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OCHROBACTRUM SP. RED-3: A NOVEL BACTERIUM FOR BIOREMEDIATION OF TEXTILE DYE RED ED3B

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Abstract

The textile dye Red ED3B is widely used in several textile industries and is a toxic azo dye. Owing to its complex structure, it persists in environment for longer period of time and is detrimental for environment and all living beings. Bacterial strains were isolated from the dye contaminated soil having high potential to decolorize this dye. 5 bacteria were isolated from acclimatized soil samples among which, *Ochrobactrum sp. RED-3* showed decolourization of Red ED3B dye which was identified by Morphological, Biochemical and 16srRNA sequencing. The optimization of various parameters such as temperature, pH, inoculums size dye concentration, carbon and nitrogen sources was studied in order to achieve maximum dye degradation. Under optimum conditions *Ochrobactrum sp. RED-3* exhibited excellent ability to degrade Red ED3B at Room temperature (97.35%), pH 9.0 (98.07%), 2% inoculum size (95.71%), dye concentration upto 800ppm (64.32%), 1% Starch (97.18%) and 1% Peptone (89.49%) as carbon and nitrogen source. Further metabolic pathway for Red ED3B degradation was predicted on the basis of GC-MS reports which confirms complete breakdown of complex dye into low molecular and non-toxic metabolites. Owing to highest efficiency for removal of Red ED3B dye, the isolate *Ochrobactrum sp. RED-3* can be exploit for complete bioremediation of dye from textile effluents.

Key words- *Ochrobactrum sp. RED-3,* decolorization, biodegradation, Red ED3B

1. Introduction:

Pollution due to textile waste water is the major problem of growing environmental concern. The textile effluent contains large amount of various dye stuffs, hazardous chemicals, surfactants and softeners which are non-biodegradable and toxic to environmental flora and fauna. These dyes can produce aromatic amines which are considered as carcinogenic to human being. (Banat *et.al.,* 1996, Weisburger, 2002). These dyes generally effect on photosynthetic ability of plants, depletion of oxygen, increased BOD, and reduce the soil fertility. Recently it has been studied that near about 12% synthetic dyes are lost from textile industry per year. (Srinivasan and Muruthy, 2009). Today more than 10,000 colours and pigments are available worldwide in textile industry. (Robinson *et al.,* 2001). The reactive dyes are largest group among all dyes which contain different chromophore groups such as azo, anthraquinone and triarymethane. The presence of the dyes (below 1 ppm) is clearly visible and affects the quality of water and environment. Hence it is very essential to remove these colours and toxic compounds from the textile waste water. (Ou *et al.,* 2005).

Traditionally these dyes were removed by physical, chemical and biological methods. The physical and chemical methods such as coagulation with alum, ferric chloride, magnesium carbon, chemical oxidation, electrophotocatalysis, adsorption, filtration and precipitation are available for the treatment of textile effluent. But these methods are having several limitations such as very expensive, produce large amount of secondary sludge. Biological methods using microorganisms are having advantage due to their low cost; require less experimental setup, easy to perform and no production of sludge. The biotransformation by using microorganisms for the decolorization and degradation of textile dyes is an efficient method (Casieri *et al.,* 2008; Syed et al., 2009). Hence for the decolorization of the textile dyes it is necessary to screen the microbes and to optimise the decolorization in presence of different parameters such as effect of co-substrate, pH, temperature, inoculums size dye concentration etc, (Zhang and Zheng, 2009). It has been studied that some of bacterial cultures are able to utilize dyes as carbon and nitrogen source. (Saranaik and Kanekae, 1999).

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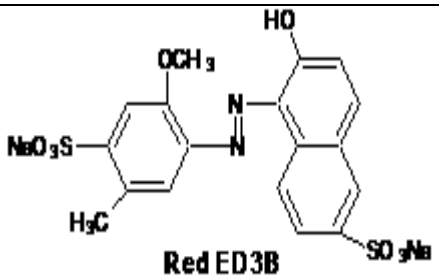
The present research work deals with the bioremediation of textile dye Red ED3B by Novel bacterium *Ochrobactrum sp. RED-3*. Various physico-chemical parameters were studied for maximum dye decolourization. The percent decolorization was measured by spectrophotometer at maximum absorbance (ƛ max) at 590 nm. Finally metabolic pathway for Red ED3B degradation by *Ochrobactrum sp. RED-3* was predicted which confirmed complete bioremediation of dye.

