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Efficiency of bacterial isolates to degrade textile dyes

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Abstract : *The increasing demand for colourfast and non fading dyes has led to the rapid growth in the use of reactive dyes, the majority of which are azo dyes which are not recyclable and scarcely biodegradable due to their complex structures. Increasing concern about their discharge into water bodies from industries has accelerated the need for new treatment schemes and to find our study three bacterial strains of genus Bacillus, Paenibacillus and Pseudomonas were isolated from dye contaminated soil. Paenibacillus and Bacillus rapidly decolorized and degraded a methyl orange azo dye Solution i.e. 47.3% and 43.6% respectively. Bacillus also degraded 26.5% Methyl red dye while Pseudomonas degraded it the least. These strains exhibited a remarkable decolourization capability at pH 7 and at temperatures 37^{0}C to 42^{0}C. Methyl orange and methyl red were found non-toxic after degradation and supported the growth of natural flora. Methyl orange and Methyl red inhibited the three selected stains at the concentration of 5 mg/ml while Congo red at the concentration of 4 mg/ml was inhibitory.*

Key words: *Bacterial strains, Degradation, Decolourization, Toxicity assay, Minimal inhibitory concentrations.*

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Introduction :

Dyes are used in wide range of industries like food, pharmaceuticals, cosmetics, leather but are of primary importance to textile manufacturing. Dyes used in textile industries have a synthetic origin and complex aromatic molecular structures which make them stable and difficult to be biodegraded. 80% of the commercial dyes used all over the world in textile industries are Azo dyes. Azo dyes, contain one or more azo bond (-N=N-) as its chemical structure and account for its major contribution in all textile dyes used. Estimations state that 10-15% of the total dye is lost during synthesis and the dyeing processes. Due to its high reactivity it is difficult to process the dye and the

total usage, nearly 10% of dyes are released into the environment as toxic wastes (Prasad, 2014).

Water pollution due to effluents from textile dyeing industries is a cause of serious concern. Bioremediation, a biodegradation process in which sites contaminated with xenobiotics are cleaned up by means of bacterial bio-geochemical processes, preferably in situ, exploits the ability of microorganisms to reduce the concentration and/or toxicity of a large number of pollutants. Microorganisms play a very important role in the biodegradation and mineralization of these dyes which is of great significance (Olaganathan *et al.*, 2012).

The biological process of biosorpation studies helps in reducing toxicity of hazardous chemical pollutants which now cause a havoc within the environment and even cause human health problems like hypertension, dizziness, fever, headache, etc. Such degradation processes are environment friendly, efficient in treatment strategy, cost effective, rapidly developing field of environment restoration and are also competitive alternative to chemical decomposition process (Aftab et al,. 2011). 15% textile industrial waste are dumped in Ganges which is responsible for serious health effects on human population (Suganya *et al.*, 2014).

The present study was undertaken to isolate aerobic microorganisms capable of decolorization/ degradation of some dyes like Methyl Orange, Methyl Red, Methyl Violet, Methyl Blue and Congo Red which are not only used in textile industries but also widely used in educational institutions.

Materials and Methods :

Sample Collection : The soil sample used in this study was collected from Dyeing unit, Bank Road, Gandhi Maidan, Patna. The sample was used within 24 hrs of collection for the processes of isolation, testing and estimation for physical characterization of sample, pH, colour, odour and dry weight of the soil sample were determined.

Isolation and Screening of Bacterial Strains: Nutrient agar media was used for isolation of bacteria by using spread plate and streak plate technique under aseptic conditions and incubated at 37^0C temperature for 24 hours until growth was observed. Along with serial dilution technique, some plates were inoculated with direct soil sample and kept in incubator at 37^0C. The isolates were purified by repeated sub-culturing and preserved on nutrient agar slants under refrigerated conditions for further use. Six strains were selected based on the differences in their cultural characteristics. The selected bacterial stains were tested for their growth in presence of different dyes introduced in Minimal salt medium which contained : $(NH_4)_2SO_4$, $CaCl_2$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $Na_2MoO.2H_2O$, Na_2HPO_4, $Na_2HPO4.7H_2O$, KH_2PO_4 (Mahmood et al., 2011). The dyes used were Methyl Orange, Methyl Red, Methyl Blue, Methyl Violet and Congo Red (Gopalakrishan et al., 2011). Three most luxurious growth showing strains were selected for further studies.

Cultural and morphological characterization of isolates: Colonies were identified on the basis of their colony morphology, colour, texture and elevation. The morphological character of isolates were observed by smears from isolated colonies, stained by Gram's method (Suganya et al., 2014).

Biochemical Characterization: For biochemical characterization isolates were tested for catalase, hydrolysis of urea, casein, starch, gelatin and cellulose, hydrogen sulphide production, nitrate reduction and sugar fermentation tests.

