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# Bioremediation of hazardous azo dye methyl red by a newly isolated *Enterobacter asburiae* strain JCM6051 from industrial effluent of Uttarakhand regions

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**ABSTRACT**

Azo dyes are often known to be carcinogenic mutagenic and recalcitrant. Dyeing effluents have emerged as a significant cause of water contamination. Dyes influence all living forms, included humans, due to their xenobiotic characteristics and toxicity, as a result, hazardous dyes from colored wastewater must be treated and removed before they are released into the ecosystem. Bioremediation is an innovative, cost-effective, and eco-friendly achievement of biotechnological novelty. Thirty dye-decolorizing indigenous strains were isolated from industrial wastewater in the present investigation from the Kashipur paper industry and SIDCUL industrial area Haridwar using nutrient broth medium amended with 100 mg/l methyl red (MR). Isolation of MR decolorizing bacteria was done by the serial dilution method followed by the spread plate method. A total of 30 isolates were isolated and subjected to primary screening which was done through the tube method. Following a primary screening, 10 potent strains were retained for further evaluation of the efficacy of color removal, designated as MRD2, MRD3, MRD4, MRD15, MRD17, MRD18, MRD19, MRD20, MRD22, and MRD28, which were presumably grouped into 10 genera according to morphology and biochemical assay. The bacterial strain MRD17 outperformed other tested strains via a decolorization assay with 74.28% degradation and decolorization of MR in 72 hours, which was further, identified as *Enterobacter asburiae* strain JCM6051 by 16S rRNA sequencing and submitted to the NCBI GenBank with accession number MT539179. In addition, the thermodynamic stability of the strain's 16S rRNA sequence was investigated using bioinformatics tools such as mfold and NEB cutter. These findings suggest that bacterial isolates might be useful in the development of an alternative and environmentally acceptable approach for decolorizing and degrading azo dyes from industrial waste.

## 1. INTRODUCTION

Around the world, more than a million tons of synthetic dyes are manufactured each year for use in the leather, textile, food, cosmetic, pharmaceutical, plastic, paint, and paper sectors with azo dyes accounting for at least 60% of that total [1]. Azo dyes (R1–N=N–R2) are well-known organic compounds produced in the industrial sector. These dyes have a chemical structure that makes it simple to add or remove functional groups, making them extremely adaptable and environmentally stable [2]. Industrial wastewater containing dye reduces water quality, which not only makes the water look less appealing moreover, it also lowers gas solubility and the quantity of sunlight that penetrates the water, impacting photosynthesis and whole aquatic ecosystems [3–5]. Aside from color, the presence of these dyes in aquatic environments offers serious environmental and health hazards owing to the toxicity of the free dyes and their transformation into mutagenic, poisonous, and carcinogenic amines, this occurs mostly as a result of anaerobic microbial reductive breakdown of the azo link or by chemicals obtained following oxidation via cytochrome P450 [6–8]. Moreover, discharging dye-containing wastewaters into water resources such as rivers, ponds, and lakes alters the pH and increases biological oxygen demand,

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total organic carbon and chemical oxygen demand [9,10]. As a result, the dyes and dye intermediates industry has been identified as one of India's 17 most polluting industries, with the Ministry of Environment, Forest, and Climate Change designating it as a "red category" [11]. For the treatment of dye wastewater, physicochemical techniques such as flocculation, adsorption, coagulations chemical oxidation, irradiation ozonization, precipitation, Fenton's oxidation, and membrane filtering are most often used [12–14]. High operational and capital expenses, the production of hazardous by-products, and sludge disposal difficulties, however, frequently restrict their use. Biological methods, such as treatment methods including a variety of bacteria, algae fungi, and yeasts were explored during the last several decades in an attempt to overcome these constraints. Biological procedures provide a number of benefits over traditional techniques, including the transformation of organic molecules to non-toxic by-products (water and carbon dioxide), relatively inexpensive, sustainability, and convenience in application [15–17]. The treatment of dye-contaminated wastewater is the major objective of this new microbial decolorization and degradation approach.

