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Biodegradation and Detoxification of a Textile Azo Dye Reactive Blue 171 by *Marinobacter sp. NB-8*

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The degradation and detoxification of an azo dye Reactive Blue 171 was carried out by the acclimatized *Marinobacter sp. NB-8* (Accession No. **HF541878**) isolated from marine environment and identified by 16s rRNA sequence analysis. Decolorization of the dye was determined by spectroscopic technique. Isolated *Marinobacter sp.NB-8* could decolorize the 1500µg/ml dye up to 92.00% in the nutrient medium within 24 hours. Slight decrease in decolorization was observed when half strength nutrient medium was used. Decolorization of the dye was studied in presence of different co-substrates *Viz*. 1% glucose, 1% yeast extract and 1% starch and found that percent decolorization was up to 92.22%, 94.00% and 92.88% respectively. Percent decolorization by cell free extract was also studied. Isolate could reduce the COD of the dye Reactive Blue 171 up to 80.78% after 24 hours. Degradation of the dye was confirmed by GC-MS analysis.

[**Keywords:** Marine Bacteria, Reactive Blue 171, Degradation, GC-MS analysis, COD reduction]

Introduction

The toxic dye effluents released by textile industries causes a great challenge to the marine life. As these dyes undergo chemical and biological changes profoundly disturbs the marine ecosystem. Their breakdown products might also be toxic to some aquatic organisms. It also greatly affects the photosynthesis of some hydrophytes by limiting light penetration, thereby deteriorating gas solubility and water quality[1]. Various conventional methods are available for the treatment of wastewater containing dyes, like flocculation, adsorption, and oxidative degradation[2,3] but these methods are highly expensive and of limited applicability. Decolorization of the synthetic dye using different microorganisms appear to be effective as it is environment friendly, less expensive and with reproducible biotransformation data[4,5.] Present article focuses on the use of marine microorganism to remediate the azo dyes contaminated environment, which are extensively used in the textile industries. Presence of high salt content in the textile dye effluents limits the development of efficient bio-treatment system to bioremediate the textile azo dyes. Taking into consideration a great need in developing bacterial strains that could thrive under high salt conditions. Salterns or Solar salt crystallizer ponds contain hyper-saline waters or the soil containing the NaCl serves as a rich source of marine microorganisms. The bacteria isolated from such saline soil will definitely yield a good collection of halophilic and halotolerant bacterial strains.

Materials and Methods

Samples– Soil samples were collected from salterns (Saltpan), areas nearby waste disposal sites of the textile industry

Dye – Reactive Blue 171 (λmax-590nm)

Soil samples were collected from salterns (Saltpan), areas nearby waste disposal sites of the textile industry and homogenized. Micro-flora from the homogenized samples, were acclimatized by adding the dye Reactive Blue 171 in increasing concentration for the period of one month. One gram of acclimatized soil was inoculated in the nutrient broth having 0.5%-20% NaCl and after incubation isolation was carried out on nutrient agar supplemented with dye Reactive Blue 171 at a final concentration of 10,000µg/ml and the same salinity. Colony showing decolorization was designated as isolate NB-8 and used for further studies.

Selected isolate NB-8 was used to inoculate in 20 ml nutrient broth and half strength nutrient broth containing 1500µg/ml concentration of dye and 10% NaCl. All these tubes were then incubated at ambient temperature for 24 hrs.

Promising isolate was inoculated in 20ml nutrient broth containing 10% NaCl concentrations, 1500µg/ml concentration of dye, 1% Glucose, 1% Starch and 1% Yeast Extract. Tubes were then incubated at ambient temperature for 24 hours and observed for decolorization of the dye.

The cells grown in nutrient broth were separated by centrifugation and used for to study the percent decolorization of Reactive Blue 171.

The percent decolorization studies were monitored by using spectrophotometer (Systronics – 106 model).The percent decolorization of the dye was determined by using following formula.

$$\text{Decolorization (\%)} = \frac{A0 - At}{A0} \times 100$$

Where, A0 - Initial Absorbance
At - Observed Absorbance

Percent COD reduction value of the dye was calculated by COD analysis using $K_2Cr_2O_7$ as a strong oxidizing agent under reflux.

Degradation of the dye by the isolate was confirmed by GC-MS analysis. The samples were prepared by extracting the products in Di-Chloro Methane.