Degradation and decolourization of dyes:

Each of the selected dye was mixed in minimal salt medium in the ratio 1:100 after which isolated strains were inoculated in the medium under aseptic conditions. The inoculated tubes were incubated at 37^0C for one and a half months. Optical Density (OD) was taken at 600nm at an interval of four days. The tubes were shaken

regularly. Controls were prepared by inoculating test cultures in basal medium without dyes. Decolorization if any was noted. Viability of strains were checked on regular intervals (Aftab *et al.*, 2011).

Toxicity assay: Replica Plating technique was employed for toxicity assay of the degraded dyes. Serial dilution of soil (from natural surrounding) was done upto 10^{-6}. Master plates were prepared by spread plate technique from 10^{-5} and 10^{-6} dilutions. Two sets of Nutrient agar media plates were poured and solidified. Replica plating was performed on them. On one set, the non degraded dye solution was introduced. On the other set the degraded dye products (collected supernatant after centrifugation to remove microbial cell cultures) were introduced. The growth on the two sets was observed after 24 hours of incubation and was compared with the master plates.

Optimization: Two parameters were taken into consideration for optimization — pH and temperature (Aftab *et al.,* 2011). Nutrient broth was prepared and pH was adjusted in four sets (5, 6.6, 7, 7.8). It was autoclaved and inoculated with test cultures. Initial absorbance was taken at 600nm and the broth tubes were incubated at 37°C for 24 hours. After incubation the final absorbance was taken at 600nm. Test cultures were streaked on Nutrient Agar media after sterilization and solidification. The plates were incubated at 4°C, 27°C, 37°C and 50°C for 24 hours. Luxuriant growth was an indicator of the favourable temperature.

Minimum Inhibitory Concentration: The viability of the strains at different concentrations of dyes was tested. Concentrations of 2 mg/ml, 4 mg/ml and 5 mg/ml were used of different dyes against the strains which were found to degrade them. The strains were inoculated under aseptic conditions in the different concentration of dye containing basal medium. Initial absorbance was noted at 600nm. The inoculated tubes were incubated for 24hrs and then final absorbance was noted (Prasad, 2014).

Results and Discussion :

Physical Properties of Soil sample collected: The pH of the sample was 6.7 with earthy odour and bluish black colour. The moisture content of 5g of the sample was 0.9 g (18%).

Cultural and morphological characterization of isolates: Three isolates selected on the basis of their growth were found to be rod to short rod in shape and creamy to light greenish in colour having opaque density with regular margin. Strain CI and C2 gave positive while strain C3 gave negative result after Gram's staining.

Biochemical Characterization: The observation of the biochemical tests performed for the selected strains is shown in table 1.

Table 1: Observation of the biochemical tests performed for C1, C2 and C3 isolates.

Biochemical Tests	C1		C2		C3	
Catalase Test	Negative		Negative		Negative	
Nitrate Production	Negative		Slightly Positive		Positive	
H_2S Production	Slightly positive		Positive		Slightly positive	
Amylase Production	Slightly positive		Positive		Positive	
Gelatinase Production	Positive		Positive		Positive	
Urease production	Positive		Slightly positive		Slightly positive	
Cellulase Production	Positive		Negative		Slightly positive	
Casein Hydrolysis	Slightly positive		Positive		Positive	
Fermentation Test	**Acid**	**Gas**	**Acid**	**Gas**	**Acid**	**Gas**
Glucose	Positive	Negative	Positive	Negative	Positive	Negative
Sucrose	Negative	Negative	Negative	Negative	Negative	Negative
Fructose	Negative	Negative	Negative	Negative	Negative	Negative

Thus on the basis of the cultural, morphological and biochemical characterization CI may be *Bacillus firmus,* C2 may be *Paenibacillus* spp and C3 may be *Pseudomonas.* However, for confirmation of the bacterial strains, molecular characterization should be done.

Degradation and decolourization of dyes: Absorbance taken at 600nm for one and a half month to check for degradation of dyes by bacterial isolates are summarized in figure I. Strain Cl was found to degrade three dyes i.e Methyl Orange, Methyl Red and Congo Red.

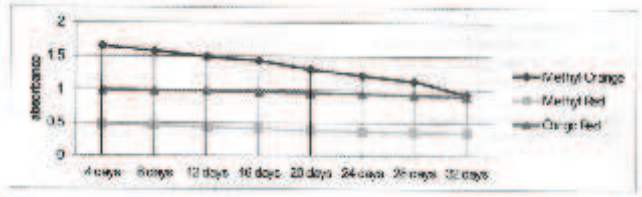

Fig. 1. Degradation trend of dyes by strain C1

Strain C2 was found to degrade two dyes i.e. Methyl Orange and Methyene Blue. The value of the absorbance is summarized in figure 2.

