Eco. Env. & Cons. 26 (November Suppl. Issue) : 2020; pp. (S439-S443)
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ISSN 0971–765X

Biodecolorization of reactive red HE7B and reactive orange 3R through Indigenous bacterial isolate *Microbacterium oryzae* strain JC8 isolated from textile effluent

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(Received 6 April, 2020; Accepted 13 June, 2020)

ABSTRACT

Residues of azo dyes are the main pollutants released from textile industries which increases the toxicity level of local water bodies. This study is focused to find out the indigenous bacterial isolates which have the potential to degrade reactive azo dyes. Total 21 bacterial isolates were isolated from textile effluent among them JC8 isolate showed the dye degrading potential which is identified as *Mycobacterium oryzae* JC8 Strain through 16S rRNA technique. GenBank accession no. MN462556 is assigned by NCBI for particular strain after submitting the data into GenBank database. 89.2% of reactive orange 3R and 84.6% of reactive red HE7B has been successfully decolorized by *Mycobacterium oryzae* JC8 Strain. Bio decolorization of these dyes were determined through UV-Vis spectrum analysis. High biodegradation rate of these isolates in natural conditions indicates the potential in treatment of azo dyes in a vast level.

Key words : *Textile industries, Reactive red HE7B, Reactive orange 3R, 16Sr RNA, Biodecolorization*

Introduction

Pollution of environment is a worldwide problem. Major component of environment (water, soil, and air) are affected through rapid industrialization and urbanization. Chemicals and other harmful agents used by these industries ultimately reach to food chain and affect the aquatic life (Moorthi *et al.,* 2007). Synthetic dyes used by these industries considered as a major pollutant for environment (Alalewi and Jiang, 2012). Among all synthetic dyes, reactive azo dyes are most extensively used in industries because of their stability and brilliance (Saratale *et al.,* 2011).

Several physical and chemical methods have been developed for mineralization of these dyes (Tiar *et al.,* 2018). These methods are costly and require high energy input. High sludge formation is also a drawback of these methods which required secondary treatment (Zille *et al.,* 2009). Microbial degradation can play a very important role in mineralization of these dyes. Many microbes such as fungi, bacteria, algae and yeast have been used to treat azo dyes (Wang *et al.,* 2009). Decolorization of reactive azo dyes using bacteria can be the best alternative of physical and chemical methods (Gudmalwar and Kamble, 2012). High growth rate and ubiquitous nature helps bacteria to achieve prominent result in degradation and mineralization in optimum condition over other microorganisms

(Maheshwari and Shivagami, 2016). This study was aimed to isolate potent indigenous bacterial isolates to decolorize reactive azo dyes i.e. reactive red HE7B and reactive orange 3R which are extensively used in sanganer textile region.

Materials and Methods

Isolation and screening of indigenous potent bacterial isolate: For isolation of indigenous reactive azo dyes decolorizing bacteria, mineral salt media (MSM) was used (Shah, 2014) with 100 mg L^{-1} of reactive red HE7B dye. 5 mL of parent effluent sample inoculated in pre sterilized 100 mL of modified MSM and flasks were kept for incubation on orbital shaker at 120 rpm and 30 °C. After 3rd day, 1 mL of incubated sample was taken out and inoculated in fresh MSM medium (Tiar *et al.,* 2018) along with 50 µl culture suspensions were inoculated into nutrient agar plate to obtained potent bacterial colonies. Bacterial isolates showed high potential in decolorizing of reactive azo dyes was coded as JC8 and selected for biodecolorization study.

Identification of potent bacterial isolate: According to Cappucino and Sherman (2008) bacterial isolates were distinguished on the basis of their cell shape, size, color and pigmentation. All biochemical tests were performed with specific requirements for each test as outlined in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). For molecular level identification of selected bacterial isolate 16s rRNA sequencing technique was used. AmpliTaq® DNA polymerase was used in sequencing of PCR product with Terminator Cycle Sequencing Kit (ABI PRISM® BigDyeTM). Blast was used to identify similarities for newly sequenced 16s rRNA sequence. Tree Dyn 198.3 software was used to tree rendering after construction of phylogeny tree through Phy ML (Dereeper *et al.,* 2008). To obtain particular accession number for bacterial isolate JC8, sequence was submitted to NCBI GenBank database.

Biodecolorization study: After screening of potent bacterial isolate, biodecolorization ability of *Microbacterium oryzae* JC8 was measured through the percent degradation method of *Adedayo et al.* (2004). Bacterial isolate JC8 was inoculated individually in 100 mL of nutrient broth medium contained 100 mg L^{-1} of dye. After every 24hr of incubation 5 mL of inoculated culture was taken out in aseptic condition to avoid cross contamination and centrifuged at 15,000 rpm for 5 minutes to exclude the bacterial slurry. Supernatant was used to measure residual dye content at ë max. Un-inoculated media with same concentration of dye (100 mg L^{-1}) was used as control.

