

Decolorization of Mordant Orange-1 by *Marinobacter algicola* MO-17

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Abstract: Problem statement: The textile industries use different types of dyes in their processing units which are liberated in natural marine water bodies. This release of dyes in turn affect the marine flora and fauna very adversely. **Approach:** The release of textile dyes into the environment is a matter of concern due to the toxicity, mutagenicity, carcinogenicity and xenobiotic nature. Hence, considerable attention has been given in determining the ability of marine microorganism in decolorization and degradation of textile dyes. **Results:** The present study deals with the decolorization of Mordant Orange-1 was carried out using the acclimatized *Marinobacter algicola* MO-17 isolated from natural marine environment. The acclimatized strain was capable of decolorizing 800 µg mL⁻¹ of the dye at high salt concentration (12%) in 24 h. The percent decolorization of the dye was moderated by spectroscopy at 385 nm (λ_{max}). **Conclusion/Recommendations:** The spectrophotometric analysis showed 78.78% decolorization in Nutrient Broth and 76.78% decolorization in Half Strength Nutrient Broth. The percent Chemical Oxygen Demand (COD) reduction of the dye by the strain was 90%. From the study performed, we conclude that, this acclimatized strain can prove better option for bioremediation of textile dyes in wastes containing high salts and in marine environment.

Key words: Marine bacteria, textile dye, decolorization, degradation, COD reduction

INTRODUCTION

Water is essential to human life and to the health of the environment. As a valuable natural resource, the sea is an immense and practically unexploited source of new potentially useful biologically active substances possessing great diversity. Large number of textile industries are located on the coastal areas due to ease of transport to the various places in world and help in building nations economy, but on the contrary the effluents released from these industries are proving a great problem for the marine life.

Large amount of water is required for the processing (dyeing) of the fabric in textile industry, which in turn releases large amount of effluent in the environment. Annually, approximately 2,80,000 tonnes of textile dyes are discharged in industrial effluent worldwide (Jin *et al.*, 2007). These dyes in large amount are liberated in marine environment. Dyes undergo chemical changes as well as biological changes in the aquatic system, consume dissolved O₂ and thus disturb the aquatic eco-system (Huang *et al.*, 2008). Consequently, survival of life fishes and other organisms becomes difficult, especially in environmental water (Rahman *et al.*, 2009). All of these

dyes persist in the environment due to their high stability against light, temperature, detergents, chemicals and microbial attack (Couto, 2009). Disposal of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration (Aksu *et al.*, 2007) and also they may be toxic to some aquatic organisms due to their breakdown products (Hao *et al.*, 2000). Dyes can be removed from wastewater by chemical and physical methods including adsorption, coagulation, flocculation, oxidation and electrochemical methods (Lin and Peng, 1994; 1996; Supaka *et al.*, 2004). However, both the physical and chemical methods have many shortcomings such as high-energy costs, high-sludge production. Hence, economical and bio-friendly approaches are needed to remediate dye containing wastewater.

Also, studies have been reported that the dye decolorization by facultative and obligate marine fungi *viz.* *Flavodon flavus*, *Gliocladium* sp., *Sordaria fimicola*, *Halosarpheia ratnagiriensis*, *Algialis grandis*, *Hypoxylon oceanicum* (Raghukumar, 2000; Raghukumar *et al.*, 2004). Among bacteria, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*,

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Bacillus subtilis, *Bacillus cereus*, *Proteus vulgaris*, *Acinetobacter sp.*, *Zooglea sp.* are dominant dye decolorizer (Sudhakar *et al.*, 2002).

Marine fungi degrade and remineralize the lignocellulose substrates by their extracellular lignin degrading enzymes. Such fungi were isolated from decaying mangrove wood, leaves, seagrass and algae (Raghukumar *et al.*, 1994). Marine microorganisms are of immense use in the decolorization of dye because most of the industrial effluents contain high concentrations of salts, especially chlorides and salts. Besides, marine microbial enzyme would act efficiently at neutral/alkaline pH and wide range of temperatures to combat the neutral/ alkaline dye effluents (Chandramohan, 1997).

Therefore, it may be economical to develop alternative means of dye decolorization such as bioremediation as it is an environmentally friendly, cost effective treatment technology (Hao *et al.*, 2000; Verma and Madamwar, 2003). Microbial decolorization and degradation is an environment friendly and cost effective means to different conventional treatment technologies (Gogate and Pandit, 2004; Uddin *et al.*, 2007).

