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Isolation and characterization of dye degrading bacteria from textile industrial waste, Panskura, West Bengal, India

KEYWORDS: Dye, Decolorizing bacteria, Paenibacillus sp., Acrolite Fast Green PGN, ABIS-ON LINE.

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ABSTRACT *The dye decolorizing bacteria Paenibacillus sp. were isolated from the textile effluent samples collected from Annapurna Cloth Printing Industry, Ghatal, Paschim Medinipur, West Bengal. The isolated HKW9 and HKS4 bacterial strain showed maximum dye decolorization property against Acrolite Fast Green PGN of 95% and 94% respectively. From the characterization (morphological and biochemical) and Advanced bacterial identification software (ABIS-ON LINE), the isolates (HKW9 and HKS4) are Paenibacillus nematophilus and Paenibacillus antarcticus respectively. High decolorization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.*

INTRODUCTION

Water is life but now a-days due to the advancement in urbanization and industrialization, it is spoiling a lot. Many contaminants present in waste water such as, acid, bases, toxic organic and inorganic dissolve solids. Among them, colors are considered the most undesirable and are mainly caused by the dyes. Dyes unusually have a synthetic origin and complex aromatic molecular structure which make them more stable to biodegrade. First synthetic dyes was reported in 1856, there are more than 40,000 dyes and pigments with some 7,000 different chemical structures, out of which 3,500 dyes are of practical use. The world wide annual production of dyes is over 7.105 tons. Consumer of the dyes includes textile, tannery, paper, pulp and leather processing industries (Rita, 2012). The effluents of those industries are highly colored and the disposal of these wastes into receiving water causes damage to the environment. In India, many pigment industry are present, most of them are located in Mumbai, New Dehli, Hydrabad and Haldia. Some industries located in India are given below:-

Kolorjet Chemicals Pvt Ltd, New Delhi-Manufacturer and exporter of chrome pigments. Also offering chrome pigments, inorganic pigments, pigment dispersions, industrial pigment dispersions and precision pigment dispersions.

Rung International, Mumbai -Leading manufacturer & exporter of anthocyanin color powder. Also deal in color pigment, color additives, food dyes, dyestuffs and food colors.

Shree Nathji Dyetuffs, New Delhi- Manufacturers of pigment powders, organic pigment powders, inorganic pigment powders, fluoroscent pigment powders, pigment emulsion pastes, pigment fine pastes, pigment red powders, pigment blue powders ,colours for cosmetic and pigment violet powders

Vega International ,Morvi -Leading manufacturer and exporter of pigment powders, ceramic pigment and indian pigments. Also offering alumina grinding balls and frits for wall tiles.

Sajan Overseas ,Ahmedabad -Manufacturing and exporter of pigment powder which includes chemical powders, pigment paste, gum powders, dye powders, color powders, zinc powders, silicon powders, silver powder and glitter powders, sulphate, pevri powder.

Shree Laxmi Industries ,Jaipur- Leading manufacturer and exporter of ultramarine blue powders and pigment powders.

Nikita Extracts, Ahmedabad -Manufacturer & exporter of color pigment. Also offering agar wood oil, ajowan oil, aniseed oil, annatto color & aromatherapy fragrance.

