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Isolation and Screening of Dye Decolorizing Bacteria from Industrial Effluent

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Abstract

Wastewater effluent from the various industries contain significant amount of synthetic dyes that require treatment to prevent ground water contamination. Malachite green and crystal violet are among millions of dyes which are being use in every day to day life of a human being. During the manufacturing and processing operation 20% of the resultant color enter in to the environment they are toxic and having extremely harmful consequence. The present study was an attempt to examine the potential of isolated bacteria for decolorization of malachite green and crystal violet (Triphenylmethane dyes) from the various effluent samples which were collected from the outlet of industries of adjoining area of Haridwar. About 50 bacterial isolates were isolated through the enrichment culture method out of 50 isolates 18 bacterial isolates capable of degrading the triphenylmethane dye efficiently were screened through clear zone method. The isolates showed the clear zone at the dye concentration of 100 mg^{-1} mediums (pH 7) and temperature 37^0C. These results signify that bacterial isolated could effectively be used in development of alternative and eco-friendly method for the decolorization of industrial effluent.

Keywords

Bacteria, Malachite Green, Crystal Violet, decolorization, industrial effluent, dyes.

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INTRODUCTION

India is the second largest exporter of dyestuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment. Many of these products are problematic because of persistence (low biodegradability) and toxicity (Sriram *et al*., 2013 & Saranraj *et al*., 2014).

Among many classes of synthetic dyes used in the textile and dyeing industries, triphenylmethane group of dyes such as crystal violet and malachite green are the largest and most versatile and play a predominant role in almost every type of application (Bumpus and Brock, 1988). Synthetic dyes like triphenylmethane dyes are toxic, carcinogenic or mutagenic and hazardous to health (Au *et al*., 1978).

Dyes are undergoing anaerobic degradation and form potentially carcinogenic compounds which will eventually end up accumulating in food chains and possibly taken by humans (Banat *et al*., 1996). Intensive irrigation of agricultural lands with water polluted with various industrial effluents severely affects soil fertility and plant growth (Nirmalarani and Janardhanan, 1988).

Bioremediation through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent (Chen *et al*.,2003). A wide variety of microorganisms are reported to be capable of decolonization of dyes (Chang and Kuo, 2000). The types of organisms being reported to have the ability to remove or degrade triphenylmethane dyes include fungi, yeast, actinomycetes and bacteria (Azmi *et al*.,1998). Fungi such as *Phanerochaete chrysosporium* are able to produce enzymes that are able to degrade dyes (Bumpus and Brock, 1988). Several types of bacteria species such as *Citrobacter sp., Bacillus sp.* and *Pseudomonas sp.* have been found to have the dye removing ability (Jang *et al*., 2005). It is also found that most strains of *Pseudomonas* sp. such as *Pseudomonas putida, Pseudomonas otitidis* and *Pseudomonas aeruginosa* possess this ability (Chen *et al*., 2007). This study is an attempt to identify bacterial strains as competent dye decolorizers so as to exploit them for the remediation of dye effluent.

MATERIAL AND METHODS

Sampling

The effluent samples were collected from four discharge points Liberty Shoes Limited Roorkee, Hanung Toys and Textiles Ltd, Common Effluent of SIDCUL industries of Haridwar and Star Paper Mill Saharanpur. Standard procedures were followed during sampling. The temperature and pH were determined at the sampling site. The pH was determined by using pH strip and temperature with laboratory thermometer. The sample were transported to laboratory at 4°C as in accordance with the standard methods.

Dyes and Culture Media

Malachite Green (MG), Crystal Violet (CV), peptone, sodium chloride, beef extract, yeast extract and agar powder were obtained from Hi-Media laboratory, India. All chemicals were of highest purity and analytical grade.

Isolation of Bacteria from Industrial Effluent

Isolation were carried out by serially diluting industrial effluent in sterile distilled water. Nutrient agar medium was used for the isolation of bacteria from the dye effluent. Serial dilutions from 10^{-1} to 10^{-7} were prepared by pipette out the 1 ml of dye effluent sample in to distilled water blank. The nutrient agar plates were prepared and labeled, then 0.1 ml of aliquot from 10-6 and 10-7 was spread on agar plate using the spread plate technique and incubated at 37^{o}C for 24hr.

