Current Research in Microbial Sciences 1 (2020) 37–43

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

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

Bioremediation of malachite green dye by two bacterial strains isolated from textile effluents

Dipankar Chandra Roy[a,b], Sudhangshu Kumar Biswas[a,c,*], Md. Moinuddin Sheam[a], Md Rockybul Hasan[c], Ananda Kumar Saha[d], Apurba Kumar Roy[e], Md. Enamul Haque[f], Md. Mizanur Rahman[a], Swee-Seong Tang[c]

[a] Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Islamic University, Kushtia-7003, Bangladesh
[b] Biomedical and Toxicological Research Institute, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh
[c] Division of Microbiology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[d] Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh
[e] Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh
[f] University of Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Keywords:
Bioremediation
Enterobacter sp.
Malachite Green dye
RAPD
Textile effluents
16S rRNA gene sequencing

ABSTRACT

Globally, water pollution from the textile industries is an alarming issue. Malachite Green dye of the triphenylmethane group is an extensively used dye in the fabric industries that is emitted through textile wastewater. This study aimed to isolate and characterize potential Malachite Green (MG) dye degrading bacteria from textile effluents. Different growth and culture parameters such as temperature, pH and dye concentration were optimized to perform the dye-degradation assay using different concentrations of MG dye in the mineral salt medium. A photo-electric-colorimeter was used to measure the decolorizing activity of bacteria at different time intervals after aerobic incubation. Two potential bacterial strains of *Enterobacter* spp. CV-S1 (accession no: MH450229) and *Enterobacter* spp. CM-S1 (accession no: MH447289) were isolated from textile effluents exhibiting potential MG dye decoloring efficiency. Further, the RAPD analysis and 16S rRNA sequencing confirmed the genetic differences of the isolated strains. *Enterobacter* sp CV-S1 and *Enterobacter* sp CM-S1 can completely decolor MG dye up to 15 mg/L under shaking condition without any requirement of sole carbon source. Thus, these two bacteria have the potency to be utilized in the textile wastewater treatment plant.

1. Introduction

Though textile industries have a contribution to the development of the global economy, water pollution by textile effluents is one of the major concerning issues over the world. One million tons of synthetic dyes are produced each year worldwide and approximately 280,000 tons of these colorants are emitted to the textile effluents annually (Jadhav et al., 2016, Periyasamy et al., 2019). Due to their non-degradable nature, these dyes inhibit the entrance sunlight in water and hamper the photosynthesis process, thus affecting the aquatic flora and fauna (Berradi et al., 2019). In addition, breathing in the evaporated dyes causes not only various allergic reactions but also hazardous for children (Kant, 2012). For instance, in Brazil, several textile dyes that contaminated drinking water source have been reported to be carcinogenic (Ot et al., 2007).

Physio-chemical techniques, for example, coagulation-flocculation, adsorption, oxidation and membrane techniques have been used for decoloring of textile dyes during the past several decades. But these methods are associated with high sludge formation, burden cost, generation of unwanted byproducts and secondary pollution (Sghaier et al., 2019). Hence, there is an urgency of an environmentally friendly, cost-effective and efficient approach of degrading textile dyes. Several microorganisms, for example, bacteria, fungi and algae can be a potential alternative of removing polluting dyes (Roy et al., 2018). Bioremediation of textile dyes by different microbial species have received much attention due to its cost-effectiveness, eco-friendly nature and public acceptability (McMullan et al., 2001). Isolation of textile dye degrading bacteria has been started since the 1970s. Bacteria use these dyes as substrates and convert them into less complex metabolites by generating various oxidoreductive enzymes (Khandare and Govindwar, 2016). Recently, different microbial species have been observed having the potentiality of degrading and decoloring textile dyes (Upadhyay, 2002, Shanooba et al., 2011, Rajeswari et al., 2011, Ali and El-Mohamedy, 2012, Forss et al., 2017, Kumar Garg et al., 2012).

* Correspondence author.
E-mail address: skbiswas@btge.iu.ac.bd (S.K. Biswas).

https://doi.org/10.1016/j.crmicr.2020.06.001
Received 3 May 2020; Received in revised form 16 June 2020; Accepted 17 June 2020

Bacterial culture capable of degrading *azo* compounds was reported for the first time in 1970s and the reported strains were *Bacillus subtilis* (Horitsu et al., 1977) followed by *Aeromonas hydrophila* (Idaka et al., 1978) and *Bacillus cereus* (Wuhrmann et al., 1980). Du *et al* (2011) reported the 90.3–97.2% biodegradtion of Malachite Green dye by *Pseudomonas sp*. strain DY1 under shaking condition, where they observed lower degradation rate in static condition (Du Sheng et al., 2011). Moreover, a crude alkaline protease isolated from *Bacillus cereus* strain KM201428 exhibited complete degradation of MG dye (Wanyonyi et al., 2017) and a newly isolated *Bacillus vietnamensis* sp. MSB17 revealed complete MG dye degrading capability (Kabeer et al., 2019). In addition to bacteria, a novel fungus species *Lasiodiplodia sp* showed MG dye degradation capability in a wide range of pH and temperature (Arunprasath et al., 2019).

The present research aimed at isolating and characterizing Malachite Green (MG) dye decoloring bacteria from the textile effluents for possible use in the industrial process of bioremediation. Two bacterial strains *Enterobacter* CV-S1 and *Enterobacter* CM-S1 were identified for Malachite Green dye degrading capability by 16S rRNA gene sequencing. 5% (v/v) inoculums *Enterobacter* CV-S1 and *Enterobacter* CM-S1 can decolorize 100% MG at a concentration of 15 mg/l within 78 h and 144 h respectively.

