Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 1366-1368

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Biodecolorization of Orange II dye by native *Bacillus* sp. and *Staphylococcus* sp. in simulated medium

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Abstract
The discharge of textile dye effluent without proper treatment poses several problems to plants, animals and humans. Biodecolorization offers an ecofriendly cost-effective clean technology for remediating such toxic color compounds from aquatic environments. In the present study, two potential bacteria were isolated from textile dye effluent employing minimal salt medium and identified as *Bacillus* sp. and *Staphylococcus* sp. following morphological and biochemical tests. The bacterial isolates were tested for orange II dye decolorization in minimal salt medium. Both the bacteria were highly tolerant to dye up to 250 mg l^{-1}. Whereas, increasing level of orange II dye exerted inhibitory effects on bacterial growth and decolorization. Maximum 68% (by *Staphylococcus* sp.) and 73% (by *Bacillus* sp.) orange II decolorization was observed at initial 100 mg orange II dye l^{-1}, pH 7.0 and 35°C under static conditions during 36 h incubation. The results indicate the possible use of these indigenous bacteria in eco-friendly sequential anoxic-oxic treatment strategy.

Keywords: Biodecolorization; *Bacillus* sp.; Orange II; *Staphylococcus* sp.; Textile effluent

Introduction
Textile dye manufacturing industry is one of the major industrial sectors in India. Azo dyes are the colored compounds due to presence of azo bond. These dyes are use in textile, cosmetic, pharmaceutical and chemical industries due to ease of production, fastness and range of colors (Claus 2002). Azo dyes constitute ~80% of annual commercial dyes produced all over the world (Daneshwar *et al*. 2007). The release of azo dyes during textile processing is problematic because of azo dyes and their degradation products such as aromatic colorless amines that are carcinogenic in nature. The colored effluents are not only aesthetically unacceptable, but also reduces the photosynthetic activity of aquatic flora, which causes death and putrefaction of aquatic flora and fauna (Sahoo and Gupta 2005; Garg and Tripathi 2011; Garg and Tripathi 2017).
Textile powerlooms and their products are raw cotton based wet fabrics, the processing of which includes desizing, scouring, bleaching, mercerizing, dyeing, printing and finishing stages (Garg and Tripathi 2013). Different types of chemicals are used during various steps of textile manufacturing. These powerlooms release wastewater into nearby streams through public drains, and also in open areas. The untreated dye effluent causes harmful effects to the natural ecosystem due to the toxicity of dyes and chemicals used during fabric processing steps. Therefore, biotreatment of such effluent offers a clean technology for environmental protection (Garg and Tripathi 2013).
Dyes and their degradation products are reported to cause adverse effects to human health and environment. Thus, there is an urgent need to restrict their release into the surrounding environment without proper treatment. The physicochemical methods employed for dye decolorization are costly and generate secondary solid waste (Garg *et al*. 2012). Conventional biological strategies alone or in combination with physical and/or chemical methods have also been attempted. However, the modern biological processes have received attention due to less sludge generation cheap bioprocessing, ecofriendly and applicability to wide range of dyes (Garg *et al*. 2017).
Keeping the above in view, an ecofrindly and cost effective strategy for dye decolorization is necessary. Thus, the present study was aimed to isolate efficient indigenous bacterial isolates from textile effluent and employ them for biodecolorization of azo orange II dye in simulated minimal salt medium.

Materials and Methods
Sampling
The textile effluent sample was collected in sterile plastic containers and glass bottles from the

discharge point of powerloom, situated at Tanda, Uttar Pradesh (India). The collected samples were transported on ice to the laboratory and stored at 4 ºC in refrigerator till further use.

Isolation, screening and identification of orange II dye decolorizing native bacteria

The native bacterial isolates was screened from the samples employing minimal salt medium (MSM) of pH 7.0 containing (g l^{-1}): K_2HPO_4 1.0, $CaCl_2$ 0.02, $FeCl_3$ 0.05, $MgSO_4$ 0.02 with carbon and nitrogen sources amended with orange II dye at 100 mg l^{-1}. Further the grown potential bacterial cultures were purified and identified using morphological and biochemical tests as per standards of Bergeys Manual of Determinative Bacteriology (Holt *et al*. 1994).

Dye decolorization trials

The dye decolorization experiments were performed in minimal salt medium (MSM) containing orange II dye at 100 mg l^{-1}. The medium was inoculated with selected *Staphylococcus* sp. and *Bacillus* sp., and incubated for 72 h at 35°C under static culture condition. The samples were drawn periodically at 24 h intervals, and analyzed spectrophotometrically for bacterial dye decolorization.

Effect of dye concentration

To determine the effect of varied initial dye concentrations on decolorization, a range (50-250 mg l^{-1}) of orange II dye were taken in MSM broth and incubated for 72 h under static culture condition.