Screening of Dye-Decolorizing Bacteria:

Fifty morphologically distinct bacterial isolates were tested for their ability to degrade the textile dyes. The isolated bacterial strains were screened out by incubating them on Nutrient Agar containing 100 mg L^{-1} Malachite Green and Crystal Violet dyes. The nutrient agar medium incubated at 37° C for 24 hrs. After the incubation, plates were observed for clear zone. The screened culture was transfer to agar slant and store 4°C for further study. 18 morphologically distinct bacterial isolates were showed best decolorization of the added dye. These efficient bacterial strains were selected for further studies.

Identification of selected isolates:

The selected isolates were examined for their morphological properties, such as size, shape, cell arrangement and staining properties. Biochemical characteristics of the isolates were evaluated by Voges–proskauer, methyl red, catalase, urease, and starch hydrolysis tests. The ability of the organisms in fermenting a number of sugars including dextrose, sucrose, mannitol and lactose were also performed. The isolates were identified up to species based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Determinative Bacteriology (Buchanan& Gibbons, 1974).

RESULT AND DISCUSSION

The present study was aimed to isolate the bacterial strains from industrial dye effluents and find out their dye degrading capacity. Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. South Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing etc. thrive as clusters. Among these the Textile industries are large industrial consumers of waters as well as producers of wastewater. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor (Jiunkins.,1982). Previous studies which have been done cleared that microbes have potential of degradation of hazardous material like petroleum oil and plastic (Dutta and Singh, 2014,

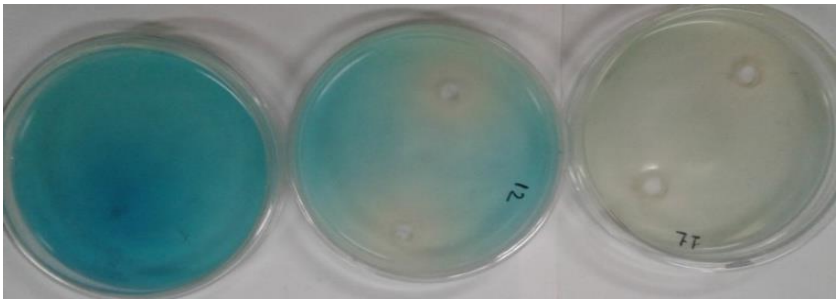
Rani and Singh, 2015). Various industrial effluent samples were collected from the adjoining area of Haridwar and used to isolate dye decolorizing microorganisms. 50 bacterial strains were isolated and purified by sub culturing on nutrient agar plates. Fifty bacterial isolates were screened for the decolorization of dye by plate assay (clear zone) and results are tabulated in Table 1 and 2. Clear zone were observed after one-day incubation (figure 1 and 2). The bacterial strains exhibiting strong decolourizing activity was also investigated (Hassan *et al.,*2013). The morphological and cultural and biochemical characteristics of screened bacterial isolates are shown in table 2 and 3. According to The Bergey's manual of determinative bacteriology and considering the physiological and biochemical tests performed, the strain was tentatively named as *Bacillus* sp strain 1DD (1) and *Pseudomonas* sp strain 4DD (12) (Buchanan& Gibbons, 1974). Out of fifty morphologically distinct bacterial isolates thirteen [1DD (1), 1DD (7), 1DD (8), 1DD (10), 2DD (5), 2DD (7), 2DD (9), 3DD (2), 3DD (3), 3DD (8), 4DD (6), 4DD (12), 4DD (15)] were decolorized the malachite green and six [1DD (2), 2DD (1), 2DD (2), 3DD (8), 4DD (8), 4DD (12)] were decolorized the crystal violet dye. The isolated bacterial strains were screened on Nutrient Agar containing 100 mg L^{-1} Malachite Green and Crystal Violet dyes. The present investigation revealed the ability of dye decolorization of bacterial strains. The morphological and cultural characteristics of screened bacterial isolates are shown in table 2. The nutrient agar medium incubated at 37° C for 24 hrs. After the incubation, plates were observed and measured clear zone. The screened culture was transfer to agar slant and store 4°C for further study.