2. Materials and methods

2.1. Sample collection

The textile effluent samples were collected in 250 ml sterilized plastic bottles from the drainage canal of two local textile industries in Kumarkhali, Kushtia, Bangladesh. Total four samples were collected in the form of untreated liquid effluent and untreated sludge namely water-1, water-2, sludge-1, sludge-2 from drainage canal that carries stagnant textile effluent for the screening of dye degrading bacteria. Physical properties such as color, temperature and pH of the samples were recorded on the sites and were stored in the laboratory at 4 °C within 12 h of collection.

2.2. Dyes, chemicals and microbiological media

Triphenylmethane dye malachite green (MG) was procured from the local market close to the thread dyeing plant of Kumarkhali, Kushtia, Bangladesh. Dye degrading mineral salt (MS) medium was prepared by adding the following components (g/L): K_2HPO_4 (2), $(NH_4)SO_4$ (0.5), KH_2PO_4 (0.2) and $MgSO_4$ (0.05); where the trace element (TE) solution was prepared by adding the following components (g/L): $FeSO_4$ (0.4), $MnSO_4$ (0.4), $ZnSO_4$ (0.2), $CuSO_4$ (0.04), KI (0.3), Na_2MoO_4 (0.05) and $CoCl_2$ (0.04) and the components of enrichment medium was MS medium with glucose (0.1%), yeast extract (0.05%), peptone (0.5%) and NaCl (0.5%). Furthermore, nutrient broth and nutrient agar medium were used for culture maintenance.

2.3. Isolation and screening of dye degrading bacteria

All samples (untreated textile effluents and sludges) were used for the isolation of dye degrading bacterial strains. A total of six morphologically distinct bacterial strains designated as CV-S1, CV-S2, CV-S3, CM-S1, CM-S2 and CM-3 were screened and tested for their ability to decolorize dyes. CV–S1, CV-S2 and CV-S3 were isolated for Crystal Violet (CV) dye degradation as described in the previously published article (Roy et al., 2018), which were further tested against MG dye. On the other hand, CM–S1, CM-S2 and CM-S3 were isolated having MG dye degradation capability using the same techniques with a few modifications. Briefly, the isolates exhibited decolorization efficiency were streaked on enrichment agar medium (2%) mixed with Malachite Green dye. The colonies of bacterial isolates showed a clear colorless zone around them were picked and cultured for further experiments.

2.4. Colony characteristics and microscopic morphology observation

After the isolation of six dye degrading bacterial strains, their colony characteristics and microscopic morphology were observed. Fresh cultures of the isolates were used to study colony characteristics, gram reaction and cell morphology. The study of colony characteristics was determined according to Engelkirk and Duben-Engelkirk (2008) (Engelkirk and Duben-Engelkirk, 2008) and the cell morphology under microscope includes smear preparation and Gram's staining was as described according to (Manual of Basic, 2003).

2.5. Growth characteristics determination

Two bacterial strains CV–S1 and CM-S1) showing the highest dye degrading efficacy were selected for further studies. Bacterial growth characteristics were determined under different pH (6.00,6.50,7.00) and temperatures (30 °C,35 °C and 40 °C) at 120 rpm in a shaker incubator. Growth characteristics were determined through growth curve analysis by turbidity measurements at different time intervals using photo-electric-colorimeter.

2.6. Sensitivity test to antibiotics

To identify drug resistance in bacteria, simple and well-standardized disc diffusion method was performed which was based on the measurement of the zone of inhibition around each antibiotic disc (Jorgensen and Ferraro, 2009). Antibiotic of different classes, Ampicillin (10 μg), Azithromycin (15 μg), Bacitracin (10 μg), Cephradine (30 μg), Ceftriaxone (30 μg), Doxycycline (30 μg), Erythromycin (15 μg), Neomycin (30 μg), Sulphamethoxazole/Trimethoprim (25 μg) and Tetracycline (30 μg) were used to test antibiotic sensitivity.

2.7. Genomic DNA extraction

The boiling method was applied to extract the genomic DNA of the isolated bacteria. Concisely, a single colony was suspended into 30 μl of distilled water from an overnight culture (at 37 °C) of LB agar plate. The suspension was then boiled for 10 min at 100 °C. The mixture was instantly transferred into ice and cooled it for 5 min. The sample was centrifuged for 10 min at 13,000 x g and the supernatant, containing DNA, was used as the template for PCR amplification (Sun et al., 2011).

2.8. Characterization by RAPD analysis

In RAPD (Random Amplified Polymorphic DNA) synthetic (10 bases long) oligonucleotide primer is used to amplify genomic DNA under low annealing temperature (BARDAKCI, 2001). The variable genes of the isolated bacterial strains were amplified using three RAPD primers: MT370563 (5´–TGCCGAGCTG–3´), MT370571 (5´–TGCGCCCTTC–3´) and MT370573 (5´–GTGAGGCGTC–3´). A total of 25 μl reaction mixture for RAPD analysis contained ddH_2O 14.75μl, $MgCl_2$ (25 mM) 2 μl, buffer (10×) 2.5 μl, dNTPs (10mM) 0.5 μl, Taq DNA Polymerase (5u/μl) 0.25 μl, DNA template 1 μl and RAPD primer (10 μM) 4 μl. The Polymerase Chain Reaction was performed by Swift^{TM} Minipro thermal cycler in the following steps: denaturing at 95 °C for 5 min, followed by 40 cycles of 40 s of denaturing at 95 °C, 60 s of annealing at 36 °C and 2 min of elongation at 72 °C with a final extension at 72 °C for 10 min.