2. Materials And Methods:

- Soil and water sample: The soil and water samples were collected from the area near by the waste disposal site of textile industry and effluent treatment plant at Solapur.
- Textile dye- Red ED3B, ƛ max 590 nm.

Table 1. Red ED3B dye Properties and Structure

Dye Properties	Dye Structure
Molecular Formula: $C_{18}H_{14}N_2Na_2O_8S_2$ **Fomula Weight:** 496.421898 **Composition:** **C** (43.55%), **H**(2.843%), **N**(5.643%), **O**(25.784%), **Na**(9.262%), **S** (12.97%) **ƛ max**- 590 nm	 Red ED3B disodium 6-hydroxy-5-[(E)-(2-methoxy-5-methyl-4-sulfonatophenyl) diazenylnaphthalene-2-sulfonate]

2.1 Acclimatization of microorganisms:

The soil and water samples from the area near by the waste disposal site of textile industry and effluent treatment plant at Solapur were collected and homogenized and mixed properly. These homogenized samples were added with increasing concentration of dye Red ED3B for the period of one month.

2.2 Isolation and screening of dye decolorizing bacteria:

Serially diluted acclimatized samples were spreaded on nutrient agar plates. The well grown, isolated colonies were further used for screening. Each and every bacterial isolate was screened using nutrient broth containing 100ppm dye. The tubes showing decolorization were used for the further studies. The isolate exhibiting highest dye decolourization in broth studies was selected and designated as RED-3 and used for further studies.

2.3 Identification and sequencing of 16S rRNA gene of the selected isolate:

The identification of the isolate was determined by 16S rRNA sequencing. The sequence was analysed for closed homology at Ribosomal Database Project (RDP II) (http://rdp.cme.msu.edu/) the sequences downloaded from the RDP II database were aligned by using CLUSTAL X2 multiple sequence alignment tools and Dambe. The phylogenic tree was constructed by neighbour joining method using Kimura-2-parmeter distances in MEGA 4.0.

2.4 Optimization of various cultural conditions:

2.4.1 Effect of Temperature, pH, Inoculum size, Dye concentration on decolourization of Red ED3B:

To study the effect of Temperature, the tubes containing 30 ml nutrient broth and 100 ppm dye concentration were inoculated with RED-3. The tubes were kept for incubation at various temperatures *viz.* Room temperature, 37^{0}C, 45^{0}C, 55^{0}C and 65^{0}C. The effect of pH was studied by using 30 ml nutrient broth with 100 ppm dye concentration and inoculated with RED-3. The pH of medium was adjusted at the range 6.0-8.0. Similarly, the effect of inoculum size was studied by inoculating RED-3 in various volumes *viz.* 1%, 2%, 3% and 4% in 30 ml nutrient broth having 100 ppm dye concentration. In order to check the efficiency of RED-3 for dye decolorization the tubes with 30 ml nutrient broth were added with various concentration of Red ED3B in the range 100 ppm to 1000 ppm. Further the tubes were inoculated with RED-3 and kept for incubation. After incubation period the tubes were observed for dye decolorization spectrophotometrically. The isolate RED-3 was examined for dye decolorization ability in presence of

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various carbon and nitrogen source. 30 ml Minimal medium containing 100 ppm dye concentration was added with different 1% Carbon sources (Glucose, Sucrose and Starch) and 1% Nitrogen source (Peptone, Yeast extract and Meat extract). Decolorization was monitored by spectrophotometer

2.5 Percent decolorization study:

The determination of percent dye decolorization was carried out at ƛ max by spectrophotometer (Systronics-106 model) and calculated by using following formula.

% Decolorization= [(A0-A1)/A0] × 100

Where, A0 = Initial absorbance

A1 = Final absorbance

2.6 Prediction of metabolic pathway

The metabolic pathway for Red ED3B degradation by *Ochrobactrum sp. RED-3* was further predicted on the basis of GC-MS analysis reports

3. Results:

3.1 Isolation and identification of isolate:

A total of 5 bacterial were isolated from acclimatized samples out of which one isolate proved excellent in screening hence used for further studies. The RED-3 isolate was identified by morphological, biochemical and 16S rRNA sequencing and confirmed as identified as *Ochrobactrum sp. RED-3*. Results are shown in Table 1, 2, 3 and figure 1.