Nonetheless, the detailed metabolic needs of various microorganisms are complicated, preventing their use in the clean-up of large amounts of colored wastewater. Bacterial systems, on the other hand, can operate quicker since they develop at a faster pace than algae and fungus. Various pure cultures of bacteria were demonstrated the capacity to degrade colors under anaerobic conditions. Bacterial degradation is primarily characterized by the reductive breakage of the azo link, which is mediated by the azoreductase enzyme system and results in aromatic amines formation. Under such conditions, azo dyes operate as artificial electron acceptors and can interact in a non-specific manner with carriers in the electron transport chain [3]. This leads to the production of a colorless amine solution. Extracellular reductive enzymes like azoreductase, as well as oxidative enzymes, including laccase, lignin, tyrosinase, and manganese peroxidases, have been found to participate in the color removal and degradation of azo dyes in several investigations [18–20]. The diverse group of microorganisms such as *Clostridium*, *Diapharobacter nitroreducens* [21], *Proteus vulgaris* [22], *Bacillus* sp. [4], *Aeromonas hydrophila*, *Proteus mirabilis*, *Pseudomonas* sp., *Shewanella* sp. use in dye decolorization and degradation [14]. In this regard, in the present study, methyl red (MR) azo dye was used for decolorization and degradation. In the current study, we assessed the isolation, screening, and characterization of most potential MR degrading bacteria isolated from industrial effluent of Uttarakhand areas. Using a UV-VIS spectrophotometer, we investigated the rate of bacterial decolorization of dyes. In addition, we also tested gene sequences for molecular identification, secondary structure stability, and thermodynamic stability.

## 2. MATERIALS AND METHODS

### 2.1. Dyestuff, Chemicals, and Microbiological Media

The dye used in the present research was MR dye which was procured from CDH, Central Drug House, New Delhi, India. The absorbance maxima ($\lambda_{max}$) for MR dye used in the present study was 410 nm. The chemicals, microbiological media, and medium ingredients used in this work were of analytical grade and available in the highest purity form. Nutrient agar (NA) and nutrient broth (NB) medium used in the decolorization study and its composition are as follows: beef extract: 3 g/l; peptone: 5 g/l; sodium chloride: 5 g/l and agar-agar: 15 g/l. Supplemented with the required amount of MR used to study the dye decolorization efficacy of bacterial strains.

### 2.2. Sample Collection and Physical Characterization of Effluent Samples

Industrial effluent samples were collected in a sterilized container. Various parameters such as temperature, pH, color, odor were monitored and stored at 4°C and processed within 48 hours of sample collection.

### 2.3. Isolation, Screening and Phenotypic Identification, Characterization of Dye Degrading Isolates

The bacterial strains were isolated from industrial effluent, the 1 ml sample was added in pre-sterilized distilled water and serially diluted. 0.1 ml aliquot samples were taken from $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions and spreaded on NA petriplates containing 100 mg/l MR using the spread plate technique. After 48 hours of incubation at 37°C, the colonies were tested for their capacity to produce a clean zone. Bacterial isolates that grew quickly at 100 mg/l dye concentrations were discovered to be potential dye decolorizers. Finally, 30 morphological distinct colonies were identified and purified using the streak plate technique. Stock cultures of screened bacterial isolates were consistently maintained on NA medium and kept at 4°C. Primary screening of 30 isolates was done in which test tubes containing 9 ml NB incorporated with 100 mg/l MR dye in it were taken. 1% inoculum of bacterial isolates was added to each tube and simultaneously, an abiotic control was set without inoculation, and incubation at 37°C for 48 hours was given. Isolates with clear promising prospects for decolorization were chosen for further testing. Following a primary screening of 30 isolates, potential 10 strains were retained for further evaluation of the efficacy of color removal designated as MRD2, MRD3, MRD4, MRD15, MRD17, MRD18, MRD19, MRD20, MRD22, and MRD28. They are further characterized according to their cultural (e.g., colonies form, coloration, texture, edge, and elevation); morphological, physiological (e.g., cell shape, size and arrangement, sporulation, motility test) and biochemical assay (e.g., Indole test, MR, VP, catalase, citrate utilization, oxidase, urease, nitrate reduction, production of $H_2S$, hydrolysis of starch and gelatin and various sugar fermentation test). By comparing the test results to the standard descriptions provided in Bergey's Manual of Determinative Bacteriology, the isolates were tentatively identified up to the genus level [23]. The bacterial strain MRD17 outperformed other tested strains via decolorization assay.

### 2.4. Decolorization Efficacy Analysis

Erlenmeyer flasks (250 ml) containing 200 ml of sterilized NB medium supplemented with MR at 200 mg/l concentration were inoculated with 2% (v/v) inoculums of each screened isolate

and cultured for 72 hours at 37°C under static conditions. To confirm that all of the decolorization was biologically mediated, a control experiment with NA containing dye without inoculum was performed in parallel. 2 ml of liquid samples were taken aseptically from the Erlenmeyer flask every 24 hours and centrifuged for 15 minutes at 4°C at 1,000 rpm for analysis. Absorbance of centrifuged cell-free supernatant samples was measured and recorded at the appropriate MR max (410 nm) using a UV-vis spectrophotometer (UV/vis spectrophotometer systronix RS 118 model, India). Based on the following equation [10]. The efficacy of color removal was expressed in percentage ratio of the decolorized dye concentration to the initial one.

