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ISOLATION OF INDIGENOUS *BACILLUS* SPP. FROM GARDEN SOIL TO DECOLORIZE SYNTHETIC TEXTILE DYES

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Textile and clothing industries are major contributor to the economic growth in Bangladesh. Establishment of a number of such industries are imparting huge amount of industrial waste containing different types of chemicals including dyes. Pollutants generated from textile industries creates a huge burden on the environment. Textile industries discharge effluents containing various harmful chemicals including synthetic dyes that are very stable and a threat to living organisms. *Bacillus* spp. are remarkable bacteria which demonstrated potential to produce diverse kinds of metabolites for different uses. This study focuses on the potential use of *Bacillus* spp. isolated from the garden soil of Stamford University Bangladesh for decolorization of BemacronBlue RS (BB) and BemacronRed RS 01 (BR) dyes. Four *Bacillus* isolates were screened out from garden soil and named as 1B, 3A, 2C and 4B. Isolates were subjected to decolorization assay with 0.002 gm/l of BB and BR dyes. *Bacillus* spp. showed great potential in decolorizing BB and BR dyes, which was initiated after 2 days of incubation. Following 8 days of incubation, decolorization of BB was, 79%, 80%, 75%, 77% and BR was, 75%, 73%, 69%, 89% by the isolates 1B, 3A, 2C and 4B, respectively. This study shed some light on the potential use of indigenous garden bacteria for decolorization of textile dyes to control environmental pollution.

Keywords: *Bacillus*, synthetic dye, optical density, decolorization, Bangladesh

INTRODUCTION

The major sources for the developing economy of Bangladesh are the textile and clothing industries. 80.7% foreign exchange and 12.36% of GDP (Gross Domestic Product) were earned from ready-made garment industries in 2016-17 (1). In Bangladesh, textile industries are using a large volume of water every day, where 90% of the water appears as waste water which may contain various types of organic, inorganic chemical compounds, including chlorinated compounds, heavy metals, pigments and textile dyes (2, 3). 10 to 15% of the textile dyes are lost in the flowing during the production and processing of the product (4). The textile, leather and food industries mostly use synthetic dyes; even these dyes are also used in agricultural research and cosmetics due to their low cost, availability and wide color range (5). Synthetic dyes are organic compounds which contain unsaturated fatty acids such as -C=C-, -N=N-, -C=N- responsible for coloring and fixation of fibers. These dyes can sustain in the environment for a long time as they are stable to light and washing (6). Synthetic dyes are xenobiotic compounds and, due to their presence in nature for a long time, can disturb light penetration and photosynthesis (7, 8).

If effluents containing dyes and other recalcitrant compounds of industries are released into the environment without any treatment, bioaccumulation of these compounds may occur in the food chain which ultimately affects human health (9, 10). Filtration, adsorption, photolysis, use of activated carbon, chemical flocculation used by industries to treat effluent before releasing into nature (2, 6, 11). But these approaches are expensive compared to biological approaches, and produce huge amounts of clay and mud that are difficult to dispose of (12). The biological approach gained interest among different industries as it is eco-friendly, has low cost and creates less clay and mud. Microorganisms including bacteria, yeasts, fungi and algae are mostly used in biological approaches as these microorganisms can decolorize dyes, even completely mineralize azo dyes that are widely used dyes in various industries (13).

There are several studies on biodegradation of synthetic textile dyes by bacteria isolated from textile effluent or sludge. *Bacillus* spp. can produce diverse metabolites and enzymes which can be applied for mitigating environmental pollutants (14, 15). Here, we would like to look at the biodegradation potential of *Bacillus* species isolated from garden soil containing various kinds of flowering plants to screen their decolorization potential against BB and BR.

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MATERIALS AND METHODS

Chemicals and Media: Textile dyes namely, BemacronRed RS (BR) and BemacronBlue RS 01 (BB) were collected directly from a textile industry located in Savar, Dhaka. The chemicals used in this work were of analytical grade. Microbiological media and medium ingredients were purchased from Oxoid, UK.

Sample collection and processing: Soil samples were collected in sterile beaker using sterile spatula at a depth of 2-3 cm from rich garden soil in Siddeshwari campus of Stamford University Bangladesh, Dhaka, Bangladesh and transported to the laboratory and stored at 4ºC before and after experiment. One gram of soil sample was dispensed into 99 ml of sterile distilled water and homogenized. One ml of homogenized soil sample was transferred into 9 ml sterile distilled water and serial dilution was carried out to 10^{-6} dilution.

