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Short Communication

Biodiversity and dye decolourization ability of an acclimatized textile sludge

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Abstract

In the present study, sludge sample from biological treatment plant of a textile industry was acclimatized for decolourization of azo dye Direct Black 38 (DB38). A continuous culture experiment showed that the acclimatized sludge could decolourize 76% of 100 mg/l DB38. Bacterial community in the sludge was analyzed using culture-independent molecular approach to get the complete picture of its diversity. RFLP analysis of its 16S rRNA gene library divided the clones into 14 distinct groups. Phylogenetic analysis of these groups showed that they belonged to five different bacterial lineages: β- and γ-*Proteobacteria* (3 and 4 respectively), *Bacteroidetes* (2), *Firmicutes* (4) and *Actinobacteria* (1). The largest number of clones was found to cluster in the γ-*Proteobacteria* (54%), followed by *Firmicutes* (19%), β-*Proteobacteria* (14%), *Bacteroidetes* (10%) and *Actinobacteria* (3%).

Keywords: Direct Black 38; 16S rRNA; Benzidine; Culturability; Phylogenetic analysis

1. Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, food, pharmaceuticals, cosmetics and other industries. Since these dyes are designed to resist fading by different physical, chemical and biological agents, they are not degraded by conventional treatment processes and enter the environment. These coloured discharges are not only aesthetically unpleasing, but also pose ecological and public health risk. Moreover, many of the azo dyes and/or aromatic amines produced by their degradation are potent carcinogens (Weisburger, 2002).

Removal of colour is normally accomplished by a combination of physical, chemical and biological processes. Biological treatment has recently gained importance due to its efficiency, lower construction and operation costs, robustness and eco-friendliness (Moharikar et al., 2005). A lot of consideration is given to the design and operational parameters (temperature, nutrients, aeration etc.) of such biological treatment plants. However, very little attention is given to the types of microorganisms present in the system, though it is known that it is the microorganisms, especially bacteria, that carry out the biodegradation. Thus, investigation of the microbial composition and functionality in effluent treatment plant (ETP) is of great importance for gaining a better understanding of wastewater treatment process.

Dye decolourizing bacteria have traditionally been detected by plating on dye-containing agar and looking for zone of decolourization (Abd El-Rahim et al., 2003). However, this approach has two major disadvantages. Firstly, it assesses the isolates based on their individual decolourization abilities i.e. it ignores the synergistic decolourization activity that a bacterial consortium might exert in the ETP (Khehra et al., 2005). Secondly, culture-based methods cannot be directly used to analyze microbial diversity because it is widely recognized that only a small proportion of total microbiota in the environmental

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samples can be cultured (Kampfer et al., 1996). The non-culturable bacteria may, however, play a vital role in the decolourization process. Introduction of PCR-based method for analysis of 16S rRNA sequences directly from environmental samples has solved this problem to a great extent (Eschenhagen et al., 2003). It provides a means to more accurately describe microbial populations and gives a clearer overall picture of microbial diversity.

Current study establishes the dye decolourization ability of a textile sludge sample followed by analysis of its biodiversity by PCR-based method. This knowledge will help in understanding and controlling the dye decolourization process, thus resulting in overall optimization.

2. Methods

2.1. Acclimatization of textile sludge

Sludge sample was obtained from activated sludge treatment plant of a textile industry located in the district of Nagpur. Direct Black 38 (DB38), a benzidine-base azo dye, was used for the acclimatization of sludge because it is readily available in the local market and its toxic and carcinogenic properties are well-established (Robens et al., 1980). Acclimatization was carried out in a continuous chemostat culture with following operational parameters: flow rate = 0.5 ml/min, hydraulic retention time (HRT) = 24 h, synthetic wastewater composition (g/l) = Na_2HPO_4 – 1.264, KH_2PO_4 – 0.326, NH_4Cl – 1, $MgSO_4$ – 0.098, $CaCl_2$ – 0.044, glucose – 1. DB38 concentration in the influent synthetic wastewater was gradually increased from 10 mg/l to 100 mg/l, in steps of 10 mg/l over a period of 30 days. Samples were collected at each step and analyzed for biomass, dye decolourization and metabolites resulting from dye decolourization.

2.2. Biomass

Twenty five millilitre of samples was filtered through 0.22 μm membrane and dried in oven. Biomass was calculated from dry weight of cells collected on the filter paper.

2.3. Decolourization

Decolourization was followed by measuring absorbance of influent and effluent at 550 nm (λ_{max} of DB38) after centrifuging them to remove the biomass. Results were expressed as % decolourization.

2.4. HPLC analysis

Samples were extracted by liquid–liquid extraction as follows. Samples were first centrifuged and clarified by passing through 0.45 μm membrane. Then they were extracted three times with equal volume of diethyl ether. The ether fractions were combined and evaporated to dryness. Finally, the residue was dissolved in 2 ml of methanol. HPLC system (Waters, USA) consisted of Waters 1525 pump and Waters 2487 photodiode array (PDA) detector set at 280 nm. Sample components were separated on Spherisorb 5-μm ODS2 column (4.6 mm × 250 mm), using a mobile phase of methanol:water (50:50) at a flow rate of 0.8 ml/min. Injection volume was 10 μl and pure benzidine (Sigma Chemicals, USA) was used as standard.

2.5. Extraction of total DNA

Total DNA was extracted from sludge sample as described earlier (Vainio et al., 1997). 10 ml of acclimatized sludge sample was collected from the chemostat when it was operating at 100 mg/l dye concentration. It was centrifuged and resulting pellet was washed twice with TE buffer (10 mM Tris, 1 mM EDTA, pH 8). It was then resuspended in 0.5 ml of TE buffer and homogenized on a vortex mixer, followed by sonication (four cycles of 15 s, 75% amplitude; Vibra Cell, Sonics, USA). Lysozyme was added to a final concentration of 2 mg/ml and it was incubated at 37 °C for 1 h. SDS and proteinase K were added to it at final concentrations of 0.5% and 0.1 mg/ml respectively, followed by incubation at 50 °C for 1 h. Resulting suspension was extracted with equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) by thorough mixing and centrifugation at 12,000*g*. Aqueous phase was transferred to fresh tube and treated with 20 μg/ml of RNase at 37 °C for 1 h. DNA was precipitated from this by adding 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of absolute ethanol, and incubating on ice for 30 min. DNA was then spooled out and washed twice in 70% ethanol before suspending in 50 μl TE buffer. The extracted DNA was purified using Ultrapure prep kit (Bangalore Genei, India).

2.6. Construction of 16 rRNA gene library

PCR amplification of 16S rRNA gene was carried out using universal primers 8f (5′-AGAGTTTGATCCTGGCTCA-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) (Hayes and Lovley, 2002). The PCR mix contained 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 1 μM of each primer, 50 ng of extracted DNA and 1 U of Taq polymerase (Bangalore Genei) in a volume of 50 μl. Amplification was carried out in a thermal cycler (GeneAmp 2700, Applied Biosystems, USA) using following temperature program: initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.5 min, and final extension of 72 °C for 15 min. Amplification of 1.5 Kb product was confirmed by running the PCR product on 1% agarose gel. It was then ligated into TA cloning vector (Bangalore Genei) and transformed into competent *E. coli XL1-blue* cells (Stratagene, USA). After blue-white screening of the transformants, 100 clones were randomly selected and their plasmid inserts were amplified using vector-specific M13