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Tistrella mobilis, a Novel Bacteria Isolated from Bagru Rajasthan Possess the Capability of Azo Dye Degradation

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Abstract: ***This study presents degradation of an azo dye, Reactive Red 120, by novel bacteria, isolated from Bagru (Rajasthan). The bacterial strain was identified as Tistrella mobilis JCM 21370 on the basis of 16S rDNA sequence. Reactive Red 120 (92.08%) was degraded in 10 days (optimum conditions include pH 7 and temperature 30^{o}C) in liquid cultures under static conditions. Tistrella mobilis is reported to be polyhydroxyalkanoate producing bacterium. The study shows bacteria is gram negative, rod shaped, possess mobility, positive for catalase and oxidase acitivity. HPLC studies shows bacterium is capable of degrading azo dyes.***

Keywords: Azo dye, Degradation, *Tistrella mobilis*, HPLC, Bagru

1. Introduction

Textile dyes are chemicals of complex aromatic structures designed to resist the impact of detergents, sunshine and temperatures [1] , so they are maximally toxic, mutagenic and carcinogenic in nature. In the textile industry, up to 200,000 tons of these dyes are lost to effluents every year during dyeing and finishing operations as a result of inefficiency in the dying process [2]. Without adequate treatment, such dyes will remain in the environment for an extended period of time [3]; therefore a complete removal of such compounds from aquatic system is required. Inorganic or organic matrice adsorption, photocatalytic or photo oxidative decolorization, microbiological decomposition, ozonization, agglomeration and chemical oxidation are broadly practiced physical, chemical and biological means for dye treatment [4]. Considering physical and chemical methods, these methods are mostly ineffecti ve, expensive; produce side reactions, high sludge, and by-products, not suited to degrade all dyes, etc [5].

On the other hand, Biological technique is one of the most cost effective treatment methods than other methods, biological treatment is awkward to the regular variation and toxoids whereas it has good affability in design and activity, it is overviewed to be proven that biological treatment is the way of eradication of color with tolerable [6]. Decolorization by biological means may take place in two ways: either by adsorption (or biosorption) on the microbial biomass or biodegradation by the cells [7]. In our study, *Tistrella mobilis* a proteobacteria of the family rhodospirillaceae isolated from Bagru (Rajasthan) possess capability to degrade azo dye.

Bagru is a village located 32km west of Jaipur (Rajasthan), its clay rich soil and hot, arid climate have for four centuries provided it with the perfect conditions for its unique style of printing and which can turn out to be inhabitants of novel micro flora, and is therefore considered ideal for study.

2. Materials and Methods

Dye and Chemicals

The Reactive Red 120 used in this study was an azo dye with linear formula ($C_{44}H_{24}Cl_2N_{14}O_{20}S_6Na_6$) was purchased from Sigma-Aldrich for decolorisation and degradation studies. All the other chemicals used were of analytical grade.

Culture medium

Bunshell and Haas medium $MgSO_4$ 0.2, K_2HPO_4 1.0, K_2HPO_4 1.0, $CaCl_2$ 0.02, $FeCl_3$ 0.05, $(NH_4)_2SO4$ 1.0 (g/l) supplemented with glucose (0.1% w/v) and yeast extract (0.05% w/v) were used for all studies. The final pH of the medium was adjusted to 7±0.2.

Isolation and characterization of the organisms

Water samples collected from dyeing houses of Bagru, Jaipur, Rajasthan (India) were used for isolation and screeing of dye decolorizing bacteria. The organisms were isolated using the pour plate and streak plate techniques using BHM amended with Reactive Red 120 (100µg l^{-1}). The morphologically distinct bacterial isolates showing clear zones around their colonies due to decolorization of dyes were further screened for their decolorizing ability. Fresh bacterial inoculums (5 ml) prepared in nutrient medium were inoculated into 100 ml (v/v) of dye containing BHM (in 250 ml Erlenmeyer flask). The flasks were incubated at 37^{o}C under static condition.

The pure stock cultures of isolates were stored at 4^{o}C on BHM supplemented with 100µg/l of Reactive Red 120 dye. The bacterial strain with efficient decolorizing ability was designated as SRB01.

