Use of Metagenomic approach to diversify bacteria from textile effluent contaminated soil

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Abstract— These Industrial dyes are released into waste water without any pre-treatment and thus polluting water and soil environment. Soil contains high level of bacteria of any environment, for the treatment of soil, soil samples collected from dyes contaminated area Kanpur and Lucknow. Meta-genomic approaches involving the extraction of DNA of pseudomonas sp. from activated sludge of textile industry and herbal care industry, was able to successfully degradation of four different dyes such as indigo dye, cotton pure dye, disperse dye and reactive dye. This group of dyes is toxic if use in more concentration. Biological degradation process is more advantages over physical and chemical degradation of soil.

Index Terms— Biodegradation, Textile dye, Herbal care dye, E.coli, pseudomonas, metagenomic approach.

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1 INTRODUCTION

The decolorization and degradation of Reactive Black 5 (RB5) azo dye was investigated by biological, photocatalytic (UV/TiO2) and combined processes. Application of Candida tropicalis JKS2 in treatment of the synthetic medium containing RB5 indicated complete decolorization of the dye with 200 mg/L in less than 24h [1]. The release of azo dye in to environment is of great concerned due to color, toxicity, mutagenicity and carcinogenicity of the dye, considerable attention has been given in evaluating the capability of microorganism in decolourisation and degradation of azo dye [2].Irpexlacteusis a white rot fungus known to decolorize various synthetic dyes. Decolorization of the azo dye Reactive Orange 16 by immobilized cultures of I. lacteuswas compared in three different reactor systems of laboratory size: small and large trickle-bed reactors and a rotating-disc reactor. The highest dye decolorization efficiency (90% in 3 days) was observed in the small trickle bed reactor [3].Azo dyes are important chemical pollutants of industrial origin. Textile azo dyes with bioaccessible groups for lignin degrading fungi, such as 2-methoxyphenol (guaiacol) and 2,6-dimethoxyphenol (syringol), were synthesised using different aminobenzoic and aminosulphonic acids as diazo components. The inocula of the best biodegradation assays were obtained from a pre-growth medium (PAM), containing one of the synthesised dyes [4]. A facultative Staphylococcus arlettae bacterium, isolated from an activated sludge process in a textile industry, was able to successfully decolourize four different azo dyes under microaerophilic conditions (decolourization percentage >97%) [5]. The treatment of dyewastewater involves chemical and physical methods such as adsorption, coagulation, oxidation, filtration and ionizing radiation. All these methods have different decolorization capabilities, capital costs and operating speed [6]. A study was performed to assess the decolorization (%) of textile reactive dyes and their biodegradation by Pleurotusostreatus. Four reactive dyes; Remazol RG, Livafix Red CA, Prucion Navy PXG and Prucian Blue PX5R were studied [7]. A new natural dye was extracted from Bisham plant then applied to cotton and linen fabrics. The thermal stability of these fabrics in their blank state then dyed by this natural dye (Commiphora-gileadensis), and then mordanted by different mordants that are; Alum (Potassium and AluminumSulfate) AL2K2 (SO4).24H2O - Chrome (Potassium di-Chromate K2Cr2O7) and Ferrous sulfate (FeSO4.7H2O) which were applied separately [8].Bacteria were inoculated on different solid media to attain biodegradability of an azo dye (Acid Orange 7). Kaolin, bentonite and powdered activated carbon (PAC) were selected to be used with cultures of Enterobacter, Pseudomonas and Morganella sp., as bacteria would be able to degrade several textile dyes [9].The disadvantages of physical and chemical treatment processes of dye wastewater are also discussed. Biological treatment processes have many advantages over the chemical and physical treatment processes such as possibilih/ of degradation qf dye molecules to carbon dioxide and water and formation of less sludge in addition to being environmently friendly. This group of dyes is toxic depending on the concentration used [10]. In this study, a defined consortium-AP of Aspergillusochraceus NCIM-1146 fungi and Pseudomonas sp. SUK1 bacterium was studied to assess its potential for enhanced decolorization and detoxification of azo dye Rubine GFL and textile effluent. Developed consortium-AP showed enhanced decolorization of dye (95% in 30 h) and effluent (98% ADMI removal in 35 h) without formation of aromatic amines under microaerophilic conditions [11] .Textile dye Reactive orange 16 was selected for biotransformation studies by Enterococcus faecalis YZ 66.Optimization of parameters for dye decolourization were studied under static anoxic condition. Under optimized condition decolourization of Reactive orange 16 by Enterococcus faecalis YZ 66 was found to be 77.73% in 80 minutes. Degradation of the dye was confirmed by UV- Visible spectophotometric, TLC, and HPLC analysis [12].Aspergillusflavuswas employed for biodegradation of two commercially used textile dyes, Bromophenol blue and Congo red. The biodegradation of these dyes by this fungus was studied in Potato Dextrose Agar (PDA) medium, while a fixed amount of dye solution (1.0 % w/v) in each case was used in culture medium. This fungus has shown positive results for the degradation/ decolourization for textile dyes [13]. Synthetic dyes, an important class of hazardous organic chemicals, can be easily removed from wastewater stream by heterogeneous photocatalysis process (UV/TiO2).

