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Decolorization and biodegradation of Reactive Blue 13 by *Proteus mirabilis* LAG

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ABSTRACT

The decolorization and biodegradation of Reactive Blue 13 (RB13), a sulphonated reactive azo dye, was achieved under static anoxic condition with a bacterial strain identified as *Proteus mirabilis* LAG, which was isolated from a municipal dump site soil near Lagos, Nigeria. This strain decolorized RB13 (100 mg/l) within 5 h. The formation of aromatic amine prior to mineralization was supported by Fourier transform infrared spectrometry (FTIR), which revealed the disappearance of certain peaks, particularly those of the aromatic C–H bending at 600–800 cm^{-1}. Gas chromatography–mass spectrophotometry (GCMS) analysis of the dye metabolite showed the presence of sodium-2(2-formyl-2-hydroxyvinyl) benzoate, with a tropylium cation as its base peak, this suggested the breakage of naphthalene rings in RB13. The detection of azoreductase and laccase activities suggested the enzymatic reduction of azo bonds prior to mineralization. In addition, phytotoxicity studies indicated the detoxification of RB13 to non-toxic degradation products by this strain of *P. mirabilis* LAG.

1. Introduction

Azo dyes are chromogenic compounds, characterized by one or more azo groups (–N=N–). More than half of dyes produced annually are azo dyes; approximately 2000 different azo dyes are currently used to dye various materials such as textiles, leather, plastics, cosmetics, and food [1]. These dyes are generally recalcitrant and their degradation products are often toxic [2]. The recalcitrant nature of wastewater, particularly those of textile industry origin, might not be unconnected with the presence of azo dyes; azo dyes account for about 50–65% of synthetic dyes [3]. Among azo dyes, sulphonated azo dyes were reported to resist biodegradation in conventional treatments [4–6].

Several methods are currently employed for the removal of colors from textile wastewater; each with its own demerits. Chemical decolorization often requires biogenic reductants, such as sulphide and ascorbate or Fe^{2+} [5,1]. These biogenic compounds are sometimes not effective and often require further treatments such as ultraviolet irradiation and peroxidation thus adding to the cost of the procedures [7,8]. Physical methods like biosorption and ion exchange have been found to be dye specific [9–11]; while flux decline, membrane fouling and cost of regular replacement are major setbacks in membrane filtration [8].

Microbial decolorization and detoxification, is a cheaper and eco-friendly alternative compared to chemical and physical methods [12]. Over the years, microbial removal of colors from textile wastewaters has focused on anaerobic sludge systems. The end products of such treatments, aromatic amines, have been reported to be more toxic than the dyes themselves [3,13]. Furthermore, anaerobic decolorizations are not necessarily results of bioremediation, since sludge often adsorbed dyes [14]. Enzyme assay are therefore expedient to verify biodegradation of dyes. Generally, color removal by bacteria has been linked to oxidoreductase enzymes such as laccase and azoreductase [15,16]. Van der Zee and Villaverde [14] reported the poor extent of decolorization of azo dyes in aerobic conditions; they however suggested combined anaerobic/aerobic treatment. This requires two separate phases: a decolorization and a detoxification phase, thus elongating the period of treatment. Microorganisms that can both decolorize and remove aromatic amines are thus an attractive option for bioremediation; as this will not only result in an ecologically friendly remediation of dyes polluted environments but also reduce the high costs associated with the currently used methods such as the advanced oxidation processes (AOPs). This paper reports the decolorization and biodegradation of a sulphonated azo dye, Reactive Blue 13, by a newly isolated strain of *Proteus mirabilis*.

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2. Materials and methods

2.1. Dyes and chemicals

Veratryl alcohol, nutrient broth and agar were obtained from Hi-Media Pvt. Ltd. (India). Catechol, o-tolidine, *n*-propanol and other fine chemicals were from SRL chemicals, India. The textile dyes: Reactive Blue 13, Reactive Yellow 42, and Reactive Red 58 were kindly donated by a textile industry in Lagos, Nigeria.

2.2. Organism and culture condition

The organism used in this study was isolated from a soil sample obtained from a municipal wastes site from Redemption city near Lagos, Nigeria. It was then maintained on nutrient agar slants at 4 °C. The pure culture was grown in 250 ml Erlenmeyer flask containing 100 ml nutrient broth (g/l): beef extract 1 g, yeast extract 2 g, peptone 5 g and NaCl 5 g at 37 °C for 24 h. The 24 h culture was kept at 4 °C and used as the seed inoculum.