Table 1: Bacterial isolates showing decolorization of Malachite Green (Average of triplicates ± SEM)

S.no	Isolate	Zone formation (in mm) (Mean± Std Error)
1	1DD(1)	22.66±0.66
2	1DD(7)	22.00±1
3	1DD(8)	17.33±0.66
4	1DD(10)	20.66±0.66
5	2DD(5)	18.00±1
6	2DD(7)	21.00±0.57
7	2DD(9)	21.66±0.88
8	3DD(2)	20.33±0.33
9	3DD(3)	21.00±0.57
10	3DD(8)	22.66±0.66
11	4DD(6)	21.00±1
12	4DD(12)	22.66±0.66
13	4DD(15)	22.33±0.66

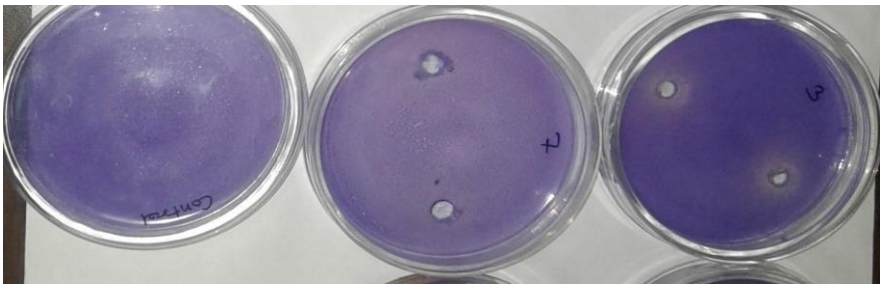
Table 2: Bacterial isolates showing decolorization of Crystal Violet (Average of triplicates ± SEM)

S.no	Isolate	Zone formation (in mm) (Mean± Std Error)
1	1DD(2)	11.00±0.57
2	2DD(1)	8.66±0.66
3	2DD(2)	10.00±0.66
4	3DD(8)	9.33±0.33
5	4DD(8)	9.33±0.33
6	4DD(12)	11.00±0.33



Control

Fig :1 Showing clear zone by isolated bacterial strain on the agar plate containing malachite green.



Control

Fig :2 Showing clear zone by isolated bacterial strain on agar plate containing crystal violet.

Table 3: Morphological and cultural characteristics of bacterial isolates.

S No	Sample	Isolate	Gram staining
1	Liberty shoes Limited Roorkee	1DD(1)	Gram positive bacilli
2	Liberty shoes Limited Roorkee	1DD(7)	Gram positive cocci
3	Liberty shoes Limited Roorkee	1DD(8)	Gram positive cocci
4	Liberty shoes Limited Roorkee	1DD(10)	Gram positive cocci
5	Hanung toy and textile Ltd Roorkee	2DD(5)	Gram positive short rod
6	Hanung toy and textile Ltd Roorkee	2DD(7)	Gram positive cocci
7	Hanung toy and textile Ltd Roorkee	2DD(9)	Gram positive bacilli
8	Common effluent of SIDCUL Haridwar	3DD(2)	Gram positive cocci
9	Common effluent of SIDCUL Haridwar	3DD(3)	Gram positive cocci
10	Common effluent of SIDCUL Haridwar	3DD(8)	Gram positive cocci
11	Star paper mill Saharanpur	4DD(6)	Gram positive bacilli
12	Star paper mill Saharanpur	4DD(12)	Gram negative rod
13	Star paper mill Saharanpur	4DD(15)	Gram positive bacilli
14	Liberty shoes Limited Roorkee	1DD(2)	Gram positive cocci
15	Hanung toy and textile Ltd Roorkee	2DD(1)	Gram negative rod
16	Hanung toy and textile Ltd Roorkee	2DD(2)	Gram positive bacilli
17	Common effluent of SIDCUL Haridwar	3DD(8)	Gram positive cocci
18	Star paper mill Saharanpur	4DD(8)	Gram positive cocci

Table – 4: Identification by biochemical characterization (Average of triplicate).