$$\text{Dye decolorization percentage} = \frac{\text{Initial absorbance} - \text{observed absorbance} \times 100}{\text{Initial absorbance}}$$

### 2.5. Molecular Identification of most Putative Dye Degrading Strain

The selected *Enterobacter* strain MRD17 was identified by 16S rRNA gene sequencing followed by reference from Biokart India Pvt Ltd Bangalore, India. Using universal primers, polymerase chain reaction (PCR) amplification of the 16S rRNA gene from a bacterial isolate was performed. Thirty-five cycles were performed with the strain-specific forward (5'-GGATGAGCCCGGCCTA-3') and reverse primer (5'-CGGTGTACAAGGCCCGG-3'). Thermal cycling consists of following steps: 3 minutes at 95°C, followed by 30 cycles of 30 seconds at 56°C, 45 seconds at 72°C, and 10 minutes at 72°C. Following ethidium bromide staining, PCR amplification was detected using agarose gel electrophoresis. After that, the PCR product was sequenced using an automated sequence technique. The same primers that were previously described were utilized for sequencing. Additionally, NCBI-BLAST was utilized to analyze the *Enterobacter* strain's sequence similarity. The 16S rRNA gene sequencing of the MRD17 strain has already been submitted to NCBI GenBank with the genbank accession number MT539179. To obtain confidence values for bootstrapping evaluation, the original data set was re-sampled 100 times using the phylogenetic bootstrap software. Using the bootstrapped data set, the phylogenetic tree was constructed using the MEGA 4 program. Using the neighbor-joining technique, multiple distance matrices were used to construct a phylogenetic tree [24].

### 2.6. Determination of Restriction Sites and RNA Secondary Structure Prediction

RNA web server operated for the prediction of the secondary structure of 16S rDNA (http://rnatbi.univie.ac.at) to examine structure stability with reference to Gibb's free energy [25] and The NEB cutter tool version 2.0 was used to locate the restriction site (nc2.neb.com/nebcutter/) [26].

### 2.7. Statistical Analysis by One-Way Analysis of Variance (ANOVA) Test

All of the experiments were done in triplicate, and the results are given as mean ± SEM. The statical analysis was carried out using one-way ANOVA in GraphPad prism 6.0, with the turkey–Kramer comparison test [27].

## 3. RESULTS AND DISCUSSION

### 3.1. Isolation and Screening of MR Decolorizing Bacteria

Azo dyes chemical structures are simple to integrate or change their functional groups, making them very flexible and environmentally stable [28]. Aside from being resistant to different degradation processes, azo dyes create hazardous chemical compounds such as aromatic amines, which are poisonous, allergic, carcinogenic, and mutagenic to living beings [29]. Hence, the biological degradation of dyes involving bacteria is considered one of the best methods. This method is cost-effective, eco-friendly that generate a significantly nontoxic intermediate compound. The isolation of a wide range of novel and diverse bacteria provides a theoretical framework for exploiting and utilizing bacteria for dye decolorization and degradation. In the present research, we have assessed the dye degradation efficiency of the bacterial isolates isolated from industrial effluents of the paper industry in Kashipur and the SIDCUL industrial area of Haridwar. The geographical coordinates of the sampling sites were mentioned in Table 1.

**Table 1:** Geographical coordinates for sampling site in Uttarakhand regions.

| Regions | Sampling site | Latitude | Longitude |
|---|---|---|---|
| Haridwar | SIDCUL Industrial area | 78.08469 | 29.93829 |
| Kashipur | Paper industry | 78.99607 | 29.21081 |

**Table 2:** Characterisation of effluent samples from two different industrial area i.e. paper industry (Kashipur) and SIDCUL industrial area (Haridwar).

| Name of parameters | Sample 1 (paper industry) Kashipur | Sample 2 (SIDCUL industrial area) Haridwar |
|---|---|---|
| Temperature | 36°C | 39°C |
| pH | 7.8 | 8.2 |
| Color | Light brown | Blackish blue |
| Odour | Unpleasant | Unpleasant |

