Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (3) : 2022 : 468-471
© Global Science Publications
ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2022.v24i03.004

EVALUATION OF BACTERIA RESPONSIBLE FOR DECOLOURIZATION OF METHYLENE BLUE DYE IN SOIL

POONAM PAL[1] AND HARDIK PATEL[2]

Environmental Science Department, Parul University, P.O. Limda, Ta. Waghodia 391760, Dist. Vadodara, Gujarat, India

(Received 18 April, 2022; Accepted 8 May, 2022)

Key words: *Methylene blue, Mycorrhizal bacteria, Decolorization, 16S rRNA sequencing*

Abstract– The discharge of textile effluents in open streams and land poses serious environmental problems. Even after physical and chemical treatment colour removal is not 100% achieved and hence it causes environmental pollution. The present study deals with examination and isolation of potential mycorrhizal soil bacteria responsible for decolorization of Methylene blue dye which is an azo dye. The results showed *Bacillus haynesii* strain NRRL B-41327 and *Bacillus licheniformis* strain DSM 13 were responsible for decolorization of Methylene blue dye in mycorrhizal soil. These gram positive, rod shaped bacteria in consortium exhibited 52.30 % colour removal efficiency. Potential of these bacteria can be further exploited for removal of residual dyes from textile effluents for restoration of ecosystem in an eco-friendly way which will be cost effective too.

INTRODUCTION

The textile and dye manufacturing are major industrial sector responsible for water and soil pollution. The progressive degradation of the environment pose adverse effect on human life. Effluents from dyeing industry alters the colour and quality of water body and has been proved hazardous to aquatic ecosystem as it reduces light penetration which is essential for photosynthesis of aquatic flora and fauna.

Textile effluent accommodate different types of dyes based on their chemical nature like cationic, basic, azo, diazo, anthraquinone base and metal complex dyes (Vasniwal *et al.*, 2016). Among the various dyes used for dyeing purposes in textile industries about 70 % of them are azo dyes (Sarkar *et al.*, 2017). Azo compounds being xenobiotic in nature are recalcitrant and persist in environment for long time as naturally they do not get decomposed or mineralized. These dyes poses azo linkages that impart intense colour to the substrate. Methylene blue is one such cationic azo dye widely used in textile industry for dyeing cotton, wool and silk (Rana Rahman and Neethu, 2017). It's a heterocyclic aromatic compound having molecular weight 373.9 and molecular formula $C_{16}H_{18}N_3SCl$ and provides blue colour when dissolved in water (Rana Rahman and Neethu, 2017). Methylene blue forms quaternary ammonium cations in aqueous solutions, has a high chroma and cause serious environmental pollution (Zhu *et al*., 2018).

Several physico - chemical treatments have been used to treat these dyes and meet emission standards. Conventional treatment methods such as coagulation, sedimentation, chemical oxidation, adsorption on activated charcoal, etc. are being used recently (Neethu and Choudhury, 2018). But due to non-biodegradable nature of methylene blue dye, they are not removed efficiently by these traditional methods. These conventional methods are costly, not very efficient, having limited applicability and produce secondary pollutants which are very difficult to dispose off (Fulekar *et al*., 2013). Hence researchers are now focusing on biological approaches for dye removal. The use of microbial technology is gaining attention worldwide because of the ability of microorganisms to degrade a wide range of recalcitrant dyes at a lower cost without producing secondary sludge.

MATERIALS AND METHODS

Enrichment of sample

One gram of collected mycorrhizal soil sample along with 0.1 g of methylene blue dye were mixed and taken in 250 ml conical flask containing 100 ml of nutrient broth. This flask was kept in orbital shaking incubator at 120 RPM at 35 °C until decolorization was observed. Three successive enrichments were carried out by inoculating 1 ml from previously decolorized media into fresh 100 ml of nutrient broth containing 0.1 g of methylene blue dye in orbital shaking incubator until decolorization was observed.

Isolation of dye degrading bacteria

Loopful of enriched sample obtained from above process was plated on to nutrient agar medium supplemented with 0.1 g of methylene blue dye using pour plate technique. Petriplates were incubated for 24 hours at 35 °C. Bacterial colonies showing clear zone around them were isolated and were purified by re-streaking on fresh nutrient agar plate and pure cultures were maintained for microscopic examination.

