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# Isolation and characterization of textile dye degrading Microbes from Ganga (Ganges) water

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**ABSTRACT**

Among the most concerned environmental pollutions that are threatening our biodiversity, water pollution is a major one where effluents from dye-based industries. Textile industries consume a huge volume of azo dyes while up to 50% of dyes find its ultimate way in the water as effluent. In India, after many festivals like Durga Puja, idols are immersed in water. The dyes by which idols are painted affect aquatic livings. The structures of azo dyes consists coupling of diazotized amine with either an amine or a phenol and also contain azo linkage. Most of these dyes are potentially toxic to aquatic life and some are even carcinogenic and mutagenic to humans. Furthermore, colour of the dyestuff interrupts the aquatic environment by reducing light penetration, gas solubility and interference of phytoplankton's photosynthesis. Unfortunately, most azo dyes are recalcitrant to aerobic degradation by bacterial cells. However, there are microorganisms that have the ability to reductively cleave azo bonds under aerobic conditions. So our goal is to isolate and identify some potential dye degrading bacteria from polluted Ganga water and characterize them biochemically.

***Key words :*** *Textile dye, Azo dye, Phytoplankton, Aerobic, Ganga Water.*

## Introduction

Textile dyeing industry is also one of the important sources of pollution. The textile industry consumes azo dyes when up to 45- 50% of the dye used ends up in water as a dye. Also, many of these dyes have toxic effects on marine life and some are even carcinogenic and mutagenic for animals. The dye containing wastewater discharged from textile industries into the aquatic resources causes reduction of sunlight penetration into waterbodies and thus decreases the dissolve oxygen content and ultimately affects the aquatic life through creating negative impact on Zooplankton, phytoplankton and other aquatic living organisms. Dye effluents are also responsible for disturbance of natural Biogeochemical cycles, which occurs in soil niche and thus create soil pollution. Azo dyes are often difficult to destroy completely, and physico-chemical treatment methods are rarely suitable for complete destruction and conversion to $CO_2$. Some characteristics of these colors such as toxicity, mutagenicity and stability to light and heat prevent the attack of microbes. Permanent removal and biodegradation is possible only after elimination and reduction of individual azo bonds. The disposal of these dyes is very important .Therefore, industrial perfumes containing azo dyes must be treated before being released into the environment to remove the toxic effects of dyes on textiles .Methods such as adsorption, and precipitation

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have major drawbacks with complex design, high consumption of chemicals and energy, and the production of large sludge. On the other hand, it has been shown that the bioremediation of industrial dyes by microbial activity will be the best solution. Because bacteria can produce a variety of primary and secondary metabolites such as enzymes, organic acids, toxins, antibiotics, and many more which can convert some of more toxic component into less toxic or nontoxic one, therefore application of bacteria or other microbes may be a potential way to degrade several textile dyes for the sustainability of aquatic life.

## Materials and Method

**Isolation of Dye degrading bacteria from Ganga Water :** For isolation of dye degrading bacteria from Ganga water we have taken sample of water from 5 different areas around Ahiritola Ghat 22.5960° N, 88.3530° E, and mixed each of them to make a single source and serial dilution followed by spread plate on Bushnell Hass Agar media supplemented with 1% Malachite Green and 1% Eriochrome Black as a carbon source. The plates were incubated at 37 deg C for 72 hours. Appearance of colony in the plate indicates that the isolates can use Malachite Green and Eriochrome Black as a carbon source and they can degrade the dyes.

**Microbiological tests:** The bacterial isolates were characterized for their Gram staining characteristics, capsule and endospore staining characteristics as per normal protocols in Microbiology.

**Biochemical tests**

**Lactose fermentation:** In order to know whether these isolates can ferment lactose or not, we use presumptive test in Lactose Broth. The appearance of Gas bubble and pH drop will indicate whether these isolates can ferment Lactose or not. All sets of Test tubes were kept at 35 °C for 48 hours.

**Phenylalanine deaminase test**

This test determines whether the microbe produces the enzyme phenylalanine deaminase, which is needed for it to use the amino acid phenylalanine as a carbon and energy source for growth. Phenylalanine agar medium was prepared by using 0.2% phenylalanine in nutrient agar. Three cultures were streaked on the agar slant. It was then incubated for 24 hrs at 37 °C. After incubation, 5 drops of 10% Ferric chloride and 5 drops of 0.1N HCl are added and the tubes are kept undisturbed for 2 to 3 minutes.

