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Full Length Research Paper

Screening and Identification of Azodye (Reactive Red RB) Degrading Bacteria *Bacillus licheniformis* Isolated from Industrial Textile Effluent

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Abstract
The aim of the study is to isolate and degrade the selective reactive red RB dye from the textile dye effluent. Biodegradation of dye by screened isolates have been attempted to test the ability to mineralized / degrade the supplemental dye in Luria bertani medium as a sole sources of carbon and nitrogen. Three dye degradable bacterial cultures was coded as RR1, RR2 and RR3. The potential strain was identified by biochemical tests and 1SrRNApartial sequencing. The results revealed that potential bacterial strain (RR3) was Bacillus licheniformis.

Keywords:*Textile effluent,Azodye, Bacterial strains, Decolourization, Gene sequencing, Biochemical characters.*

Introduction

Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade (Aksu, 2005). The three most common groups of dyes are Azo, Anthraquinone and Phthalocyanine (Axelsson et al., 2006). There are over 10,000 commercially available dyes with a production of over 7×10^5 tons per year. Azo dyes, accounts for almost 60 to 70% of all the synthetic dyes produced globally (Tripathi and Srivastava, 2012).Approximately one million metric ton of dyes are produced annually out of which azodyes represent about 70% on weight basis (Hao et al.,2000).

They are characterized by the presences of one are more azobonds (-N=N-) in association with one and more aromatic compounds.During manufacturing and usage of azo dyes an estimated amount of 10-15% is released in to the environment (Tan et al., 2000). Chung and Cerniglia (1992) reported that mutagenicity of azodyes was related to chemical structure of dye molecule.Discharge of coloured dye water into water bodies creates aesthetic problems and disturbs aquatic ecosystem. These dyes are therefore released into the environment and lead to the acute toxic effects on the flora and fauna of the ecosystem. The release of coloured effluents in water bodies reduces the photosynthesis as it impedes penetration of light in water (Slokar, 1998; Strickland, 1995). Industrial effluents, like textile wastewater containing dyes must be treated before their discharge into the environment. The dye wastewater from the textile is one of the most difficult methods to treat (Kim et al., 2004; Tantak, 2006).
Various physicochemical methods, such as flocculation, adsorption on activated carbon, ozonation, ion exchange, electro coagulation, froth flotation, membrane filtration and reverse osmosis have been used for decolourization of dyes in wastewater. However, these methods are less efficient, costly, limited applicability, and produce more waste, it is difficult to dispose. (Ogugbue et al., 2011).These methods are quite expensive, have operational problems and generate huge quantities of sludge (Kapdan and Kargi, 2002; Sandhaya et al., 2005).Biological processes provide alternative technologies that are cheaper and environmentally friendly (Asadet al., 2007).Many microorganisms belonging to the different taxonomic groups of bacteria have been reported for their ability to decolourize azo dyes (Feng et al., 2012).

In the present study, we focused on the isolation and identification of dye decolorizing microorganisms from contaminated soils in textile industry and analyzed the ability of these isolates to degrade Reactive red RB dye.

Materials and Methods

Dye

Reactive red RB dye is a activated Vinyl compound, a monoazo, and is one of the dye used for colouring textile fibers and was provided by a dyeing unit, Satravada, Chittoor district, Andhra Pradesh, India.

Soil sample

The soil sample were collected in a polythene bag from textile dye effluent contaminate site, small scale textile industryat Satravada, Chittoor District, Andhra Pradesh, India.Samples were taken at a depth of 10-15 cm by using sterile spatula.Then the sample was brought immediately to the laboratory and were carried out for various microbiological studies.

Identification and screening of dye degrading bacteria from textile effluent

The isolation of bacterial strains were carried out by serially diluting of soil samples in sterile distilled water and subsequently plating on nutrient agar medium using spread plate technique.Well grown colonies were picked and further purified by streaking

on nutrient agar. The isolated bacterial strains were maintained on nutrient agar slants and stored in refrigerator at 4°C.Identification of the bacterial isolates was carried out by the routine bacteriological methods such as preliminary tests like colony morphology, motility, Indole, Methyl red, Voges- proskauer, Citrate, oxidase and sugar fermentation tests.Each strain was then inoculated in to nutrient broth and incubated at 37°C for 24 hours. Each isolate was streaked on Luria bertani medium amended with dye, incubated at room temperature and observed for clear zone around the colony and they were selected for further use.

