Materials Today: Proceedings 36 (2021) 167–170

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

Efficacy of dye degradation of contaminated soil microbial isolates

M. Biruntha [a,*], J. Archana [a], K. Kavitha [a], K. Vanimuthu [a], B. Karunai Selvi [b], J. Arockia John Paul [c], R.M. Vithyavathy [d], K. Kaviyarasu [e], G. Ramalingam [f]

[a] Vermiculture Technology Lab, Department of Animal Health and Management, Alagappa University, Karaikudi 630 003, Tamil Nadu, India
[b] Department of Botany, V. V. Vanniaperumal College for Women (Autonomous), Virudhunagar 626 001, Tamil Nadu, India
[c] Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur 630 211, Tamil Nadu, India
[d] Department of Bioinformatics, Alagappa University, Karaikudi 630 003, Tamil Nadu, India
[e] Nanosciences African Network (NANOAFNET), Materials Research Department (MRD), iThemba LABS-National Research Foundation (NRF), 1 Old Faure Road, 7129, PO Box 722, Somerset West, Western Cape Province, South Africa
[f] Quantum Materials Research Lab(QMRL), Department of Nanoscience and Technology, Alagappa University, Karaikudi 630003, India

ARTICLE INFO

Article history:
Received 3 December 2019
Received in revised form 10 February 2020
Accepted 17 February 2020
Available online 16 March 2020

Keywords:
Aspergillus niger
Microbial decolurization
Mega X
Bootstrap
Phylogenetic tree

ABSTRACT

Microbial decolourization of textile dyes is cost effective and eco-friendly method. Two bacterial species (*Bacillus cereus* and *Bacillus flexus*) and one fungal species *Aspergillus niger* were isolated from the dye spilled soil and identified through biochemical and molecular characterization by gene sequencing. Phylogenetic tree was constructed with blast result of closely related sequences using Mega X software with 500 bootstrap. The phylogenetic tree of isolated species showed great diversity with *Bacillus* and *Aspergillus* species. *A. niger* showed 98% of dye degradation capacity, *B. cereus* has very poor ability to degrade dye when compared with *B. flexus*. The sequences of *B. cereus* (MH299975), *B. Flexus* (MH299976) and *A. niger* (MH299977) were submitted to NCBI gene bank for global retrieval.

Selection and peer-review under responsibility of the scientific committee of the NANOSMATAFRICA-2018.

1. Introduction

Environmental pollution due to dye has been recognized as one of the most serious problem because of 200,000 tons of textile dyes are discharged as industrial effluents every year [1]. The environmental management of dye effluents posing a major global thrust towards protection of biodiversity and health of the ecosystem [2]. The subsequent discharge of textile effluent accumulate mainly in water bodies and leads to adverse effects in terms of dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), colour, etc., [3]. However, in developing nations, the problem converts severe due to poor environmental legislation, substandard work conditions, and inefficient waste treatment systems [4]. In textile industries azo dyes make up approximately 70% of altogether dye stuffs during dye processing Unit [5,6].

Microorganisms have developed enzyme system for the decolorization and mineralization of azo dyes under certain environmental conditions [7]. Several Bacteria and white rot fungi like *Phanerochaete chrysosporium* and *Trametes versicolor*, due to their efficient lignolytic enzymatic systems, have been reported to degrade or sequester azo, heterocyclic, reactive or polymeric dyes [8]. Hence, the present study focused on the isolation of textile dye degrading bacteria and fungi from a dye contaminated environment, molecular characterization and analysis of their ability to degrade textile dyes into non-toxic product.