**IMViC test**

The purpose of this test is to determine whether a bacteria is faecal or non faecal. Tryptophan broth, MR-VP broth and Simmons's citrate agar medium were prepared for iodole production test, Methyl Red test, Voges Proskauer test and citrate utilization test respectively. Kovac's reagent and methyl red were used as indicator for Indole test and MR test respectively. Barritt's reagent was used to test VP reaction. After 24 hrs of incubation indicators were added to observe the result.

**Amylase test**

The purpose of the test is to see if the organism can produce amylase or not. Starch agar medium was prepared and three test cultures were streaked over the solid agar surface. Iodine solution was added after 24 hours of incubation at 37 °C on the edge of agar plate.

**Protease production test**

This test is used to check whether the organism is able to produce extracellular protease or not. For this protease agar media (Composition:peptone-0.5 g, Nacl-0.5 g, Beef extract-0.15 g, yeast extract-0.15 g, casein peptone-1 g, agar-20 g, bromophenol blue-0.0045 g, distilled water-1l) was prepared and sterilised. Aliquote of each 24 hour culture were inoculated and incubated for 24 hours at 37 °C. A colour change from green to blue indicates protease positive as the media contains bromophenol blue as a pH indicator.

**Catalase test**

The purpose of the test is to observe if the organism can secrete catalase enzyme or not. Three cultures are taken in each grease free slide and $H_2O_2$ was added to it.

**Urease test**

In order to test urease production by the isolates, urea agar media was prepared and sterilised. Aliquote of each 24 hour culture were streaked and incubated for 24 hours at 37 °C. A colour change from yellow to pink indicates urease positive as the media contains phenol red as a pH indicator.

### Dye degradation Assay method

BHM agar plates with 50 mg/l of dye concentration was prepared. All the isolates were grown in BHM broths having Glucose as a carbon source and then after 24 hours of incubation at 37 °C, the bacterial broth was centrifuged at 10,000 rpm for 10minutes. The pellet was discarded and the supernatant was kept. Now, a well was bore and the 50 microliter supernatant was pipetted in the well and was incubated at 37 °C for 4 days to see the zone formation.

In order to check the ability of dye degradation, all the 9 isolates were grown in BH broth having 50 mg/l concentration of Malachite Green and Eriochrome Black separately in different conical flasks. These were incubated for upto 96 hours at 37 °C. 3 ml of Cell suspension were collected at 24, 48, 72, 96 hours of incubation from each isolates and after centrifugation at 1000 rpm for 10 mins, the supernatant were collected and used (without further dilution) for taking absorbance at respective wavelengths with respect to control where only BH broth enriched with respective dye was taken. The percentage of Dye decolorization was estimated using the formula:

$$\text{Percentage of Decolorization} = \frac{(\text{Initial absorbance- final absorbance})}{\text{Initial Absorbance}} \times 100$$

## Results and Discussion

### Isolation of Dye degrading bacteria from Ganga Water

We have isolated 9 different bacterial colonies named A-I from BH agar media enriched with Malachite Green and Eriochrome Black and were studies for further analysis.

### Microbiological tests

Except culture C And E, all other isolates show Gram Positive in nature. Fig. 1 and 2 show Gram staining results under 450 X magnification. All of them shows prominent capsules via negative staining with nigrosin. Among all, 4 isolates, A, E, F, G shows endospore at 450 × magnification via Dorner method. The details of morphology, Gram Charecter, Capsule and endospore formation is given in table number 1

**Biochemicals tests:** Different biochemical tests like

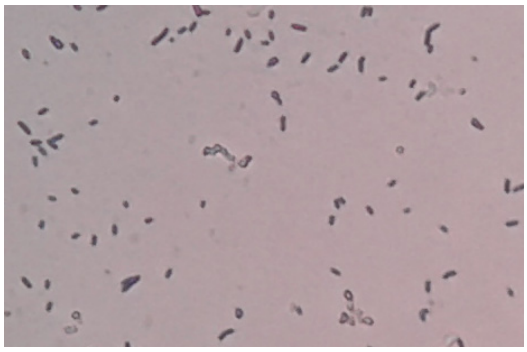


**Fig. 1.** Gram staining of Culture A (450 × magnification)

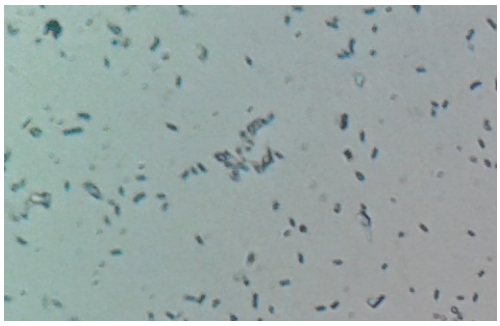


**Fig. 2.** Gram Staining of Culture B (450 X magnification)

fermentation of Lactose, IMViC test, Phenylalanine deaminase, Catalase, Amylase, Urease test etc. Were performed and results are given in Table number 2. Results for amylase test were given in Fig 3 whereas IMViC test of isolates A and D is given in Fig 4.

