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Increasing Efficiency of the Dye Degrading Bacteria by Plasmid Transfer Method

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ABSTRACT

Background: Two local strains of *Bacillus* sp. were isolated from industrial effluent and screened for dye degradation from industrial effluent. *Bacillus* sp. and *E. coli* isolated from effluents had a low capacity but high growth rate and these are aerobic microorganisms.

Methods: Both bacterial strains were used on the basis of resistant to either co-Trimoxzo or cephalexin antibiotics for the screening of transformed bacteria. *Bacillus* sp. and *E. coli* were sensitive and *Pseudomonas* sp. SUK1 was resistant to the same antibiotic respectively. *Pseudomonas* sp. SUK1 was used a as source of the plasmid (donor strain) to transform *Bacillus* sp. and *E. coli*. Competent bacterial cells were prepared by cold $CaCl_2$ treatment while Heat shock method was used for transformation. Transformed bacteria were screened by using the antibiotic sensitivity test and agarose gel electrophoresis. These techniques lead to increased dye degradation capacity.

Results: Transformed *E. coli* was isolated and screened according to their antibiotic resistance and also found increased Red BLI (89.60%) dye degrading capacity in aerobic condition.

Conclusion: The transformed bacteria can degrade 50 mg l^{-1} of individual dyes and even a mixture of dyes (which was actually the condition in the effluent of textile dye industry) within 16 hrs.

Key-words: *Bacillus* sp., Dye degradation, *E. coli, Pseudomonas* sp. SUK1, Plasmid, Transformation

INTRODUCTION

Textile dyeing effluents containing recalcitrant dyes are polluting water due to their color and by the formation of toxic or carcinogenic intermediates Such as aromatic amines from azo dyes [1,2]. Azo dyes are aromatic compounds with one or more-N= N-Groups and also the largest class of synthetic dye used at commercial level [3] such as textile, food, paper making and cosmetic industries [4,5]. Microbial consortium was effectively used for degradation of different types of dyes [6]. In last decade more focus was given for the dye decolorization with fungal systems [7].

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The general mechanism of bacterial dye degradation has triggered by azoreductase enzyme and related transport proteins. But unfortunately studies at genetic level for dye degrading proteins are rare. Thus far very few articles in literature were found dye decolorization by gene or plasmid transfer. Exogenous DNA transfer in to the recipient cell through DNA transformation can permanently or transiently alter the heredity [8]. To enhance dye degradation capacity of bacteria at genetic level, two strategies are generally preferred; (i) Using strains containing additional corresponding genes integration with chromosome and (ii) Using Plasmid-containing strains or transformation of plasmid [9]. In this paper, we aimed to introduce a plasmid isolated from *Pseudomonas* sp. SUK1, *E. coli* and *Bacillus* sp. to obtain high efficient dye degrading, aerobic, and fast growing transformed organism.

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MATERIALS AND METHODS

This research work was carried out from January 2014 to November 2014. *Pseudomonas* sp. SUK1, which was a potential dye degrader under anoxic condition [10] was obtained from the Department of Biochemistry, Shivaji University, Kolhapur, India and used in this study. *Bacillus* sp. was isolated from the effluent of textile dye industry using minimal agar medium. Luria Bertani (LB) broth and agar were used to propagate *Pseudomonas* sp. SUK1 strain at 37^0C with anoxic condition. Bacterial cell lysis was performed by alkaline lysis method [11]. LB agar medium was supplemented with antimicrobial sensitivity testing with Himedia Combi VII octa disc for antibiotic resistance test and for the selection of transformed bacteria. LB agar plus 50 mg l^{-1} dye was used to detect the dye decolorization capacity i. e. degradation of the dye after incubation at 30°C for 24 hrs in anoxic condition. Nutrient agar was used for the growth of recipient and transformed bacteria also for dye degradation.

A loopful of microbial culture was inoculated in 250 ml capacity Erlenmeyer flask containing 100 ml nutrient broth. After 24 hrs, dye was added at concentration 50 mg ml^{-1}. Aliquots of 3 ml was withdrawn from culture media at different time intervals, centrifuged at 5000 rotation per minute (rpm) for 15 min and separated the bacterial cell mass.

Decolorization of dye was determined by measuring the absorbance of medium at respective optimal wavelength via colorimetric assay and percent decolorization was calculated as follows-

Dye Decolorization (%) =

$$\frac{\text{Initial absorbance - Observed absorbance}}{\text{Initial absorbance}} \times 100$$

Plasmid isolation was done using the miniprep method [11]. Plasmid analysis was performed on agarose gel electrophoresis and visualization by using Ethidium bromide as a staining dye [12]. Isolated plasmid DNA was eluted from agarose gel by melting agarose after visualization of DNA band. The isolated plasmid was transformed into competent cells of *E. coli* and *Bacillus* sp. by cold calcium chloride ($CaCl_2$) treatment method [13]. The transformant was selected on LB agar plates containing antibiotics disk and screened through antibiotic sensitivity test [14]. The transformant microbial colonies were designated as *E. coli* X1. In another LB agar plates containing antibiotics for *Bacillus* sp. was not show any growth on LB agar plate containing antibiotic disc.