2. Materials and methods

2.1. Sample collection and isolation of microorganism

The soil samples were collected from dye spilled areas of textile industry in Tirupur, Tamil Nadu, India**.** Acid red textile dye used in this experiment was purchased from local suppliers. Soil samples were serial diluted (10^{-1} to 10^{-9}); inoculated on the petriplate and kept for 24–48 h at 37 °C for the growth [9]. The bacteria and fungal colonies were isolated from the soil samples using nutrient agar and potato dextrose agar media respectively. Selected isolates were characterized by colony morphology on nutrient agar, Gram staining, and morphological characteristics described by Bergey's manual [10] and biochemical tests viz., indole test, starch hydrolysis, citrate test, catalase test and oxidase

* Corresponding author.
E-mail address: biruntha6675@gmail.com (M. Biruntha).

https://doi.org/10.1016/j.matpr.2020.02.692
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the NANOSMATAFRICA-2018.

test were performed with isolated dye utilizing bacteria [11]. The macroscopic features of the hyphal mass, morphology of cells and spores, were used for identification of fungi [12].

2.2. Molecular identification of the isolates

The molecular characterization of isolates was done using DNA sequencing and Phylogenetic analysis. Sequencing reactions were performed using an ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme). Single-pass sequencing was performed on each template using below 16 s rRNA universal primers (8F-5′AGAGTTTGATCCTGGCTC AG3′and1541R 5′AAGGAGGTGATCCAGCCGCA3′) and 18 s rRNA universal primers (5′-GTAGT CATAT GCTTG TCTC-3′) and NS8 (5′-TCCGC AGGTT CACCT ACGGA-3′). The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer. Sequences from the 16S rRNA and 18S rRNA of bacteria and fungi respectively were used to construct the Phylogenetic tree analysis.

2.3. Dye degradation assay

The biodegradation assay was performed with 1.0% concentration of commercial dye in 100 ml of nutrient broth containing 3 ml (about 2×10^8 cells/ml) of the each isolate was inoculated [13]. Each isolate was subjected for biodegradation assay including one control without addition of an inoculum. The level of dye degradation was determined using the gravimetric analysis.

3. Results and discussion

From the soil sample (Fig. 1), the isolated colonies were identified based on the morphology and biochemical characteristics. Based on biochemical test (Table 1), the isolated bacterial S1, S2, and fungal F1 were confirmed as *B. cereus*, *B. flexus* and *A. niger* respectively (Fig. 2).

3.1. Phylogenetic analysis for isolated bacteria and fungi

The phylogenetic dendrogram of 16S rRNA implies the genetic diversity of *Bacillus* species in the contaminated soil and used to distinguish closely related species. The *Bacillus cereus* clustered with 10 different species and *Bacillus flexus* clustered with 9 different species by neighbour joining construction using Mega X software using 500 bootstraps. The *Bacillus cereus* was closely related to *B. paramycoides*, *B. wiedmannii*, *B. thuringiensis* and distantly related to *B. toyonensis*. The *Bacillus flexus* was closely related to *B. paraflexus*, *B. megaterium* and distantly related to *B. oceanisediminis* and *B. depressus*. The phylogenetic topography showed the *Bacillus cereus* and *Bacillus flexus* are out grouped each other indicates the highly diverse of *Bacillus* species. The Phylogenetic dendrogram of 18S rRNA of *Aspergillus niger* was closely related to *Aspergillus carbonarius*, *Aspergillus cremeus* and distantly related to *Aspergillu scervinus* and *Aspergillus candidus*. The sequences of *B. cereus* (MH299975), *B. Flexus* (MH299976) and *A. niger* (MH299977) were submitted to NCBI gene bank. Already identification work was carried out by gene sequencing and the work has been reported [14].

3.2. Biodegradation of dye by gravimetric method

Degradation of dye using *Bacillus cereus, Bacillus flexus* and *Aspergillus niger* were analysed by gravimetric method (Fig. 3). The reduction of dye colour was observed after 7th day of incubation. The reductive decolourisation efficiency of block B and congo red dyes by *Bacillus* sp.,and *Pseudomonas* sp., under aerobic conditions in the presence of additional carbon sources has also been reported [15]. Among the isolates, *Aspergillus niger* has 98% dye

Fig. 1. Dye contaminated soil collected from Tirupur.

Table 1
Biochemical test for bacterial identification.