S. No	Biochemical test	1DD (1)	1DD (7)	1DD(8)	1DD(10)	2DD(5)	2DD(7)	2DD(9)	3DD(2)	3DD(3)	3DD(8)	4DD(6)	4DD(15)	1DD(2)	2DD(1)	2DD(2)	3DD(8)	4DD(8)	4DD(12)
1	**Methyl red**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
2	**Voges proskauer**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	**Catalase test**	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
4	**Urease**	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
5	**Starch hydrolysis**	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
6	**Fermentation (Dextrose)**	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
7	**Fermentation (Sucrose)**	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
8	**Fermentation (Lactose)**	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
9	**Fermentation (Mannitol)**	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve

(+) means positive growth/present;(-) means no growth /absent

CONCLUSION

Due to industrial effluent from textile and other dye stuff using industries to neighboring water bodies and waste water systems facing health concerns. Most of the dyes are carcinogenic in nature. The ability of the microbes to carry out dye decolourization has received much attention.

The present study reveals that the 18 isolated bacterial strains have decolorizing capacity which can destroy the recalcitrant nature of the dye and made them easily biodegradable.

REFERENCES

1. Au, W., Pathak, S., Collie, C.J. and Hsu, T.C., 1978.Cytogenetic toxicity of gentian violet and crystal violet on mammalian cells in vitro. *Mutation Research/Genetic Toxicology*, *58*(2-3), pp.269-276.
2. Azmi, W., Sani, R.K. and Banerjee, U.C., 1998. Biodegradation of triphenylmethane dyes. *Enzyme and microbial technology*, *22*(3), pp.185-191.
3. Banat, I.M., Nigam, P., Singh, D. and Marchant, R., 1996. Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource technology*, *58*(3), pp.217-227.
4. Buchanan R. E. and Gibbons N. E.,1974Bergey's Manual of Determinative Bacteriology, 8th edition, The Williams and Wilkins Company, Baltimore.
5. Bumpus, J.A. and Brock, B.J., 1988. Biodegradation of crystal violet by the white rot fungus Phanerochaetechrysosporium. *Applied and Environmental Microbiology*, *54*(5), pp.1143-1150.
6. Chang, J.S. and Kuo, T.S., 2000. Kinetics of bacterial decolorization of azo dye with Escherichia coli NO3.*Bioresource Technology*, *75*(2), pp.107-111.
7. Chen, C.C., Liao, H.J., Cheng, C.Y., Yen, C.Y. and Chung, Y.C., 2007. Biodegradation of crystal violet by Pseudomonas putida. *Biotechnology letters*, *29*(3), pp.391-396.
8. Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J., 2003. Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology*, *101*(1), pp.57-68.
9. Dutta S, Singh P. Laboratory scale experiment on the bioremediation of refined petroleum hydrocarbon by using CO2 evolution method. Int J Basic Appl Biol. 2014; 1(1): 6-10.
10. Jang, M.S., Lee, Y.M., Kim, C.H., Lee, J.H., Kang, D.W., Kim, S.J. and Lee, Y.C., 2005. Triphenylmethane reductase from Citrobacter sp. strain KCTC 18061P: purification, characterization, gene cloning, and overexpression of a functional protein in Escherichia coli. *Applied and Environmental Microbiology*, *71*(12), pp.7955-7960.
11. Jiunkins R (1982) Pretreatment of textile waste water, Proceedings of 37th Industrial waste Conference. Purdue University, Lafayette, USA.
12. Nirmalarani, J. and Janardhanan, K., 1988. Effect of South India Viscose factory effluent on seed germination seedling growth and chloroplast pigments content in five varieties of Maize (Zea mays I) Madras. *Agric. J*, *75*, pp.41.
13. Rani A, Singh P. Biodegradability of polyethylene by *Aspergillus Niger*. World Journal of Pharmaceutical Research.2015;4(3) 1621-1626.
14. Saranraj, P. and Stella, D., 2014. Impact of sugar mill effluent to environment and bioremediation: a review. *World ApplSci J*, *30*(3), pp.299-316.
15. Saxena, R.M., Kewal, P.F., Yadav, R.S. and Bhatnagar, A.K., 1986.Impact of tannery effluents on some pulse crops.*Ind. J. Environ. Health*, *28*(4), pp.345-348.
16. Sriram, N., Reetha, D. and Saranraj, P., 2013. Biological degradation of Reactive dyes by using bacteria isolated from dye effluent contaminated soil. *Middle–East Journal of Scientific Research*, *17*(12), pp.1695-1700.