J. Indian Chem. Soc.,
Vol. 96, April 2019, pp. 425-428

# Exploring different sources for Malachite Green decolorizing bacteria

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*Manuscript received online 01 February 2019, accepted 14 March 2019*

A total of ninety strains of bacteria were isolated from several different sources like effluents from tanneries, textile industries, jute mills and dairies; from water samples from a pond, an urban canal, a river and an estuary; from soil samples, and from two common aquatic weeds. All these bacterial strains were checked for their ability to degrade Malachite Green which is heavily used as a colorant in textile and leather industries and is highly toxic, having established carcinogenic and cytotoxic properties. Qualitative assays on solid medium was done to choose dye degraders preliminarily, followed by quantitative assays in liquid medium to estimate their degradation potential in terms of percentage decolorisation. Bacteria showing dye degradation potential have been mostly isolated from industrial effluents, but our study suggests that even some strains isolated from natural water sources have excellent potential as dye degraders and could be effectively used to decolorize waste water in future.

Keywords: Malachite Green, isolation, decolorisation, bacteria.

## Introduction

Dyes are one of the major harmful components of effluents of several different industries[1]. Leather, textile, printing, food processing are the most rigorous users of dyes[2] and also belongs to the list of the greatest contributors of coloured effluents. Most of the dyes have complex molecular structures and synthetic origins which give them high stability and thus become difficult to remove[3] and are recalcitrant xenobiotics[4]. Malachite Green (MG) is a triarylmethane dye. It is available mainly in two forms of salts: hydrochloride and oxalate. Here Malachite Green oxalate has been used. Apart from its widespread use in textile industries, it is also commonly used as a parasiticide in aquaculture since it is capable of preventing fungal attacks and protozoan infections[5]. However, Malachite Green has been reported to show high levels of toxicity in both fish and humans on prolonged exposure. Mutations, cancers and developmental abnormalities have been attributed to this dye[6]. Attempts have been made to decolorise Malachite Green using bacteria *Kocuria rosea* (complete decolorisation in 5 h)[7], using fungus *Cunnighamella elegans* (nearly 85% decoloriastion in 24 h)[8], *Aspergillus flavus* and *Alternaria solani* (both species showed more than 96% decolorisation within 6 h)[9] and even by some waterborne pathogenic strains of *Mycobacteria*[10], among many others. In the present study, we have isolated bacteria from several different sources and have tried to explore their potential to decolorise this dye. Previously most of the dye degrading strains have been isolated from industrial effluents since it is presumed that they are better adapted in the toxic environment of the effluents[11–14]. We tried to find out whether bacteria present in any other natural source, like soil, endophytes of a few aquatic plants, or bacteria living in some natural water sources could also have similar dye degrading potential.

*Materials and methods:*

*Dyes and chemicals:*

The triarylmethane dye Malachite Green oxalate was purchased from Sisco Research Laboratory. All other chemicals and media components used were obtained from Himedia and were of analytical grade.

*Growth medium:*

Nutrient broth medium (Composition: Peptone 10 g/L; Beef extract 10 g/L; Sodium chloride 5 g/L; pH adjusted to 7) from Himedia Laboratories and Minimal Salt medium [MSM] ($Na_2HPO_4$: 1.8 g/L; $(NH_4)_2HPO_4$: 0.72 g/L; $K_2HPO_4$: 1.8 g/L; $MgSO_4.7H_2O$: 0.144 g/L; $FeSO_4.7H_2O$: 0.0072 g/L; $MnSO_4.7H_2O$: 0.00504 g/L; KCl: 0.144 g/L; sucrose: 7.2 g/L;

pH adjusted to 7) was used for the initial isolation of bacterial strains and all subsequent experiments.

*Isolation of bacteria:*

Effluents were collected aseptically in sterile containers for initial isolation of microorganisms and for their subsequent use; they were stored in refrigerator at 4ºC. These samples were procured from several different industries in and around Kolkata, including, but not limiting to the ones that use dyes : Jeans dying factory, Kolkata [A]; Domestic baatik unit from Madhyamgram, North 24-Parganas [B]; Small printing press from Jadavpur, Kolkata [C]; three tanneries from Park Circus area, Kolkata [D, W and I]; Tannery from Bhangar, South 24-Parganas [X]; Textile mill from Gangulibagan, Kolkata [Y]; Textile mill from Uluberia, Howrah [Z]; A large dairy industry from Dankuni, Hooghly [F]; A jute mill from Kolkata [G]; and lastly, small-scale cottage cheese manufacturing unit also from Kolkata [H]. For enrichment of total population of dye degrading isolates in the samples, 1 mL of the sample was aseptically added to 100 mL of nutrient broth and minimal salt medium. The flasks were incubated in shaking condition at 150 rpm at 28ºC for 6 days. The enriched cultures were serially diluted up to$10^{-3}$ dilution and the diluted cultures were spread plated aseptically and incubated at 28–30ºC for 3 days.