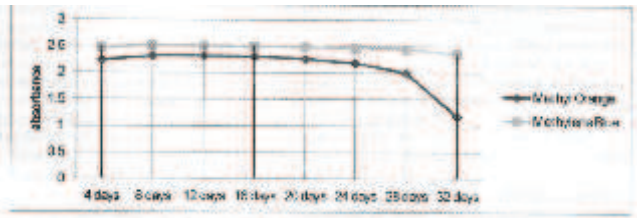

Fig 2: Degradation trend of dyes by strain C2 *(Paenibacillus)*

Strain C3 was found to degrade two dyes i.e Methyl Orange and Methyl Red. The value of the absorbance are summarized in figure 3.

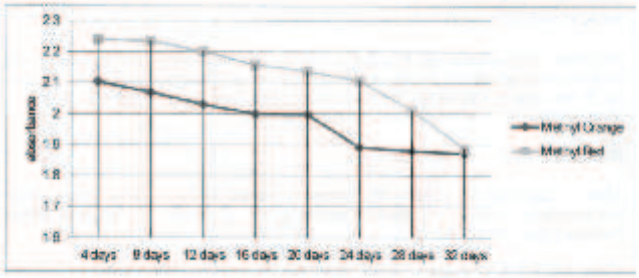

Fig 3: Degradation trend of dyes by strain C3 (*Pseudomonas*)

Decolourization: Methyl Red turned maroon in due course of experimental proceedings while methylene blue and methyl orange turned greyish black and brown respectively.

Optimization: 37°C was found to be most favourable temperature for all the three strains. However, a slightly lower optimum temperature has been reported by Guo *et al.* (2008) for strain Cl *(Bacillus). Besides* this pH 7 was found to be suitable for all the isolated strains.

So, from this study, it could be concluded that neutral pH supported bacterial activity to decolorize the selected dyes. Similar results have been observed by earlier workers (Mali *et al.,* 1999; Chang *et al., 2000).*

Toxicity Assay: The degraded dye product supported growth of natural flora of microbes which non degraded dye products did not. The toxicity of methyl orange, methyl red also decreased considerably.

Minimum Inhibitory Concentration: The minimum inhibitory concentration for both Methyl Orange and Methyl Red was found to be near 5 mg/ml. For Congo Red and Methylene Blue it was near 4 mg/ml, which were degraded by strain C I and strain C2 respectively. C I *(Bacillus)* was found to degrade 43.6% Methyl Orange, 26.5% Methyl Red and 9.8% Congo Red. *C2 (Paenibacillus)* was found to degrade 47.3% Methyl Orange and 4.4% Methylene Blue and C3 *(Pseudomonas)* was found to degrade 11.2% Methyl Orange and 15.9% Methyl Red.

Conclusion :

Manmade activities are disturbing the environment which is caused by the disposal of non degradable organic and inorganic wastes into the natural ecosystem. Degradation by microorganisms as an alternative has proved to be very effective due to their natural processing. Dyes being xenobiotics with complex structures are not much prone to these microbes but some of the isolates have appreciable degrading potential. Methyl orange shows a high rate of degradation by these isolates. Applied industrially these strains can be used to treat effluent extensively.

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References :

Aftab U., Riaz Khan M., Mahfooz M., Rehman A., Hassan Aslam S (2011). 'Decolourization and degradation of textile azo dyes by *Corynebacterium* sp. isolated from industrial effluent.' *Pakistan J Zool.,* 43(I): 1-8.

Chang, J.S., T.S. Kuo, Y. Qu, J. Guo, P. Wang and Zhang 'H. (2000.) 'Azo dye decolorization with a mutant *Escherchia coli* strain.' *Biotechnology Letters,* 22: 807- 8j2.

Gopalakrishnan R., Sellappa S (2011). 'Decolourisation of Methyl Orange and Methyl Red by Live and Dead Biomass of Fungi.' *Asian J Exp. BioI. Sci.,* 2(4): 569-574.

Guo, 1., J. Zhou, D. Wang, C. Tian, P. Wang and Uddin S A (2008). 'A novel moderately halophilic bacterium for decolorizing azo dye under high salt condition'. *Biodegradation* 19: 1519.

Mahmood S., Arshad M., Khalid A. and Huma N Z (20 II). 'Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater.' *Soil Environ.* 30(1): 712.

Mali, PL.,.Mahajan MM, Patil DP and Kulkarni MY (1999). 'Biodecolorisation of members of triphenylmethane and azo groups of dyes'. *Journal of Scientific and Industrial Research 59:221*224.

Olaganathan R, Jamila P (2012). 'Biodegradation of textile dye Anthraquinone Vat Blue 4 by *Pseudomonas aeruginosa.' International Journal of Advanced Biotechnology and Research.* 3(4): 743-751.

Prasad M P (2014). 'Studies on the degradation of textile dye by *Pseudomonas aeruginosa ' Research Journal of Recent Sciences* I (I): 59-62.

Suganya K., Revathi K., Anuradha V (2014). 'Decolourization of dyes using bacteria isolated from textile dye effluent. *'Asian Journal of* Pharmaceutical Sciences & technology 4(2): 68-71.