Physical and biochemical characterization

Various morphological characters of the bacteria like size, shape, texture, colour, opacity were noted. Other biochemical and microbiological characters were performed as described in Bergey's Manual of Determinative Bacteriology. Tests such as gram staining, indole test, methyl red test, catalase test, voges – proskauer test, oxidase test and citrate test were performed.

Decolorization assay by consortium

This experiment was performed to know the ability of bacterial isolate to decolourize the dye. For this, consortium was prepared by mixing all pure cultures obtained from above step in 100 ml nutrient broth containing 0.1 g of Methylene blue dye in conical flask along with control flask (bacteria was not inoculated). These conical flasks were kept on orbital shaker at 100 RPM for 5 days until decolorization was observed at normal room temperature. A small aliquot of this media was extracted after every 24 hours and was subjected to centrifugation at 3000 RPM for 30 minutes. The supernatant was collected and initial and final absorbance was measured using spectrophotometer at 665 nm (λ max). Decolorization of the media indicates the degradation of the dye by the bacteria. The efficiency of degradation of the dye can be calculated as follows -

$$\%\ Degradation = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}}\ x\ 100$$

Identification of efficient dye decolorizing bacteria

The bacteria strain showing maximum decolorization values were selected for further experiment and to prepare pure culture. A well isolated colony of bacteria was identified presumptively as per Bergey's Manual of Determinative Bacteriology on the basis of physical, biochemical and microbiological characteristics. Amongst all isolates, two most efficient dye degrading bacterial cultures were further identified on the basis of 16S rRNA sequencing using universal primers. Pure cultures were given to Biokart India Pvt. Ltd., Bengaluru. The result obtained were compared with database of known sequences at NCBI using BLAST program.

Results and Discussion

Many azo dyes are used in textile and dying industry whose effluents lead to water and soil pollution by releasing intense colour and foul smell. Physico-chemical methods are present to treat these effluents, but they have certain limitations. Hence the use of microbial technology is gaining attention worldwide.

Biodegradation of commercially available dye namely methylene blue was studied against bacterial isolates which have been isolated from mycorrhizal soil sample by pour plate method. The selected bacterial isolate were investigated for their

Table 1. Physical colony characteristics of selected bacteria

Sr. No.	Characters	Organism 1	Organism 2
1	Size	3-4 mm	2-3 mm
2	Shape	Rod shape	Rod shape
3	Texture	Smooth	Smooth
4	Colour	Creamy white	Creamy white
5	Opacity	Translucent	Translucent

Table 2. Biochemical characteristics of selected bacteria

Sr. No.	Characters	Organism 1	Organism 2
1	Gram staining	+	+
2	Indole test	-	-
3	Methyl red test	-	-
4	Catalase test	+	+
5	Voges – proskauer test	+	+
6	Oxidase test	-	+
7	Citrate test	+	+

physical and biochemical characterization as follows table-1 & Table-2.

Decolourization study with consortium

Microorganisms in consortium acts as good candidates for decolourization of dye. An experiment was set up to know the ability of isolated bacteria to decolourize methylene blue dye. All bacterial pure culture suspension were mixed together in uniform quantity in 100 ml nutrient broth containing 0.1 g of methylene blue dye in conical flask on orbital shaker for 5 days. Decolourization of methylene blue dye was observed as shown in Fig. 1.

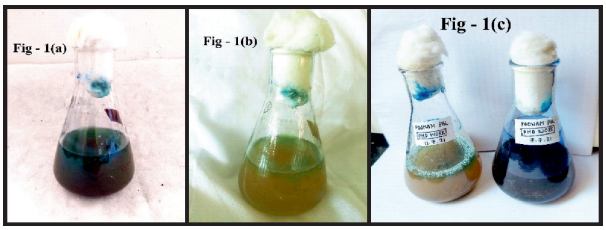


Fig. 1. (a) Blank, (b) Decolourized dye, (c) Comparison between blank & decolourized dye after 5 days