Biodecolorozation analysis: After biodecolorization dye molecule breakdown into new substrate, possibly low molecular weight which leads to removal of color. UV- Vis spectral analysis was used to detect the decolorization and degradation of reactive azo dyes.

Statistical analyses: All the data were presented as mean ± SD values of particular experiment (Singh *et al.,* 2014). Significance of decoloriaztion percentage of dyes analyzed through one way ANOVA with Tukey-Kramer post hoc test at P-value < 0.05.

Results

This is the very first approach in decolorization of reactive azo dyes by bacterial isolate *Microbacterium oryzae*. Reactive azo dyes which are widely used in Sanganer region are almost mineralized by *Microbacterium oryzae* JC8 strain.

Bacterial Isolate JC8 found gram positive in gram reaction. In microscopic view JC8 was observed as rod shaped. It was found positive for methyl red, Citrate, Starch, Catalase and lipid utilization and found negative for Indole, Voges Proskauer, Urease, Oxidase and sulphide test. After construction of phylogenetic tree JC8 was identified as *Microbacterium oryzae*. After successful submission of sequence into NCBI, GenBank assigned the MN462556 accession number to *Microbacterium oryzae* JC8 strain.

Reactive orange 3R was declorized the most 89.28% followed by reactive red HE7B 84.66% (Figure 1). *Microbacterium oryzae* JC8 degrade mono azo reactive orange 3R almost 7% higher in comparison of di-azo reactive red HE7B after 24 hr of incubation. Dcolorization of azo dyes are dependent upon their structures. At first two days highest decolorization was observed for reactive orange 3R 21.35 % and 22.41 %. But maximum decolirization in a day was recorded for reactive red HE7B at third day 25.05% (Figure 1).

UV-Vis spectra of reactive red HE7B (Figure 2) and reactive orange 3R (Figure 3) control dye sample were compared with decolorized broth spectra of 1st day and 5th day. Decrement of absor-

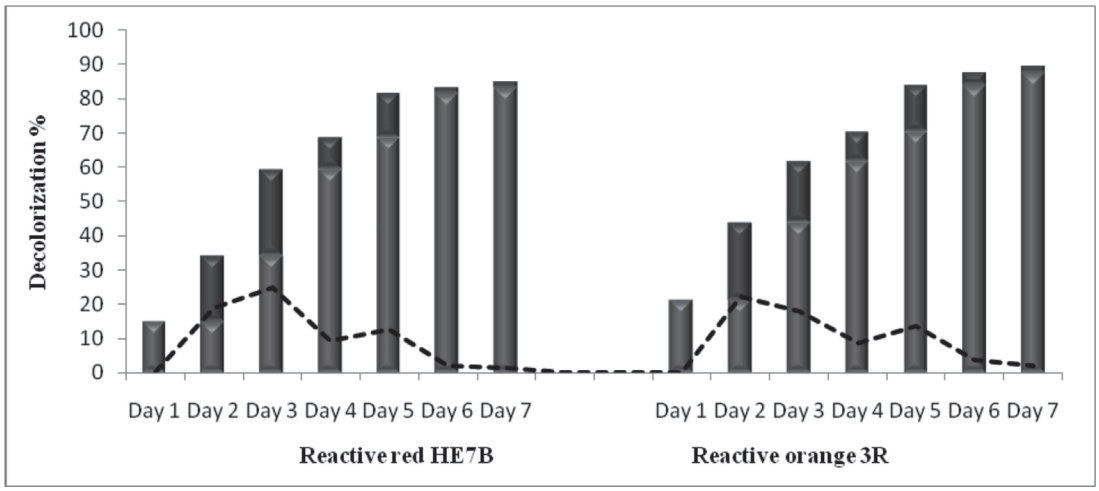

Fig. 1. Decolorization of reactive red HE7B and reactive orange 3R through *Microbacterium oryzae* JC8 (incubated at 37 °C and initial dye concentration used was 100 mg L^{-1}at pH 7)

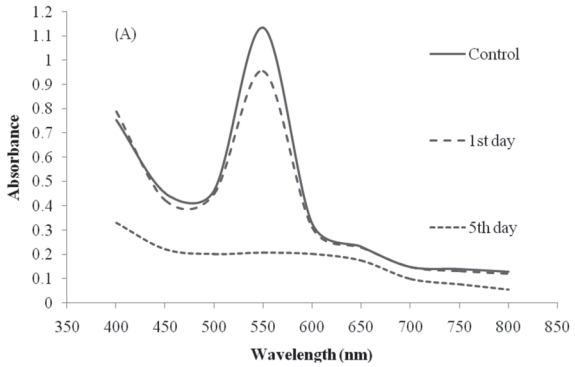

Fig. 2. UV- Vis spectra of reactive red HE7B dye and metabolite of 1st day and 5th day