Table 1: Morphological identification of *Ochrobactrum sp. RED-3*

Bacterial isolate	Size (mm)	Shape	Colour	Margin	Elevation	Consistency	Opacity	Gram Nature	Motility
RED-3	1	Circular	Colourless	Entire	Low convex	Smooth	Opaque	Gram Negative	Non-Motile

Table 2: Biochemical characters of *Ochrobactrum sp. RED-3*

Bacterial isolate	Utilization of						Hydrolysis of		
	Glucose	Fructose	Sucrose	Mannitol	Lactose	Maltose	Casein	Starch	Gelatine
RED-3	A, G	A, G	A, G	A, G	-	+	-	+	-

Where: A: Acid**; G:** Gas**; '-'** = Negative test**; '+'=** Positive test

Table 3: Biochemical characters of *Ochrobactrum sp. RED-3*

Bacterial isolate	Enzyme Activity			Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate reduction
	Catalase	Urease	Oxidase					
RED-3	+	+	+	-	+	-	+	+

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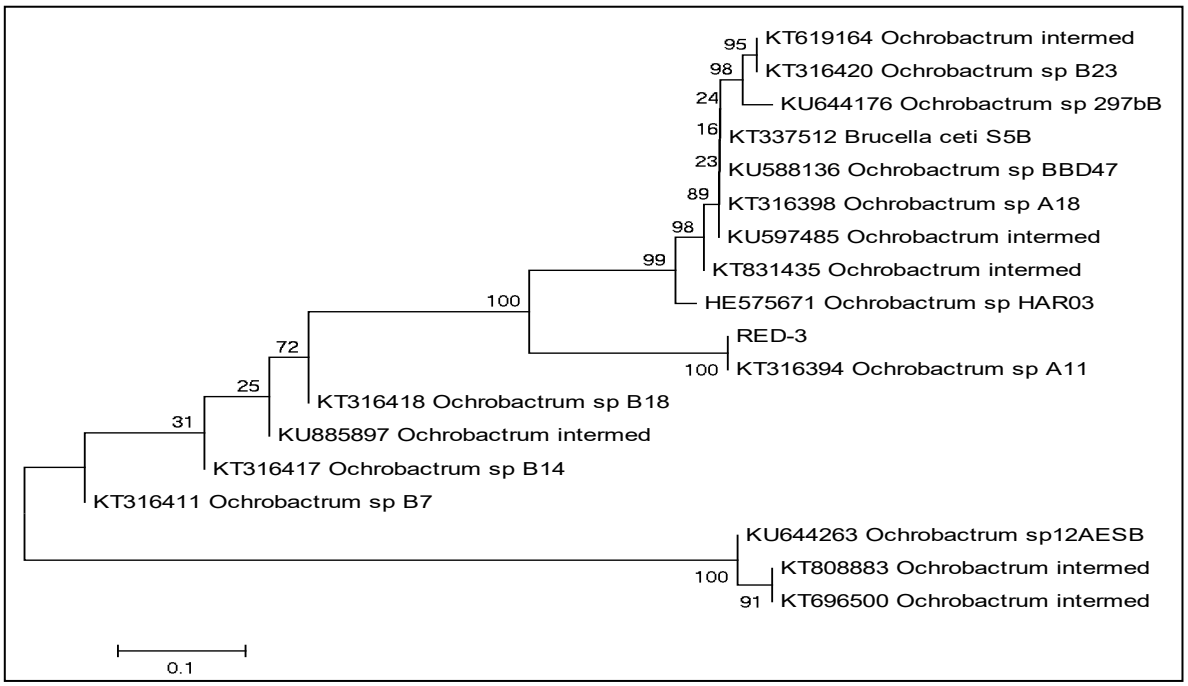

Figure 1 – Phylogenetic Tree of RED-3. The 16S rRNA sequence showing relationships among RED-3 and closest type strain species of Ochrobacterum. The percent numbers at the nodes indicate the level of bootstrap support based on neighbor-joining analyses of 1,000 replicates. The scale bar (0.1) indicates the base pair substitutions per site i.e. the genetic distance. On the basis of 16S rRNA identification the isolate RED-3 was identified as Ochrobacterum sp. RED-3.