These dyes have considerable complex structural diversity [14]. Effluent from textile industries were treated with enzyme from white rot fungi isolated from outskirts of Mumbai and identified as Polyporusrubidus
in our laboratory. Decolorisation of 4 Reactive dyes commonly found in the effluents such as Reactive bue, Reactive orange, Ramazol black and Congo red was examined by treatment with enzyme from Polyporusrubidus [15]. This azo dye was decolorized and degraded completely by Streptomyces krainskiiSUK–5 at 24 h in shaking condition in the nutrient medium at pH 8. Induction in the activity of Lignin Peroxidase,and NADH-DCIP Reductase and MR reductase represents their role in degradation [16]. The potential of Trametesvillosaand Pycnoporussanguineusto decolorize reactive textile dyes used for cotton manufacturing in the State of Minas Gerais, Brazil, was evaluated. Growth and decolorization halos were determined on malt extract agar containing 0.002g L-1 of the dye [17]. Removal mechanisms of Acid Red 131, Acid Yellow 79 and Acid Blue 204 dyes with different chemical groups under anaerobic process using mixed anaerobic granular sludge were studied. The UV-visible spectrum obtained and dissolved residual chemical oxygen demand measured at the end of incubation suggest that Acid Red 131 and Acid Yellow 79 were biodegraded and no further degradation of dye metabolites have occurred [18]. Synthetic dyes are widely used in textile, paper, food, cosmetic and pharmaceutical industries. The textile industry accounts for two thirds of the total dyestuff market. During dyeing process approximately 10-15% of the dyes used are released into the wastewater [19]. The potential of a sequential anoxiceaerobic bioreactor to decolorize and degrade azo dye C.I. Acid Red 88 (AR-88) was evaluated. An up flow fixed-film column reactor (UFCR) having polyurethane foam (PUF) as immobilization support was built using a consortium based on four acclimatized bacterial strains belonging to Stenotrophomonassp [20].Azo dyes are widely used in textile industries. Removal of the color from textile waste water is a striking issue. To curb this issue, biological treatment can be employed rather than physico- chemical processes. In this work, Bacillus subtilisis used to degrade the reactive dye – RED M5B [21]. Uncontaminated and Vat blue 4 contaminated soil were screened for heterotrophic bacterial population and the bacterial density were found to be 19.3 X 104 and 5.5 X 104 CFU/gm respectively. The bacterial genera of dye contaminated soil was dominated by Pseudomonas sp. (32.5 %) followed by Bacillus sp. (27.5 %) [22].Textile waste water is a highly variable mixture of many polluting substance ranging from inorganic compounds and elements to polymers and organic products. To ensure the safety of effluents, proper technologies need to be used for the complete degradation of dyes. Traditionally, treatments of textile waste water involve physical or chemical methods. But both physical and chemical methods have many short comings [23].Azo dyes, which are widely used in textile industries when left in water bodies without any treatment cause environmental pollution and in turn are toxic,carcinogenic and mutagenic. The efficient treatment of the sludge from the industries is not economical and a challenging task [24].
Decolorization of Disperse textile dye Brown 21, a very important commercial dye in textile industries was investigated. A dye decolorizing bacterium was isolated from effluent collected from a GIDC, Pandesara, Surat, India. Various physicochemical parameters like pH, temperature, carbon sources and nitrogen sources were optimized for maximum decolorization of dye [25].

