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BIODEGRADATION OF METHYLENE BLUE BY *ENTEROBACTER* SP. FROM PALK STRAIT, SOUTHEAST COAST OF INDIA

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ABSTRACT

Methylene blue is a heterocyclic aromatic chemical compound; it has many uses in a range of different fields, such as biology, chemistry, textile industries, etc. Colour stuff discharged from these fields poses certain hazards and environmental problems. Marine sediment samples were collected from Palk Strait, southeast coast of India. A total of 10 morphologically different bacterial strains were isolated and enrichment method using as screening medium supplemented with methylene blue (0.01%). All the strains were tested by their ability to degrade methylene blue at different concentrations (10 -150 mg/ml of screening medium). Of these ten isolates, strain M10 degraded maximum of 100 mg/ml of methylene blue (plate method). The potential strain was identified as gram negative, rod shape, non-motile bacteria. Based on the biochemical test it may be confirm as *Enterobacter sp*. This bacterium inoculated in Methylene blue, Malachite Green, Eosin Yellow dyes in different concentration, the percentage of degradation after 48 hours *viz.*, 67.30%, 53.01%, and 72.30%. Present investigation, resulted that rapid microbial degradation of textile dyes this type of bacterium may be degrade the any type of synthetic dyes in variable and extensively may be used in the textile industry.

Key Words: Biodegradation, Marine Sediment, Methylene blue, Malachite Green, Eosin Yellow, *Enterobacter sp*.

INTRODUCTION

Dyes are organic compounds used as coloring agents in chemical, textile, pulp and paper, printing, cosmetic, leather and food industries (Gulanz *et al*., 2004; Kumar and Porkodi, 2007). In process of washing and finishing colored products, waste water contaminated with dyes is generated. The contaminated waste waters are hazardous which is great to threat to environment (Zhao *et al.*, 2008; Ugurlu, 2009). The removal of textile dyes from waste water is a one of the most important environmental issues to be solved today discharge of hazardous waste water without additional treatment can seriously damage the environment. The colored discharged effluents inhibit penetration of sunlight and oxygen which are crucial requirements of aquatic life (Vadivelan *et al.*, 2007). Biodegradation of dyes is not an easy process due to their toxic and complex aromatic structure (Ozer *et al.*, 2007 ;Yasin *et al.*,2007). In typical dyeing processes, 50-100% of the dye is fixed on the fiber, and the unfixed dyes are discharged in *sp*ent dye-baths or in the

wastewater from subsequent textile-washing operations (Jiraratananon *et al*., 2002). Methylene blue (MB) is commonly used in textile and paper industries it may deleteriously affect humans, fish, algae, and submerged plants in various ways Bana *et al.*,2007; Nilratnisakorn *et al.*, 2008). MB is a heterocyclic aromatic compound with the molecular formula $C_{16}H_{18}ClN_3S$. At room temperature, it appears as a solid, odorless, dark green powder, which gives a dark blue color when dissolved in water. Various methods, such as membrane filtration, ion exchange, chemical coagulation, oxidation-reduction, and adsorption onto activated carbon, are available for removing or decolorizing dye-bearing wastewaters. However, these methods are very expensive (Crini, 2006; Kumar*et al.*, 2007). The utilization of alternative low-cost materials with high adsorption activity to solve environmental problems has received considerable attention over the recent years.

MATERAIAL AND METHODS

Chemicals (**Dyes**)

Methylene Blue (MB), Malachite green, Eosin yellow was obtained from Sisco Research Laboratories Pvt Ltd, Mumbai, India. The chemicals used were of the highest purity available and of an analytical grade.

Sediment Sample Collection

Present study, for the isolation of dye degrading bacteria, soil samples were collected from Thondi cost, southeast cost of India. Sediment samples were collected in the clean polyethylene bags and trans*p*orted to the laboratory by keeping them in ice box and processed within 3 hours and microbial analysis were carried within 4 hours.

Screening of dye degrading bacteria

5 grams of sediment sample was diluted to 50% seawater and homogenized thoroughly and store at 4^0C. Simultaneously screening agar medium was prepared supplemented with Methylene blue at minimal concentration (0.01%). After preparation 0.1ml of sample were inoculated into the plate and *sp*readed sterile L-road. After then all the plates were incubated at room temperature for 48-72hrs. One uninoculated plate was kept as a control.

Screening Medium Composition (Chen *et al*., 2003)

Yeast extract-10gm/lit; NaCl- 5gm/lit; Peptone-5g/lit; Agar-10gm/lit; Methylene Blue-0.1gm/lit; pH-7.0.