3.2 Optimization of various cultural conditions:

3.2.1 Effect of Temperature, pH, Inoculum size, Dye concentration on Red ED3B decolourization

The isolate RED-3 exhibited complete decolorization of Red ED3B at Room temperature (97.35%) and at pH 9.0 (98.07%). The isolate RED-3 exhibited maximum decolorization (95.71%) when inoculated with 2% inoculums size upto 800ppm dye concentration (64.32%). Above 800ppm concentration the efficiency of dye decolourization was slightly decreased because of increasing concentration of dye. The isolate RED-3 exhibited highest decolorization of Red ED3B in presence of 1% Starch (97.18%) as carbon source 1% Peptone (89.49%) as Nitrogen source. The results are shown in

Figure 2.

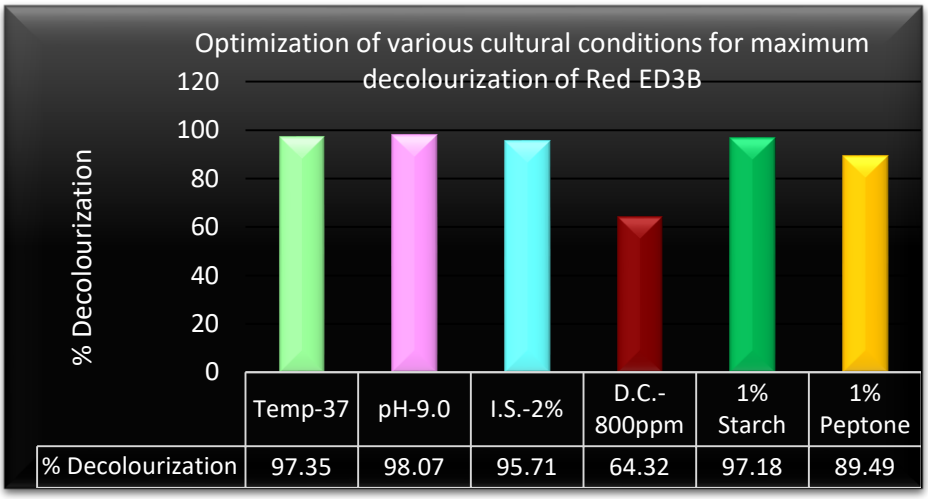

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Figure 2: Optimization of Various Cultural conditions for maximum decolourization of Red ED3B by *Ochrobactrum sp. RED-3*

3.3 Prediction of metabolic pathway

The metabolic pathway for Red ED3B degradation by *Ochrobactrum sp. RED-3* was further predicted on the basis of GC-MS analysis reports (Figure 3).

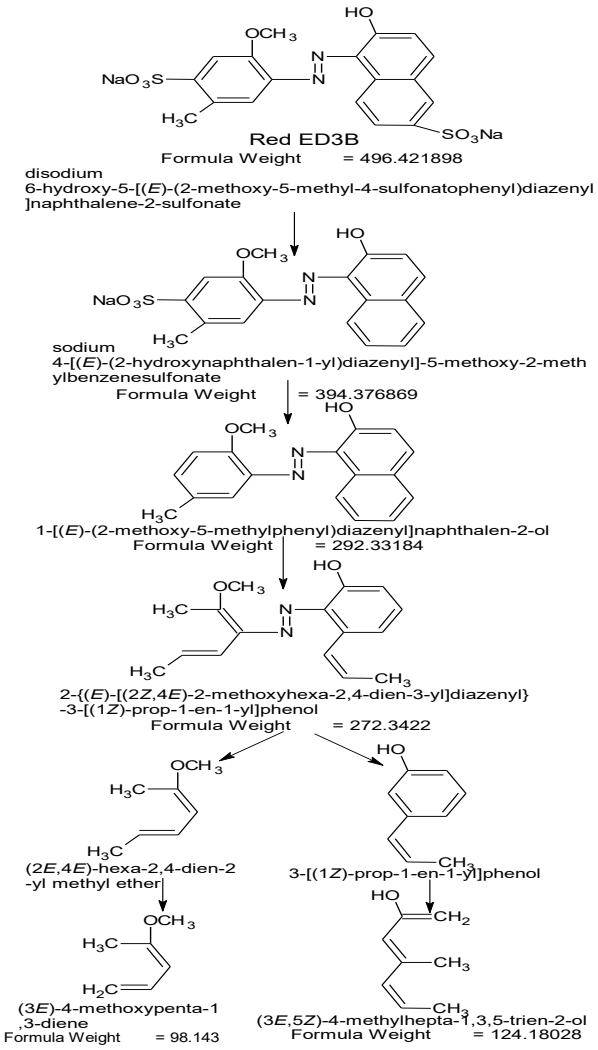

Figure 3: Metabolic Pathway of Red ED3B degradation by *Ochrobactrum sp. RED-3*

4. Discussion:

It has been proved that microflora from dye contaminated or acclimatized soil is naturally resistant to the toxicity and chemicals (Khehra *et al.*, 2005). Most of the bacteria isolated from textile waste were reported to effective removal of dye from textile waste (Dawkar V.V., 2008). The textile dye Red ED3B was having ƛ max 590 nm. The dye decolorization assay was performed by using spectrophotometer at its ƛ max. It was previously suggested by Asad *et al.,* (2007) that decolorization of dye could be due to microbial adsorption of dye or due to degradation.