Analytical determination

Dye decolorization assay

The extent of orange II dye decolorization was determined as per the method of Zimmermann *et al*. (1982). The samples were centrifuged at 10000 rpm for 10 min in a cooling centrifuge (4°C). The supernatant was analyzed spectrophotometrically at 482 nm against control (uninoculated dye medium) for estimating per cent orange II biodecolorization (Garg and Tripathi 2013). The extent of dye decolorization was calculated using the following formula:

$$\text{Decolorization extent (\%)} = \frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}} \text{ X 100}$$

Statistical analyses

Each experiment was performed in triplicate. The standard deviation (SD) was calculated using Microsoft Excel, and the results are presented as mean ± SD value.

Results and Discussion

Characterization of textile orange II dye decolorizing bacteria

Thirteen bacterial cultures were isolated from the textile dye effluent sample on simulated minimal agar medium amended with orange II dye. However, only two bacterial cultures RML1 and RML2 were selected (based on their higher tolerance dye tolerance) for further studies. Both the cultures were identified using morphological and biochemical tests as per the Bergey's Manual of Determinative Bacteriology (Holt *et al*. 1994) in our laboratory (not shown). On the basis of microbiological and biochemical tests the isolates were identified as *Bacillus* sp. and *Staphylococcus* sp., respectively. Further both the native potential bacterial isolates were used for dye decolorization study in simulated minimal salt medium containing orange II dye.

Effect of dye concentration

Optimization of dye concentration should always be considered as a prerequisite for biodecolorization studies. The results revealed that the extent of dye decolorization decreased with increase in orange II concentration (50-250 mg l^{-1}) by both the bacterial isolates. However, complete biodecolorization was observed at 50 mg l^{-1} dye concentration by both *Bacillus* sp. and *Staphylococcus* sp. Whereas maximum 68% (by *Staphylococcus* sp.) and 73% (by *Bacillus* sp.) orange II dye biodecolorization was observed at initial 100 mg orange II dye l^{-1}, pH 7.0 and 35°C under static conditions during 48 h incubation. Dye decolorization was sharply decreased when the concentration increased from 150 to 250 mg l^{-1} in minimal salt medium.

Table 1: Effect of orange II dye concentration on its decolorization by *Staphylococcus* sp.

Dye concentration (mg l^{-1})	Percent decolorization by *Staphylococcus* sp.					
	Time (h)					
	0	12	24	36	48	60
50	0	24.2	53.7	84.0	99.3	99.5
100	0	11.5	39.7	68.0	67.5	67.0
150	0	7.3	31.8	43.5	44.0	44.7
200	0	2.9	8.5	22.0	15.2	15.5
250	0	0	7.0	8.5	8.0	7.3

*Values were statistically significant at ≤ 0.05.

Table 2: Effect of orange II dye concentration on its decolorization by *Bacillus* sp.

Dye concentration (mg l^{-1})	Percent decolorization by *Bacillus* sp.					
	Time (h)					
	0	12	24	36	48	60
50	0	21.5	60.0	82.0	98.5	99.0
100	0	19.8	55.9	78.0	77.2	76.5
150	0	7.5	35.0	40.2	41.0	37.0
200	0	0	2.5	4.0	5.2	3.9
250	0	0	0	0	0	0

*Values were statistically significant at ≤ 0.05.

Similar to our observation, Garg *et al*. (2012) also reported that that when dye concentration increased, the extent of dye biodecolorization by *Pseudomonas putida* isolate was decreased. The decreased color removal might be due to the toxicity of dye molecule at higher levels. Many researchers reported bacteria that were able to perform azo dye(s) decolorization (Garg *et al*. 2011, 2012, 2014, 2015; Garg and Tripathi 2013; Grekova-Vasileva *et al*. 2009; Saratale *et al*. 2012). The dye concentration can influence the extent of dye biodecolorization through a combination of factors including the toxicity of dye at higher concentrations, and ability of enzyme to recognize the substrate efficiently at very low concentrations that may be present in some wastewaters (Garg and Tripathi 2013). Ghodake *et al*. (2011) reported 71% decolorization of initial 100 mg amaranth l^{-1} by *Acinetobacter calcoaceticus* within 72 h incubation. Further increase in dye concentration was inhibitory for biodecolorization. Other researchers also reported that the extent of dye decolorization was inversely related with dye concentration in simulated MSB and textile effluent (Dayaram and Dasgupta 2008; Garg *et al*. 2012; Garg and Tripathi 2013).

Conclusions

Two efficient dye decolorizing bacteria were isolated and identified from textile dye effluent. High degree extent of orange II biodecolorization was showed by both the indigenous bacterial cultures at 100 mg dye l^{-1} concentration under static culture conditions. The strain was tolerant to orange II dye up to 250 mg l^{-1}, while the maximum 68% (by *Staphylococcus* sp.) and 73% (by *Bacillus* sp.) orange II decolorization was observed at initial 100 mg orange II dye l^{-1}. Whereas complete dye decolorization was observed at 250 mg l^{-1} concentration. The increasing concentration of dye was inhibitory for orange II dye biodecolorization. The dye decolorization by native bacterial cultures indicates the possible use of these indigenous isolates for ecofriendly biodecolorization of azo dyes under natural environmental conditions.

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