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2 Material and Method

2.1 Soil Sampling

In this study, E.coli and Pseudomonas sp. used were isolated from sludge of Kanpur and Lucknow textile and herbal care industry.

2.2 DNA Extraction

DNA was isolated from one gram of soil. Extraction was done using 10 % SDS and incubation for 1 hour 30 min at 65°C. Phenol-Chloroform-Isoamylalcohol was used to obtain the isolated DNA.The precipitated DNA was reconstituted using 1X TE buffer.Quality and quantity of DNAextracts were also verified on 1.8% agarose gels stained with ethidium bromide.

2.3 Elution of Digested PUC 18 from Gel

The excised DNA was luted from the gel by using column method. 10mM Tris buffer was used as elution buffer and after inubation at high temperature the purified DNA was stored at -20°C before used for further process.

2.4 Ligation of Purified DNA Bam HI /Hind III Fragment in to PUC 18 and its cloning

A ligation mixture of total volume 20 µl was prepared and ligation was done at -20°C.Competent cell preparation was done using 0.1 M CaCl2 and 40% glycerol.Transformation done by heat shock method.

2.5 Amplification by PCR using Universal PCR

The PCR reactions were performed using a 15µl reaction mixture containing 10X Taq DNA Polymerase buffer with 2mM $MgCl_2$, 2.5 Mm dNTPs, 5µM primers, 4 µl Taq DNA polymerase, 30 to 40 ng template DNA and sterile distilled water. After completion samples were stored at 4 °C. This PCR amplified products were separated by electrophoresis on 1.5% agarose gel with 1X TBE buffer containing Ethidium Bromide (EtBr) and then visualized in Gel documentation system.

2.6 Sequencing of the fragment from uncultured *Pseudomonas*

Amplified fragment from uncultured *Pseudomonas sp.* were sequenced.The determined sequence of pseudomonas sp. was 986 bp long.They were 100%identical to published Uncultured Pseudomonas sp gene for 16S ribosomal RNA.The sequenced gene was aligned using multiple sequence alignment tool CLUSTAL W. Then using this data phylogenetic tree for the target gene was obtained.

3 Result and Discussion

Based on the experiment for degradation of Indigo dye, Cotton pure dye, Reactive dye and Disperse dye,Pseudomonas sp. bacterial isolated from dye contaminated soil through metagenomic approach.Environmental biotechnology relies upon the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria plays acental role (Liu S, Suffita JM (1993) andStolz A (2001). Molecular studies revealed its characterisation as 16S rDNA amplification has 986bp nucleotides in length identified as Pseudomonas sp.and this 16S rDNA nucleotide sequence has been deposited in Genbank and assigned accession number AB841260,AB841261 (Pseudomonas sp.) Comparison of 16S rDNA sequences with sequences deposited in NCBI showed that isolate strain was most closely related to auncultured bacterial Pseudomonas sp.16S ribosomal RNA gene partial sequence shown sequence similarity of 95%. The phylogenetic tree showed the grouping of Pseudomonas sp. (Figure 1).

