Characterization of Some Bacterial Isolates Collected From Water Samples of Hooghly River.

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Abstract

Few bacterial strains were isolated from the water samples collected from two very overcrowded sites of Hooghly River. Out of a number of strains, only four dominant strains namely PD 1, PD 3, PC 1 and PC 4 were selected and their relative growth rates were measured. Most of these strains were Gram positive, aerobic, motile rod growing preferably at 37C at pH 6-7 but strain PC 1 was found to be a psychotropic, pigment producing alkalophilic bacterium showing resistance against some common antibiotics.

Key words: Antibiotic resistance, Bacterial isolates, Ganga water, Water pollution

Introduction

Water playing a vital role in chemical metabolism has immense importance on socio economic and physiologic aspects. Pollution caused by effluents from industries, domestic run outs and other anthropogenic causes becomes a great threat to the public health. There has been growing concern about the need to protect the environment from the various forms of pollution caused by growing populations, industrialization, use of modern agricultural methods etc. (Hunt and Wilson 1986). Microorganisms often play a major role in determining the extent of this pollution (Higgins and Burns 1975).

The River Hooghly is the main outlet of effluent from famous Dakshineswar temple as well as highly congested domestic and industrial areas of Uttarpara carries a rich variety of micro organisms. A thorough analysis of bacterial community of these sites are warranted to get a clear picture about the rate of contamination by toxic metals and role of this water in spreading communicable diseases.

Present work deals with collection and isolation of dominant bacterial strains from two significant sites of river Hooghly and preliminary characterization of the dominant bacterial strains.

Materials and Methods

Collection of water samples: Water samples from Hooghly river from two sites contaminated by temple runout and industrial effluents (namely near Dakshineswar, Kolkata and Uttarpara, Hooghly) in West Bengal were collected with sterile containers and transported to the laboratory immediately for analysis.

Isolation of organisms: The samples were serially diluted (10 fold) before plating on sterile nutrient agar plates for the isolation of bacterial isolates (Panneerselvam and Arumugam, 2012).

Cultivation of bacterial strain : The selected strains were cultivated in 100 ml Erlenmeyer flasks each containing 20 ml Basal Medium (BM) composed of (gl-1): peptone 0.9; (NH4)2 HPO4 0.4; KCl 0.1; MgSO4.H2O 0.1and glucose 0.25 (pH 6) at 4-37°C for 24 hrs.

Growth measurement of bacterial culture: 10 ml of samples were withdrawn at regular interval of time at room temperature and turbidity is measured by Spectrophotometer (Schimadzu, Japan) at 650 nm.(Noisommit-Rizzi *et al*, 1996)

Antibiotic sensitivity test: Antibiotic susceptibility of the isolates was tested according to the NCCLS by disc diffusion method with an inoculum of 10^8 cfu, and agar dilution method with 10^4 fu/spot (Deb Mandal *et al*,2011). The antibiotics (content per disc) used in the study were Chloramphenicol (30 ìg); Streptomycin (30 ìg); Novobiocin (20 ìg); Tetracyclin (30 ìg); and Kanamycin (30 ìg).

Photomicrographic Study: Both the unstained strain (phase contrast) and Gram stained strain were visualized under Axioscop-40 (Zeiss) microscope at 100X.

Effect of temperature, pH on the growth : The strain was grown in different flasks containing media with various initial pH (4-9) at 37°C and at various cultivation temperature (4- 50°C) maintaining initial pH of the medium at 6.0 to check the optimum pH and temperature for growth respectively

Chemicals: All the chemicals used are of analytical grade.

All the above experiments were done in triplicate and average value was considered.

Results and discussion

A number of bacterial colonies were found to appear on dextrose agar plate, a report almost similar to that of Saha *et al*, 2009.

Among the various colonies formed 8 distinct colonies were selected for further study. These colonies were named PD1,PD2,PD3,PD4 (Site 1) and PC1,PC 2,PC 3,PC 4 (Site 2) were isolated from the agar plates inoculated of which PD1 and PD 3 and PC 1 and PC 4 were found to be more in number (Fig 1) and hence were taken for further study.

The characteristic features showed that most of the bacterial strains were aerobic, Gram positive motile rods (Table 1). These strains were cultivated on dextrose agar plate of which PD 1 and PC 4 showed remarkable halos indicating glucose clearing zones around their colonies (Fig 2).