**Table 3:** Primary screening of MR decolorizing and degrading bacteria of isolated strains by tube method.

| S. No | 12 hours | 24 hours | 36 hours | 48 hours |
|---|---|---|---|---|
| MRD1 | − | − | − | + |
| MRD2 | + | + | ++ | +++ |
| MRD3 | +++ | +++ | +++ | +++ |
| MRD4 | + | ++ | +++ | +++ |
| MRD5 | − | − | − | + |
| MRD6 | − | − | − | + |
| MRD7 | − | − | − | + |
| MRD8 | − | − | − | + |
| MRD9 | − | − | − | + |
| MRD10 | − | − | − | + |
| MRD11 | − | − | − | + |
| MRD12 | − | − | − | + |
| MRD13 | − | − | − | + |
| MRD14 | − | − | − | + |
| MRD15 | + | + | ++ | +++ |
| MRD16 | − | − | − | + |
| MRD17 | +++ | +++ | +++ | +++ |
| MRD18 | + | ++ | +++ | +++ |
| MRD19 | + | + | ++ | +++ |
| MRD20 | + | + | ++ | +++ |
| MRD21 | − | − | − | + |
| MRD22 | + | ++ | +++ | +++ |
| MRD23 | − | − | − | + |
| MRD24 | − | − | − | + |
| MRD25 | − | − | − | + |
| MRD26 | − | − | − | + |
| MRD27 | − | − | − | + |
| MRD28 | + | ++ | +++ | +++ |
| MRD29 | − | − | − | + |
| MRD30 | − | − | − | + |

− = no decolorization and degradation, + = slightly decolorization and degradation, ++ = moderately decolorization and degradation and +++ = maximum decolorization and degradation.

It was investigated that the effluent sample from Kashipur was light brown in color had an unpleasant odor. The temperature was 36°C and the pH was 7.8. The sample of SIDCUL area was blackish blue, with an unpleasant odor. The temperature and pH were determined to be 39°C, 8.2, respectively, and are shown in Table 2. A total 30 morphological distinct colonies were isolated from the paper industry (Kashipur) and SIDCUL industrial area (Haridwar) on the basis of clear zone formation around the colonies in the dye containing medium. In previous studies, many researchers isolated potential dye decolorizing bacterial isolates from the textile dye effluent, activated sludge, soil contaminated with dye collected from waste disposal sites, lake mud and waste water treatment plant which indicated the natural adaptation of these isolates to high dye concentrations and their survival in the presence of hazardous chemicals [30–33]. Further primary screening was performed to evaluate the decolorization ability of each isolate, which was tested in dye (100 mg/l) containing a liquid medium. The isolates that demonstrated color removal after 48 hours of incubation at 37°C were classified as MR decolorizers, and the findings are shown in Table 3. Ten active isolates were screened based on primary screening and designated as MRD2, MRD3, MRD4, MRD15, MRD17, MRD18, MRD19, MRD20, MRD22, and MRD28. Screened isolated strains were subjected to dye decolorization assay. The bacterial strain MRD17 outperformed other tested isolates via decolorization assay with a decolorization percentage of 74.28% within 72 hours. When the current study's results are compared to prior research, no additional carbon

**Table 4:** Morphological and cultural characteristics screened isolates of MR decolorization and degradation.

| S. No | Sample | Isolates | Colony characteristic | Gram staining | Motility |
|---|---|---|---|---|---|
| 1 | SIDCUL industrial area (Haridwar) | MRD2 | Medium, circular, entire, smooth, opaque | Gram positive rods | Non motile |
| 2 | SIDCUL industrial area (Haridwar) | MRD3 | Large, round, circular, entire mucoid, opaque | Gram negative rods | Motile |
| 3 | Paper industry (Kashipur) | MRD4 | Small, irreguar, circular, mucoid, opaque | Gram negative rods | Motile |
| 4 | SIDCUL industrial area (Haridwar) | MRD15 | Medium, entire, mucoid, translucent | Gram negative rods | Motile |
| 5 | Paper industry (Kashipur) | MRD17 | Large, round, entire, smooth, opaque | Gram negative rods | Motile |
| 6 | Paper industry (Kashipur) | MRD18 | Small, circular, mucoid transclucent | Gram negative rods | Non motile |
| 7 | Paper industry (Kashipur) | MRD19 | Medium, round, entire, smooth opaque | Gram negative cocci | Non motile |
| 8 | SIDCUL industrial area (Haridwar) | MRD20 | Small, circular, entire, smooth translucent | Gram negative rod | Non motile |
| 9 | Paper industry (Kashipur) | MRD22 | Small, circular, entire, opaque | Gram negative rod | Non motile |
| 10 | SIDCUL industrial area (Haridwar) | MRD28 | Small, round, irregular, mucoid, opaque | Gram negative cocci | Non motile |

source was supplied except the medium, and the decolorization assay showed maximal decolorization of 74.28% in 72 hours as compared to WFB3c, WFB4g, and SFB5c, which showed 60.07% in 48 hours [33].