The isolation and screening of dye decolorizing bacteria was carried out by using nutrient medium containing 100ppm concentration of Red ED3B. The 16S rRNA gene sequence of isolate *Ochrobactrum sp. RED*-3 derives the phylogenetic relationship. The phylogenetic tree was constructed by Neighbour-Joining Method which was previously suggested by Felsenstein J. (1993). At Room temperature the isolate *Ochrobactrum sp. RED-3* showed maximum decolorization of the dye at pH 9, under static condition. The

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rate of metabolic reactions is affected by temperature. As the dye decolorization is metabolic process, the temperature above certain level causes thermal inactivation of proteins. The isolate showed effective decolorization of dye at temperature below 65^0C. But the maximum decolorization (97.35 %) was at Room Temperature.

Azo dyes are deficient in carbon source so the decolorization carried only with metabolite condition (Chang *et al.*, 2004). The previous study reveals that Peptone is an ideal source of nitrogen, in presence of which bacteria exhibit maximum decolorization of the dye. (Praveen and Bhat, 2012). In current research the isolate showed maximum dye decolourization (89.49%) in presence of 1% Peptone. This result was exactly in accordance with results of Bhosale and Ghumbre, (2022), who reported 98% decolourization of Blue RGB in presence of 1% Peptone. The present study exhibit that addition of 1% Starch could increases the percent decolorization. This was earlier suggested by Jang *et al*., (2007). It was studied that addition of 0.1% of starch as carbon source increases the rate of decolorization of Ranocid fast blue dye. (Chen *et al.*, 2005). The isolate *Ochrobactrum sp. RED*-3 was able to decolorize Red ED3B upto 10000ppm concentration of dye. The decolorizing ability of isolate was highest upto 800ppm. This study proved that the metabolites formed after decolorization of Red ED3B were less toxic than original dye. Similar reports were suggested by Dhanve *et al*. in 2009 in case of Reactive yellow 84A dye and its metabolites formed after decolorization. The same report was revealed by Saratale *et al*. (2009) that the textile dye Navy Blue HER inhibits 90% germination of *Sorghum vulgare* and *Phaseolus mungo* seeds when treated with 1500 ppm concentration of Navy Blue HER. But when same concentration of degraded dye products was used, it showed 100% germination of seeds.

The GC-MS analysis was carried out to find the byproducts formed after dye degradation and metabolic pathway for dye degradation was predicted. The intermediate carbocation is highly reactive and is often attacked by nucleophiles like –OH or –SO3 or halogen ions by nucleophillic attack which causes asymmetric cleavage of the azo bond. This is similar to the cleavage of azo bond of Red ED3B by *Ochrobactrum sp. RED*-3. The probable prediction of dye degradation pathway shows the breakdown of N=N by enzymatic action of isolate. The cleavage of Azo bond can be carried out by symmetrically or asymmetrically by an active site of an enzyme (Pasti *et al.,*1992).

5. Conclusion:

The dyes are used for colouring the fabrics in textile industries which are carcinogenic, mutagenic, and toxic to all living beings. Hence it is mandatory to remove these dyes by using suitable method. The present research work deals with the effective bioremediation of such harmful textile dyes by using promising bacterial isolates. Red ED3B dye is being widely used in textile industries and it is highly carcinogenic in nature. This research work is reveals that *Ochrobactrum sp. RED*-3 was able to completely decolorize and degrade Red ED3B by using very easy and cheap but effective techniques. Thus, it could be concluded that the isolate *Ochrobactrum sp. RED*-3 can be successfully used for the treatment of textile effluent. As per the best of our knowledge, this report is the first which reveals that the *Ochrobactrum sp. RED*-3 is a novel bacterium for effective bioremediation of textile dye Red ED3B which will lead to the green technology.

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