Isolated species	*Bacillus cereus*	*Bacillus flexus*
Colony shape	Rod	Lobate
Colony morphology	Smooth	White
Gram stain	+	–
Motility	+	–
Catalase test	+	+
Oxidase test	–	–
Citrate utilization	+	+
Indole test	–	+
Starch hydrolysis	+	+

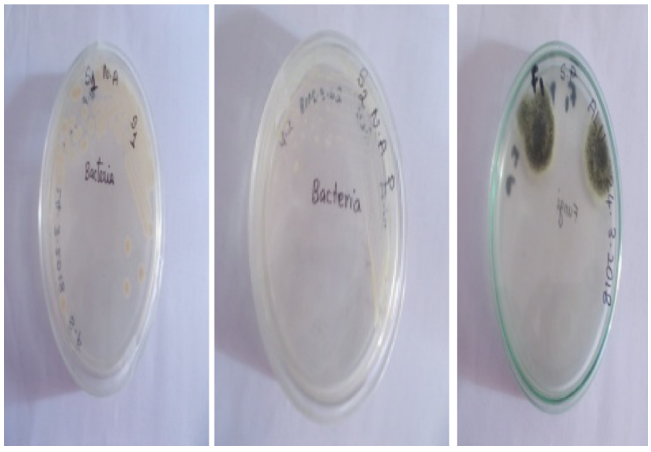

Fig. 2. Isolated microorganism from dye contaminated soil.

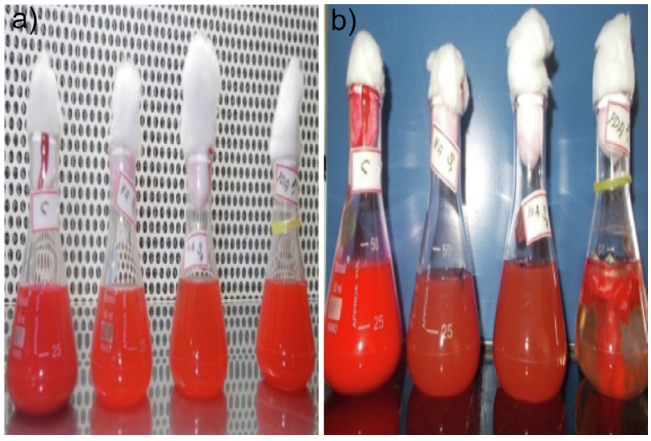

Fig. 3. Reduction of acid red dye by S1- *Bacilus cereus*, S2- *Bacillus flexus* F1 – *Aspergillus niger* and C- control (without culture) for 7 days. Colour reduction was observed in A) before degradation B) after degradation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

degrading capacity whereas the *Bacillus cereus* has very poor capacity to degrade dye in the contaminated soil. The *Bacillus flexus* has 33% of degrading efficiency is represented in Fig. 4. Likewise, the *Pseudomonas* sp. was found to be more efficient in dye decolorization and facile conditions showed the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents and to degrade the toxic chemicals present in textile wastewater before releasing it into the nearby local environment [16,17].

4. Conclusion

From these result it is concluded that *Aspergillus niger* have high ability to degrade the textile dye. *Bacillus cereus* has very less capacity of degradation when compared to other species. Moreover it is determined that the closely related species for isolates using phylogenetic analysis. Finally the sequence of isolated species was submitted to gene bank with the accession number of MH299975 for *Bacillus cereus*, MH299976 for *Bacillus flexus* and MH299977 for *Aspergillus niger* and may be used for further universal retrieval.

CRediT authorship contribution statement

M. Biruntha: Conceptualization, Data curation, Formal analysis, Project administration, Funding acquisition. **J. Archana:** Investigation, Writing - original draft, Data curation. **K. Kavitha:** Investigation, Methodology, Resources, Software. **K. Vanimuthu:** Writing - original draft, Validation. **B. Karunai Selvi:** Formal analysis, Software, Writing - review & editing. **J.A. John Paul:** Writing - review & editing. **R.M. Vithyavathy:** Writing - review & editing. **K. Kaviyarasu:** Visualization. **G. Ramalingam:** Data curation, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to RUSA Scheme Phase 2.0Grant [F-24-51/2014–U, Policy (TNMulti-Gen), Dept of Edn., Govt. of India, dated 09.10.2018] for their financial support. The authors are also grateful for the research collaboration among institutes and universities.