### 3.2. Morphological, Physiological, Biochemical Characterization and Identification

The selected bacterial isolates were characterized on the basis of their cultural, morphological physiological characteristics as mention in Table 4, and biochemical assay was presented

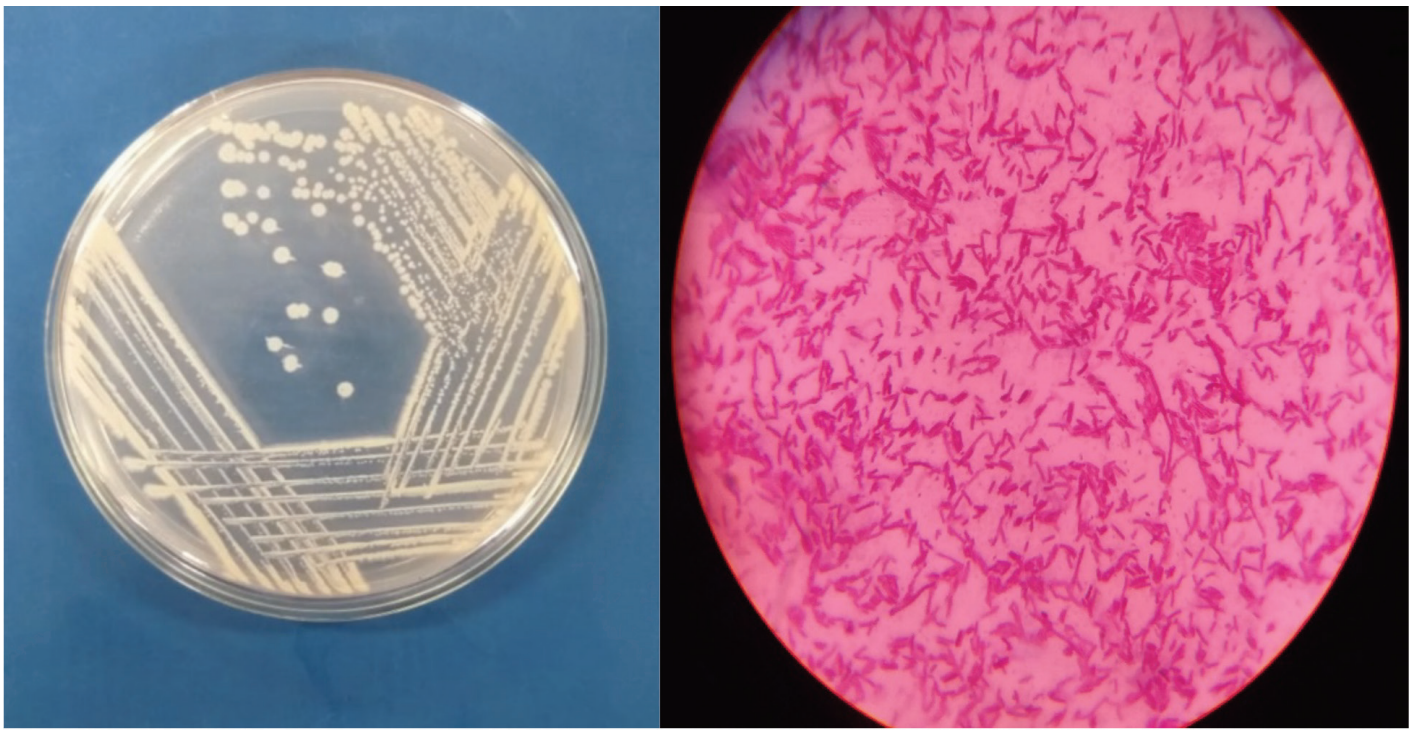


**Figure 1:** Morphological (a) and microscopic (b) view of *E. asburiae* JCM6051 (MRD17).

**Table 5:** Biochemical characteristics of screened isolated after primary screening.

| Parameters studied | MRD2 | MRD3 | MRD4 | MRD15 | MRD17 | MRD18 | MRD19 | MRD20 | MRD22 | MRD28 |
|---|---|---|---|---|---|---|---|---|---|---|
| Indole test | – | − | − | − | − | − | − | + | − | − |
| MR test | – | − | − | − | − | − | − | + | − | − |
| VP test | + | − | − | − | − | − | + | + | − | + |
| Citrate | + | + | + | + | + | + | − | + | + | + |
| Catalase | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | − | − | + | − | + | + | + | + | − |
| Nitrate | + | + | + | | + | − | – | + | | − |
| Gelatine hydrolysis | + | + | + | + | + | − | + | + | + | − |
| Starch hydrolysis | + | − | − | − | − | − | − | − | − | − |
| Urease | – | − | + | + | − | − | − | − | + | + |
| Glucose | + | − | + | + | − | − | + | + | + | + |
| Lactose | + | + | − | – | + | − | − | − | − | + |
| Sorbitol | + | + | − | – | + | − | − | − | − | + |
| Mannitol | + | + | − | + | + | − | − | + | + | + |
| Ramnose | + | + | − | – | + | − | − | – | − | + |
| Sucrose | + | + | − | – | + | − | − | + | − | + |
| Fructose | + | + | − | – | + | − | − | − | − | + |
| Maltose | + | + | − | – | + | − | − | − | − | + |
| Galactose | + | + | − | | + | − | − | − | − | + |
| Identified sp. upto genus level | *Bacillus* sp. | *Enterobacter* sp. | *Proteus* sp. | *Pseudomonas* sp. | *Enterobacter* sp. | *Alcaligens* sp. | *Neisseria* sp. | *Aeromonas* sp. | *Pseudomonas* sp. | *Klebsiella* sp. |