Swalorporation Ltd., Haldia - Dimethanate Fenithrothion, Ethion, Malathion**.**

Different synthetic dyes are used for various industrial applications in huge quantity and improper disposal of those dyes into environment causes a serious damage. Depending on exposure time and dye concentration, dyes can have acute and chronic effects on exposed organisms and the presence of very small quantities of dyes in water (less than 1 ppm) is highly visible due to their brilliance. The greatest environmental concern with dyes is their absorption and reflection of sunlight entering water. Light reflection diminishes photo synthetic activity of algae and seriously influence on the food chain. Many dyes and their breakdown product are carcinogenic, mutagenic and toxic to life. Textile dyes can cause allergies such as contact dermatitis and respiratory disease, allergic reaction in eyes, irritation to mucous membrane and the upper respiratory tract. Reactive dyes form covalent bonds with cellulose woolen and PA fibers. It is assumed that in the same way reactive dyes can bind with -NH2 and –SH group of proteins in living organisms. A lot of investigations of respiratory disease in workers dealing with reactive dyes have been made. Certain reactive dyes have caused respiratory sensitization of workers occupationally exposed to them. Removal of color from dye bearing waste water in a complex problem because of difficulty in treating such waste waters by conventional treatment method. Photo oxidation, activated carbon, reverse osmosis, ion exchange membrane filtration and flocculation are applied for color removal from textile effluents. These physico-chemical methods are less effluent, costly with limited applicability and produce wastes, which are difficult to dispose off. In

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some cases, traditional biological procedures were combined with chemical or physical treatment processes to achieve better decolorization.

Biotechnological tools also have been applied for the degradation of various textile dyes and it was found that up to 70% color removal was noticed with different micro flora. As viable alternative biological processes have received increasing interest owing to their cost effectiveness ability to produce loss sludge and environment benignity. Efforts to isolate bacterial culture capable of degrading azo dyes started in the 1970s with reports of a *Bacillus subtilis* (Chang J.S., Chou C. and Chen S.Y. 2001). Bacterial isolates from soil and sludge sample belonging to Bacillus sp. *Alcalgenes sp. Aeromonas sp.* was found to have high dye decolourization ability (Aksu Z. and Do¨nmez G. 2003). Cyanobacteria like *Gloeocapsa sp. Phormidijun ceylanicum sp* decolorized acid red 97 and FF sky blue dye more than 80% after 26 days (Claus, H. 2002). Decolourization of direct Yellow and Emo red dyes by bacteria and actinomycetes were studied (Chang J.S. and Kuo T.S. 2000).

In recent years a number of studies have focused on some microorganisms that are able to degrade and absorb dyes from wastewater. A wide variety of microorganisms are capable of decolorization of a wide range of dyes some of them are as bacteria: *Escherichia coli* NO3 (Chang J.S. and Kuo T.S. 2000), *Pseudomonas luteola* (Chang J.S., Chou C. and Chen S.Y. 2001), *Aeromonas hydrophila* (Chen K.C., Wu J.Y., Liou D.J. and Hwang S.C.J. 2003); fungi: *Aspergillus niger* (Fu Y. and Viraraghavan T. 2002), *Phanerochaete chrysosporium, Aspergillus terricola* (Saikia N. and Gopal M. 2004), *P. chrysosporium* (Fournier D., Halasz A., Thiboutot S., Ampleman G., Dominic M. and Hawari J. 2004); yeasts: *Saccharomyces cerevisiae, Candida tropicalis, C. lipolytica* (Aksu Z. and Do¨nmez G. 2005); algae: *Spirogyra* species (Gupta V.K., Rastogi A., Saini V.K. and Jain N. 2006); *Chlorella vulgaris* (Acuner E. and Dilek F.B. 2004), *C. sorokiniana* (De-Bashan L.E., Moreno M., Hernandez J.P. and Bashan Y. 2002), *Lemna minuscula* (Valderama L.T., Del Campo C.M., Rodriguez C.M., de-Bashan E. L. and Bashan Y. 2002), *Scenedesmus obliquus, C. pyrenoidosa and Closterium lunula* (Yan H. and Pan G. 2004).

Therefore, biological method have been successfully used to clean up dye from a textile effluent but their applications in land remediation are still in the stage of infancy. Thus ,this study aims to isolate and charecterised the potential bacteria for decolorization effluent containing a textile.

MATERIALS AND METHODS

Sample collection

Water samples were collected from the effluent of Annapurna cloth printing industry private limited at Ghatal (kasibazar), West Bengal. Soil samples were collected from drainage canal that carry textile effluent located 100 meters away from industry. All samples were collected in sterile glass-screw cap tubes and preserved at 4°C in refrigerator and samples were tested within 24 hrs of collection.