Spectrophotometric analysis of methylene blue dye

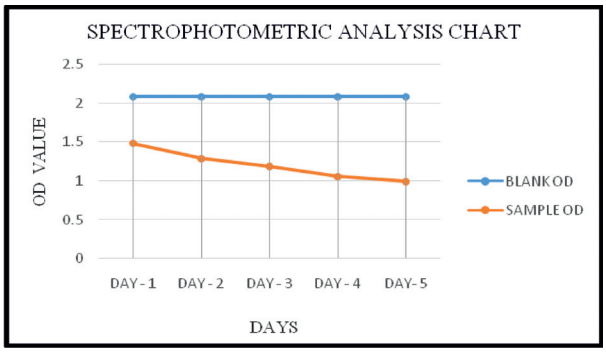
The study was carried out for 5 days and percentage of decolourization was monitored everyday using UV-visible spectrophotometer. The maximum absorption wavelength for methylene blue dye was found to be 665 nm which was taken as reference to carry out further photometric analysis of the culture samples. There was gradual decrease in the absorbance value as shown in the table below and graphically represented in Graph 1.

Table 3. Absorbance value of dye decolourization

Days	Blank OD (Nm)	Sample OD (Nm)	Decolouri-Zation (%)
Day – 1	2.080	1.476	29.03
Day – 2	2.080	1.282	38.36
Day – 3	2.080	1.182	43.17
Day – 4	2.080	1.052	49.42
Day – 5	2.080	0.992	52.30

Identification of bacterial strain responsible for degrading methylene blue dye

Identification of bacterial isolates which can degrade or decolourize methylene blue dye was done using 16S rDNA sequence analysis. Sequencing machine used was ABI 3130 Genetic analyser. The gene



Graph-1. Spectrophotometric analysis chart

amplification was performed using 16S forward 5′-GGATGAGCCCGCGGCCTA-3′ and 16S reverse 5′-CGGTGTGTACAAGGCCCGG-3′ primer. Results of phylogenetic relationships revels that the strain of organism-1 showed 99.61% sequence similarity with *Bacillus haynesii* strain NRRL B-41327 and strain of organism-2 showed 99.85% sequence similarity with *Bacillus licheniformis* strain DSM 13.

Phylogenetic tree of *Bacillus haynesii* strain NRRL B-41327 and *Bacillus licheniformis* strain DSM 13 are shown in Fig-2 and 3 respectively.

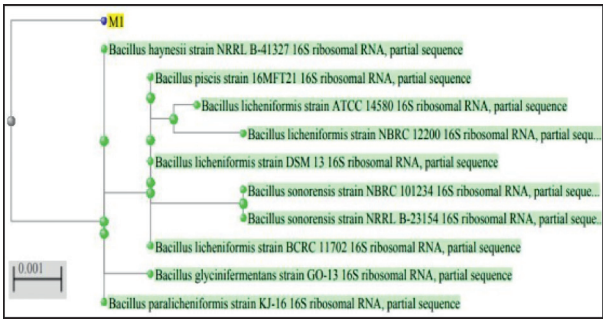


Fig. 2. Phylogenetic tree of *Bacillus haynesii* strain NRRL B-41327

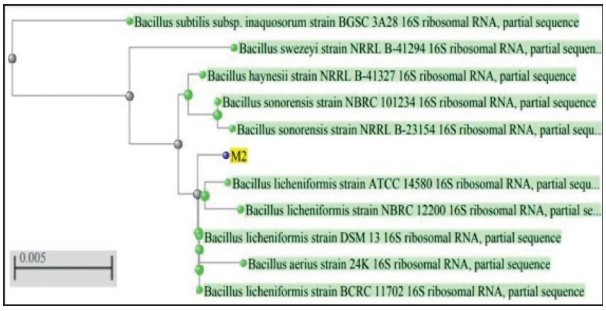


Fig. 3. Phylogenetic tree of *Bacillus licheniformis* strain DSM 13.