Decolourization activity

Decolourization assay was measured in the terms of percentage decolourization using UV-Visible Spectrophotometer (Thermoscientific). The decolorizing activity was expressed in terms of the percentage decolourization was calculated as (Sarate et al., 2009; Sahasrabudhe and Pathode, 2012b).

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Identification of the Isolate by 16S rRNA

The 16S rRNA sequence analysis is now used as a frame work for the modern classification of bacteria. Further the strains were amplified by PCR and then confirmed by molecular based 16SrRNApartial sequencing accomplished atBioaxis DNA Research Centre, Hyderabad. Sequence was further subjected to BLAST analysis from the NCBI data base to obtain the sequence similarity with related organism. The sequence was aligned with closely related sequences retrieved from EMBL by using CLUSTAL W programme (Thompson et al., 1994). The Screened potential bacterial isolate was characterized by 16SrRNA partial sequences were analyzed using a BLAST search algorithm to estimate the degree of similarity to other rRNA sequences obtained from the NCBI/ Genbank. The Phylogenetic tree for the datasets was constructed to access the relationship between the organisms using the PHYLIP analysis programme. The bacterial strain was deposited in Genbank, NCBI and accession numbers was obtained (Goda et al., 2010).

Results and Discussion

General properties of dye

The general properties of selected reactive red RB dye showed (Table 1).

Table 1. General properties of Reactive red RB dye

Name of the commercial dye	Remazol red 198
C.I Number	181055
C.I Name	Reactive red 198
No. Of azo bonds	Monoazo
γ max (nm)	518 nm
Molecular weight (g/mol)	967.5
Chemical formula	$C_{27}H_{18}C_lN_7Na_4O_{15}S_5$

Isolation and screening of dye degrading bacteria

Total seven isolates was isolated from textile dye effluents and screened for dye degradation. Among seven isolates three showed efficient decolourization. Each colony was coded as RR1, RR2 and RR3. These strains cultures were grown in Luria bertani medium amended with reactive red RB dye (500mg/l) and screened for dye decolourization. Strainsthatshowed that high decolourizing potential was selected for further optimization study. Based upon the growth characteristics, staining reactions and biochemical tests (Martins *et al.* 2006) as showed in (Table 2and 3). The isolates were identified based on biochemical tests according to Bergey's Manual of Determinative bacteriology (Holt et al., 1994).Pure cultures were maintained on Luria bertani agar slants and preserved under refrigeration at 4^{o}C for further use (Hayase et al.,2000; Kumar et al., 2005; Chen et al., 2002).

Table 2. Morphological characteristics of the Reactive red RB dyebacterial isolates

Colony characteristics	RR1	RR2	RR3
Configuration	Swarming	Swarming	Irregular
Pigment	Opaque	Opaque to grey	White to brown
Gram staining	Positive	Positive	Positive
Cell shape	Cocci	Short rods	Curved rods
Motility	-	+	+
Spore	-	+	+

Table 3. Biochemical characterizationof Reactive red RB bacterial isolates

Biochemical tests	RR1	RR2	RR3
Oxidase	+	-	-/+
Catalase	+	+	+
Indole	-	-	-
Methyl red	+	+	+
Voges- proskauer	+	-/+	+
Citrate	-	-/+	+
Urease	-/+	-	-/+
Nitrate reduction test	+	-/+	+
Acid from glucose	+	+	+
Acid from lactose	-	+	+
Acid from sucrose	+	+	+
Acidf rom mannitol	-	+	-
Gelatine liquefaction	-	+	+
Strachhydrolysis	-	-	+
Casein hydrolysis	+	+	+
H_2S production	-/+	-	+
TSI	+	+	+

Degradation of dye in liquid broth

Biodegradation of dye by screened isolates showed the ability to mineralize/ degrade the supplemental dye in Luria bertani medium as a sole source of carbon and nitrogen sources as energy.Decolourization studies were followed in static condition by three bacterial isolates such as RR1, RR2 and RR3 by using various carbon, nitrogen sources at different pH, temperatures and dye concentrations. All these bacterial isolates were inoculted in to 250ml conical flask containing 100ml of Luria bertani broth amended with 600ppm concentration of dye was inoculated for each isolate separately. Control and Inoculated medium was incubated at 37°C for 6 days (Fig 1). About 5 ml samples were withdrawn aseptically and centrifuged at (Hitachi) 10,000 rpm for 10 minutes at 4°C (Khehraet al., 2005). The clear supernatant was used for measuring absorption at 518 nm using UV-Vis spectrophotometer (Thermo scientific). The results were noted based on turbidity and optical density (OD) value by using following formula (Jacob Thomson, 1998). Decolourization activity (%) was calculated by the following formula and all assays were done in triplicates and the Mean value was used for statistical analysis.