S.No.	Experimental set up	Dye degradation	
		OD	in %
1	Blank	3.122	-
2	*Bacillus cereus*	3.112	0.32
3	*Bacillus flexus*	2.091	33
4	*Aspergillus niger*	0.053	98

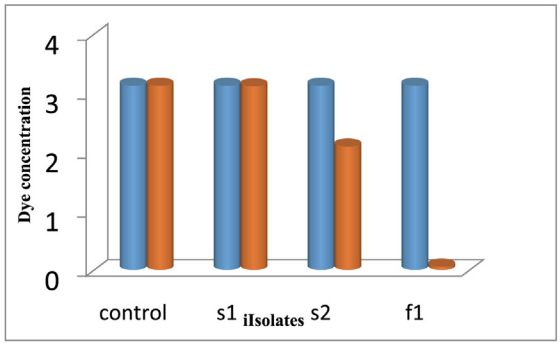

Fig. 4. Dye degrading efficiency of isolates in percentage of dye degradation and reduction concentration of dye.

References

[1] F.M. Drumond Chequer, G.A.R. de Oliveira, E.R. Anastacio Ferraz, J. Carvalho, M. V. Boldrin Zanoni, D. de Oliveir, P. Textile Dyes. 6 (2013) 151–167.
[2] B.J. Brüschweiler, S. Kung, D. Bürgi, L. Muralt, E. Nyfeler, Regul. Toxicol. Pharm. 69 (2014) 263–272.
[3] R.G. Saratale, S.S. Gandhi, M.V. Purankar, M.B. Kurade, S.P. Govindwar, S.E. Oh, G.D. Saratale, J. Biosci. Bioeng. 115 (2013) 658–667.
[4] D. Rawat, V. Mishra, R.S. Sharma, Chemosphere 155 (2016) 591–605.
[5] Rajat Pratap Singh, Pradeep Kumar Singh, Ram Lakhan Singh, Toxicol. Int. 21 (2014) 160–166.
[6] R.G. Saratale, G.D. Saratale, J.S. Chang, S.P. Govindwar, J. Taiwan Inst. Chem. Eng. 42 (2011) 38–157.
[7] Ewa Zabłocka-Godlewska, Wioletta Przystaś & Elżbieta Grabińska-Sota 229 (2018) 176.
[8] M. Solís, A. Solís, H. InésPérez, N. Manjarrez, M. Flores, Process Biochem. 12 (2012) 47.
[9] S. Boonchan, M.L. Britz, G.A. Stanley, Appl. Environ. Microbiol. 66 (2000) 1007–1019.
[10] J.G. Holt, N.R. Kreig, P.H.A. Sneath, J.T. Stanely, S.T. Willams, Bergey's Manual of Determinative Bacteriology, 9th ed., Lippincott, Williams & Wilkins, Baltimore, 1994.
[11] D.L. Balkwill, W.C. Ghiorse, Appl. Environ. Microbiol. 50 (1985) 580–588.
[12] J.B. Morton, Mycorrhiza 2 (1993) 97–109.
[13] K. Lakshman, T.R. Shamala, Enzyme Microbial Technol. 39 (2006) 1471–1475.
[14] Anupama Mittal, Padma Singh, Ind. J. Experim. Biol. 47 (2009) 760–765.
[15] Eman Zakaria Gomaa, Microbiol. J. 6 (2016) 15–24.
[16] Satyendra Kumar Garg, Manikant Tripathi, Res. J. Microbiol. 12 (2017) 1–19.
[17] E. Arivuselvi, G. Ramalingam, J. Adv. Res. Appl. Sci. 4 (2017) 7426–7431.