Dye collection: Textile dye, Acrolite Fast Green PGN was collected from Annapurna cloth printing industry private limited.

Isolation of bacterial colony from collected sample

The bacterial colony were isolated from textile dye effluent and soil sample by serial dilutions and plating method through appropriate dilutions on modified Zhou and Zimmermann(ZZ) agar medium (M. Ponraj1, K. Gokila1 and Vasudeo Zambare 2011).

Screening of dye decolourizing bacteria

The dye decolorizing bacteria were screened using modified method of Sapna and Sandeep (2012). Decolorization activity was performed in 100ml of Nutrient Agar media containing 0.02g of Acrolite Fast green PGN and 10% (v/v) inoculums of each isolate colony. Uninoculated dye served as control. Inoculated medium and control was incubated at 30°C for 6 days under shake culture condition. About 2 ml samples were withdrawn aseptically and centrifuged at 8000rpm for 15 minutes. The clear supernatant was used for measuring absorption at 600nm using UV-vis spectrophotometer. The percent decolorization of effluent was determined by using the formula**.**

D = [(Ao-A1) / Ao]×100 ; Where, D- Decolorization in %; Ao-initial absorbance; A1-final absorbance.

BIOCHEMICAL TEST

Casein hydrolysis test

The organisms were spread on the NA-Casein medium and incubated for 48hrs at 37°C. The halo zones on the plate indicate the utilization of casein from the medium (Cappuccino and Sherman, 2005)

Starch hydrolysis test

The organism was spot inoculated on the NA-Starch medium and incubated for 48hrss at 37°C. The plates were then treated with iodine cubes and halo zones were observed which indicates the utilization of starch by both the organism (Cappuccino and Sherman, 2005).

Gelatin hydrolysis

The organism was spot inoculated on the NA-gelatin medium and incubated for 48hrs at 37°C. Gelatin hydrolysis was indicated by clear zones around gelatinase-positive colonies. In some cases, plates are flooded with HgCl2 to precipitate unhydrolysed gelatin making the clear zones. Results are often observed within 5-10 min after flooding with $HgCl_2$ (Cappuccino and Sherman, 2005).

IMViC test

Indole test

The organisms were taken and inoculated in freshly prepared and sterilized peptone water. The tubes were incubated for 24hrs at 370C. 4-5 drops of Kovac's Reagent was added to the tubes. No color change was seen as both the organisms are indole negative.

Methyl red test

The organisms were taken and inoculated in MR-VP broth for 24hrs at 37°C. The MR Reagent was added to the tubes and interpretation was taken, red color indicated as positive result.

Voges-proskauer test

The organisms were taken and inoculated in MR-VP broth and incubated at 370C for 24 hrs. 5-6 drops of Barritt's reagent A was added and then 2-3 drops of Barritt's reagent B was added to the tube, red color indicated as positive result.

Citrate utilization test

The organisms were inoculated on Simmons citrate agar slants and incubated for 24-48 hrs at 37°C. The color change from green to blue was noted as positive but here no color change was observed in case of both the organisms.

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Catalase test
The organism was taken in a slide and few drops of 3% H_2O_2 was added to it. Effervescence Evolved indicates the production of H_2O_2.

Carbohydrate fermentation
An inoculum from a pure culture is transferred aseptically to a sterile tube of sugar (Maltose, Glucose, Fructose, Mannitol and Galactose) broth. The inoculated tube is incubated at 35°C-37° C for 24 hours and the results are determined. A positive test consists of gas formed, indicating a pH change to acidic.

Species identification
Phenotypic analysis of the isolates was done by advanced bacterial identification software (ABIS ONLINE).

ABIS online is software developed as a lab tool for bacterial identification. Bacteria identification results are purely informative and are not intended to be an official point of view. Because of the permanent changes in bacterial nomenclature and classification, som e bacterial names and taxa may not comply with the Approved Lists of Bacterial Names

RESULTS
Physico-chemical characterization of collected samples
The samples were collected in sterilized container from respective sites. The color, Nature of samples was recorded and tabulated (Table-1).