Recovery and preparation of Stock Culture

After inoculation, morphologically different colonies were observed on screening medium and then colonies with zone of clearance on screening medium were selected as dye degrading strains (Chen *et al*., 2003). All the selected colonies were transferred into nutrient agar plates. After the inoculation all the strains were subcultured and maintained as stock culture on nutrient agar slants for further.

Degrading of Multi-dye degradation by potential strain in shake flask method

The screening medium was prepared with increasing concentration of Methylene blue, Malachite green, Eosin yellow (10 to 150mg/100ml) in conical flask. After the sterilization 1 ml broth culture of potential strain was inoculated and placed on rotary shaker at 120 rpm for 24-48 hours at room temperature.

Assay of Methylene Blue decolorization by the potential strains: (Suneja *et al*., 2004)

Decolorization was expressed in terms of percentage of decolorization. Dye decolorization by the potential isolates in the broth cultures was assayed by using colorimetric method. Prior to this, the absorption maximum of the Methylene blue supplemented broth was determined. The absorption maxima were 540 nm for dye incorporated broth and hence decolorization was assayed at 540 nm. The culture broth was centrifuged at 1000rpm for 10 minutes in and the bacterial cells were removed in pellets. The

supernatant was analyzed calorimetrically at 540nm to measure the percentage of dye decolorization. Then the percentage of dye degradation was calculated by applying the absorbance value below formula described by Sani and Banerje, 1999.

$$\text{Percentage at decolorization} = \frac{\text{Initial absorbtion} - \text{Final absorbtion}}{\text{Initial absorbtion}} \times 100$$

Finally the percentage of dye decolorization was calculated.

Identification of potential strain

Phenotypic characteristic such as microscopic appearance (grams staining, motility), cultural and biochemical characteristics of potential strain was studied by adopting standard procedures. Then the potential strain was identified based on the studied phenotypic properties.

RESULTS AND DISCUSSION

Synthetic dyes are artificially produced by which a large group of organic chemicals which encountered in practically all *sp*heres of our daily life. In this the usage of organic chemical may be have on undesirable effect not only on the human being as well as environment, in this correlation several researchers has studied about the many physical and chemical methods, including adsorption, photodegradation, precipitation, filtration and processing units have been used for the treatment of dye-containing effluent. Currently, microbial biodegradation has become a promising approach or dye treatment because it is cheaper effective and more environmentally friendly but this study related with biological degradation by using microbial culture is to restricted (Zhou and Zimmermann, 1993). In view of this the present study was made on attempt to identify the possible biodegradation of industrial dyes by using marine associated bacterial isolates. The colony morphology was observed after five days of incubation total of ten morphologically different colonies were observed on isolation medium. A clear zone of decolorization was observed around the colonies and named as strain M1, M2, M3 till M10. All the strains were recovered using nutrient agar plates and transferred to the nutrient agar slants as stock for further studies. After the incubation of screening medium plates with increased concentration of selected dye Methylene blue the following results were obtained (Table 1)

Table 1. Dye degradation of different isolated bacterial strains.

Concentrations of Dye *(%)*	Dye Degradation by Selected Strains								
	M1	M2	M3	M4	M5	M6	M8	M9	M10
0.01	+	+	+	+	+	+	+	+	+
0.02	+	+	+	+	+	+	+	+	+
0.03	-	+	+	+	+	+	+	+	+
0.04	+	-	+	+	+	+	+	-	+
0.05	+	-	+	+	+	-	+	+	+
0.06	+	-	-	+	+	-	+	+	+
0.07	-	+	-	-	-	-	-	+	+
0.08	-	-	+	-	+	-	-	-	+
0.09	-	-	-	-	-	-	-	-	+
0.10	-	-	-	-	-	-	-	+	+

+ Culture growth present; - Culture growth absent

Based on the above results strain M10 was selected as a potential strain and also selected for further studies.

