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MOLECULAR CHARACTERIZATION AND AZOREDUCTASE ACTIVITY OF *PSEUDOMONAS STUTZERI* ISOLATED FROM TEXTILE DYE EFFLUENT

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ABSTRACT

Azo dyes are extensively used in textile and other industrial processing. Besides its toxic nature, its removal from the wastewater is a challenging task for the researchers. A screening study on Reactive Black B azo dye decolourizing microorganism followed by biochemical and molecular characterization resulted in the identification of organism as *Pseudomonas stutzeri* (KR013216). Azoreductase enzyme was found to be the core enzyme involved in the decolourization mechanism.

Key words: Azo dyes, *Pseudomonas stutzeri*, Reactive Black B, Azoreductase.

INTRODUCTION

For the past few decades water pollution is one of the serious threats to our society. Major contribution in water pollution is done by textile industries. Dyes constitute *chromophores* and *auxochromes*[1]. The problem of azo dyes is the pollution, it causes due to toxic heavy metals[2]. Effluents containing these azo dyes are directly released into the environment without any pretreatment[3]. Therefore, to reduce the environmental impact various stratagies are employed for the treatment of textile waste water[4-6]. Many physico chemical techniques involved possess major disadvantages. As an alternative to this, biological treatment methods with the involvement of microorganisms are nowadays employed for the deolourization and degradation of textile effluents[7].

Microorganisms found to be able to decolorize textile dyes includes bacteria, fungi and yeasts[8-10]. Key enzymes linked to color removal by bacteria are oxidoreductase enzymes such as laccase and azoreductase[11,12]. *Pseudomonas* species, particularly *P. putida* and *P. aeruginosa species* are well-known to produce extracellular azoreductase[13].

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Azoreductases are found to have a variety of applications including textile dye effluent treatment, hair dye bleaching, inkjet manufacturing processes, synthetic dyes preparation etc.[14-16] The present study aim for the isolation and characterization of azo dye degrading bacteria from textile effluent.

EXPERIMENTAL

Materials and methods

Microorganism and chemicals

Dye effluent samples were collected in sterile containers from textile industry, Tirupur, Tamil Nadu, India. Reactive Black B dye was purchased from Himedia (Mumbai, India).

Isolation and screening

The effluent sample was subjected to serial dilution and spread plated on nutrient agar at temperature-25ºC and pH7.4. Further the colonies were sub cultured on nutrient broth in same culture conditions and isolated pure colonies were subjected to screening experiments by the addition of 4 mg/L Reactive black dye in the nutrient agar medium for 24 h. Based on greater clearance zone, single colonies were picked for further studies.

Identification of dye decolorizing bacteria

Biochemical and Molecular characterization of the isolate was carried out by 16S rRNA sequencing and phylogenetic were constructed.

Decolorization potential assay

The isolate, identified as *Pseudomonas stutzeri* was tested for decolorization of azo dye (Reactive Black B). Dye decolourization experiment was carried out in 250 mL flask containing 100 mL of textile dye solution (0.1%). Followed by sterilization, 1% inoculum was added and it was kept at 130 rpm in shaker at 37°C. 10 mL of samples were withdrawn at 24 hours intervals and then it was centrifuged for 20 min at 5000 rpm and the supernatant was collected.

Determination of the colour of the samples

The % decolorization of supernatant was assessed by using UV-Vis spectro-photometer at 597 nm and it was determined by –

$$\% \text{ Decolourization} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

RESULTS AND DISCUSSION

Isolation and characterization of dye degrading microbe

Among the samples collected, strain which was found to produce greater zone of clearance on azo dye containing nutrient agar medium was selected. From the isolated positive organisms, hyperactive strain was selected based on the degradation of Reactive Black B in the agar medium and dye broth. The selected most active strain was used for further studies because of their superiority in decolorizing azo dyes when compared to other strains. Fig. 1 depicts the bacterial strain with maximum decolourization capacity, Fig. 2 shows the result obtained after gram staining.

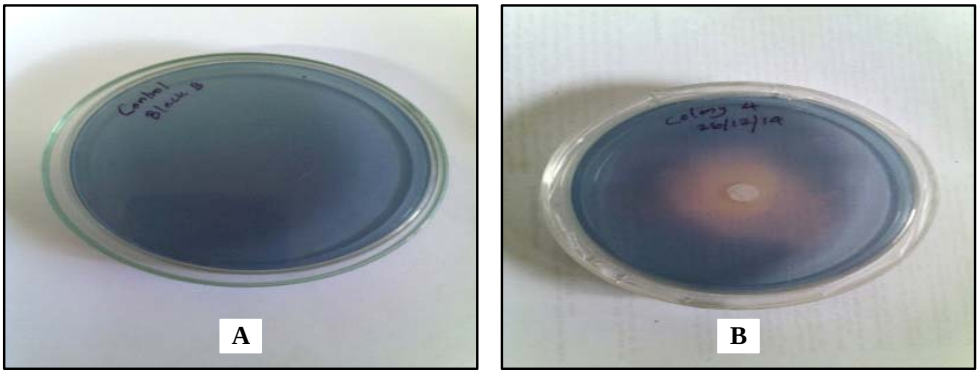

Fig 1: Isolated bacterial sample with dye decolourizing capability A: Control B: Zone of clearance of isolated bacteria in Black B dye plate