Screening and identification of *Bacillus* spp.: Screening and identification of *Bacillus* spp. were performed following the previously described procedure with minor modifications (16). In brief, serially diluted bacterial cultures (100 µl) were spreaded on nutrient agar media and incubated at 37ºC for 24 h. Subsequently, isolated colonies were streaked on starch agar media containing starch as the only carbon source for starch hydrolysis test to detect their amylolytic activity. The plates were incubated at 37ºC for 24-48 h. Following incubation, plates were flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5 g potassium iodide solution, and 125 ml of water, kept at room temperature) to identify clear zone around the colony. Deep blue color around the growth indicates negative result that is no amylolytic activity where clear zone of starch hydrolysis was produced by amylase producers. The pure cultures showing clear zones were subcultured at regular interval and maintained on to nutrient agar slants at 4ºC. Isolated amylase producing bacteria were presumptively identified by Gram staining, cultural and biochemical characteristics. The biochemical tests that were performed includes methyl red (MR), voges-proskauer (VP), citrate utilization, indole production, H_2S production, motility, gelatin hydrolysis, sugar hydrolysis, oxidase, catalase and carbohydrate fermentation.

Dye decolorization assay: Decolorization experiment of those two selected dyes was performed by using 0.002 gm/L of dye in 15 ml test tubes containing 10 mL of nutrient broth. A 100 µL of 24 h old bacterial culture corresponding to Mcfarland standard 0.5 was used as inoculum to inoculate the dye supplemented broth. The inoculated test tubes were incubated at 37ºC for 2, 4, 6 and 8 days in a shaking water bath at 80 rpm. Following incubation, decolorization of dyes by selected isolates was determined at their specific maximum wavelength in the culture supernatant using a UV-spectrophotometer. After incubation at each time period, samples were centrifuged at 10,000 rpm for 10 min and the supernatants were subjected to UV-spectrometry and the absorbance was recorded. The uninoculated media with BR and BB dyes were served as respective blank for the dye decolorization assay. The percentage of dye decolorization was calculated as stated before (17).

$$\text{Decolorization (\%)} = \frac{\text{(Initial OD} - \text{Final OD)}}{\text{Initial OD}} \times 100$$

RESULTS

Isolation and characterization of *Bacillus* spp.:

Among all the isolates collected from soil samples, a total of four isolates of *Bacillus* spp. (1B, 2C, 3A, 4B) were identified. All isolates were subjected to characterization by morphological (Table 3), cultural (Table 4) and biochemical tests (Table 5) and found to belong to the genus, *Bacillus*.

Table 1: Microscopic observation of the *Bacillus* isolates.

Microscopic observation	1B	2C	3A	4B
Shape Arrangement	long rod single, chain	long rod single, chain	long rod single, chain	long rod single, chain
Gram Reaction	positive	positive	positive	positive
Spore Staining	Spore present	Spore present	Spore present	Spore present

Decolorization of dye by the isolates:

In our present study, the selected isolates were tested independently for their ability to decolorize 0.002 gm/L of the two textile dyes. The experiment was performed in a time-dependent manner for 2, 4, 6 and 8 days. Decolorization percentage of the BB dye with the isolated *Bacillus* spp. is showed in Figure 1. In most of the cases, isolates showed a time-dependent decolorization of BB dye from 2 to 8 days. After 8 days of incubation, BB dye showed nearly 79%, 80%, 75%, 77% decolorization by the isolate 1B, 3A, 2C and 4B, respectively.

Decolorization percentage of BR dye by the *Bacillus* isolates is presented in Figure 2. Here, a time-dependently induced dye decolorization was observed. Following 8 days incubation, 75%, 73%, 69%, and 89% BR dye decolorization was observed in a time-dependent manner by the isolates 1B, 3A, 2C, and 4B.

Table 2: Colony characteristics of the *Bacillus* isolates on nutrient agar media.

Characteristics	1B	2C	3A	4B
Size	Large	Large	Medium	Large
Shape	Irregular	Round	Round	Round
Margin	Lobate	Entire	Entire	Undulate
Elevation	Flat	Umbonate	Umbonate	Flat
Consistency	Butyrous	Butyrous	Butyrous	Butyrous
Texture	Smooth	Smooth	Smooth	Smooth
Opacity	Opaque	Opaque	Opaque	Opaque
Odor	Earthy	Earthy	Earthy	Earthy
Pigmentation	Creamy white	Creamy white	Creamy white	White

Table 3: Biochemical characteristics of the *Bacillus* isolates.

Tests	Media	Characteristics			
		1B	2C	3A	4B
Starch hydrolysis	Starch agar plate	+++	+++	++	+
Methyl red test	GPB broth	-	+	-	+
Voges-proskauer test	GPB broth	-	-	-	-
Citrate utilization	Simmons citrate agar slant	-	-	+	-
Indole production	1% peptone	-	-	-	-
H_2S production	2% peptone	-	-	-	-
Motility test	MIU media	+	+	+	+
Gelatin hydrolysis	Gelatin media	-	-	-	-
Sugar hydrolysis	Triple sugar iron agar slant	A/A	A/A	A/A	K/A
Oxidase	Nutrient agar	+	+	+	+
Catalase	Nutrient agar	+	+	+	+
Carbohydrate fermentation	Glucose	+	+	+	+
Carbohydrate fermentation	Fructose	-	+	+	+
Carbohydrate fermentation	Maltose	+	+	+	+
Carbohydrate fermentation	Lactose	+	-	+	+
Carbohydrate fermentation	Dextrose	-	+	+	+
Carbohydrate fermentation	Sucrose	-	+	+	+

Note: GPB= Glucose Phosphate Broth, MIU= Motility Indole Urea, A=Acid, K=Alkaline.