Microbial toxicity of the dye Reactive Blue 171 was studied on three ecologically important microorganisms *Viz. Rhizobium sp., Pseudomonas sp.* and *Azotobacter sp.* by the Agar Well Assay.

Results and Discussion

The promising organism was identified by using different biochemicals and 16s rRNA sequence analysis. Promising organism produced catalase and nitrate reductase enzymes and able to ferment Glucose, Lactose, and Sucrose. The phylogenetic tree was developed by using MEGA 4.0. **(Fig. 1)**

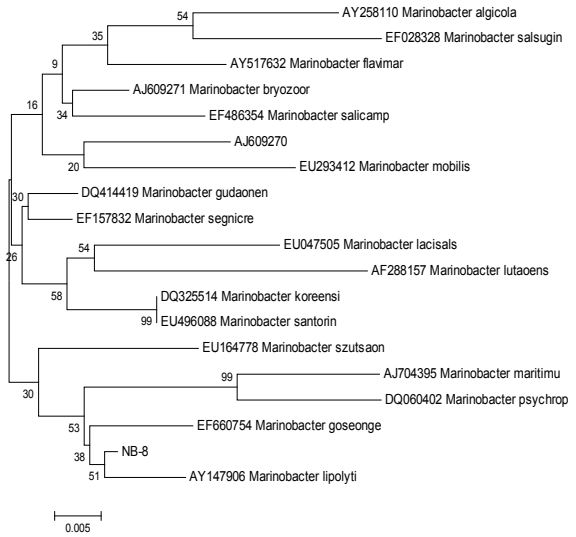

Fig. 1 – Phylogenetic analysis of the 16S rRNA sequence of *Marinobacter sp. NB-8*. The percent numbers at the nodes indicate the level of bootstrap support based on Maximum Composite Likelihood analysis of 1000 replicates. The scale bar indicates the base pair substitutions per site.

The selected isolate *Marinobacter sp. NB-8* was examined for its decolorization ability in nutrient broth, in half (½) strength nutrient broth and in cell free extract containing 10% NaCl and the initial dye concentration of 1500µg/ml (**Fig. 2**).

This results were in accordance with Jahir Alam Khan (2011)[6] who studied a moderately halotolerant microbe identified as *Bacillus megaterium* showed 64.89% dye degradation in nutrient medium containing high salt concentrations. Sylvie *et al*., (2008)[7] reported a halophilic bacteria biofilm from saltern which showed removal of more than 99% of the phenol from a synthetic wastewater containing 15% NaCl. Tarun Agarwal and Rachana Singh (2012)[8] also reported the moderately halotolerant strain of bacterial genus *Klebsiella*, able to degrade the azo dyes Acid Orange 7 and Congo red up to 88.45% and 79.64% respectively.

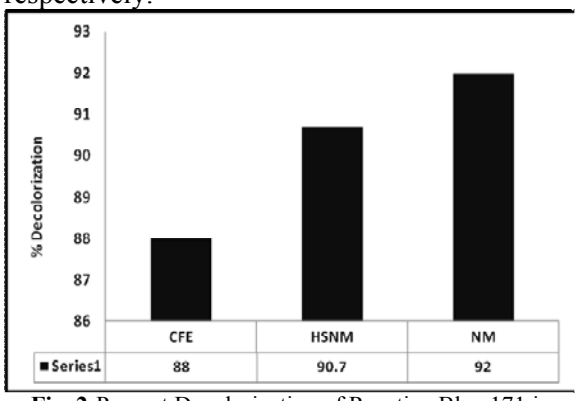

Fig. 2-Percent Decolorization of Reactive Blue 171 in Different Nutrient Media in 24 hours at 590nm λmax

Where, **CFE** – Cell Free Extract
HSNM – Half Strength Nutrient Medium
NM – Nutrient Medium

Bibi, R., *et al*., (2012)[9] reported maximum decolorization of Reactive Black-5 by the selected five strains of bacteria and was observed when yeast extract at the rate of 4g/L was applied to the liquid medium containing 100mg/L dye. In accordance with other reports, the best decolorization was achieved with the yeast extract (Chen *et al*., (2003)[10]; Kodam *et al*., (2005)[11]; Moosvi *et al*., (2005)[12]. As per Fig. 3 it is evident that decolorization was excellent in presence of yeast extract.