Soil samples were collected from area: soil drenched by domestic waste-water [soil 1] and soil drenched by dairy effluent [soil 2]. Soil suspensions were made (1 gram soil in 100 mL sterile distilled water). Then they were serially diluted upto$10^{-6}$ dilution and plated on solid nutrient agar and minimal salt medium for isolation of single colonies for pure cultures.

Three natural water sources: A pond from Shantiniketan, Birbhum [Water source 1] , River Kopai, Birbhum [Water source 2], The Hugli-Matlah estuary, South 24-Parganas, near Bay of Bengal [Water source 3] and water from an urban sewage [Us] canal at Keshtopur, Kolkata were also explored for isolation of dye degrading bacteria. Isolation was done using standard spread plate method.

Bacteria were also isolated from two common aquatic weeds: Water hyacinth (*Eichhornia crassipes*) [Plant 1] and Duck Weed (*Lemna trisulca*) [Plant 2]. The endophytic bacteria used in this study were isolated from different parts of these two healthy growing aquatic weeds. The plant samples were washed under running tap water (*pre-treatment)* to remove debris and air-dried before being cut into pieces of 5 $cm^2$ in diameters. In order to eliminate epiphytic microorganisms (*surface sterilization)*, the samples were immersed in 0.5% (v/v) sodium-hypochlorite for 1 min, and then washed in sterile distilled water, followed by washing in 70% (v/v) ethanol for 1 min. Finally, samples were rinsed twice with sterilized distilled water for 1 min each. The surface sterilized samples were blot-dried using sterile filter paper and then aseptically cut into 2 $cm^2$ in diameters. The samples were then transferred aseptically onto nutrient agar and minimal salt medium plates and incubated at room temperature for a period of 1 week. The plates were observed daily, and colonies were subcultured individually.

*Screening of MG decolorizing bacteria:*

*(i) Qualitative screening:* All the isolated strains were inoculated into Mineral Salt plates amended with Malachite Green. These were incubated at 37ºC for 48–72 h. Strains which had zone of clearance on the otherwise green plates were selected as potential dye-degraders. All the selected bacterial strains were maintained as pure cultures at 4ºC and used for further quantitative studies.

*(ii) Quantitative screening:* All the strains selected by qualitative tests were subjected to quantitative decolorisation assays in liquid medium. Primary inoculums of each of the selected strains (liquid culture grown upto an optical density of 1 at 600 nm) were used to inoculate flasks containing MG-supplemented (40 mg/L) MSM. These flasks were incubated at 37ºC for 7 days and the colour change was noted at regular intervals using UV-Vis spectrophotometer (Agilent Cary 60, UV-Vis).

*Decolorisation assay:*

In order to compare the decolorisation ability of the different selected strains, the percentage degradation of dye was calculated and compared to the corresponding control. The decolorisation efficiency of different isolates was expressed according to the following equation[15]:

$$\text{Percentage decolorisation} = \frac{(I - F)}{I} \times 100$$

where, $I$ = initial absorbance and $F$ = final absorbance.

The isolates which showed degradation of the dyes over 50% were chosen to proceed with further work.

*Time associated degradation and observation of pH:*

Degradation pattern of MG was studied for a period of 7 days with the chosen strains. During this period, the pH of the growth medium was also monitored to understand whether any acidity or alkalinity of the bacterial strains was responsible for the leaching out and subsequent decolorisation of the dye.

## Results and discussion

*Isolation and qualitative dye degradation:*

A total of 90 bacterial strains were isolated from the above mentioned sources. All isolates were screened for dye decolorisation on solid agar medium amended with 20 mg/L. Out of the ninety bacterial strains isolated from twelve effluent samples, three natural water sources, two soil samples, two aquatic plants and an urban canal, only nine strains (strain X3 from effluent X; strain 57 from soil 2; strains P6, P9 and P12 from water source 1; strains Us1 and Us2 from sewage sample Us; and strains H1 and H2 from effluent H) gave preliminary positive result for MG decolorisation.

*Quantitative dye degradation:*

Quantitative dye degradation analysis was performed with all the nine strains that could preliminarily degrade MG (Fig. 1). Strains P6, P9 and P12 showed decolorisation of MG dye more than 50%. From the quantitative assay these three strains were selected for further work. All these 3 bacterial strains had been isolated from natural water sources. It may be mentioned here that, the strain P9, which showed highest decolorisation of Malachite Green was isolated from natural water source 1, i.e. a local freshwater pond in Shantiniketan in Birbhum, West Bengal.

*Time associated degradation and observation of pH:*

Optimum decolorisation could be noted during the 3–5 days' time span for almost all the strains. Particularly, for strain P9, above 80% decoloriastion of the MG dye could be attained from the very first day and it subsequently increased to above 90% on the 5th day. However, from the 6th day, decolorisation seemed to reach a saturation point and did not change much from then onwards.