**Dye Degradation Assay** : Although except culture

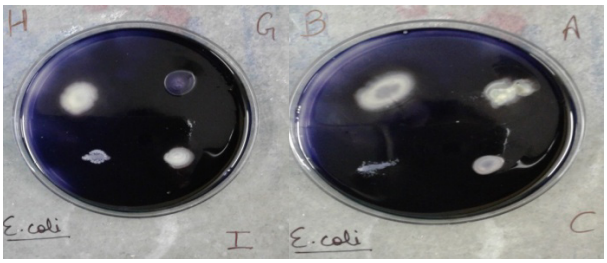



**Fig. 3 & 4.** Amalylase tests of isolates.

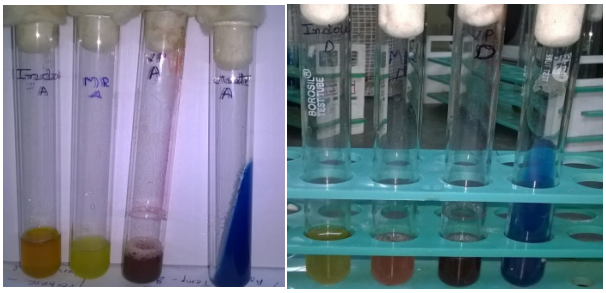


**Fig. 5 & 6.** IMViC test of Isolates A & D.

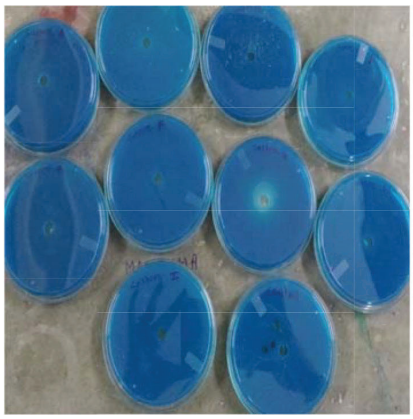


**Fig. 7.** Plates showing Halo zone by Different isolates

**Fig. 8.** Broth showing colour changes at 96Hrs.

G (Fig. 7)a significant zone didnot appeared in plates having Malachite Green , but during measurement of optical density we found all the isolates can degrade a good percentage of Malachite Green dye at different time intervals. Maximum degradation has been mediated by the culture G with over 60 perent degradation at 96 hrs. Results are given in Fig. 8. The details were given in the Table number 3.

During measurement of optical density we found all the isolates can degrade a good percentage of Eriochrome Black Dye at different time intervals. Again maximum degradation has been mediated by the culture G with over 82 perent degradation at 96 hrs. The details were given in Table 4. The corresponding pics are provided in Figs. 9 and 10.

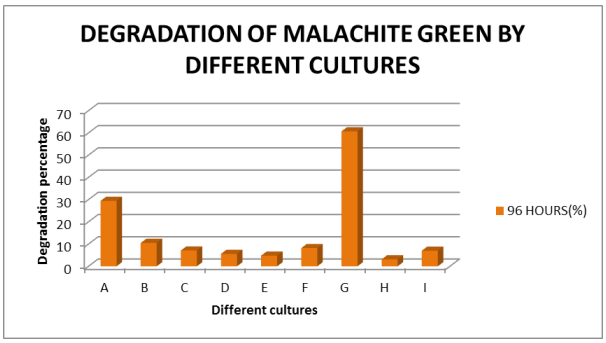
India is a land of festivals and Indian festivals are known for their idols and aesthetics. A major part of the aesthetic of the idols finds roots in the use of synthetic dyes, mostly Azo dyes. They are used in

**Table 1.** Morphological and Staining characteristics of 9 isolates.

| Cultures | Morphology | Gram Staining | Capsule | Endospore |
|---|---|---|---|---|
| *A* | *Rods* | + | + | + |
| *B* | *Rods* | + | + | – |
| *C* | *Short Rods* | – | + | – |
| *D* | *Short Rods* | + | + | – |
| *E* | *Rods* | – | + | + |
| *F* | *Rods* | + | + | + |
| *G* | *Coccus* | + | + | + |
| *H* | *Coccus* | + | + | + |
| ***I*** | ***Coccus*** | **+** | **–** | **+** |