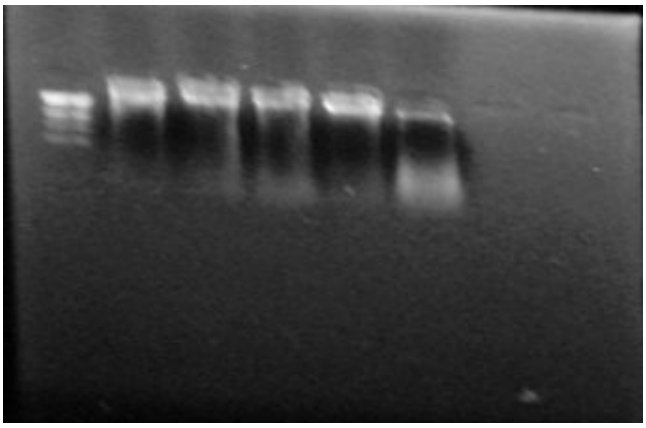


Fig 2 (a): Genomic DNA of the soil contaminated with Bacteria on 0.8% Agarose gel

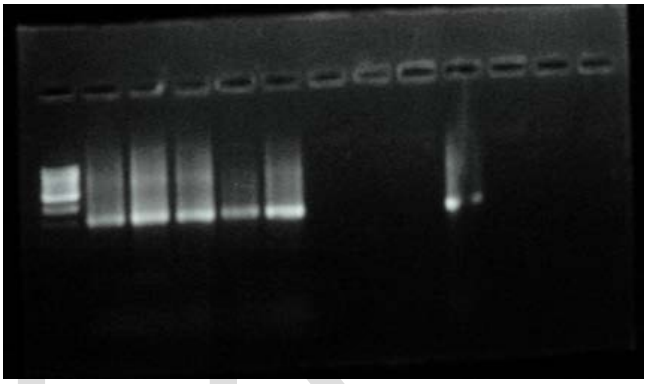


Fig 2 (b): Electrophoresis of the soil contaminated with Bacteria and its amplification by PCR on 1.5% gel