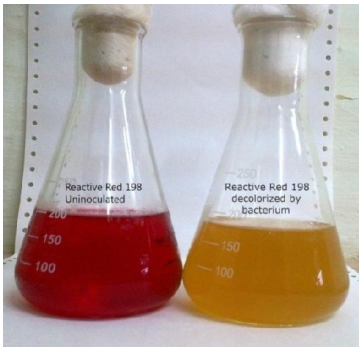

Figure 1. Degradationof RR3 in liquid broth

Identification of the bacteria by 16S rRNA

The molecular identification of the potential strain was carried out by amplifying the 16S rRNA gene was described by (Shivaji et al., 2000). It was confirmed and characterized as RR3 (*Bacillus licheniformis*)by using partial sequencing of 16S rRNA and BLAST analysis. The sequence 16S rRNA gene showed 99% similarity with species of *Bacillus*. The sequence was further used for BLAST analysis from NCBI database to obtain 99% sequence similarity of related organisms and aligned by using CLUSTAL W programme. Phylogenetic tree was constructed with PHYLIP analysis by using Neighborjoining method (Patil et al., 2008). The tree was drawn to the scale, with branch lengths in the same as those of the evolutionary distance used to infer the phylogenetic tree (Dhanve et al., 2009).The phylogeneticrelationship between the isolated bacterial strains and other related bacteria found in the Gen bank database (Fig 2).The sequences were searched on BLAST tool (blast n) towards nr/nt nucleotide sequence database in NCBI (www.ncbi.nlm.nih.gov). The results were similar by Mabrouk and Yusef (2008) studied decolourization of Fast Red by *Bacillus subtilis* HM and the degradation product was *p*- aminoazobenzene (Zissi et al., 1997). Other strains of *Bacillus sp.* which were able to decolourize or degrade dyes are ADR isolated from soil for C.I. Reactive orange 16 (Telke et al.,2009).

Nucleotide sequence accession number

The strain was deposited in nucleotide sequence data base with accession numberis KF908819 (T. Madhuri et al., 2013)isolate was identified as *Bacillus licheniformis*.This process was carried by City for Scientific Research and Technology Applications, Genetic Engineering and Biotechnology Research Institute, the Environmental Biotechnology Department, New Borg El-Arab City, Alexandria, Egypt.

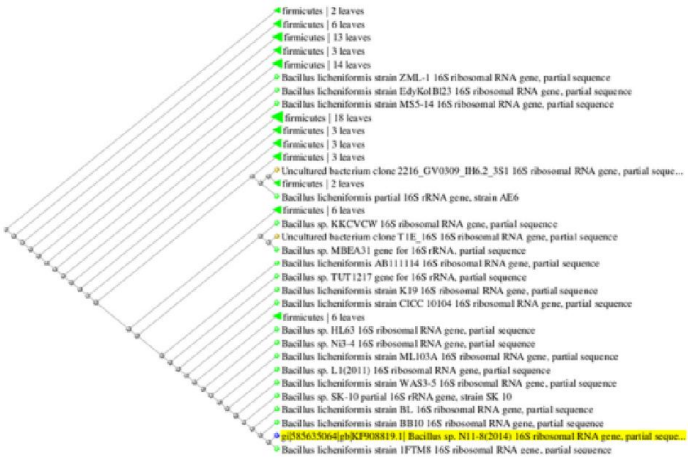

Figure 2. Phylogenetic relationship of *Bacillus sp.* based on partial 16s rRNA sequence

Conclusion

The present study concludes that the three isolates were isolated from the textile industry effluent and screened for reactive red RB dye degradation. Among these isolates potential isolate RR3 was selected on the basis of highest decolourizing ability and it is identified as *Bacillus licheniformis* by partial gene sequence of 16S rRNA. Sequencewas further subjected to BLAST analysis and phylogenetic tree.

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