2.9. Phylogenetic identification through 16S rRNA gene sequencing

16S rRNA genes were amplified using the bacteria-specific universal primers, a forward primer F27 (5´–AGAGTTTGATCCTGGCTCAG–3´; Tm: 61 °C); and a reverse primer R1391 (5´–GACGGGCGGTGTGTRCA–3´; Tm: 67.4 °C). The recipe of 25 μl reaction mixture was: ddH_2O 14.75 μl, $MgCl_2$ (25 mM) 2 μl, buffer (10×) 2.5 μl, dNTPs (10mM)

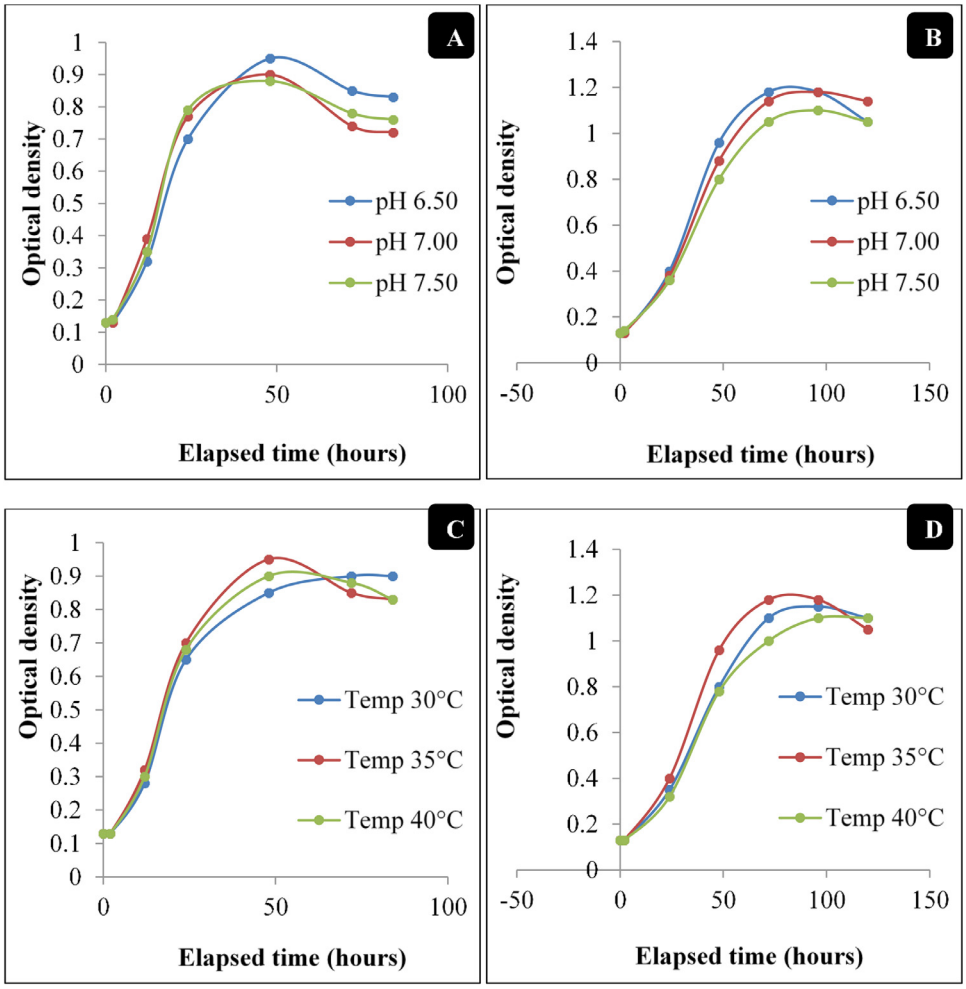

Fig. 1. Effect of pH and temperature on bacterial growth: A, Effect of pH on the growth of CV-S1; B, Effect of pH on the growth of CM-S1; C, Effect of temperature on the growth of CV-S1 and D, Effect of temperature on the growth of CM-S1.

0.5 µl, Taq DNA Polymerase (5 u/µl) 0.25 µl, DNA template 1 µl, forward primer (10 µM) 2 µl and reverse primer (10 µM) 2 µl. The thermal cycle for PCR amplification was the same as mentioned in the section "Characterization by RAPD analysis" except annealing temperature which was 65 ˚C instead of 36 ˚C. The amplified PCR product was purified from agarose gel by using gel purification kit according to manufacturer protocol. The sequence was generated from the purified 16S rRNA gene using the DNA sequencer instrument (Model: Genetic Analyzer 3130). The similarity in nucleotide sequences generated from the 16S ribosomal RNA amplification was analyzed through NCBI BLAST (http://www.ncbi.nlm.nih.gov) by aligning the homologous sequences. Neighbor-joining method was used to determine an evolutionary relationship by using online software: PhyML (v3.0 aLRT), Muscle (v3.7), Gblocks (v0.91b), and TreeDyn (v198.3) on the platform of Phylogeny.fr (Dereeper et al., 2010, Edgar, 2004).

2.10. Assay of Malachite Green dye degradation

The rate of MG dye degradation was expressed as percentage. Using a photoelectric colorimeter the degradation was calculated by observing the decrease in absorbance at absorption maxima (λ max). Further the dye degradation (DD) assay was determined based on initial optical density (IOD) and final optical density (FOD) by the following equation (Chen et al., 2003). The concentration of dye in the supernatant was observed by absorbance at 660 nm.

$$\mathrm{DD}\,(\%) = \frac{IOD - FOD}{\mathrm{IOD}} \times 100$$

Different parameters like inoculum size, pH, temperature and initial dye concentration were optimized as previously described by Roy et al. (2018). Briefly, a volume of 10 ml solution containing MS medium enriched with trace element solution and MG dye was placed in 50 ml test tube. The MG dye of 15, 30 and 50 mg/L concentrations were inoculated with the freshly grown 5% bacterial inoculums under aerobic shaking condition (120 rpm) at pH 6.50 and temperature 35 ˚C. Furthermore, under the optimized conditions the dye degradation rates by the bacterial strains were observed at different time intervals until complete degradation. Each treatment was performed three times and the mean value was recorded for analysis.