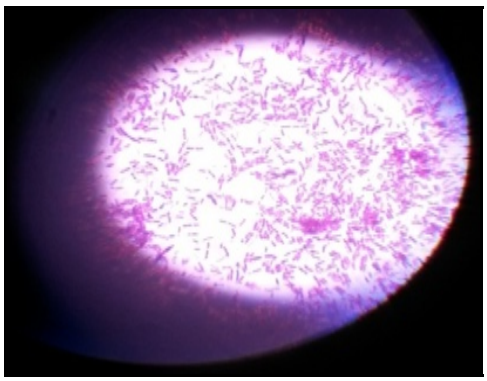

Fig. 2: Morphology of isolated bacteria *Pseudomonas stutzeri*

Table 1 provides the morphological and biochemical properties, which reveal that isolated organism belongs to *Pseudomonas spp*. However previous reports indicate that, though several microorganisms acquire the dye degradation ability but only few organisms can sustain in the effluent conditions[17].

Table 1: Biochemical characteristics of isolated bacteria *Pseudomonas stutzeri*

Test	Observations
Cell shape	Rod
Cell motility	Motile
Gram staining	Negative
Citrate	Positive
Indole	Negative
Methyl red	Negative
Voges proskauer	Negative
Catalase	Positive

Bacteria have been reported to be highly substrate specific to the dye which they are adopted. In earlier report, there are five bacterial constituents *B.Pumilus, B.megaterium, B.cereus, B.vallismortis* and *B.subtilis* that were examined to decolorize certain dyes like Congo red, Bordeaux, Ranocid Fast Blue and Blue-BCC and all isolates decolorized about 72%[18].

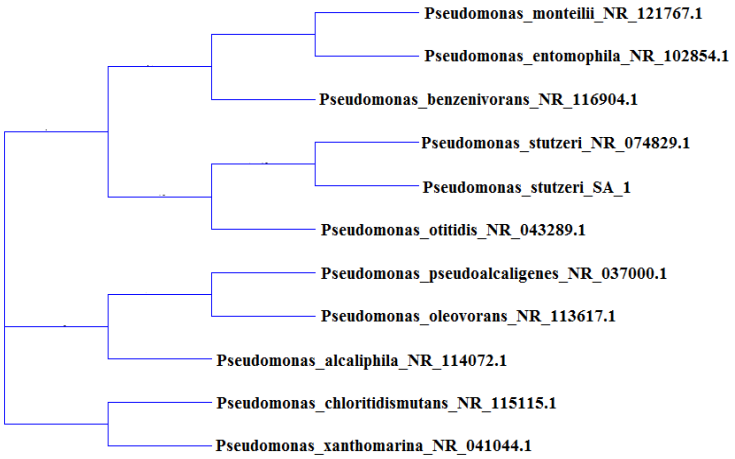

Fig. 3: Phylogenetic tree

The organism was subjected to 16S rRNA sequencing. From this, the nucleotide sequence of the organism was obtained. The sequence was substituted in an online tool Phylogeny fr. to analyze its phylogenetic tree. The organism was identified to be *Pseudomonas stutzeri*, shown in Fig. 3. The sequence obtained was submitted in NCBI with accession number KR013216.

Decolourization potential assay

The isolate *Pseudomonas stutzeri* was found to degrade Reactive Black B dye to a greater extent up to 93.5%. The percentage decolorization obtained in 96 hrs was highly significant. Table 2 represents the percentage decolourization obtained by *Pseudomonas stutzeri* in 4 consecutive days. Fig. 4 represents the decolourization by *P.stutzeri* in LB broth. Reduced breaking of azo bond is the first step in azo dye degradation by bacterial strains. The existence of azo reductases in obligate aerobic bacteria was first proven in *Pseudomonas K22* and *Pseudomonas KF46*. Earlier, various extensive studies were also performed to find out the characteristics of microorganisms in dye decolorization[19-21].

Table 2: Percentage dye decolourization of Black B by *Pseudomonas stutzeri*

S. No.	No of days	O.D at 597 nm for control	O.D at 597 nm for test	% Dye decolourization for control	% Dye decolourization for test
1	0	3.817	3.817	0.00	0.00
2	1	3.817	1.584	0.00	58.5
3	2	3.817	0.747	0.00	80.4
4	3	3.817	0.405	0.00	89.3
5	4	3.817	0.248	0.00	93.5

Fig. 4: Control and test showing the decolorizing capacity of *Pseudomonas stutzeri* in dye containing broth

CONCLUSION

The increase in population has in turn led to the increase of pollutants. Textile industries are involved in the release of harmful waste water into the ecosystem. Major disadvantage behind the conventional methods is that they can lead to the development of concentrated sludge, which can again cause serious environmental issues. So, as an alternative, enzymes were found favourable in the treatment of textile effluent, mainly due to its characteristic features like broad specificity, stability, decolourization capability and non pathogenic nature can therefore be utilized on a large scale. Azo dyes are the most commonly used synthetic dye and they were thus used in this study to investigate the ability of azoreductase to degrade them under aerobic conditions. Identification and characterizational study of the isolated *Pseudomonas stutzeri* proved that azoreductase can breakdown azo compounds under aerobic conditions.

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