In the present study, a bacterium was isolated from marine water capable of decolorizing Mordant Orange-1 textile dye. This strain was studied for decolorization of the dye Mordant Orange-1 in various different conditions like in complete nutrient medium, in half strength nutrient medium and in presence of different co-substrates. The decolorization of the dye was monitored spectrophotometrically (Systronics-106) at its specific absorbance maxima (λ_{max}) 385 nm. Percent COD reduction of the dye was calculated.

MATERIALS AND METHODS

- Soil-Soil from nearby salterns (salt pans), compost, textile sludge from sludge drying beds of textile industry, ETP.
- Textile Dye- Mordant Orange-1 (λ_{max} -385 nm). Analytical Grade dye purchased from Sigma-Aldrich (USA).
- Marine water from different places.

Acclimatization and isolation of microorganisms:

Soil samples from nearby salterns (salt pans), compost and textile sludge from sludge drying beds of textile industry were collected and mixed properly. The microflora from the homogenized samples were acclimatized by adding increasing concentration of NaCl (0.5-12%) and after a week, the dye in increasing concentration was added to this soil for the period of one month. One gram of acclimatized soil was

inoculated in nutrient media containing 0.5-12% NaCl. Isolation of promising bacteria was carried out on nutrient agar having the same NaCl concentration and Mordant Orange-1 at a final concentration of 10,000 $\mu\text{g mL}^{-1}$. The colony showing zone of decolorization of the dye on the plate was designated as MO-17 and was selected for further studies.

Decolorization of dye in nutrient broth: The selected culture was then inoculated in 25 mL nutrient medium (Peptone -1.0 g, NaCl -0.5 g, Beef Extract -0.3 g, Distilled Water -100 mL, pH -7.2) containing 12% NaCl concentration and 10,000 $\mu\text{g mL}^{-1}$ concentration of dye. These tubes were then incubated at ambient temperature for 24 h and observed for decolorization of the dye. Percent decolorization was determined.

Decolorization of dye in half strength nutrient broth: Isolated culture was then inoculated in 25 mL half strength nutrient medium (Peptone -0.5 g, NaCl -0.25 g, Beef Extract -0.15 g Distilled Water -100 mL, pH -7.2) containing 12% NaCl concentration and 1ml of 10,000 $\mu\text{g mL}^{-1}$ concentration of dye. These tubes were then incubated at ambient temperature for 24 h and observed for decolorization of the dye.

Decolorization studies in presence of different Co-substrates: The isolate was inoculated in 25 mL nutrient broth (Peptone -1.0 g, NaCl -0.5g, Beef Extract -0.3 g, Distilled Water -100 mL, pH -7.2) containing 12% NaCl concentration, 1ml of 10,000 $\mu\text{g mL}^{-1}$ concentration of dye and 1% Glucose. Tube was then incubated at ambient temperature for 24 h and observed for decolorization of the dye. Additionally, nutrient medium containing 1% Starch and 1% Yeast Extract with the same dye and NaCl concentration were also used to test the ability of the isolate MO-17 to decolorize the dye Mordant Orange-1.

Percent decolorization study: The percent decolorization studies were done by using spectrophotometer. The percent decolorization was calculated by using the formula as:

$$\text{Percent decolorization} = \frac{\text{Initial absorbance} - \text{final absorbance}}{\text{Initial absorbance}} \times 100$$

Percent COD reduction: Percent COD reduction was determined by reflux method using Potassium dichromate as a strong oxidizing agent.

RESULTS

Isolation and identification: The organism was isolated from marine environment on nutrient agar with 12% salt concentration. It was then identified by using biochemical tests and 16s rRNA analysis technique. The biochemical results showed, Oxidase, Catalase positive but unable to ferment Glucose, Sucrose, Lactose and Maltose. The phylogenetic tree was developed by using Neighbor joining method by Kimura-2-parameter with 1000 replicates in MEGA 4.0 (Fig. 1).

Percent decolorization of dye in nutrient medium:

The promising isolate MO-17 was studied for its ability to decolorize the dye Mordant Orange-1 in nutrient medium containing 12% NaCl concentration and 1ml of 10,000 $\mu\text{g mL}^{-1}$ concentration of dye. The percent decolorization results of dye Mordant Orange-1 in nutrient broth is given in Table 1.

Percent Decolorization of Dye in Half (½) strength nutrient medium:

The promising isolate MO-17 was studied for its ability to decolorize the dye Mordant Orange-1 in half strength nutrient medium containing 12% NaCl concentration and 1 mL of 10,000 $\mu\text{g mL}^{-1}$ concentration of dye.