Genomic DNA extraction and I6S rRNA gene amplification

The bacterial strain SRB01 (fresh single colony) was inoculated in 20 ml of nutrient broth and incubated at 37^{O}C for 48 hrs in shaker incubator (150 rpm). The DNA was isolated using CTAB method. The concentration of DNA in suspension was estimated by spectrophotometric measurement at A_{200}. Amplification of 16S rRNA was obtained for each bacterial isolates by PCR amplification employing forward primer (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer (5'-CTT GTG CGG GCC CCC GTC AAT TC-3'). PCR reactions were performed in a reaction mixture of 50 µl containing 20 µl T_{aq} DNA polymerase, 22 µl PCR grade water, 1.0 µl of each forward primer and reverse primer, 1.0 µl of $MgCl_2$ and 5.0 µl of genomic DNA. The PCR reaction mixture kit was purchased from Kappa. The reaction was performed in a conventional PCR with initial denaturation at 93^{O}C for 3 mins, followed by 38 cycles each of 95^{O}C for 30 sec, 60^{O}C for 30 sec, and 72^{O}C for 50 sec and final elongation at 72^{O}C for 3 min. The presence and yield of PCR amplified product (16S rRNA gene) was ascertained on 2.0% agarose gel (w/v) prepared in 1% TBE. Gel electrophoresis was carried out at 90 V for 45 mins in 1X TBE Buffer, Staining was done with ethidium bromide and gel was visualized using UV transilluminator.

Sequencing of 16S rDNA and BLAST analysis

The PCR product obtained were of high quality and were got sequenced 1st BASE DNA Sequencing Division. The received sequences in FASTA format was cleaned up to remove the misleading sequences and was improved upon using Chromas software. The improved consensus sequences were blasted using BLASTn [8] of NCBI (http://blast.ncbi.nlm.nih.gov/Blast) and the specie against which highest total score was exhibited, was considered as the species identified. Evolutionary analysis was conducted by Neighbor joining method.

Determination of degradation of Reactive Red 120 using HPLC

HPLC analysis was performed model Agilent 1500 series, by using C-18 column (150mm × 4.6mm) version. The compounds were eluted with an isocratic elution of Water: Methanol (90:10 v/v) at the flow rate of 1ml/min and absorbance recorded at 254nm. The temperature of the column was kept 30^{O}C and the injected volume was 20µl. The mobile phase (solvent) was prepared using water and methanol in the ratio 90:10 and degassed using sonicator. The HPLC (Aligent Series) of Reactive Red 120 before and after degradation (after 10 days) with *Tistrella mobilis* JCM 21370 was performed.

3. Results and Discussion

Isolation and identification of dye degrading bacteria

Isolation of bacteria was carried out from water samples collected from different dyeing houses and textile dye wastewater sites of Bagru. Bacterial strain SRB1 with efficient decolorizing ability against Reactive Red 120 was isolated using BHM with glucose and yeast extract as co-substrate. SRB1 strain was observed to be gram negative, rod shaped, possess mobility, positive for catalase, oxidase and indole activity, positive for gelatin hydrolysis and negative for urea hydrolysis.

Identification of bacterial strain SRB1 was done on the basis of 16S rRNA gene sequence. The sequence of SRB1 strain showed maximum similarity (99%) with *Tistrella mobilis* during similarity search using BLAST tool and identified as *Tistrella mobilis* JCM 21370 (Table 1) . The 16S rDNA partial sequence of *Tistrella mobilis* JCM 21370 (SRB1) has been deposited in GenBank under the accession number NR_028632.1. In phylogenetic analysis (Fig 1), SRB1 strain fall in cluster of *Tistrella* sp. The evolutionary history was inferred using the neighbor joining. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitution per site.

High Performance Liquid Chromatography (HPLC) analysis of Reactive Red 120 degradation

The HPLC of control (BHM amended with 100µl/l Reactive Red 120 dye) showed highest peak at retention time 1.494 with peak area 2634.83057. Limit of detection (LOD) and Limit of Quantification (LOQ) of the HPLC process was determined. Areas of peak in sample with approximately same retention time were determined; *Tistrella mobilis* peak area was 1043.005533 at retention time 1.481 (Fig 2). This indicates 92.08% of dye has been degraded within 10 days.

Earlier studies have reported *T. mobilis* to be a polyhydroxyalkanoate producing bacterium [9]. Its habitat has been reported as wastewater [10] and deep sea [11]. Some earlier studies in some other context isolated strain reportedly from Indian Ocean[10] and Atlantic Ocean [12]. Since this bacteria release didemnin products via a unique port assembly line [13], most of the research on this bacterium has been carried out in the field of medicine (cancer treatment).

In our knowledge, this is the first report investigating the role of *T. mobilis* in textile dye degradation.

4. Conclusions

Microorganisms represent half of the biomass of our planet, yet we know as little as 5% of the microbial diversity of the biosphere [14]. Based on the above study, it may be concluded that

1. *Tistrella mobilis* JCM 21370 possess the ability to degrade azo dye, although its enzyme system and mechanism need to be studied.

2. Bagru has been a hub of printing and dyeing since past 350 years. Not much of the research has been reported on its environmental conditions and diversity of microbial flora it possess. As being an area where more of natural

dyes and some synthetic dyes are in practice, it can come out as habitant of novel microorganisms.