in Table 5. All these characteristics were compared with the standard description of Bergey's Manual of Determinative Bacteriology [23] and isolates were provisionally identified up to the genus level and named as *Bacillus* sp., *Enterobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Alcaligenes* sp., *Neisseria* sp., *Aeromonas* sp., *Pseudomonas* sp., and *Klebsiella* sp. Screened isolates were streaked on NA slants and cultured for 48 hours at 37°C. The pure culture is then kept in a refrigerator at 4°C and subculture on a regular basis. The bacterial strain MRD17 outperformed other tested isolates via decolorization assay. Morphological, physiological, and biochemical characterizations revealed that MRD17 is a Gram-negative, rod-shaped bacterium with a white, large, round, entire, smooth, opaque colony, and entire margin. The cell was discovered to be non-motile, non-spore-forming, and white in color. MRD17 was able to grow at temperatures as high as 35°C and at pH levels as low as 3% NaCl. MRD17 had a favorable effect on citrate utilization, catalase, nitrate reduction, gelatine hydrolysis, and could use a variety of carbon sources, including glucose, lactose, sorbitol, mannitol, rhamnose, sucrose, fructose, maltose, and galactose. As a result of these morphological, biochemical, and physiological characteristics, isolates may be assigned to the genus *Enterobacter*. MRD17 was shown morphologically and microscopically in Figure 1. The foregoing findings imply that the isolated indigenous strain MRD17 might be employed in the biological treatment of industrial effluent containing MR and used on an industrial scale for wastewater treatment plants. Figure 2 depicted decolorization activity of MR by MRD17 isolate.

### 3.3. Molecular Identification of Dye Decolorizing Bacteria

The taxonomic position of the bacterium MRD17 was established using 16S rRNA gene sequencing. The NCBI GenBank database was used to analyze the strain MRD17's 16S rRNA gene sequence. The phylogenetic tree of the strain MRD17 was built using the neighbor-joining technique from the genomes of closely related strains (Fig. 3). The stain exhibits 98.07% sequence homology to *Pantoea agglomerans* strain JCM1236 for the 16S rRNA gene. The 16S rRNA gene sequence of *Enterobacter asburiae* strain JCM6051 was also submitted in GenBank with the accession number MT539179. An earlier report showed that some species of *Enterobacter* have a role in the biodegradation of dyes [34]. But to the best of our knowledge, there was no literature available on the use of *E. asburiae* strain in MR decolorization and degradation.

### 3.4. RNA Secondary Structure Prediction and Determination of Restriction Sites

The stability of 16S rRNA sequence of the strain was studied, the concept of studying free energy associated with the folding of 16S rRNA gene sequence might provide preliminary information to make a contemporaneous prediction on stabilities of the genes.

**Figure 2:** Decolorization activity of MR by MRD17 isolate.

The 16S rRNA folding was designed to better understand the gene sequence's thermodynamic stability (Fig. 4). For instance, the free Gibbs energy value of 16S rRNA in its folded state was found to be −485.89 kcal/mol. This analysis demonstrates that the lowest energy level of the 16S rRNA sequence, which mandated excellent nucleotide folding stability in microorganisms. The restriction site analysis of the 16S RNA sequence of strain JCM6051 revealed a GC-AT content of 55% and 45%, respectively. Sites of restriction were depicted in Figure 5.

### 3.5. Statistical Analysis by One-Way ANOVA Test

Primary screening clearly showed that all 10 isolated strains were potentially active and completely decolorize the MR dye within 48 hours. Screened isolated strains were subjected to dye decolorization assay and the results were statistically analyzed by one-way ANOVA using GraphPad prism 6.0, with turkey–Kramer comparison test and found that *p*-value is 0.02 which was less than 0.05; hence, the value was significant and the results were mentioned in Figure 6.

