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Diversity of culturable sodium dodecyl sulfate (SDS) degrading bacteria isolated from detergent contaminated ponds situated in Varanasi city, India

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ABSTRACT

In the present investigation, an attempt has been made to isolate and identify SDS-degrading bacteria from different detergent contaminated ponds situated in Varanasi city, UP, India. Initial survey of ponds indicated that these ponds were contaminated with detergents. Employing enrichment technique in minimal medium (PBM) with SDS as a sole carbon source, a total of 24 isolates were recovered from 7 detergent contaminated ponds. Studies on rates of SDS degradation indicated that the rate of SDS degradation varied from 97.2% to 19.6% after 12h incubation under identical conditions. An estimation of alkyl sulfatase activity indicated that the activity varied from 0.168 ± 0.004 to 0.024 ± 0.005 μmol SDS/mg protein/min. Molecular characterization of these isolates was performed on the basis of ARDRA and ERIC PCR, which indicated that these isolates were broadly divided in 8 groups. Some selected isolates were identified on the basis of 16S rDNA sequencing. It was found that these isolates belonged to *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, *Pseudomonas stutzeri*, *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida* and *Pseudomonas otitidis* respectively. Among these isolates *P. aeruginosa*, *P. putida* and *P. otitidis* have been previously shown to degrade and metabolize SDS, the rest of the isolates appear to be new.

1. Introduction

In India, man-made ponds have been used as an alternate source of drinking water. However, these ponds are also employed for washing of clothes and bathing purposes by washer men and local people (Prakash et al., 2009). Many ponds are situated in vicinity of temples and are used for bathing purposes for people who visit the temples for worshipping and also for disposal of wastes originating from the temples (Sharma et al., 2009). In certain ponds, sewage water and waste water from nearby cottage industries are also discharged. A detailed survey of few ponds located around the city was made by Tyagi et al. (2006), which revealed that most of the ponds are eutrophic. However, data related to amount of detergents present, if any in pond water is lacking. Furthermore, no attempt has been made to study detergent degrading bacteria. In a different study, Ghose et al. (2009), reported the presence of high amount of anionic detergents in surface and underground water in greater Kolkata, India.

Sodium dodecyl sulfate (SDS) is an anionic detergent widely used in household products and in Industry (Karsa, 1992). It has been reported that the presence of anionic detergent especially SDS in environment arises mainly from disposal of domestic and industrial wastes (Fendinger et al., 1994). SDS is shown to be toxic to health and survival of aquatic animals (Ribelles et al., 1995; Rosety et al., 2001; Rocha et al., 2007). It was realized that removal of detergent from environment was a necessity (Singer and Tjeerdema, 1993). Certain bacteria such as *Pseudomonas* sp., *Bacillus cereus*, *Acinetobacter calcoaceticus* and *Pantoea agglomerans* have been isolated which utilize SDS as a carbon source for growth (Payne and Feisal, 1963; Thomas and White, 1989; Singh et al., 1998; Abboud et al., 2007). However, little if any, work has been done on molecular diversity of SDS-degrading bacteria. Recent advances in studies pertaining to microbial ecology have led to the development of new approaches for the characterization of microbial communities (Zhou et al., 1997). Most common PCR-based genomic fingerprinting methods are Amplified Ribosomal DNA Restriction Analysis (ARDRA) (Porteous et al., 1994) and Repetitive (Rep) PCR i.e. Enterobacterial Repetitive Intergenic Consensus (ERIC) elements (Versalovic et al., 1991; Hulton et al., 1991). Although, PCR based fingerprinting methods are suitable for characterization of bacterial communities, however, these techniques cannot be employed for the identification of bacteria (Ventura et al., 2001). 16S rDNA sequence analysis is currently the most widely used method for bacterial identification (Lawongsa et al., 2008). In the

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present study, an attempt has been made to survey various ponds situated in Varanasi city with a view to access the level of anionic detergents in pond water. The next approach was to isolate and screen the SDS-degrading bacteria present in pond water. Molecular diversity among various isolates was accessed by PCR based techniques such as ARDRA and ERIC-PCR. Identification of selected isolates was performed on the basis of 16S rDNA sequencing.

2. Materials and methods

2.1. Chemicals and culture media

Sodium dodecyl sulfate (SDS), methylene blue and other biochemicals were purchased from Sigma–Aldrich, MO, USA. All other inorganic chemicals were of analytical grade and obtained from SISCO Research Laboratories Pvt. Ltd. and HiMedia Pvt. Ltd., Mumbai, India. Luria-Bertani (LB) medium contained (g/l): tryptone 10.0, NaCl 5.0, yeast extract 5.0, pH 7.0. The Phosphate Buffered Medium (PBM) contained (g/l): K_2HPO_4 1.0, KH_2PO_4 1.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 0.20, NaCl 0.5 and $CaCl_2$ 0.02, pH 7.5. The medium also contained trace elements (1 ml of stock) having (g/l): $FeCl_3 \cdot 6H_2O$ 0.24, $CoCl_2 \cdot 6H_2O$ 0.04, $CuSO_4 \cdot 5H_2O$ 0.06, $MnCl_2 \cdot 4H_2O$ 0.03, $ZnSO_4 \cdot 7H_2O$ 0.31 and $Na_2MoO_4 \cdot 2H_2O$ 0.03. SDS (1 g/l) was added separately after autoclaving the medium.