Table-1 Physical and chemical Characteristics of water samples

SL NO	SAMPLE	NATURE OF SAMPLE	COLOR	P^H
1	Waste Water	Liquid	Dark green	8.2
2	Soil	Solid	Light green	8.0

Isolation of dye decolorizing bacteria
The bacterial colony was developed after 24hr incubation and twenty two colonies (ten colony from soil and designated as HKS1-10 and twelve colony from water and designated as HKW1-12) were selected for the determination of dye decolonization activity. All 22 isolates were tested individually for their ability to decolorize Acrolite Fast Green PGN at the concentration 50mg/L. All isolates of both samples decolorize the dye with different capacity from 45% to 95% but HKS4 and HKW9 showed maximum decolorizing property as 94% and 95% respectively, (Table 2 and 3).

Table-2 Decolorization of Acrolite Fast Green PGNby bacterial isolates from soil sample

Sample	Bacterial isolates	Decolorization(%)
S O I L	HKS 1	80
	HK S2	45
	HK S3	55
	HK S4	94
	HK S5	85
	HK S6	80
	HK S7	80
	HK S8	80
	HK S9	60
	HKS10	85

Table-3 Decolorization of Acrolite Fast Green PGN by bacterial isolates from water sample

Sample	Bacterial isolates	Decolorization(%)
WASTE WATER	HKW1	70
	HKW2	85
	HKW3	90
	HKW4	85
	HKW5	90
	HKW6	60
	HKW7	90
	HKW8	80
	HKW9	95
	HKW10	65
	HKW11	80
	HKW12	85

Selectetion of more effective dye decolorization bacterial isolates
Eight potential isolates namely; (Soil-HKS4, HKS5, HKS6, HKS10), (Water-HKW3, HKW5, HKW7, HKW9) showed good Decolorization efficiency about 76% to 95%. Dye degrading isolates were identified on the basis of morphological and biochemical character (Table 4 and 5).

Table-4 Gram's staining property and morphological characteristics of bacterial isolates from soil and waste water sample

Sample	Isolates	Gram reaction	Shape
S O I L	HKS4	Gm (-ve)	Rod
	HKS5	Gm (-ve)	Round
	HKS6	Gm (-ve)	Rod
	HKS10	Gm (-ve)	Rod
W W A A S T T E E R	HKW3	Gm (-ve)	Rod
	HKW5	Gm (-ve)	Rod
	HKW7	Gm (-ve)	Rod
	HKW9	Gm (-ve)	Rod

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Table-5 Colony characteristics of dye decolorizing bacterial isolates from soil sample and waste water on Zhou and Zimmermann agar media

Sample	Isolates	Colony characteristics							
		Size	Shape	Margin	Elevation	Surface texture	Consistancy	Opacity	Pigmentation
SOIL	HKS 4	M	Round	Irregular	LowConvex	Smooth	B	TP	LC
	HKS 5	S	Round	Even	Convex	Smooth	B	OP	LC
	HKS 6	S	Round	Even	Convex	Smooth	B	OP	LY
	HKS10	S	Round	Irregular	Flat	Rough	B	OP	LC
WWAASTTER	HKW3	M	Round	Irregular	Flat	Smooth	B	OP	LC
	HKW5	M	Round	Irregular	Flat	Rough	B	OP	LC
	HKW7	S	Rough	Even	Convex	Smooth	B	OP	LO
	HKW9	B	Round	Even	Flat	Smooth	B	OP	LC

Abb:-S-Small, M-Moderate, L-Large, B-Buterious , LY-Light Yellow, LC-Light Cream, LO-Light Orange, TP-Transperent, OP-Opaque,

Table-6 Fermentation property of bacterial isolates from soil and waste water sample