2.3. Screening and identification of organism

The organism was first screened for the ability to decolorize azo dyes using 60 mg/l dye mixtures consisting of 20 mg each of Reactive Yellow 42, Reactive Blue 13, and Reactive Red 58 in 1 l nutrient broth. Colony morphology, microscopic identification and biochemical characterization was used to identify the strain to the species level using Cowan and Steel Manual [17]. The 16S rDNA analysis was performed by BAC full length service from Laragen Inc., Los Angeles, USA. The 16S rDNA sequence was initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov) using BLAST (blastn) tool and corresponding sequences were downloaded and evolutionary history was inferred using the Neighbor-joining method [18]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches [19]. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages [20]. The clock calibration to convert distance to time was 0.01 (time/node height). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [21] and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA4 [22].

2.4. Decolorization experiments

Approximately 0.05 g dry cell mass (DCM) were added into 100 ml, 100 mg/l Reactive Blue 13 in nutrient broth. The reaction was done at both static anoxic and shaking (120 rpm) conditions. 6 ml samples were withdrawn at 1 h intervals for 6 h. 3 ml of samples were centrifuged at 5000 rpm for 15 min to remove biomass. Using the remaining 3 ml, biomass was determined according to Dong et al. [23] by measuring optical density at 600 nm:

$$OD_{600} = OD_{sample} - OD_{supernatant} \quad [23]$$

The relationship between the DCM of the *P. mirabilis* and OD_{600} was found to be 1.0 OD_{600} = 10.7678 g DCM/l. An analogue of Beer–Lambert law ($OD_{600} = \varepsilon C$) was used to determine an extinction factor, this showed that the organism is related to biomass by the equation:

$$\text{Biomass concentration (g DCM/l)} = \frac{OD_{600}}{0.09286}$$

Percentage decolorization was calculated as

$$\%\,\text{Decolorization} = \frac{A_o - A_t}{A_t} \times 100$$

where A_o and A_t are initial and final absorbance units respectively.

2.5. Effect of initial pH and incubation temperature on decolorization

Static culture experiments were conducted at various initial pH values between 4 and 10 with the temperature maintained at 37 °C using water bath. The experiment was later repeated with varied temperature between 15 °C and 45 °C with the initial pH of 7.0. Approximately 0.02 g DCM of the bacteria was added to 50 ml of the solution (100 mg/l of RB13) in 100 ml conical flasks. All experiments were done in triplicates and abiotic controls were used as 0% decolorization. The extents of decolorization were determined after 3 h at the wavelength of maximum absorbance of the solution (λ_{max} = 546 nm).

2.6. Biodegradation experiments

Biodegradation was monitored using UV–visible and Fourier transform infrared (FTIR) analyses of the metabolites of Reactive Blue 13. Determination of the identities of the metabolites of RB13 degradation was carried out using gas chromatography–mass spectrophotometry (GCMS) spectra of the dye metabolites. The UV–visible spectral analyses were done for both the biotic and abiotic solutions (control) of the reactive dye at specific intervals using Shimazu UV-vis (UV-1650PC) and changes in their absorption spectrum (200–1000 nm) were recorded. For FTIR and GCMS analyses, the 24 h metabolites produced during the biodegradation of Reactive Blue 13 dye was extracted with equal volume of ethyl acetate. The extract was dried in a rotary evaporator and re-dissolved in HPLC grade methanol for GCMS analysis. Solid extracts, obtained by allowing the re-dissolved extract to air dry overnight in an inoculating hood, were used for FTIR analyses. FTIR spectra of Reactive Blue 13 and its metabolic extract was done using FTIR model 800 (Shimadzu, Japan). The scans were done in the mid IR region of 400–4000 cm^{-1} with 16 scan repeat. The GCMS scans were done with a QP2010 mass spectrometer (Shimadzu, Japan) at ionization voltage of 70 eV with a temperature programmed mode (Resket column 0.25–60 mm; XTI-5). Degradation products were identified by mass spectra and fragment patterns.