Effect of temperature on growth: Screened bacterial strains were allowed to grow within a varied range of temperature from 7°C to 37°C; of which 37°C was most preferred by strain PD1, PD3 and PC 4. This observation was similar to that reported by Saha *et al,* 2009 But surprisingly, PC 1 was found to be moderately psychrophilic as it showed best at 7°C.

Effect of pH on growth: It was found that the bacterial strain isolated from site 1 grew better in acidic pH 5-6 whereas strains isolated from site 2 grew better at pH 7-8. These differences were probably attributed by the pH differences in the aquatic habitat caused by the effluents changing the pH of the river water. Strain PC 1 was found to be alkalophilic in nature and grew well at pH 8.

Effect of antibiotics: Among all working strains, the strain PC1 was found to be resistant to Streptomycin and Kanamycin but susceptible to Novobiocin, Chloramphenicol and Tetracycline (Table 2). It could be presumed that somehow the strain achieved resistance against some amyloglycoside antibiotics. Such antibiotic resistance in bacteria associated withhas been a global concern and became a great threat to the public health (Sudha *et al,* 2001).

Growth kinetics of the bacterial strains indicated that all the strains had achieved a stationary phase of growth within 12 hours of incubation and had a short lasting lag phase. Among these strains, PD 3 had a high growth rate (Fig 6).

Conclusion

Among the strains isolated, PC 1 was found to be a psychotropic, pigment producing alkalophilic Gram positive , aerobic motile rod and showed resistance against some common antibiotics. Hence it could be utilised for further studies on its pathological and industrial significances.

	Characteristic features of the strains			
	PD 1	PD 3	PC 1	PC4
Shape	Round	Rod	Rod	Rod
Gram Staining	Negative	Positive	Positive	Positive
Colony character	Colourless Smooth outline	Cream coloured Smooth outline	Pigmented Smooth outline	Colourless Irregular outline
Nature	Aerobic	Aerobic	Aerobic	Aerobic
Motility	-	+	+	+
pH preference	Acidic	Acidic	Alkaline	Near neutral

Table 2. Relative antibiotic sensitivity of the isolated bacterial strains

Antibiotics	Sensitivity			
	PD1	PD3	PC1	PC4
Kanamycin	+	+	-	+
Strepromysine	+	+	-	+
Chroramphenicol	+	+	+	+
Tetracycline	+	+	+	+
Novobiocin	+	+	+	+

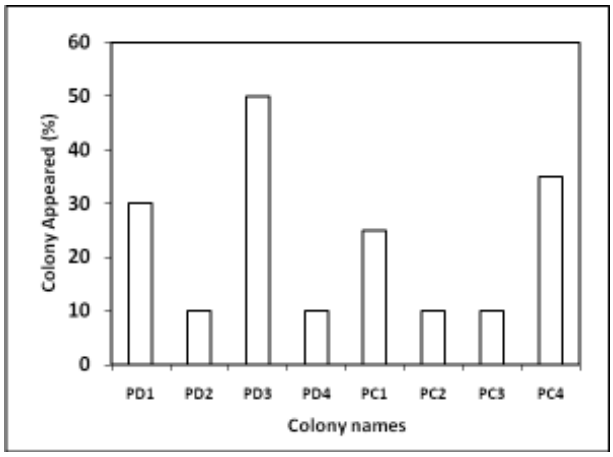

Fig 1. Relative number of bacterial strains appeared on plates

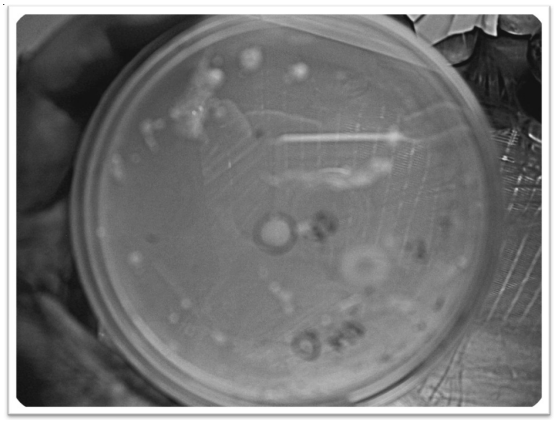

Fig 2. Appearance of halos around the colonies of PD 1 and PC 4 strains.