2.11. Statistical analysis

All the experiments in this study were triplicated and the findings were reported as Mean± standard deviation (SD).

3. Results and discussion

3.1. Physical properties of textile effluents

Textile wastewater contains a high amount of unused dyes that create abnormal coloration (Errais et al., 2010). Visually the physical appearance of the collected three textile effluent samples were black colored and one was turquoise blue. Besides, textile effluents have a wide PH and temperature range (Yaseen and Scholz, 2019). The pH determination of wastewater is crucial as it is a significant parameter that determines and influences the treatment process (Mandal, 2014).

During collection, the pH of the tested sample was slightly acidic to neutral (6.5–6.7) which was within the permissible limit of WHO and IS 10500:1991. Temperature affects various chemical and biological processes in water and is an important parameter (Bhatia et al., 2018). Due to winter season, the temperatures of the present collected samples were around 18 °C.

3.2. Colony characteristics and microscopic morphology

The colony morphology or appearance of the colonies varies from one species to species and colony features serve as an essential indicator in the identification of bacteria (Engelkirk and Duben-Engelkirk, 2008). In this present investigation, though CV-S1 and CM-S1 were isolated with different samples, similar colony characteristics were observed.

3.3. Growth characteristics

The growth pattern of bacteria can vary vastly species to species (Brown et al., 2011). The growth curve of the microbial culture was analyzed to study the population growth by plotting the optical density in the *Y*-axis and the incubation time in the *X*-axis. Both the bacteria showed growth variations at different temperatures and pH (Fig. 1). Highest growth was observed at pH 6.50 and temperature 35 °C.

3.4. Sensitivity to antibiotics

Globally, multi-drug resistant bacteria is an emerging public health crisis (Sheam et al., 2020). Wastewater treatment plant serves as a remarkable source of antibiotic-resistant intestinal bacteria and antibiotic-resistant genes, which may transfer to other environmental bacteria (Osińska et al., 2019). Hence, the determination of the antibiotic resistance profile of selected bacteria is necessary for large scale implementation of these bacteria in treatment plants. Based on the zone of inhibition, both CV-S1 and CM-S1 were found sensitive to azithromycin, cefriaxone, sulphamethoxazole/trimethoprim and tetracycline, intermediate sensitive to ampicilin, doxycycline and neomycin and resistant to bacitracin, cephradine and erythromycin.

3.5. RAPD analysis

The two strains of MG dye degrading bacteria were characterized genetically by the RAPD technique using the MT370563, MT370571 and MT370573 primers (Fig. 2). Electrophoresis of the amplified variable genes using 1.4% agarose gel showed different DNA banding patterns. In case of primer MT370563, CM-S1 showed three bands around 800 bp, 600 bp and 400 bp, whereas the CV-S1 showed two bands around 800 bp and 400 bp. In case of primer MT370571, CM-S1 and CV-S1 both showed three similar bands around 1500 bp, 800 bp and 400 bp. On the other hand, in case of primer MT370573, CM-S1 showed only one band around 800 bp, whereas the CV-S1 showed four bands around 800 bp 600 bp 300 bp and 400 bp. It revealed that the two isolated bacterial strains were genotypically different. Among three RAPD primers, primer MT370571 showed no variation between CM-S1 and CV-S1 where the primer MT370573 showed more variation than and MT370571. RAPD is a PCR based method that is used to identify polymorphism in genomic DNA, thus useful in ascertaining dissimilarity in closely related bacterial strains (Permaul et al., 1996). This molecular characterization of potential strains is helpful in identification and diversity analysis among bacterial isolates (Spratt, 2004, Agrawal et al., 2015).

3.6. Phylogenetic identification of dye degrading bacteria

16S ribosomal RNA (rRNA) gene sequencing is an effectual strategy for bacterial identification as well as phylogenetic relationship analysis (Patel, 2001). According to the isolation source, the highest homology

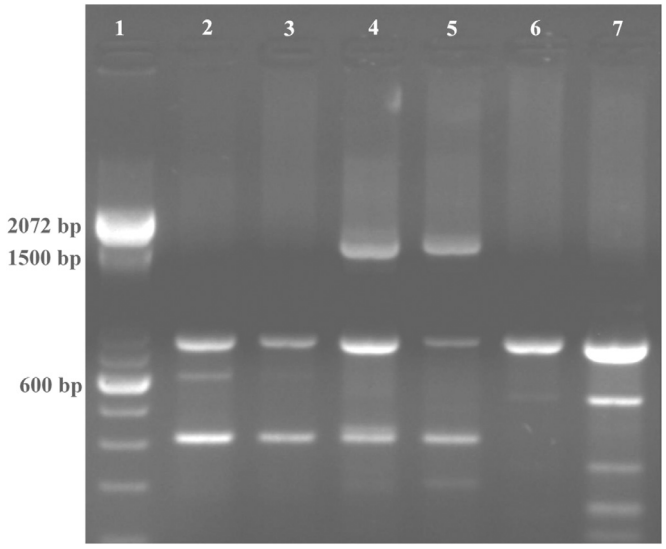

Fig. 2. Observation of genotypic variation of two isolates using three RAPD primers: Lane 1, Marker; lane 2 and lane 3, strain CM-S1 and strain CV-S1 respectively with primer MT370563; lane 4 and lane 5, strain CM-S1 and strain CV-S1 respectively with primer MT370571; lane 6 and lane 7, strain CM-S1 and strain CV-S1 respectively with primer MT37073.

(99%) for CV–S1 was *Enterobacter* sp. HSL69 and for CM–S1 was *Enterobacter* sp. HSL99. In order to perform a better classification, the phylogenetic tree was constructed. The evolutionary relationship of the dye degrading bacterial strains with other relevant bacteria were presented in the (Fig. 3). The homology stipulated that the isolated strain CM–S1 was in the phylogenetic branch of the genus *Enterobacter*, and the strain CV–S1 constructed a new branch.