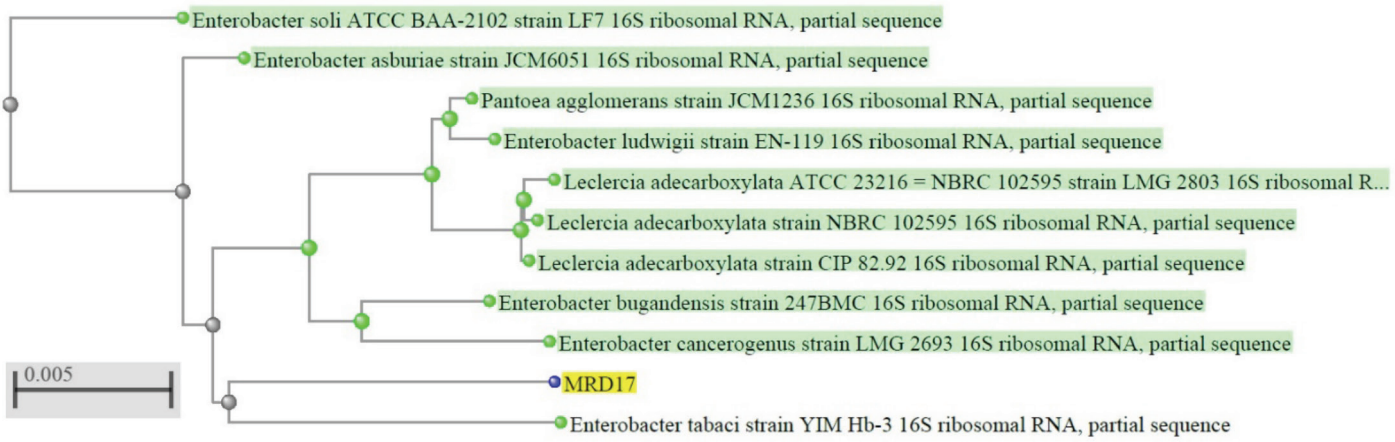


**Figure 3:** Phylogenetic tree of the *E. asburiae* strain JCM6051 and related organisms, tree conducted using neighbor-joining method with weighted version weighbor with alphabet size 4 and length size 1,000.