2.7. Enzyme assays

Activities of azoreductase, veratryl alcohol oxidase, lignin peroxidase, laccase and tyrosinase were assayed spectrophotometrically. All enzyme assays were carried out at room temperature (~30 °C) with 24 h nutrient broth culture supplemented with 100 mg/l dye (induced) or without dye (control). Cultures were centrifuged at 10,000 rpm to pellet out microbial cells. The cell free supernatants of the centrifuged cultures were used as sources of extracellular enzymes. To obtain intracellular enzymes, the harvested cells (pellets) were suspended in 50 mM potassium phosphate buffer (20 ml), homogenized, placed on ice-bath and ultrasonic waves were applied at 15 W (40–0 mA) for 30 s. Five strokes were given at 2 min intervals at 4 °C. The cell lysate was centrifuged at 10,000 rpm for 15 min at 4 °C. The clear supernatant obtained was used as crude source of intracellular enzymes.

Azoreductase activity was assayed by monitoring the removal of Methyl Red at 430 nm [2]. The reaction mixture contained 0.6 ml 50 mM phosphate buffer (pH 7.4); 0.25 mM Methyl Red, 0.12 mM $NADH_2$ or $NADPH_2$, 0.8 ml distilled water to give a final volume of 2 ml. Veratryl alcohol oxidase activity was determined as described

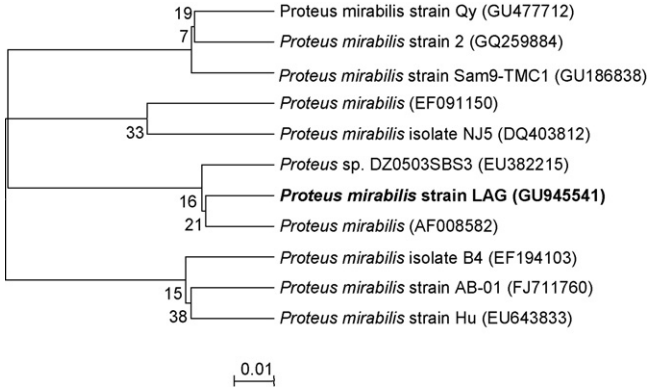


Fig. 1. Phylogenetic analysis of 16S rDNA sequence of bacterial isolate *Proteus mirabilis* strain LAG. Distance tree constructed using Neighbor-joining method by using MEGA4. The sequences have been retrieved from NCBI database, showing the phylogenetic relationships of *P. mirabilis* strain LAG and other species of genus *Proteus*. Numbers at nodes show the level of bootstrap support based on data for 1000 replication. Bar, 0.01 substitutions per nucleotide position and numbers in parenthesis represent GenBank accession numbers.

by Tien and Kirk [24]. The oxidation of veratryl alcohol to veratryl aldehyde at 310 nm was monitored in 2 ml reaction mixture of containing 0.2 ml veratryl alcohol (10 mM), 1.6 ml citrate buffer (pH 3.0) and 0.2 ml of the crude enzyme; to give a final concentration of 1 mM veratryl alcohol. Lignin peroxidase activity was determined as the H_2O_2-dependent oxidation of veratryl alcohol to veratrylaldehyde. The increase in absorbance at 310 nm due to the oxidation of veratryl alcohol to veratrylaldehyde was monitored [20]. The reaction mixture of 2 ml contained, 0.2 ml veratryl alcohol (10 mM), 1.6 ml citrate buffer (pH 3.0) and 0.2 ml enzyme. The reaction was initiated with the addition of 100 μl of 0.5 mM H_2O_2, and monitored over 3–5 min. Laccase activity was determined spectrophotometrically as the oxidation of 4,4-diamino-3,3-dimethylbiphenyl (o-tolidine) to its aldehyde form at 310 nm [25]. The reaction mixture of 2 ml contains 1 mM o-tolidine (ethanolic solution) in 1.6 ml of 0.1 M acetate buffer (pH 4.0). The reaction was initiated with the addition of 0.2 ml crude enzyme and monitored for 2 min. Tyrosinase activity was determined in a reaction mixture of 2 ml, containing 0.01% catechol in 0.1 M phosphate buffer (pH 7.4) by measuring liberated catechol quinone at 495 nm [26]. One unit of enzyme activity was defined as a change in absorbance units/min/mg of protein. Specific activity was expressed in units per mg protein. All values reported are means of triplicates.