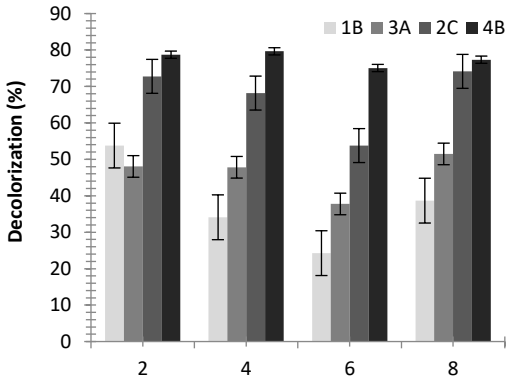

Figure 1: Decolorization of BB dye by the isolates 1B, 3A, 2C and 4B following 2, 4, 6, and 8 days.

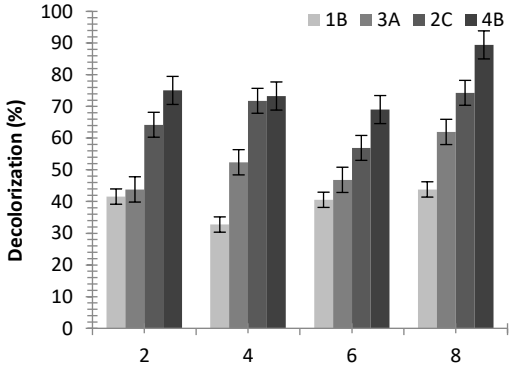

Figure 2: Decolorization of BR dye by the isolates 1B, 3A, 2C and 4B following 2, 4, 6, and 8 days.

DISCUSSION

In this study, soil samples were collected from the garden containing various types of flowering and fruit plants. We believed that the soil would be a good choice to isolate *Bacillus* spp. as our previous study showed the potential of *Bacillus* spp. collected from the same garden of Stamford University Bangladesh had good potential in Crystal Violet dye docolorization (18). Following morphological, cultural and biochemical analysis, we presumptively, isolated four *Bacillus* isolates for this study.

All isolates showed the potential decolorizing capability of the two textile dyes, BB and BR. With a careful optimization of environmental conditions and incubation period, the potential can excel. The effects of pH, temperature, initial dye concentration and inoculum size greatly influence dye degradation capability. It was previously observed that dye degradation rate was remarkably decreased when higher concentrations of dye were used. Similarly, with reducing inoculum size, the degradation rate also reduced and the most significant result was obtained when 10% inoculum was used (19).

In our study, dye decolorization is synonymous with dye degradation as following centrifugation, bacterial isolates were pelleted and retained their original color, which indicated that the color of the dyes did not adsorb onto bacterial cells. Therefore, decolorization was due to degradation, not because of absorption into bacterial isolates. There was neither growth nor decolorization shown in the control tubes. Thus, the decolorization was due to the metabolic activity of the isolates.

Our spectrophotometric data showed a great potential of BR and BB dye decolorization by the isolates. This is explained by Bheemaraddi *et al.* who elucidated that the organisms are capable of utilizing the dyes as the sole source of carbon with minimal nutritional requirements under static conditions (20, 21). Only a few organisms are capable of utilizing the synthetic dyes present in the environment. The decolorization rate can be induced by performing the optimization experiment with various time, temperature, pH, inoculum size and can achieve a optimal condition for performing the experiment with great success. Furthermore, a consortium of isolates showed increased dye decolorization of Methyl red and Carbol fuchsin up to 100% and 96%, respectively, within only 24 h (6). Therefore, mix consortia can be a good choice to achieve increased dye decolorization in a short time. Here, co-metabolic activities might be involved to make accessible of the dye to one organism that is otherwise unable to degrade the dye through partial metabolism by other organisms.

CONCLUSION

The natural color is replaced by synthetic colors due to rapid commercial industrialization. They are used in tanning, textiles, printing, leather and many more industries. Their massive use is harmful for micro- and macro-biota due to severe water pollution. Although there is containment to expose the harmful effluent in nature after physical and chemical treatment. But, many industries dispose of their effluent with no treatment or partial physical and chemical treatment. These problems can be reduced by introducing microbes to degrade the residual dyes present in the effluent with minimal cost. The potential of isolated dye degrading microbes can be utilized in this regard following detailed studies on process parameters for bioremediation and on genes responsible for degrading the dye and further genetic manipulation to improve the potentiality of these organisms to solve the pollution problems caused by synthetic dyes.

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