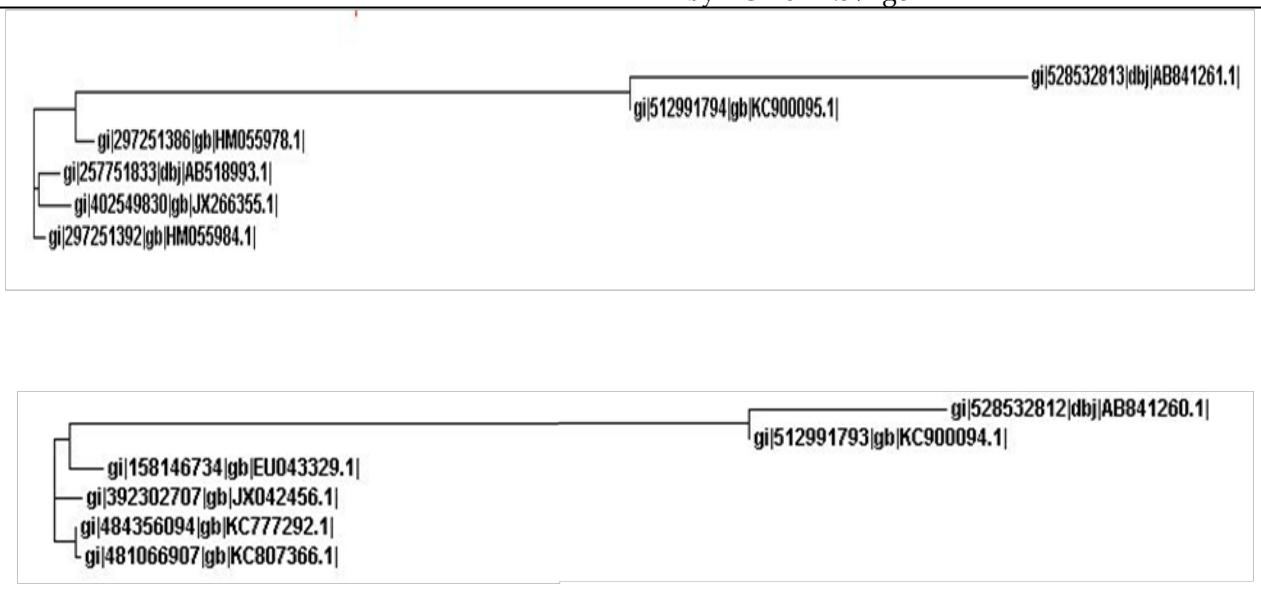
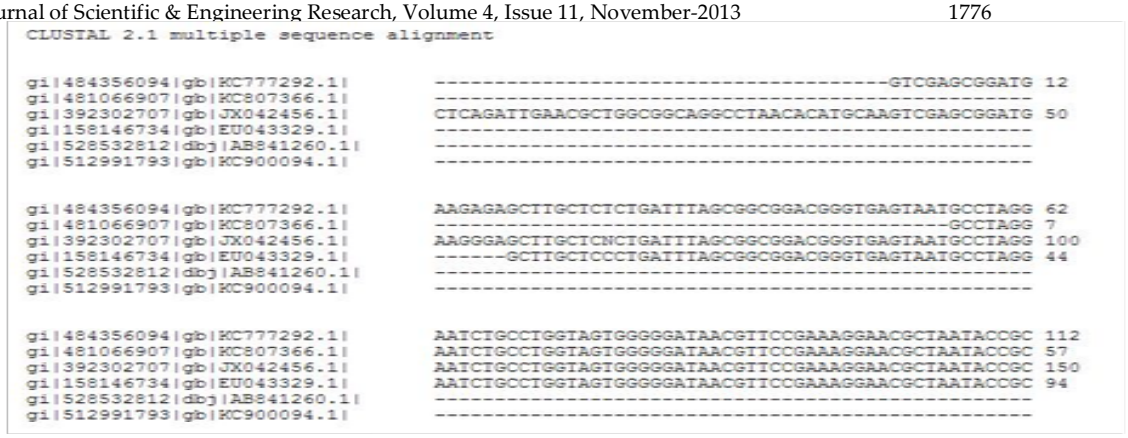
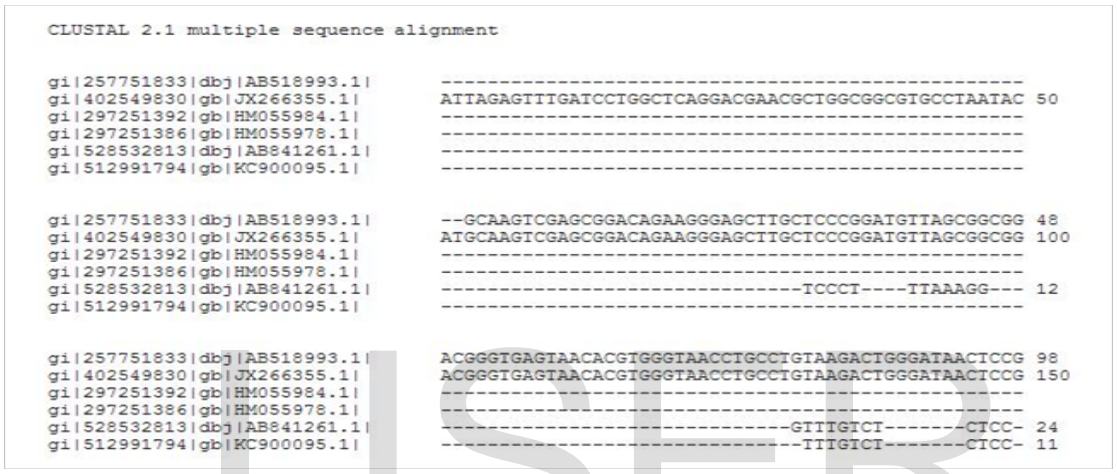


Fig 1: Phylogenetic analysis of sequenced gene





4 CONCLUSION

This study revealed the molecular identity of bacterial strain Pseudomonas sp. An isolate from dye polluted site and its potentials in diversify of textile and herbal care dyes, such as reactive dye, disperse dye, cotton pure dye and indigo dye, which are commonly used in the industries. Biological treatment is the only way for ultimate controlling of dye contaminated soil of textile and herbal care industries; however, more and more research and development works are needed to develop a viable alternative process for the treatment of dye contaminated soil andwaste water.

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