Isolation and Characterization of Heavy Metal Resistant Bacteria from Barak River Contaminated with Pulp Paper Mill Effluent, South Assam

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Received: 23 December 2011/Accepted: 7 May 2012
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Abstract A group of 15 heavy metal resistant bacteria were isolated from Barak River contaminated with paper and pulp effluents. These isolates displayed different degrees of chromium tolerance. Four isolates showed 34 %--49 % of growth at a concentration of 4.0 mM of Cr6+ and subjected to chromium reduction assay under aerobic condition. The isolate E (4) showed highest reduction (34.38 %) followed by E (3) and K(6)PA6, both showed 28.75 % reduction and then D (2) (27.5 %) after 72 h of incubation. These 4 isolates also showed different degrees of resistance to other heavy metals like Ni, Cu, Co and Cd. Antibiotic sensitivity profile of these selected bacterial strains was determined against 10 different antibiotics. Isolate E (4) appeared to be most susceptible being inhibited by eight antibiotics and resistant to penicillin G and ampicillin. The isolate E (3) was resistant to as many as five antibiotics and showed susceptible responses to the rest of the antibiotics. Both the isolates K(6)PA6 and D (2) were resistant to four antibiotics and showed intermediate to susceptible responses to the rest of the antibiotics.

Keywords Barak River · Metal tolerance · Bioremediation

Water the most important resource of nature is increasingly becoming a scare resource. Rivers are playing an important role as major water resource in this planet. Unfortunately, rivers are being polluted by indiscriminate disposal of sewage and industrial waste, which also affect physico-chemical properties and microbiological quality (Koshy and Nayar 1999). Most of the rivers in urban areas of the developing world are the end points of effluents discharged from the industries. Paper pulp industries are the sixth largest effluent generating industries of the world (Ugurlu et al. 2007). These effluents have been found to contain approximately 700 organic and inorganic compounds (Tambekar et al. 2008). Some of these substances have been classified as carcinogenic and mutagenic (Karrash et al. 2006).

Microorganisms are considered to be the best indicators of water pollution. In general, they are very sensitive to low concentration of heavy metals but rapidly adapt to the specific habitat conditions. The microorganisms have acquired a variety of mechanisms for adaptation to heavy metals. Among the various adaptation mechanisms, metal sorption, mineralisation, uptake and accumulation, extracellular precipitation and enzymatic oxidation or reduction to a less toxic form and efflux of heavy metals from the cell has been reported (Joshi-Tope and Francis 1995).

Many reports are available on antibiotic and heavy metal resistant bacteria isolated from different polluted environment (Rabanshi 2008; Ezaka and Anyanwa 2011) but no report is available from Barak River contaminated with pulp mill effluent. The present study was an attempt to evaluate the status of heavy metal resistant bacteria.

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Published online: 22 May 2012
isolated from Barak River water contaminated with paper mill effluent.

**Materials and Methods**

Water samples were collected from Panchgram site (near Hindustan Paper Corporation) of Barak River during monsoon season into pre-sterilised plastic bottles.

For the isolation and enumeration of bacteria, each water sample was serially diluted in sterile distilled water and plated on nutrient agar and different selective medium. All the media were prepared with the addition of distilled water and autoclaved properly. The plates were prepared 24 h prior to sampling. The bacterial population in different samples was estimated by spread plating method on nutrient agar and selective medium plates with 1 mL of suitable dilutions. Bacterial inoculated plates were incubated at 37°C for 24–48 h and final counts of colonies were noted. All trials were performed in duplicate. Colonies differing in morphology were isolated in pure form and maintained on nutrient agar slants with proper indexing.

Dissolved oxygen was determined by modified Winkler’s azide method, pH by digital pH meter, alkalinity and FCO2 were measured by titrimetric method. All the physico-chemical parameters were analysed following the standard protocols (APHA 1998). The water quality was determined by the standard most probable number (MPN) method using the three tube test with lactose broth was employed. Fermentation tubes were inoculated with 10, 1, and 0.1 mL aliquots of water sample (APHA 1998). The tubes were incubated at 37°C for 24 h. Positive tubes producing acid and gas were used in estimating the presumptive MPN/100 mL. Confirmed test was carried out by transferring a loopful of broth from a positive tube into Brilliant green lactose bile (BGLB) broth, followed by incubation at 37°C for 24–48 h. The tubes were observed for gas formation. Completed test was performed by plaing a loopful of broth from a positive BGLB tube on to an Eosine Mehylene Blue (EMB) Agar plate. The plates were incubated at 37°C for 24–48 h and observed for dark red colonies with metallic green sheen. Final faecal coliform or *E. coli* count as MPN/100 mL was calculated based on the completed test.

The isolates were tested for their resistance to chromate by growth in nutrient broth tubes containing various concentrations of chromium (0.1, 0.5, 2, 4 mM) as K₂CrO₄. These tubes were inoculated with freshly grown culture of the isolates and incubated at 30–37°C for 48 h. The bacterial growth was determined by measuring the optical density using spectrophotometer at 540 nm. Relative growth of the isolates was expressed as the percentage of those obtained in untreated control which was taken as 100 %. Chromate reductase activity of the bacterial isolates was assayed following the standard procedure (Park et al. 2000). For reduction assay, nutrient broth medium containing 100 μM of Cr (VI) was inoculated with isolates and incubated up to 72 h at 37°C. Samples of inoculated medium were collected during the incubation period after every 24 h interval.

The resistance of the selected isolates against other heavy metals was also tested in the nutrient broth. The other heavy metals that were tested include Cd, Co, Ni and Cu. These metals were used as their chloride salts. The relative resistance of the isolates was determined from the percent inhibition of growth over the control. Growth was also determined by measuring the optical density at 540 nm.

For biochemical characterisation the isolates were tested for catalase activity, indole production, methyl red test, Voges-Proskauer test, citrate utilisation test, MacConkey agar test and fermentation of eight different sugars. These biochemical tests were done by using the biochemical test kits provided by HiMedia Pvt. Ltd. Identification of the bacterial isolates was carried out according to Bergey’s Manual of Determinative Bacteriology (Holt et al. 1994).

To determine the antibiotic sensitivity of the bacterial isolates, antibiotic discs (Hi-media) were placed on freshly prepared lawns of each isolates on nutrient agar plates. The plates were incubated at 30–35°C for 24 h. The diameter of the inhibition zones was measured to the nearest mm and the isolates were classified as resistant (R), intermediate (I) and susceptible (S). Disks containing the following antibiotics were used: Tetracycline (30 μg), Rifampycin (30 μg), Streptomycin (10 μg), Vancomycin (30 μg), Penicillin G (10 units), Ampicillin (10 μg), Chloramphenicol (30 μg), Gentamycin (10 μg), Erythromycin (15 μg) and Polymyxin B (300 units).

All experiments of physico-chemical parameters were performed in triplicates and statistical analysis was performed according to the standard method (Steel and Torrie 1992). The results are given as mean ± SE values.

**Results and Discussion**

The physico-chemical parameters and bacteriological load at the site Panchgram of river Barak has been presented in Table 1. The pH value of the water sample was 6.25 ± 0.55, which is slightly acidic. The value of DO, FCO₂ and alkalinity was recorded as 5.36 ± 0.14, 4.3 ± 1.51 and 14.0 ± 2.64 mg/L respectively. The values of total faecal coliform and total viable count (TVC) was recorded as 350 MPN/100 mL and 42 (× 10³) mL⁻¹.

15 pure cultures of bacterial isolates different in their morphology were isolated in pure form and were subjected to assessment for relative Cr resistance. The chromium concentrations used during screening ranged from 0.1 to