Sample	Isolate	Fermentation									
		Glucose		Maltose		Mannitol		Fructose		Galactose	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
SOIL	HKS 4	-	-	+	-	-	-	+	-	-	-
	HKS5	+	-	+	-	+	-	+	+	-	-
	HKS6	+	-	+	-	+	-	+	+	+	-
	HKS10	+	-	+	-	+	-	+	-	-	-
WWAASTTER	HKW 3	+	-	+	-	+	-	+	+	+	-
	HKW5	+	-	+	-	+	-	+	-	+	-
	HKW7	+	-	+	-	+	-	+	-	-	-
	HKW 9	+	-	+	-	+	-	+	+	+	-

Table-7 Biochemical property of bacterial isolates from soil and waste water sample

Sample	Isolates	Hydrolysis			Indole production	Methyl Red	Voges proskaur	Citrate utilization	Catalase Test
		Casein	Starch	Gelatin					
SOIL	HKS4	-	+	-	-	+	-	-	-
	HKS5	-	+	-	-	+	-	+	-
	HKS6	-	+	-	-	+	-	-	-
	HKS10	-	+	-	-	+	-	+	-
WWAASTTER	HKW3	-	+	-	-	+	-	+	-
	HKW5	-	-	-	-	+	-	-	-
	HKW7	-	+	-	-	+	-	+	-
	HKW9	-	+	-	-	-	-	-	-

Species analysis using advanced bacterial identification software (ABIS ONLINE)

From the software analysis, it was concluded that the isolate bacteria (HKS4 and HKW9) showed maximum decolorizing property (94% and 95%) are-

Paenibacillus antarcticus(HKS4) ~79%
Paenibacillus nematophilus(HKW9) ~79%

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DISCUSSION

The textile and dyeing industry are one of the industries, which contribute mainly to the soil and water pollution. Large amount of dye containing effluents are discharged into water bodies by these industries carrying pollution problem. This pollution problem is a topic of great public and government concern today, forced by legislation. The industrial units are now looking forward to cost effective solutions for reduction of pollution loads to meet the regulatory requirements. The bacterial cultures which have high Decolorization property were identified by microscopic (Table-4), colony character (Table-5), biochemical characters (Table-6 and 7) and Soft ware programming (ABIS ONLINE).

The present study report the high Decolorization of textile dye effluent by bacteria with maximum Decolorization effect. The dye decolorizing bacterial *Paenibacillus sp.* were isolated from the textile effluent samples collected from Annapurna Cloth Printing Industry, Ghatal, Paschim Medinipur, West Bengal. HKW9 (*Paenibacillus antarcticus)* and HKS4 (*Paenibacillus nematophilus)* bacteria strain showed maximum dye Decolorization (Acrolite Fast Green PGN) of 95% and 94% respectively.

The Gram's staining indicated that all isolates are Gram negative and rods shape except one isolate (Table-4). The additional information from Gram staining was in the form of cell morphology and arrangement. The growth pattern of these isolates on ZZ agar media was small round.

The isolation of different microorganisms from the sample indicates the natural adaptation of microorganisms to survive in the presence of toxic dyes. The difference in their rate of Decolorization may be due to the loss of ecological interaction, which they might be sharing with each other under natural conditions. Similar result also obtained by Saikia N. and Gopal M. (2004).

The difference in Decolorization pattern is due to the dissimilarity in specificities, structure and complexity, particularly on the nature and position of substituent in the aromatic rings and the interaction with azo bond with different dyes as reported by many authors (Carliell, C. M., Barclay, S. J., Naidoo, N., Buckley, C. A., Mulholland D. A. and Senior, E. 1995). The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as dye containing effluent.

CONCLUSSION

The textile dye (Acrolite Fast Green PGN)) is degradable under aerobic conditions with a concerted effort of bacteria isolated from textile dye effluent. The results reported here warrant further investigation (Molecular level) to establish the usefulness of these isolates for bioremediation and biodegradation application such as effluent containing dye. High Decolorization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

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