Though identification of bacterial strains through 16S rRNA gene sequencing vary between genera and species, up to 90% bacterial strains at the genus level and 86% at species level can be authentically identified by this process (Winand et al., 2020). 98.65% similarity is recognized as the threshold for species identification via 16S rRNA gene sequencing (Kim and Chun, 2014). Therefore, based on this consideration, the isolates were identified as *Enterobacter* sp. CV-S1 and *Enterobacter* sp. CM-S1. The newly constituted branch confirms that the identified strain CV-S1 is a new strain of the genus *Enterobacter*. (Roy et al., 2018)

3.7. Malachite green dye degradation

As pH 6.5 and temperature 35 °C were optimized for dye degradation and at these defined pH and temperature, 5% inoculums of *Enterobacter* sp. CV-S1 was able to decolor 15 mg/L MG dye within 78 h (Fig. 4), whereas 5% inoculums of *Enterobacter* sp. CM-S1 was able to decolor the same amount within 144 h (Fig. 5). The dye degradation curves (Fig. 6) revealed that the two bacterial isolates *Enterobacter* sp. CV-S1 and *Enterobacter* sp. CM-S1 completely decolored MG dye (15 mg/L) after 78 h and 144 h respectively.

Joshi and Mhatre (2015) also observed maximum MG dye degradation by *Enterobacter* sp. at pH 7.00 and temperature 37 °C which was close to this pH and temperature (Joshi and Mhatre, 2015). In addition, Du et al. (2011) observed that the optimum pH was 6.6 and the temperature was 28 °C–30 °C for the MG dye degradation by *Pseudomonas* sp. strain DY1 (Du Sheng et al., 2011). The deterioration of Malachite green was studied at various increasing concentrations of dye i.e. 15, 30 and 50 mg/L. It was found that the rate of degradation was decreased with increasing concentration of dye (Table 1) and a similar trend was observed by Wanyonyi et al. (2017) (Wanyonyi et al., 2017).

Another interesting finding is that during MG dye degradation no supplementary carbon and protein source was added in MS medium. In

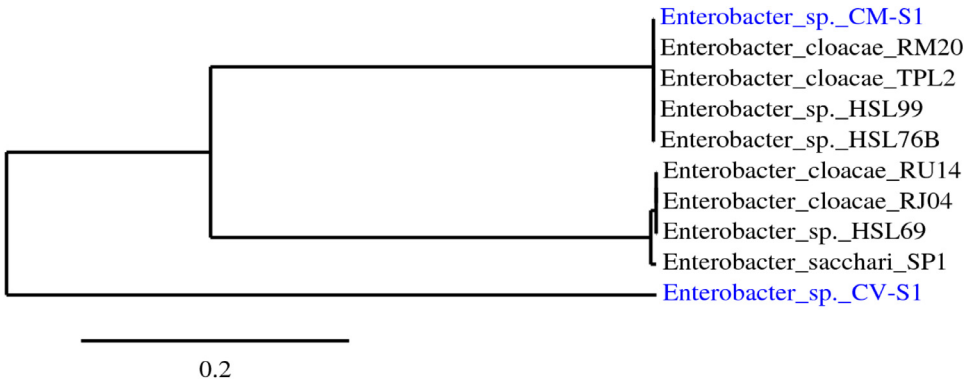

Fig. 3. CM-S1 and CV-S1 were the isolated dye-degrading bacteria where the phylogenetic tree was reconstructed through maximum-homology method executed in the PhyML program (v3.0 aLRT) (Dereeper et al., 2010), (Edgar, 2004)).

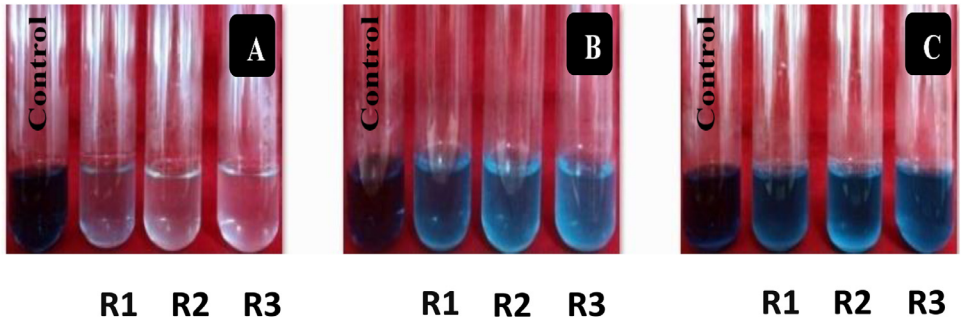

Fig. 4. Degradation of Malachite green dye by *Enterobacter* sp. CV-S1: **A,** 15 mg/L; **B,** 30 mg/L; **C,** 50 mg/L. C, represents Control, R1, R2 and R3 represent Replication 1, Replication 2 and Replication 3, respectively.

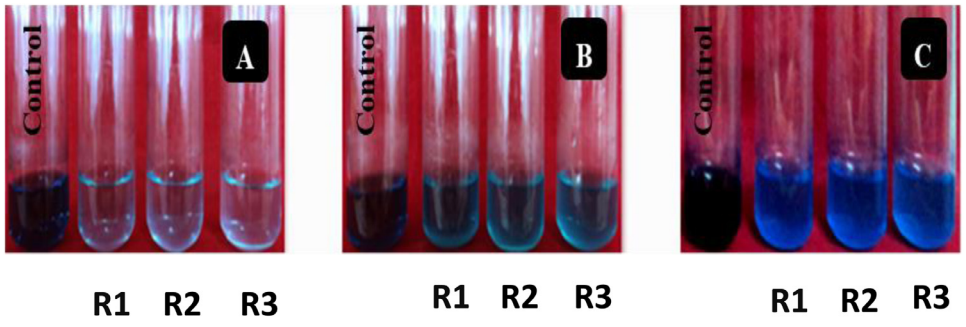

Fig. 5. Degradation of Malachite green dye by *Enterobacter* sp. CM-S1: **A,** 15 mg/L; **B,** 30 mg/L; **C,** 50 mg/L. R1, R2 and R3 represent Replication 1, Replication 2 and Replication 3, respectively.