2.2. Study sites

Detailed survey of various ponds located around Varanasi city, revealed that seven ponds namely, Assi (AS), Sundarpur (SP), Niralanagar (NN), Naipura (N), Kandwa (K), Pisachmochan (PM) and Jawaharnagar (JN) were extensively used for bathing and washing purposes and received either sewage water or industrial effluents or both. All these ponds were found to retain water throughout the year. However, the level of water decreases significantly during summer season.

2.3. Estimation of anionic detergent from pond water

Amount of anionic detergent in water samples was determined by the methylene blue active substance (MBAS) assay (Hayashi, 1975). Calibration curve was prepared by using standard solutions (based on weight) of pure SDS (Sigma–Aldrich, USA).

2.4. Enrichment and Isolation of SDS-degrading bacteria

For the enrichment of SDS-degrading bacteria, 1 ml of pond water was added to 100 ml sterilized PBM supplemented with SDS (1 g/l) in a culture flask and incubated at 30 °C and 120 rpm in a shaker (Orbitek LT, Scigenics Bioteck. Pvt. Ltd., Chennai). After 3–4 days of growth, 1 ml culture was transferred to fresh PBM supplemented with SDS (1 g/l). Repeated sub-culturing (at least 3–4 times) resulted in the enrichment of putative SDS-degrading bacteria. Spreading on solid PBM agar-plates containing SDS as a sole carbon source, resulted in the formation of minute colonies. Based on colony morphology different SDS-degrading bacteria were isolated.

2.5. Test for SDS degradation

Briefly, overnight grown cultures in LB medium were centrifuged at 8000 rpm for 5 min at room temperature, washed with PBM and suspended in 100 ml of PBM containing SDS. Initial OD (at 600 nm) of each isolate was adjusted to approximately 0.025. The cultures were incubated at 30 °C with shaking at 120 rpm. Samples from each flask were removed at desired intervals, and assayed for residual SDS and growth (Ellis et al., 2002).

2.6. Preparation of crude cell extracts

Crude cell extracts were prepared following the method of Ellis et al. (2002). Bacterial cells grown in PBM containing SDS as a sole source of carbon were harvested after mid logarithmic phase of growth by centrifugation at 10,000 rpm for 20 min at 4 °C in a Sorvall RC-5B superspeed refrigerated centrifuge (Du Pont Instruments, USA). Cell pellets were washed twice with 10 mM Tris–HCl by centrifugation and suspended in 10 mM Tris–HCl (pH 7.5). The cells were ruptured by sonication for a total duration of 3 min, consisting of intermittent sonication for 30 s on and 30 s off, operated at level 5 and a 25% duty cycle in a Branson Sonifier 450 (Branson Ultrasonics Corp., USA). To minimize heat inactivation of enzymes, samples were sonicated after placing eppendorf tubes in ice box. Cell debris was removed by centrifugation at 12,000 rpm for 10 min at 4 °C. The cell extracts were stored at −20 °C prior to assay for alkyl sulfatase activity.

2.7. Assay of alkyl sulfatase

Alkyl sulfatase activity in crude cell extracts was estimated as per the method of Ellis et al. (2002). Briefly, crude cell extract (10–50 μl) were incubated at 30 °C in 1 ml of 10 mM Tris–HCl (pH 7.5) containing SDS at a final concentration of 50 μg/ml. Samples (100 μl) were removed at fixed intervals and assayed for residual SDS by MBAS method as described earlier. One unit of enzyme activity was defined as the amount of enzyme which converted 1 μmol of SDS per minute under the assay conditions. The experiments were performed in triplicate and result was presented as mean ± standard deviation.

2.8. Biochemical characterization of SDS-degrading isolates

All isolates were characterized by conventional biochemical tests, i.e. Gram stain, motility, pigment production, growth, presence of enzymes such as catalase, oxidase, urease, nitrate reductase, gelatin and starch hydrolysis (Shaw and Latty, 1982). Utilization of carbohydrates, amino acids was tested in PBM as described by (Kämpfer et al., 1991; Molin and Ternstrom, 1982).

2.9. Isolation of genomic DNA

Genomic DNA of all the isolates was extracted by DNeasy Tissue Kit (Qiagen, Gmbh, Hilden, Germany) according to the instructions of manufacturer. The genomic DNA was electrophoresed on a 0.8% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH-8.0) containing ethidium bromide (0.5 μg/ml) and visualized in a gel documentation unit (Bio-Rad Laboratories, USA).

2.10. Amplification of full length 16S rDNA

16S rDNA (1.5 kb) was amplified using universal primer pair For 5′-AGA GTT TGA TYM TGG CTC AG -3′, and Rev 5′-CTA CGG CTA CCT TGT TAC GA -3′. Amplification was performed in a final volume of 50 μl. The PCR reaction mix included; 1.5 U of *Taq* DNA polymerase (Bangalore Genei), 1× PCR buffer with 1.5 mM $MgCl_2$, 300 ng of each forward and reverse primers (Integrated DNA Technologies, USA), 125 μM of each dNTP's (Bangalore Genei, India) and 50 ng template DNA. Thermal cycle for the amplification was set as; 3 min at 95 °C, 30 cycles of 1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C followed by 5 min at 72 °C and storage at 4 °C. Amplification was performed in a PTC-100 Thermal Cyclar (MJ Research, Inc., Walton, USA). The samples were analyzed by agarose (1.5%) gel electrophoresis and visualized on gel documentation unit (Bio-Rad Laborotories, USA).