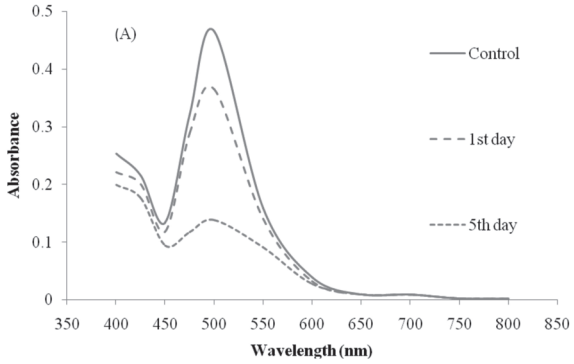

Fig. 3. UV- Vis spectra of reactive orange 3R dye and metabolite of 1st day and 5th day

bance in 1st day samples showed the initiation of biodecolorization and in 5th day samples maximum absorbance peak were almost disappeared which indicate the mineralization of reactive azo dyes intotheir simplest form.

Discussion

21 bacterial isolates were screened to identify potent isolate for reactive azo dyes decolorization. Various studies have been reported in terms of efficient azo

Table 1. Decolorization of reactive azo dyes by bacterial isolate *Microbacterium oryzae* JC8 Strain

Days/ Reactive dyes	Degradation %			
	Reactive red HE7B		Reactive orange 3R	
	$\overline{X}$ + SE	SD	$\overline{X}$ + SE	SD
Day 1	14.93 ± 0.10	0.17	21.35 ± 0.09	0.16
Day 2	34.12 ± 0.05	0.09	43.76 ± 0.11	0.19
Day 3	59.17 ± 0.12	0.19	61.65 ± 0.05	0.09
Day 4	68.65 ± 0.07	0.11	70.17 ± 0.03	0.03
Day 5	81.34 ± 0.03	0.03	83.82 ± 0.14	0.29
Day 6	83.21 ± 0.21	0.24	87.44 ± 0.08	0.13
Day 7	84.66 ± 0.07	0.11	89.28 ± 0.13	0.27

*SD –Standard Deviation, $\overline{X}$ - Mean of 3 replicates, SE – Standard Error

dye degrading bacterial isolates from textile effluent such as *Brevibacterium* sp by Franciscon *et al.* (2012); *Enterobacter* sp. by Roy *et al.* (2018), consortium of *Bacillus subtilis, Bacillus cereus*, *Bacillus mycoides*, *Bacillus* sp., *Pseudomonas* sp., and *Micrococcus* sp. by Mahmood *et al*. (2015). Micrococcus luteus by Hassen *et al.* (2013).

Although this is the first report regarding the *Microbacterium oryzae* for biodecolorization of reactive azo dyes but several other *Microbacterium* spp. have been reported earlier for degartion of dyes and other organic molecules. Roat *et al.,* (2016) studied the biodegradation abilities of *Microbacterium* spp. for diazo dye reactive blue 160. According the report *Microbacterium* spp. has great potential in degradation and detoxification of diazo dyes. Franciscon *et al.,* (2012) reported about the degradation and decolorization of four azo dyes reactive yellow, reactive black, direct blue and reactive red through *Microbacterium* sp. VN1. Apart from dye degradation *Microbacterium* spp. also reported for degradation of low density polyethylene and biofilm. Saggu *et al.,* (2019) reported that protease enzyme which was extracted from the *Microbacterium* sp. SKS10 were capable in biofilm removal in efficient manner.

Less decolorization of diazo dye in comparison of mono azo dye indicate that chemical structure plays a significant role in decoloriztion of azo dyes as also reported by the Paszczynski *et al.,* (1992). According to Perace *et al.,* (2003) dyes which posses more azo bond are hard to degrade in comparison of less azo bond dyes which is accordance to this study.

Biodecolorization of these dyes was conformed through the UV-Vis spectral analysis. Jadhav et al., (2007) reported the biodecolorization of reactive red dye mediated by *Rhizobium radiobacter* bacterium through UV-VIS spectral analysis. Degradation of reactive red 2 was also confimed through UV-VIS spectroscopic analysis which was mediated by Pseudomonas sp (Kalyani *et al.,* 2009). These findings are accordance to our study for reactive azo dye decolorization analysis by UV-VIS spectroscopy. Sahasrabudhe and Pathade (2011) confirmed the biodegradation of reactive orange 3R through UV-VIS spectra analysis. Biodegradation of dye was mediated by the bacterium *Enterococcus faecalis.* In recent study Slosarcikova *et al.* (2020) reported the reactive orange 3R degradation mediated by *Pleurotus ostreatus* after analyzing of UV-VIS spectra of degraded product.

Conclusion

Present study confirms that indigenous bacterial isolates have the potential to degrade reactive azo dyes. These isolates can be used as a bioremediation tool in degradation of other harmful chemicals at vast levels to reduce the environmental risk.

Acknowledgement

This work was carried out and completed in the Department of Microbiology of JECRC University, Jaipur.

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