Characterization and identification of potential strain

There are many methods for identifying bacteria. Traditionally an observational and biochemical approach has been used. Simply looking at a bacterial colony growing on an agar plate can give an experienced researcher clues to a bacterium's identity. Bacteria are categorized as "Gram Positive" or "Gram Negative" according to whether or not they are stained by a chemical dye, a common biochemical techniques (Mahalakshmi *et al.,* 2011). All the phenotypic and biochemical characteristics of strain M10 were given in Table 2. Based on the characteristics the Potential strain M10 was comes under *Enterobacter sp.*

Table 2. Phenotypic and biochemical characterization of M10 strain

S.NO	Biochemical Test	Positive	Negative
1	ONPG	-	-ve
2	Lysine Utilization	+ ve	
3	Ornithine Utilization	+ ve	
4	Urease	+ ve	
5	Phenylalanine Determination	+ ve	
6	Nitrate Reduction	+ ve	
7	H2S Production	+ ve	
8	Citrate Utilization	+ ve	
9	Vogus Prosker's		-ve
10	Methylene Red		-ve
11	Indole	+ ve	
12	Malonate Utilization	+ ve	
13	Esculine Hydrolysis	+ ve	
14	Arabinose	+ ve	
15	Xylose	+ ve	
16	Adonitol		-ve
17	Rhamnose	+ ve	
18	Cellobiose	+ ve	

19	Mellbiose	+ ve	
20	Saccharose	+ ve	
21	Raffinose	+ ve	
22	Trehalose	+ ve	
23	Glucose	+ ve	
24	Lactose	+ ve	
25	Catalase	+ ve	
26	Growth under Anaerobic condition	+ ve	
27	Gram Staining		-ve
28	Cell wall Structure	Bacilli	
29	Motility	+ ve	

+ ve ;Positive: -ve; negative

Degradation of Multi-dyes by potential Strain M10 flask method and assay of decolorization

There are many reports on decolorization of textile dyes in flask method by microorganisms (Suneja *et al.,* 2004). Present experiments focus, after incubation of screening medium with increased concentration of Methylene blue, Malachite Green, Eosin Yellow in shake flask method, the following results were obtained. From this observation, the strain M10 can able to degrade at the maximum *viz.,* 34.29%, 40.12%, 54.50% in dye (0.13) concentration. But the percentage of degradation was decreased when increase the concentration of dyes (Table. 3). In optimum pH 7.0 the *Enterobacter sp*. was degraded Methylene blue, Malachite Green, Eosin Yellow the percentage *viz.,* 67.30%, 53.01%, 72.30% (Table. 4). The results suggest that the optimum pH for the biodegradation of methylene blue, malachite green, and eosin yellow was degraded maximum at the pH of 7 that may be due to the bacteria and caused release of enzymes or redox mediators to cause dye reduction, or the dye may be reduced by alkaline hydrolysis.

Table 3. Decolorization of textile dyes in flask method after 48^0C

Concentration	**Percentage of Degradation**		
	Methylene Blue (%)	Malachite Green (%)	Eosin Yellow (%)
0.07	67.30	53.01	72.30
0.08	36.56	52.84	70.70
0.09	36.65	51.12	66.04
0.10	36.14	49.99	63.50
0.11	35.20	48.18	54.78
0.12	34.70	45.05	58.50
0.13	34.29	40.12	54.50

Table 4. Effect of pH on decolorization of dyes by *Enterobacter sp*. at 37 ^{o}C

Decolorization Dyes (%)	P^H			
	7.0	8.0	9.0	10.0
Methylene Blue	67.30	50	31.2	25.3
Malachite green	53.01	40.3	10.5	8.6
Eosin yellow	72.30	61	53.1	26.2

The decolorization mechanism of *Enterobacter sp*. involved dye adsorption and dye reduction. Its decolorization depends on dye concentration, glucose, and pH of the dye medium (Forgacs *et al.*, 2004). These bacteria may decolorize the dye under reduction either by electron trans*p*ort from the cellular metabolic pathways of flavins or quinines, or by external redox mediators as well as by azoreductase enzymes (Pandey *et al.*, 2007) Therefore, *Enterobacter sp*. was the best decolorizer It may remove dye by initial adsorption together with decolorizing by electron trans*p*ort under aerobic conditions and by azoreductase enzymes. It's concluded from the presence findings that the isolated *Enterobacter* bacterial species from the sediment samples, Thondi cost southeast cost of India showed potential biodegradation effect on textile dye degradation. Further studies are highly required to identify the chemical constitution for the biodegradation is highly necessary.

CONCLUSION

The present study revealed the ability of *Enterobacter sp*. to decolorize Methylene blue, Malachite Green, and Eosin yellow. Results obtained this work showed that this *Enterobacter sp.* possessed high decolorization efficiency against multi-dyes. In conclusion this type of bacterium may be degrade the any type of synthetic dyes in variable and extensively may be used in the textile industry.

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