**Table 2.** Different Biochemical and Enzymatic Assay

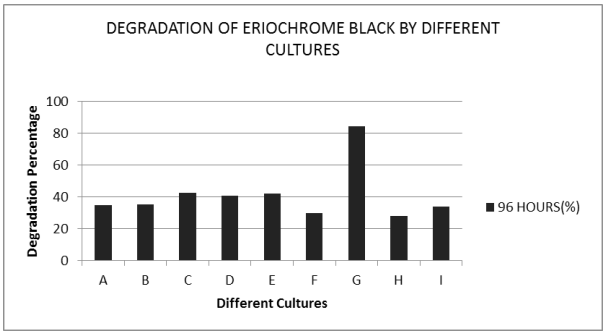
| Cultures | Indole | Mr | Vp | Citrate | Catalase | Urease | Amylase P | Phenylalanine Deamination | Protease | Lactose fermentation |
|---|---|---|---|---|---|---|---|---|---|---|
| A | – | + | + | + | + | + | – | – | + | – |
| B | – | + | + | + | + | + | – | – | – | – |
| C | – | + | + | + | – | + | – | – | – | – |
| D | – | + | + | + | – | + | – | – | – | – |
| E | – | + | + | + | + | + | – | – | – | – |
| F | – | + | + | + | + | – | – | – | – | – |
| G | – | + | + | + | – | – | + | – | – | – |
| H | – | + | + | + | – | + | + | – | – | – |
| I | – | + | + | + | – | + | + | – | – | – |




**Graph 1.** Degradation percentage of Malachite green by the isolates


**Fig. 9 and 10.** After 96 hrs of incubation all isolates shows good percentage of degradation

painting the idols,the textiles and other decorative items etc. After celebration these compounds are dumped into large waterbodies where they form a major polluting source. Therefore it is very essential to isolate and characterize dye degrading microbes and their application in water bodies. We have isolated 9 different bacteria from Ahiritola Gath after Durga Puja festival, 2021. These isolates can degrade a significant amount of Malachite Green dye and Eriochrome Black dye. Several microbiological and biochemical tests have been conducted to characterize them. Among all isolates, Sample G bacteria show highest decolorizing activity by degrading




**Graph 2.** Degradation percentage of Eriochrome Black by the isolates.

**Table 3.** Degradation potential of Malachite Green by isolates at different time intervals

| Cultures | 24 hours (%) | 48 hours (%) | 72 hours (%) | 96 hours (%) | Zone Formation |
|---|---|---|---|---|---|
| A | 5 | 12.6 | 22.7 | 29.4 | – |
| B | 0 | 4.8 | 5.2 | 10.5 | – |
| C | 0 | 4.3 | 5.1 | 7.1 | – |
| D | 1.1 | 2.5 | 4 | 5.6 | – |
| E | 0.5 | 3.06 | 3.9 | 4.8 | – |
| F | 2 | 3.2 | 7.9 | 8.2 | – |
| G | 16.9 | 33.8 | 54.2 | 60.7 | + |
| H | 0 | 1.6 | 2.4 | 3.1 | – |
| **I** | **0** | **3.09** | **6.5** | **7** | **–** |

**Table 4.** Degradation potential of Eriochrome Black Dye by isolates at different time intervals

| Cultures | 24 hours (%) | 48 hours (%) | 72 hours (%) | 96 hours (%) | Zone Formation |
|---|---|---|---|---|---|
| A | 12.09 | 17.6 | 29.02 | 35.02 | – |
| B | 26.2 | 26.3 | 31.5 | 35.2 | – |
| C | 25.2 | 30.2 | 36.2 | 42.3 | – |
| D | 28.03 | 32.02 | 40.2 | 40.9 | – |
| E | 19.02 | 22.2 | 25.2 | 42.2 | – |
| F | 10.2 | 19.3 | 27.01 | 30.02 | – |
| G | 34 | 52.6 | 73.6 | 82.2 | – |
| H | 10.4 | 16.2 | 20.6 | 28.01 | – |
| I | 9.2 | 16 | 26.8 | 34.1 | – |

both dyes at 96 hours of incubation. A clear zone has been observed by the same bacterial culture against Malachite Green which seems to be a carcinogenic compound. Therefore we can conclude these isolates show efficient dye degrading ability which can be put on water bodies so that water can be cleaned up to sustain life and to restore ecological balance. Further characterization of those bacteria in form of 16s rRNA identification and dye degradation enzymatic assay can also be perfomed.

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**Declaration:** The author declares no conflict of interest.

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