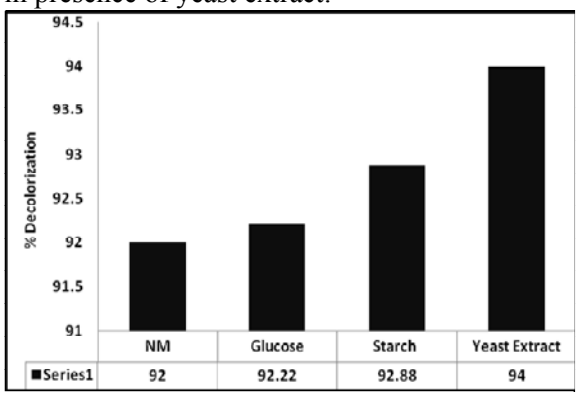

Fig. 3-Percent Decolorization of Reactive Blue 171 in Nutrient Media containing 1% Co-substrates in 24 hours at 590nm λmax

Where, NM – Nutrient Medium

The percent COD reduction of the dye Reactive Blue 171 by *Marinobacter sp.NB-8* was found to be 80.78% after 24 hours (Vigneeswaran, *et al*., 2012)[13].The GCMS analysis report (**Fig. 4**) showed that the dye Reactive Blue 171 degraded into smaller fragments having different molecular weights. Thorat and Kale (2010)[14] also reported that the degradation products of the dye were of much lower mass than the original compounds.

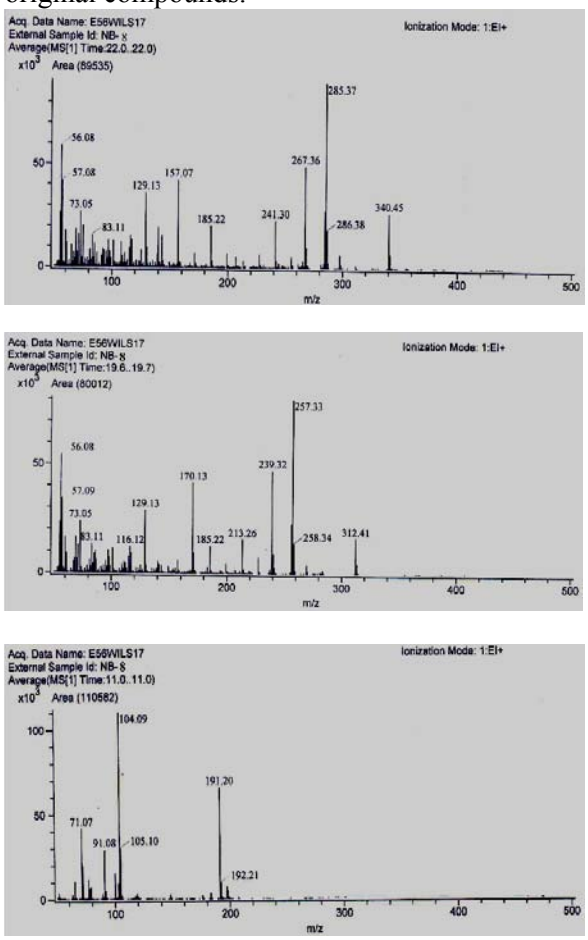

Fig. 4 – GC-MS Analysis Report of Reactive Blue 171 by *Marinobacter sp.NB-8*

The results showed that the wells which were poured with decolorized broth had no zone of inhibition and wells with original dye solution had zone of inhibition indicating that the biodegraded or decolorized products were non-toxic to the tested beneficial bacterial flora of the soil our result was in strong agreement with Mali *et al*., (2000)[15], Kalyani *et al*., (2009)[16] and Mane *et al.*, (2008)[17].

Conclusions

Reactive dye present in the industrial effluents is responsible for polluting the aquatic life. Halophiles can be directly used without any pretreatment of the saline effluents. The use of such marine microorganism able to degrade reactive dye in presence of salt could help prevent costly dilution to lower the salinity, or the removal of salt by conventional methods before biological treatment and can be used for the treatment of effluents containing high salt content. In the present study, *Marinobacter sp.NB-8* could effectively degrade and detoxify the reactive dye Reactive Blue 171 present in textile waste effluent.

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