Microorganisms and Cultivation- *E. coli* X1, transformed bacteria harboring plasmid was cultivated in nutrient agar containing antibiotic co-Trimoxzo at the concentration 200 ug l^{-1}.

Measurement of dye concentration- Azo dye used in this study was Red BLI obtained from Manpas and Textile Dye Industry, Ichalkaranji, Maharashtra, India. The concentration of azo dye was measured by using double beam spectrophotometer and absorbance of supernatant of the media at 540 nm [15].

Batch decolorization operations- In typical batch decolorization tests early stationary phase culture or 24 h grown *E. coli* X1 mixed with dye (Red BLI) to undertake bacterial decolorization under static condition with monitoring of dye at designated time interval. Unless stated otherwise, the dye concentration was 50 mg l^{-1}. Physiological conditions like pH and temperature of the reaction solution were maintained 7 and 30°C respectively [16].

RESULTS

The isolated bacterium was successfully transformed with the plasmid DNA of *Pseudomonas* sp. SUK1. Isolated plasmid and genomic DNA profile were observed on agarose gel electrophoresis (Fig. 1). The isolated plasmid was approximately 2000 base pairs (bp) in size after comparing with mid-range DNA ladder of 1000 bp. Antibiotic sensitivity assay shown co-Tromoxil can be used for selection of transformant. Therefore co-Tromoxil and other antibiotics was screened and used for detection of transformant (Fig. 2). Antibiotic resistant and sensitivity pattern was shown that recipient microorganism acquired resistant for co-Tromoxil lead to confirm the transfer of plasmid (Table 1). This technique was very useful in the process of gene transfer. Antibiotic screening markers are useful in genetic engineering and molecular biology.

Transformed microorganism *E. coli* X1 showed very high efficiency for dye degradation in aerobic conditions with an ambient temperature of 30°C in around 16 h with 96% degradation of Red BLI, 95% for Navy Blue-HER, 94% for Golden Yellow-HER as per Table 3. Also, it decolorized a mixture of 7 dyes with 82.54% decolorization (Fig. 3).

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Batch decolorization of dye Red BLI by the wild type *E. coli* had shown much less degradation of 42.22 % (Table 2). Comparatively, transformed *E. coli* was able to decolorize Red BLI with 89.60% decolorization and also few other dyes with higher efficiency (Table 3).

Table 1: Antimicrobial sensitivity testing against microorganisms

Antibiotics	Microorganisms		
	***Bacillus* sp.**	***E. coli***	***Pseudomonas* sp. SUK1**
Amoxycillin (10 mcg)	R	S	S
Cloxacillin (5 mcg)	R	R	R
Erthromycin (15 mcg)	S	S	S
Tetracycline (10 mcg)	S	S	S
Penicillin (2 mcg)	R	R	R
Co-Trimoxzo (25 mcg)	S	S	R
Penicillin V (3 mcg)	R	R	S
Cephalexin (30 mcg)	S	S	R

R= Antibiotic resistant, S= Antibiotic susceptible

Table 2: Dye decolorizing Capacity of *E. coli* (wild type)

Dye	Time (h)		Time (h)	
	24 h	**Decolorization (%)**	**48 h**	**Decolorization (%)**
Golden Yellow-HER	0.174	28.68	0.127	47.95
Green HE-4BD	1.011	3.80	1.003	4.57
Navy Blue-HER	0.480	29.20	0.333	50.88
Yellow 4G	0.183	29.06	0.150	41.86
Red HEA	0.198	26.39	0.163	39.40
Reactive Orange TGLL	0.355	18.58	0.246	43.58
Red BLI	0.520	42.22	0.490	45.55

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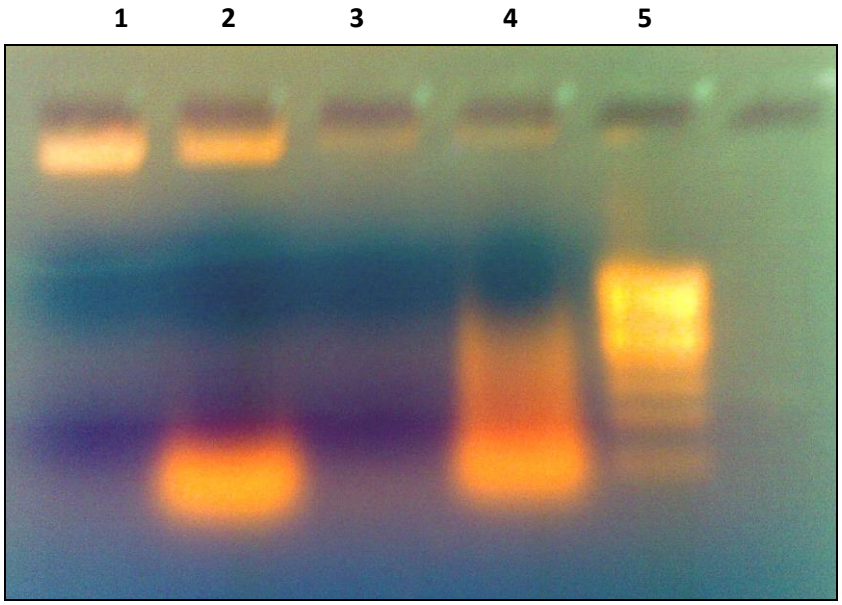