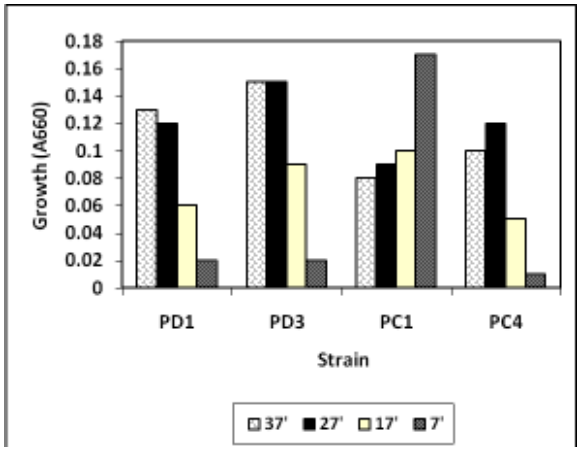

Fig 3. Effect of temperature on bacterial growth.

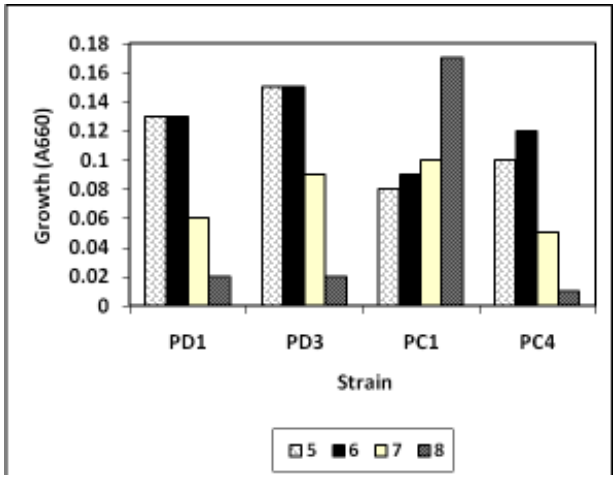

Fig 4. Effect of pH on bacterial growth.

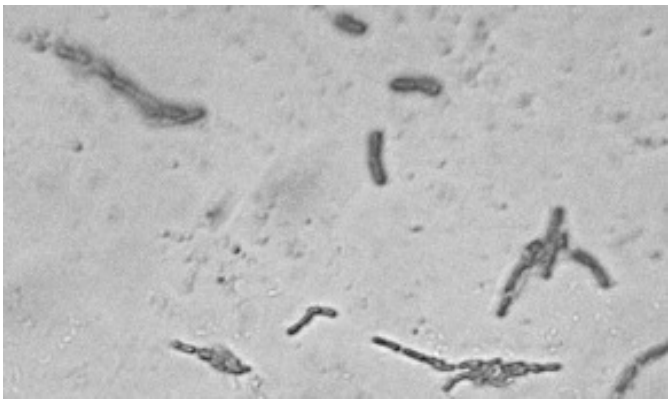

Fig 5. Photomicrograph of strain *Bacillus* sp PC 1.

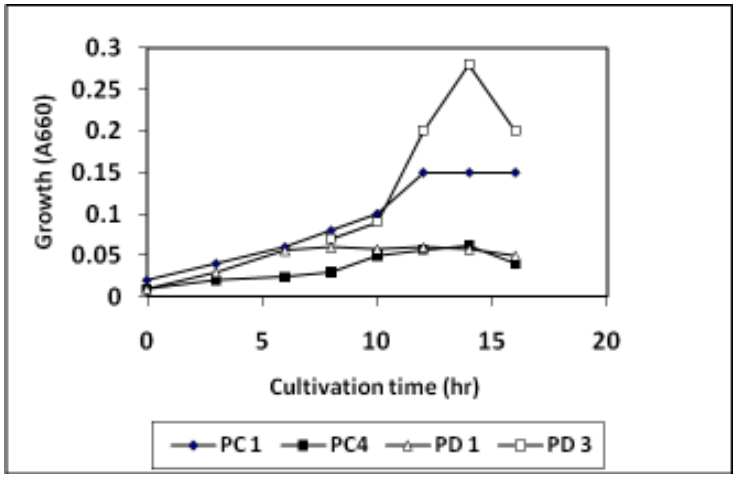

Fig 6. Kinetics of growth under optimum condition by the working strains.

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Table 1. Characteristic features of the strains.