Table 1: Identification of bacterial isolate inferred from 16S rRNA gene sequences by BLAST

Name of Isolates	Maximum Score	Maximum Identity	Query Coverage	Description Name	Gene Bank Accession no
SRB1	1489	99%	96%	*Tistrella mobilis strain JCM 21370*	NR_028632.1

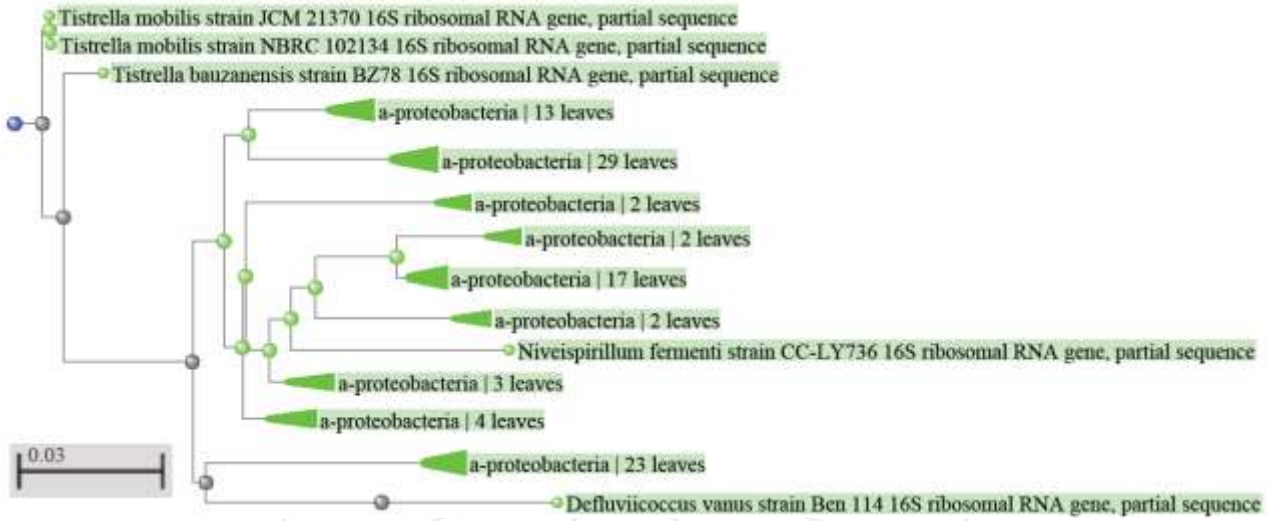

Figure 1: BLAST tree view of potential identified bacterial isolate *Tristella mobilis* JCM 21370

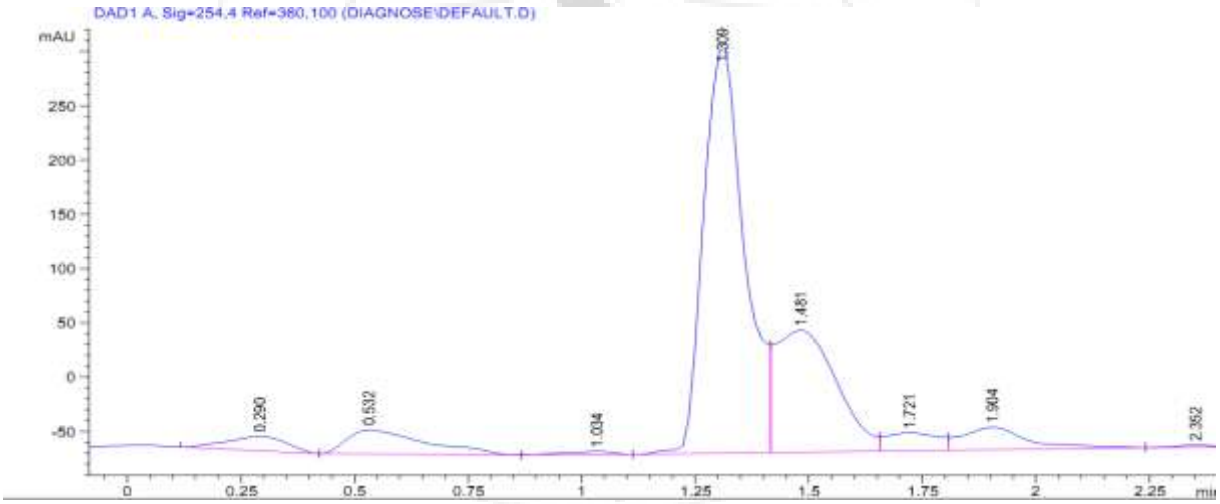

Figure 2: HPLC chromatogram of Reactive Red 120 dye treated with *Tistrella mobilis* JCM 21370

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