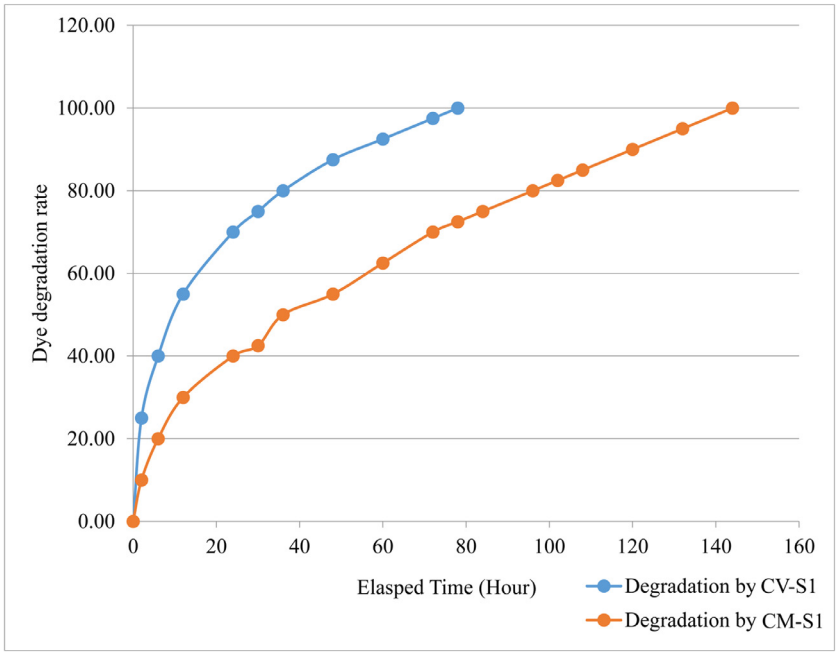

Fig 6. MG dye (conc. 15 mg/L) degradation curve by two bacterial strains.

Table 1
Different concentrations (mean value of 3 replications) of MG dye degradation by *Enterobacter* sp. CV-S1 and *Enterobacter* sp. CM-S1.

Isolates	Dye Concentration(mg/l)	Optical Density (Initial)	Optical Density (Final)	Degradation (%)	Duration of observation(hours)
Enterobacter sp. CV-S1	15	0.2 ±0	0±0	100	78
	30	0.22±0	0.05±0	77.27	
	50	0.24±0	0.09±0	62.5	
Enterobacter sp. CM-S1	15	0.2±0	0±0	100	144
	30	0.22±0	0.09±0	59.09	
	50	0.24±0	0.11±0	54.17	

the previous researches, carbon or protein-enriched media were used for MG dye degradation by bacteria (Ramezani et al., 2013, Sneha et al., 2014, Parshetti et al., 2006, Lal and Srivastava, 2011). Indeed, these previously isolated microorganisms required the sole carbon source to accelerate the decoloring capacity. For instance, Ali et al (2009) observed MG dye degradation for 6 d by two fungal strains *Aspergillus flavus* and *Alternaria solani*, which was up to 30μM (≈10.95mg/L), when MG was a sole source of carbon. But degradation had increased up to 50μM (≈18.25mg/L) when an extra carbon source was added in the medium (Ali et al., 2009). *Enterobacter* sp. CV-S1 and *Enterobacter* sp. CM-S1 identified in this study had shown a complete degrading capability as they utilized up to 15mg/L MG dye as a carbon source.

4. Conclusion

Although microbial degradation or decolorization of textile effluent is a challenging process, some microbes recently have been explored as the potential scavengers of textile dye and textile effluent. In the current study, two MG dye degrading bacterial strains, *Enterobacter* sp. CV-S1 and *Enterobacter* sp. CM-S1 were identified through advanced molecular techniques. The maximum growth of these two strains was observed at pH 6.50 and temperature 35°C. These isolates were able to completely decolor MG dye up to 15mg/L without any supplementary carbon source. These observations reveal the potential usage of two bacterial strains in the wastewater treatment plants in the near future.

Declaration of Competing Interest

The authors don't claim any conflict of interest.

Author contribution

DCR (Researcher), SKB (Associate Professor) and AKS (Professor) contributed to study the design and did the study. DCR (Researcher), MMS (Researcher), MRH (Researcher), MEH (PhD) collected the data. DCR (Researcher), SKB (Associate Professor), MMR (Professor), AKR (Professor) contributed to data analysis and interpretation. DCR (Researcher), SKB (Associate Professor) and MMS (Researcher) drafted the article with the help of all authors. SST (Senior Lecturer), AKR (Professor), AKS (Professor), MMR (Professor) reviewed the manuscript critically. SKB (Associate Professor) and AKS (Professor) supervised the project. All authors read and approved the final version.

Acknowledgments

We want to acknowledge Zulkar Nain (East West University, Dhaka, Bangladesh) for his help in the statistical analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2020.06.001.