Fig. 1: DNA profile of *Pseudomonas* sp. SUK1 on 1% Agarose gel electrophoresis
Lane 1: Control DNA (48kb), **Lane 2:** Chromosomal DNA, **Lane 3:** Blank,
Lane 4: Plasmid DNA (~2000bp), **Lane 5:** 1000 bp ladder DNA

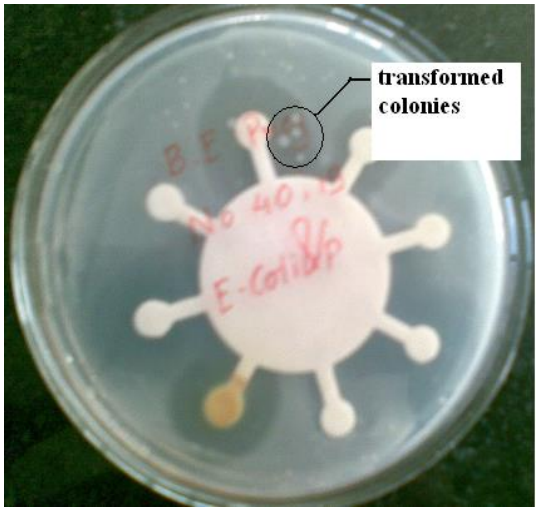

Fig. 2: E. *coli* genetically transformed colonies obtained with antibiotics as screening markers

Table 3: Decolorization of dyes by transformed *E. coli*

Dye	Wavelength (nm)	Initial Reading	After 24 h	Decolorization (%)
GoldenYellow-HER	620	1.55	0.11	94.53%
Green HE-4BD	550	1.90	0.27	85.50%
Navy Blue-HER	430	1.88	0.12	95.10%
Yellow 4G	420	1.79	0.14	91.63%
Red HEA	680	1.91	0.15	92.00%
Reactive Orange TGLL	620	1.56	0.45	71.23%
Red BLI	540	1.80	0.16	96.11%

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Fig. 3: Degradation of mixture of 7 dyes by transformed *E. coli* X1 (82.54 %)

DISCUSSION

Pseudomonas sp. SUK1 is a facultative anaerobe and requires anaerobic conditions for degradation of dyes, whereas the transformed organisms can grow and degrade dyes aerobically. While *E. coli* was able to grow in effluent conditions but cannot degrade the dye efficiently. Therefore by transformation, fast growing ability of *E. coli* under effluent condition and the dye degrading capacity of *Pseudomonas* sp. SUK1 was combined in transformed bacteria which can be used directly in the effluent to treat it by degrading the dyes efficiently. Horizontal mobility of plasmid was a very often phenomenon in nature [17,18]. Most of *Pseudomonas* strains were carrying degrading plasmid for example Tol and IncP Plasmid [19]. Similarly, resistant plasmids were good source of screening marker as well as a useful bacterial DNA vector [20]. Bacterial plasmids pGNB1 and NAH7 can efficiently degrade dyes as well as transferable [21,22]. Advancement in this process was transfer of degrading plasmid in bacteria to make more efficient dye degrading transformant which was a prominent alternative as compared to conventional biodegradation process. Horizontal mobility of plasmid was used widely for finding multidrug resistance in pathogenic microorganisms. Therefore transformant *E. coli* X1 dye degrading efficiency was increased by more than two fold for textile dye Red BLI.

CONCLUSIONS

Therefore results indicated that transformed bacteria will be good and effective strain for biotransformation of the textile dyes. Plasmid transfer is also a natural process.

The transformed bacteria can degrade 50 mg l^{-1} of individual dyes and even a mixture of dyes (which is actually the condition in the effluent of textile dye industry) within 16 hrs. Thus, the transformed bacteria have in situ application where both the organisms were difficult to use.

In future genetically modified strains can be used more efficiently for effective and diverse carcinogenic dye degradation as compare to the wild type alternative source. Efforts need to be focused on dye degrading enzyme system manipulation at genetic level will be more efficient method in biodegradation. Enzyme immobilization and making a multi enzyme nano-flower system will be effective method for biodegradation. This will be the economical, as well as widely suitable method for small and large industrial scale effluent treatment plants.

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CONTRIBUTION OF AUTHORS

All authors equally contributed in this article.

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