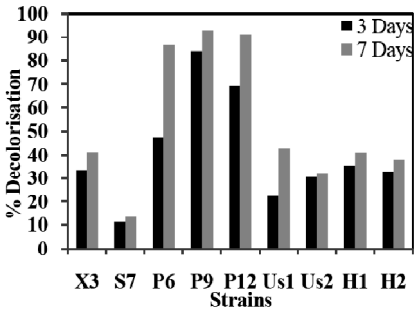


**Fig. 1.** Quantitative decolorisation of MG by the selected bacteria.

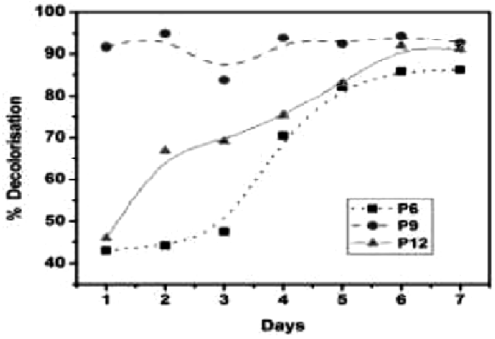


**Fig. 2.** Dye degradation pattern of selected isolates over 7 days.

It is clear that none of the bacterial strains altered the pH of the medium enough so as to enable leaching out of the dyes (Table 1). So, the bacteria were decolourising the dyes and that the dye was not being faded due to any acidic or alkaline properties of the strains.

**Table 1.** Monitoring pH and dye degradation by the 3 strains

| Sl. No. | Strain | Malachite Green | | | |
|---|---|---|---|---|---|
| | | Day 3 | | Day 7 | |
| | | Percentage decolorisation | pH | Percentage decolorisation | pH |
| 1. | Un-inoculated control for MG | 0 | 7.5 | 0 | 7.5 |
| 2. | P6 | 45.92 | 7.6 | 83.21 | 7.7 |
| 3. | P9 | 81.6 | 7.6 | 90.11 | 7.8 |
| 4. | P12 | 38.29 | 7.4 | 51.39 | 7.5 |

## Conclusions

In this quest, it was established that even natural water can be a prospective source of dye degrading bacteria, which are at par or even better in some cases in dye-degradation, than their counterparts present in effluents. One strain isolated from a freshwater pond in West Bengal, could decolourise Malachite Green more than 90%. Dye decolorisation was actually taking place, and not leaching/ fading due to any alkaline or acidic metabolite released the bacterial strains, since there was more or less no change in

pH of the culture medium throughout the entire study. These strains can be further optimized and various consortia can be made to explore their ability to work synergistically.

## Acknowledgement

The authors express their gratitude to the Department of Biotechnology, Govt. of West Bengal for funding the work.

### References

1. J. P. Jadhav, D. C. Kalyani, A. A. Telke, S. S. Phugare and S. P. Govindwar, *Bioresour. Technol.*, 2010, **101**, 165.
2. V. K. Garg, M. Amita, R. Kumar and R. Gupta, *Dyes Pigm.*, 2004, **63**, 243.
3. Z. Aksu, *Process Biochem.*, 2005, **40**, 997.
4. A. Pandey, P. Singh and L. Iyengar, *Int. Biodeterior. Biodegradation.*, 2007, **59**, 73.
5. R. A. Schnick, *Prog. Fish Cult.*, 1988, **50**, 190.
6. S. Srivastava, R. Sinha and D. Roy, *Aquat. Toxicol.*, 2004, **66**, 319.
7. G. Parshetti, S. Kamle, G. Saratale and S. Govindwar, *Acta Chimica Slovenica*, 2006, **53**, 492.
8. C. J. Cha, D. R. Doerge and C. E. Cemiglia, *Appl. Environ. Microbiol.*, 2001, **67**, 4358.
9. H. Ali, W. Ahmad and T. Haq, *Afr. J. Biotechnol.*, 2009, **8**, 1574.
10. J. J. Jones and J. O. Falkinham, *Antimicrob. Agents Chemother.*, 2003, **47**, 2323.
11. P. Nigam, I. M. Banat, D. Singh and R. Marchant, *Process Biochem.*, 1996, **31**, 435.
12. M. S. Khehra, H. S. Saini, D. K. Sharma, B. S. Chadha and S. S. Chimni, *Dyes Pigm.,* 2005, **67**, 55.
13. H. A. Modi, G. Rajput and C. Ambasana, *Bioresour. Technol.*, 2010, **101**, 6580.
14. S. Asad, M. A. Amoozegar, A. Pourbabaee, M. N. Sarbolouki and S. M. M. Dastgheib, *Bioresour. Technol.,* 2007, **98**, 2082.
15. R. G.Saratale, G. D. Saratale, D. C. Kalyani, J. S. Chang and S. P. Govindwar, *Bioresour. Technol.,* 2009, **100**, 2493.