References

Agrawal, P.K., Agrawal, S., Shrivastava, R., 2015. Modern molecular approaches for analyzing microbial diversity from mushroom compost ecosystem. 3 Biotech 5, 853–866. https://doi.org/10.1007/s13205-015-0289-2.
Ali, N.F., El-Mohamedy, R.S.R., 2012. Microbial decolourization of textile waste water. J. Saudi Chem. Soc. 16, 117–123. https://doi.org/10.1016/j.jscs.2010.11.005.
Ali, H., Ahmad, W., Haq, T., 2009. Decolorization and degradation of malachite green by aspergillus flavus and alternaria solani. African J. Biotechnol 8, 1574–1576. https://doi.org/10.5897/ajb2009.000-9233.
Arunprasath, T., Sudalai, S., Meenatchi, R., Jeyavishnu, K., Arumugam, A., 2019. Biocatalysis and agricultural biotechnology biodegradation of triphenylmethane dye malachite green by a newly isolated fungus strain. Biocatal. Agric. Biotechnol 17, 672–679. https://doi.org/10.1016/j.bcab.2019.01.030.
BARDAKCI, F., 2001. Random amplified polymorphic DNA (RAPDs). Turk J. Biol. 25, 185–196. https://doi.org/10.1385/1-59259-038-1:675.
Berradi, M., Hsissou, R., Khudhair, M., Assouag, M., Cherkaoui, O., El, A., El, A., 2019. Heliyon Textile fi nishing dyes and their impact on aquatic environs. Heliyon 5. https://doi.org/10.1016/j.heliyon.2019.e02711.
Bhatia, D., Sharma, N.R., Kanwar, R., Singh, J., 2018. Physicochemical assessment of industrial textile effluents of Punjab (India). Appl. Water Sci 8, 83. https://doi.org/10.1007/s13201-018-0728-4.
Brown, P.J.B., Kysela, D.T., Brun, Y.V., 2011. Polarity and the diversity of growth mechanisms in bacteria. Semin. Cell Dev. Biol 22, 790–798. https://doi.org/10.1016/j.semcdb.2011.06.006.
Chen, K.C., Wu, J.Y., Liou, D.J., Hwang, S.C.J., 2003. Decolorization of the textile dyes by newly isolated bacterial strains. J. Biotechnol. 101, 57–68. https://doi.org/10.1016/S0168-1656(02)00303-6.
Dereeper, A., Audic, S., Claverie, J.M., Blanc, G., 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol. Biol 10, 8. https://doi.org/10.1186/1471-2148-10-8.
Du Sheng, L., Gang, W., Wang, B., Zhao, X.J.Y., Chen, Y., 2011. Biodegradation of malachite green by Pseudomonas sp . strain DY1 under aerobic condition : characteristics , degradation products , enzyme analysis and phytotoxicity. Ecotoxicology 438–446. https://doi.org/10.1007/s10646-011-0595-3.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.
Engelkirk, P.G., Duben-Engelkirk, J.L., 2008. Laboratory diagnosis of infectious diseases : essentials of diagnostic microbiology. Wolters Kluwer Health/Lippincott Williams & Wilkins.
Errais, E., Duplay, J., Darragi, F., 2010. Textile dye removal by natural clay – case study of fouchana Tunisian clay. Environ. Technol 31, 37–41. https://doi.org/10.1080/09593330903480080.
Forss, J., Lindh, M.V., Pinhassi, J., Welander, U., 2017. Microbial biotreatment of actual textile wastewater in a continuous sequential rice husk biofilter and the microbial community involved. PLoS One 12, e0170562. https://doi.org/10.1371/journal.pone.0170562.
Horitsu, H., Takada, M., Idaka, E., Tomoyeda, M., Ogawa, T., 1977. Degradation of p-Aminoazobenzene by Bacillus subtilis. Eur J Appl Microbiol 4. doi:10.1007/bf00508109.
Idaka, E., Ogawa, T., Horitsu, H., Tomoyeda, M., 1978. Degradation of azo compounds by aeromonas hydrophila var . 24B. J. Soc. Dye. Colour 91–94. https://doi.org/10.1111/j.1478-4408.1978.tb03398.x.
Jadhav, I., Vasniwal, R., Shrivastava, D., Jadhav, K., 2016. Review article microorganism-based treatment of azo dyes. J. Environ. Sci. Technol. 9, 188–197. https://doi.org/10.3923/jest.2016.188.197.
Jorgensen, J.H., Ferraro, M.J., 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis 49, 1749–1755. https://doi.org/10.1086/647952.
Joshi, P.A., Mhatre, K.J., 2015. Advances in applied science research. Adv. Appl. Sci. Res 6, 55–88.
Kabeer, F.A., John, N., Abdulla, M.H., 2019. Biodegradation of malachite green by a newly isolated Bacillus vietnamensis sp . MSB17 from continental slope of the Eastern Arabian Sea : enzyme analysis , degradation pathway and toxicity studies. Bioremediat. J. 0, 1–9. https://doi.org/10.1080/10889868.2019.1671790.
Kant, R., 2012. Textile dyeing industry an environmental hazard. Nat. Sci 4, 22–26.
Khandare, R.V, Govindwar, S.P., 2016. Microbial degradation mechanism of textile dye and its metabolic pathway for environmental safety,. Environ. Waste Manage.. https://doi.org/10.1201/b19243-15.
Kim, M., Chun, J., 2014. 16S rRNA gene-based identification of bacteria and archaea using the EzTaxon server, 1st ed Elsevier Ltd https://doi.org/10.1016/bs.mim.2014.08.001.

Kumar Garg, S., Tripathi, M., Singh, S.K., Tiwari, J.K., 2012. Biodecolorization of textile dye effluent by pseudomonas putida SKG-1 (MTCC 10510) under the conditions optimized for monoazo dye orange II color removal in simulated minimal salt medium. Int. Biodeterior. Biodegrad 74, 24–35. https://doi.org/10.1016/j.ibiod.2012.07.007.

Lal, N., Srivastava, A.K., 2011. Decolorization of malachite green by newly isolated bacillus strain MTCC -3330. Arch. Environ. Sci 5, 71–76.

Mandal, H.K., 2014. Influence of wastewater PH on turbidity. Int. J. Environ. Res. Dev 4, 105–114.