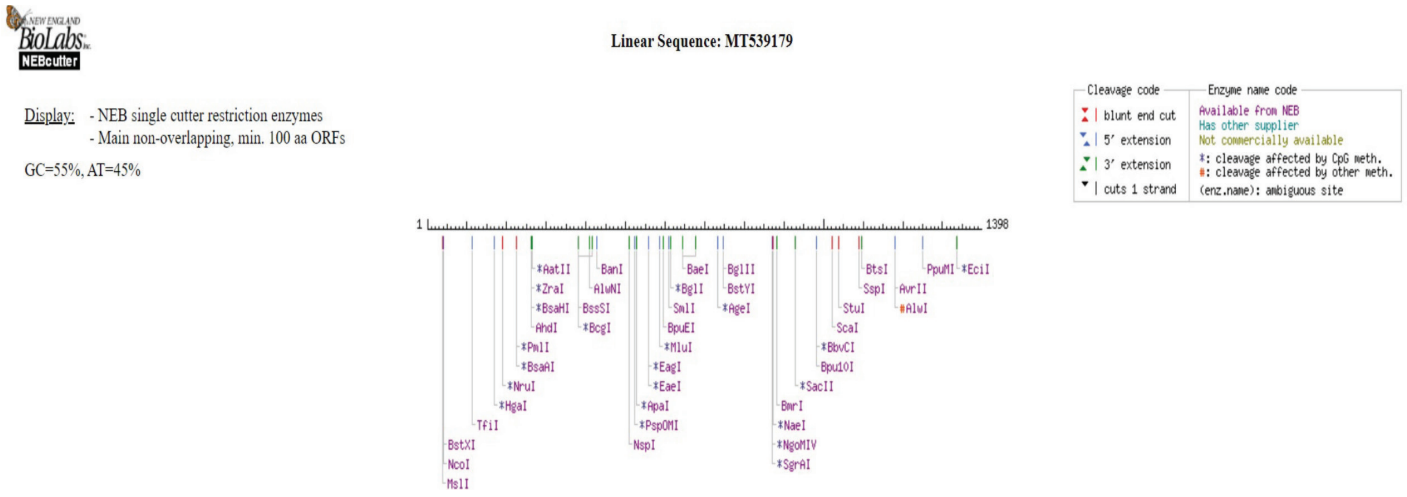


**Figure 4:** Restriction sites on 16S rRNA sequence of *E. asburiae* strain JCM6051.

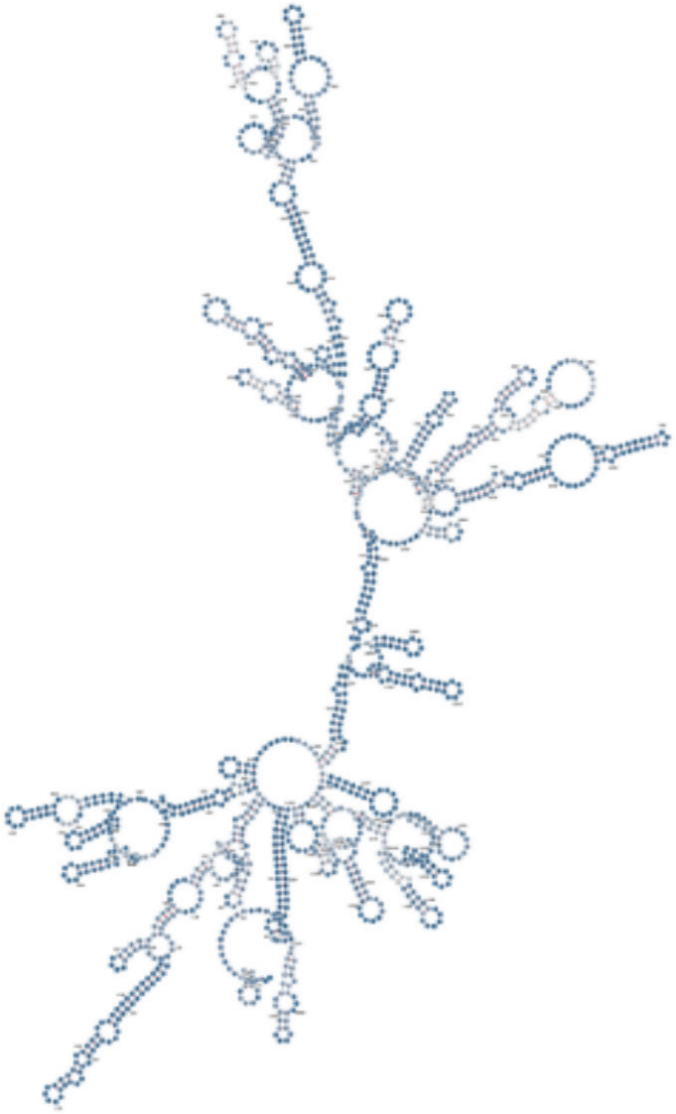

**Figure 5:** Predicted secondary structure of 16S rRNA isolated from *E. asburiae* strain JCM6051 (Gibb's free energy −485.89 kcal/mol).

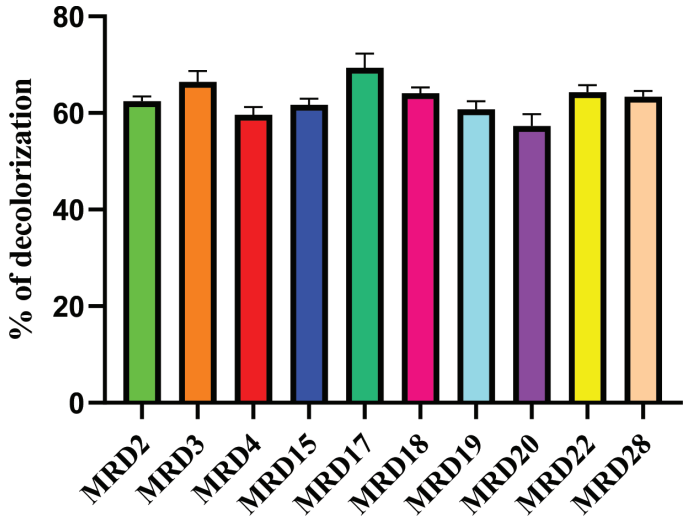


**Figure 6:** Dye decolorization assay of screened isolates.

## 4. CONCLUSION

Dyeing wastewater poses a significant danger to long-term environmental development, and remediation is a challenging feat. Taking this into consideration, the current study explored the isolation, screening, and identification of indigenous bacterial isolates and found that isolates *Bacillus* sp., *Enterobacter* sp., *Proteus* sp., *Proteus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Alcaligenes* sp., *Neisseria* sp., *Aeromonas* sp., *Pseudomonas* sp. *And Klebsiella* sp. Were involved in MR color removal and degradation. Out of which *Enterobacter sp.*, i.e., MRD17 depicted as most putative decolorizer and degrader which was further phylogenetically identified as *E. asburiae* strain JCM6051, which was newly isolated as the strain was not reported earlier in MR decolorization and degradation. The biological treatment is a low-cost and environmentally friendly approach; hence, it would be sufficed to conclude that our bacterial strain significantly applied in bioremediation of treatment of wastewater treatment plant.

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## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included within this research article.

## PUBLISHER'S NOTE

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