 95% ethanol was added to precipitate the polysaccharide. The pellet was washed with absolute alcohol and then dried to remove ethanol vapors Dried pellet was dissolved in sterilized distilled water and used as sample.

Hydrolysis of polysaccharide

10 ml of the sample was hydrolyzed by heating with 1 ml of 0.2 N HCl for 4 hours in boiling water bath.

GENERAL TEST FOR DETECTION OF CARBO HYDRATE

1. Molish test

Two drops of 20% L-napthol solution (in ethanol) was added and mixed to 2 ml of a 0.1% solution of the sample. 2 ml of concentrated H_2SO_4 was poured to the side of the tube.

2. Test for monosaccharide and oligosaccharide

Nitrochromic acid test

4 ml of concentrated nitric acid and 5 drops of 5% potassium chromate solution was added to 10 ml of sample.

Test For Polysaccharide

1. Iodine test

2 ml of sample was taken in a test tube and few drops of 0.01 N iodine was added.

2. Chromatographic method

For descending chromatography different solvent systems such as BAW, BEW and isopropanol: n-butanol: water (70:10:20) were used. The solvent was allowed to travel for 16-48 hours. The paper was sprayed with aniline phthalate solution and incubated at 110°C for 10 minutes.

Figure 1: Antibiotics sensitivity test.

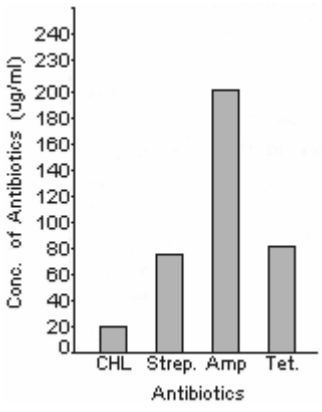


Table 1: Morphological and biochemical characters of the isolate.

Organism	Strain code	Gram staining	Shape	Molitity	Oxidase	Glucose	Lac	Pigmentation
P. aeruginosa	CMG 58	-ve	Rod	+ve	+ve	+ve	-ve	Green

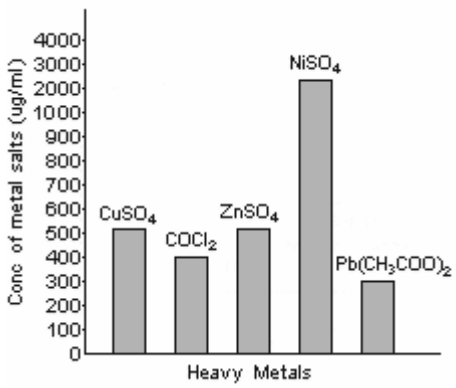
Lac: Lactose fermentation.

Table 2: Resistance to heavy metals and antibiotics.

Strain Code	Chl 20 ug/ml	Strep 75 ug/ml	Amp 200 ug/ml	Kan 75 ug/ml	$CuSO_4$ 500 ug/ml	Cod_2 400 ug/ml	$ZnSO_4$ 500 ug/ml	$NiSO_4$ 200 ug/ml	pb (CH_3 ono) 300 ug/ml
CMG 58	S	R	R	R	R	R	R	R	R

Key: Kan: Kanamycin Chl: Chloramphenicol Amp: Ampicin Strep: Streptomycin

Figure 2: Resistance to heavy metals



RESULTS

Organism

Purified strain was identified on the basis of Gram's reaction, morphological and biochemical test, organism was identified as *Pseudomonas aeruginosa* and designated as CMG 58 (Table 1).

Antibiotics sensitivity test

The isolate showed resistance to Ampicillin (200 ug/ml), Kanamycin (75 ug/ml), Streptomycin (75 ug/ml) but found to be sensitive to Chloramphenicol (20 ug/ml). (Figure 1, Table 2).