Table 1: Percent Decolorization in Nutrient Broth and Half (½) Strength Nutrient Broth containing 12% salinity by *Marinobacter algicola* MO-17 in 24 h

Decolorization in nutrient broth (%)	Decolorization in Half Strength Nutrient Broth (%)
78.78	76.78

Table 2: Percent Decolorization in Nutrient medium containing different Co-substrates at 12% salinity by *Marinobacter algicola* MO-17 in 24 h

1% Glucose (%)	1% Yeast extract (%)	1% Starch (%)
77.81	79.32	76.70

The results of percent decolorization of dye Mordant Orange-1 in nutrient broth is given in Table 1.

Percent decolorization of dye in presence of different co-substrates:

The promising isolate MO-17 was further studied for its ability to decolorize the dye Mordant Orange-1 in nutrient medium containing 12% NaCl concentration, 1% Glucose, 1% Starch and 1% Yeast extract as co-substrates and 1ml of 10,000 $\mu\text{g mL}^{-1}$ concentration of dye. The results of percent decolorization of dye Mordant Orange-1 in presence of different co-substrates is given in Table 2.

Percent COD reduction of dye: The Percent COD reduction of the dye Mordant Orange-1 after decolorization by *Marinobacter algicola* MO-17 was 90%.

DISCUSSION

The isolate decolorize the dye substrate and decolorizing efficiency was dependent on the growth of the isolate in the tubes. There was neither growth nor decolorization in the control tubes. This showed that the decolorization was due to the metabolic activity of the organism.

Halophiles have been reported to be involved in the dye decolorization (Khalid *et al.*, 2008a). It has been investigated that the decolorization of azo dyes by a member of the genus *Shewanella*: *Shewanella putrefaciens* strain AS96 under hypersaline conditions (Khalid *et al.*, 2008b; Ammozegar *et al.*, 2010).

It has also been reported that reported halophilic microorganism *Shewanella putrefaciens* to be capable of the complete removal of Reactive Black-5, Direct Red-81, Acid Red-88 and Disperse Orange-3 (all 100 mg L^{-1}) within 8 h in presence of 40 g L^{-1} NaCl (Ammozegar *et al.*, 2010).

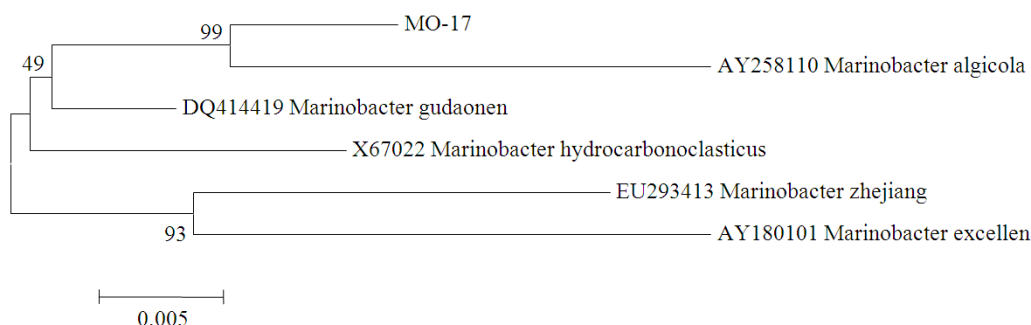


Fig. 1: Phylogenetic Tree of the isolate MO-17 was drawn using Neighbor joining method by Kimura-2-parameter with 1000 replicates in MEGA 4.0

In the present study, the extreme halophilic *Marinobacter algicola* MO-17 isolated from acclimatized soil decolorized the dye Mordant Orange-1 to an extent of 78.88% in nutrient medium, 76.78% in half strength nutrient medium. This rate of decolorization may be due to the high metabolic diversity being observed in the halophiles due to their extremophilic nature (Oren *et al.*, 1992; Ventosa *et al.*, 1998). Decolorization in marine water medium; indicate that the organism can be used for effluents containing high salt content.

CONCLUSION

The isolated strain MO-17 was identified as a member of the genus *Marinobacter* by 16 s rRNA sequence analysis and biochemical tests. The decolorization of the dye in half strength nutrient broth was comparatively less than in Nutrient broth but this method will be cost effective. The COD of the dye was reduced considerably after the treatment of *Marinobacter algicola* MO-17.

The results of the present study indicate that the isolate from Marine environment *Marinobacter algicola* could decolorize the dye Mordant Orange-1 efficiently and the organism can be used for the bioremediation of textile dye Mordant Orange-1 in Marine Environment.

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