Aligned sequence data of *Bacillus haynesii* strain NRRL B-41327 and *Bacillus licheniformis* strain DSM 13 are shown in Fig - 4 and Fig - 5 respectively :-

Fig. 4. Aligned sequence data of *Bacillus haynesii* strain NRRL B-41327

Fig. 5. Aligned sequence data of *Bacillus licheniformis* strain DSM 13

CONCLUSION

Due to scarcity of water in arid and semi-arid regions, people are bound to reuse industrial wastewater such as textile wastewater. Also the catchment area near these streams are used for farming which pollutes the soil and enters the plants also grown in that region. This critical issue can be overcome by the use of microbes capable of degrading colored pollutants in an eco-friendly manner. Isolating single bacterial culture for decolourization of azo dye in large scale application will not be more effective. In a consortial system consisting of a mixture of defined microbial population, a large variety of enzymes get released and attack the same chemical structure in different ways, resulting in faster degradation of the complex chemical structure (Sarkar *et al.,* 2017).

Using this concept, methylene blue dye was being decolorized by consortium of *Bacillus haynesii* strain NRRL B-41327 and *Bacillus licheniformis* strain DSM 13 extracted from mycorrhizal soil. This microbial degradation will offer an easy, cheaper and effective method for decolourizing methylene blue dye effluents released from textile industries.

ACKNOWLEDGEMENT

The research work has been carried out in Parul Institute of Applied Sciences laboratory. The authors are grateful to Gujarat Government for providing financial aid under SHODH Scheme.

REFERENCES

Daneshvar, N., Ayazloo, M., Khatee, A. R. and Pourhassan, M. 2007. Biological decolorization of dye solution containing Malachite Green by microalgae *Cosmarium* sp. *Bioresource Technology.* 98(6): 1176-1182.

Fulekar, M., Wadgaonkar, S. and Singh, A. 2013. Decolourization of Dye Compounds by Selected Bacterial Strains isolated from Dyestuff Industrial area. *Int. Journal of Advancements in Research & Technology.* 2(7): 182-192.

Gayathi, R., Kavi, A., Meena, P., Karthikeyan, S. and Ramesh babu, N. G. 2014. Biodegradation of Azo Dyes by Using Soil Bacteria. *Int. Journal of Engineering Research & Technology*. 3(1): 738-741.

Hsueh, C., Chen, B. and Yen, C. 2009. Understanding effects of chemical structure on azo dye decolorization characteristics by *Aeromonas hydrophila. Journal of Hazardous Material.* 167(1-3): 995–1001.

Neethu, N. and Choudhury, T. 2018. Treatment of methylene blue and methyl orange dyes in wastewater by grafted titania pillared clay membranes. *Recent Patents on Nanotechnology.* 12(3): 200-207.

Pearce, C. I., Lloyd, J. R. and Guthrie, J. T. 2003. The removal of color from textile wastewater using whole bacterial cells: a review. *Dyes Pigment*. 58(3): 179–196.

Rana Rahman, M. and Neethu, M. K. 2017. Degradation of methylene blue in textile waste water using activated sawdust and eggshell biosorbent. *Int. Journal Of Advanced Research And Innovative Ideas In Education*. 2(4): 7-16.

Sarkar, S., Banerjee, A., Halder, U., Biswas, R. and Bandopadhyay, R. 2017. Degradation of synthetic azo dye of textile industry : A sustainable approach using microbial enzyme. *Springer*. 2(2): 121-131

Shafqat, M., Khalid, A., Mahmood, T., Siddique, M. and Habteselassie, M.Y. 2017. Evaluation of bacteria isolated from textile wastewater and rhizosphere to simultaneously degrade azo dyes and promote plant growth. *Journal of Chemical Technology And Biotechnology.* 92(10): 2760-2768.

Stolz, A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiology and Biotechnology.* 56(1-2): 69–80.

Vasniwal, R., Jadhav, I., Shrivastava, D. and Jadhav, K. 2016. Biodegradation and Decolorization of Azo Dye (Deep Red Glx) by Alkaliphilic *Bacillus cereus* Strain BPL Isolated from Textile Effluent Contaminant Soil. *International Journal of Engineering Research & Technology.* 5(9): 1-8

Zhu, C., Feng, Q., Ma, H., Wu, M., Wang, D. and Wang, Z. 2018. Effect of methylene blue on the properties and microbial community of anaerobic granular sludge. *Bio Resources.* 13(3): 6033-6046.