Resistance to heavy metals

Surprisingly the isolate CMG 58 showed to exhibit resistance to inorganic salts of CuSO4 (500 ug/ml), $CoCl_2$ (400 ug/ml), $ZnSO_4$ (500 ug/ml), $NiSO_4$ (2000 ug/ml) and $Pb(CH_3COO)_2$ (300 ug/ml) which suggests that the isolate is multiple metal resistant (Figure 2, Table 2).

Assessment of accumulation of Ni salt

19.87% (per ug of cells) accumulation was observed in minimal media after 48 hours that decreased to 6.67% and became constant after 4 days to approximately 0.3% (Figure 3, Table 3).

Analytical analysis

Molish test showed the presence of a purple ring at the junction of two layers, this indicated the presence of carbohydrates. Further a blue coloration was obtained with nitrochromic test which suggested that the carbohydrate may be of low molecular weight.

Chromatographic analysis

The hydrolyzed and un-hydrolyzed samples of the isolate produced spots corresponding to glucose. In addition to this an unidentified spot of pentose was also observed.

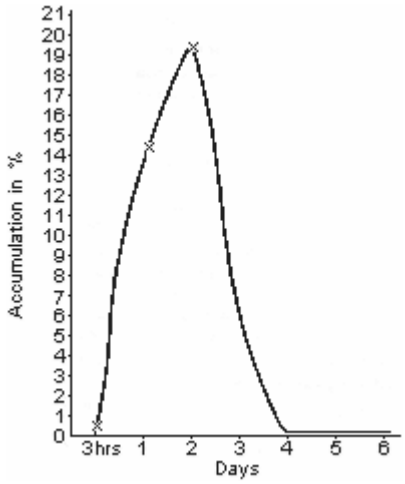
Table 3: Assessment of Nickel accumulation by *Pseudomonas aeruginosa.*

Organism	Strain code	Time Interval	% of accumulation
P. aeruginosa	CMG 58	3 hrs.	0.37 %
		1 day	14.7 %
		2 days	19.87 %
		3 days	6.67 %
		4 days	0.31 %
		5 days	0.3 %
		6 days	0.3 %
		7 days	0.24 %

DISCUSSION

A wide range of bacteria are known to produce large mucoid colonies on agar plates with highly viscous growth in broth (7). The soil isolate showed mucoidness on agar

Figure 3: Assessment of Nickel accumulation in percentage.



plates. It was identified as Pseudomonas aeruginosa designated as CMG 58 Mucoidness indicated the presence of polysaccharide which was confirmed by the analytical methods. These mucoid colonies showed more mucoid growth on MacConkey's agar than nutrient agar, mucoidness on MacConkey's agar was further increased by adding NaCl. Role of sodium chloride in increasing polysaccharide is not known.

The isolate was found to be resistant to streptomycin, ampicillin and Kanamycin but sensitive to Chloramphenicol. In addition to antibiotic resistance, the isolate also showed multiple metal resistance. These results are surprising because usually metal resistance has been observed in the isolates from industrial effluent (6). However, there had been few reports of metal resistance in clinical isolates (2). It is possible that CMG 58 might have acquired metal resistance from other organisms through conjugation or metal resistance might have been induced due to the presence of high concentrations of metal salts in the soil. Whether these antibiotic resistance and metal resistance markers are present on chromosome or plasmid is not known.

Strain CMG 58 showed maximum accumulation of nickel at 48 hours that decreases rapidly after 3rd day. This might be due to the decrease in number of viable cells count.

The polysaccharide produced by the organism consist of glucose and an as yet unidentified pentose. As Bacterial exopolysaccharide has been reported to help in bio-sorption of metal salts, therefore there is a possibility that the exopolysaccharide produced by the strain might be involved in metal accumulation.

Thus the strain CMG 58 can be exploited for the production of polysaccharide commercially. It has metal resistance and antibiotic resistance markers it can be used as a novel organism for genetic studies, the studies on metal accumulation are in progress.

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