Manual of Basic Techniques for a Health Laboratory, 2003, 2nd ed World Health Organization, Geneva https://doi.org/10.3109/00313028209061302.

McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I.M., Marchant, R., Smyth, W.F., 2001. Microbial decolourization and degradation of textile dyes. Appl. Microbiol. Biotechnol. 81–87. https://doi.org/10.1007/s002530000587.

Osińska, A., Korzeniewska, E., Harnisz, M., Felis, E., Bajkacz, S., Jachimowicz, P., Niestępski, S., Konopka, I., 2019. Small-scale wastewater treatment plants as a source of the dissemination of antibiotic resistance genes in the aquatic environment. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2019.121221.

Ot, R., Lima, D., Paula, A., F, D.M., De Palma, D., De Arag, G., 2007. Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source. Mutat. Res 626, 53–60. https://doi.org/10.1016/j.mrgentox.2006.08.002.

Parshetti, G., Kalme, S., Saratale, G., Govindwar, S., 2006. Biodegradation of malachite green by Kocuria rosea MTCC 1532. Acta Chim. Slov. 53, 492–498.

Patel, J., 2001. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol. Diagnosis 6, 313–321. https://doi.org/10.1054/modi.2001.29158.

Periyasamy, A.P., Rwahwire, S., Zhao, Y., 2019. Environmental Friendly Textile Processing. Springer Nature Switzerland AG.

Permaul, K., Pillay, D., Pillay, B, 1996. Random-amplified polymorphic DNA (RAPD) analysis shows intraspecies differences among Xanthomonas albilineans strains. Lett. Appl. Microbiol. 23, 307–311. https://doi.org/10.1111/j.1472-765X.1996.tb00196.x.

Rajeswari, K., Subashkumar, R., Vijayaraman, K., 2011. Biodegradation of mixed textile dyes by bacterial strains isolated from dyewaste effluent. Res. J. Environ. Toxicol 5, 97–107. https://doi.org/10.3923/rjet.2011.97.107.

Ramezani, S., Pourbabaee, A.A., Daneshmand, H., 2013. Biodegradation of malachite green by klebsiella terrigenaptcc 1650: the critical parameters were optimized using taguchi optimization method. J. Bioremediation Biodegrad. 04, 1–6. https://doi.org/10.4172/2155-6199.1000175.

Roy, D.C., Saha, n, A.K., Sikdar, B., Rahman, M., Roy, A.K., Prodhan, Z.H., Tang, S.-S., 2018. Biodegradation of crystal violet dye by bacteria isolated from textile industry effluents. PeerJ 6, e5015. https://doi.org/10.7717/peerj.5015.

Sghaier, I., Guembri, M., Chouchane, H., Mosbah, A., Jaouani, J, A., Neifar, U, M., 2019. Recent advances in textile wastewater treatment using microbial consortia,. J. Text. Eng. Fash. Technol. 5, 134–146. https://doi.org/10.15406/jteft.2019.05.00194.

Shanooba, P., Dhiraj, P., Yatin, P., 2011. Microbial degradation of textile industrial effluents. African J. Biotechnol. 10, 12657–12661. https://doi.org/10.5897/AJB11.1618.

Sheam, M., Haque, Z., Nain, Z., 2020. Towards the antimicrobial , therapeutic and invasive properties of Mikania micrantha Knuth : a brief overview. J. Adv. Biotechnol. Exp. Ther. 3, 92–101. https://doi.org/10.5455/jabet.2020.d112.

Sneha, U., Poornima, R., Sridhar, S., 2014. Optimization and decolorization of malachite green using pseudomonas putida. J. Chem. Pharm. Res. 6, 50–57.

Spratt, D.A., 2004. Significance of bacterial identification by molecular biology methods. Endod. Top 9, 5–14. https://doi.org/10.1111/j.1601-1546.2004.00106.x.

Sun, Q., Lan, R., Wang, Y., Zhao, A., Zhang, S., Wang, J., Wang, Y., Xia, S., Jin, D., Cui, Z., Zhao, H., Li, Z., Ye, C., Zhang, S., Jing, H., Xu, J., 2011. Development of a multiplex PCR assay targeting O-antigen modification genes for molecular serotyping of Shigella flexneri. J. Clin. Microbiol. 49, 3766–3770. https://doi.org/10.1128/JCM.01259-11.

Upadhyay, R.S., 2002. Microbial bioremediation of textile effluents. Prog. Ind. Microbiol. 36, 331–348. https://doi.org/10.1016/S0079-6352(02)80018-2.

Wanyonyi, W.C., Onyari, J.M., Shiundu, P.M., Mulaa, F.J., 2017. Biodegradation and detoxification of malachite green dye using novel enzymes from bacillus cereus strain km201428: kinetic and metabolite analysis. Energy Procedia 119, 38–51. https://doi.org/10.1016/j.egypro.2017.07.044.

Winand, R., Bogaerts, B., Hoffman, S., Lefevre, L., Delvoye, M., Van Braekel, J., Fu, Q., Roosens, N.H.C., De Keersmaecker, S.C.J., Vanneste, K., 2020. Targeting the 16s rRNA gene for bacterial identification in complex mixed samples: Comparative evaluation of second (illumina) and third (oxford nanopore technologies) generation sequencing technologies. Int. J. Mol. Sci 21, 1–22. https://doi.org/10.3390/ijms21010298.

Wuhrmann, K., Mechsner, K., Kappeler, T., 1980. Investigation on rate-determining factors in the microbial reduction of azo dyes. Microbiol. Sew. Ind. Wastes 8.

Yaseen, D.A., Scholz, M., 2019. Textile dye wastewater characteristics and constituents of synthetic effluents : a critical review. Int. J. Environ. Sci. Technol 16, 1193–1226. https://doi.org/10.1007/s13762-018-2130-z.