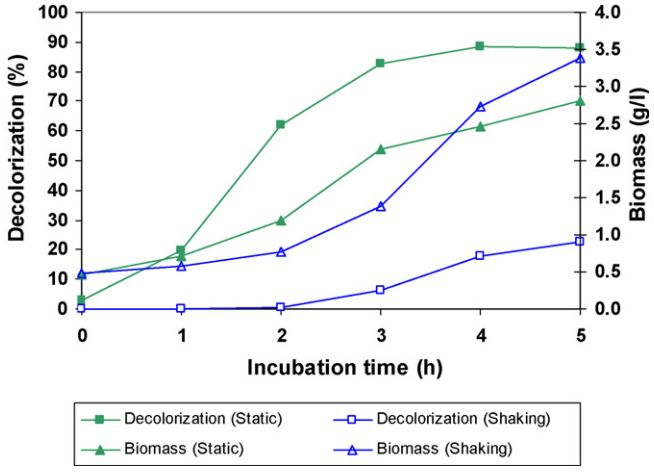


Fig. 2. Decolorization of Reactive Blue 13 under static anoxic and shaking condition. 100 ml of the dye solution in nutrient broth (100 mg/l) was inoculated with ~0.05 g of the organism.

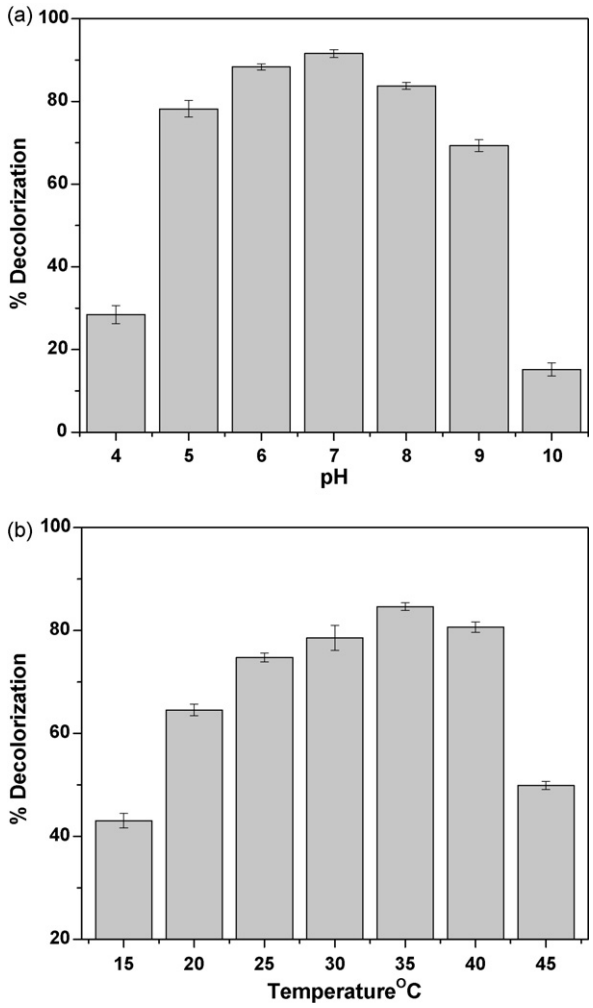


Fig. 3. (a) Effect of pH at 30 °C and (b) effect of temperature at pH 7 on the decolorization of RB13.

2.8. Phytotoxicity studies

Ethyl acetate extract of Reactive Blue 13 metabolic products was dried and dissolved in water to a final concentration of 1000 ppm.

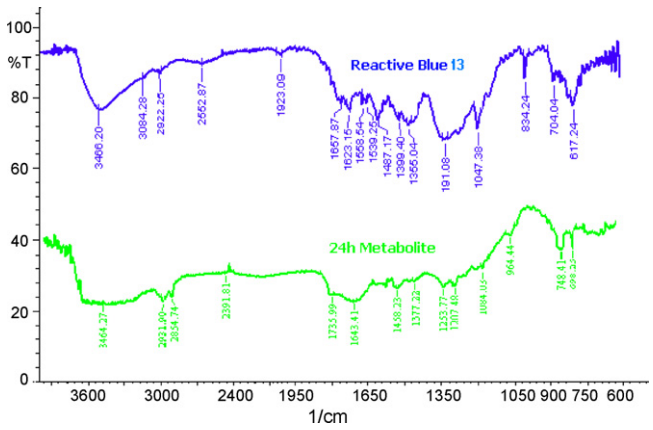


Fig. 4. FTIR spectra of Reactive Blue 13 and its 24 h metabolite.

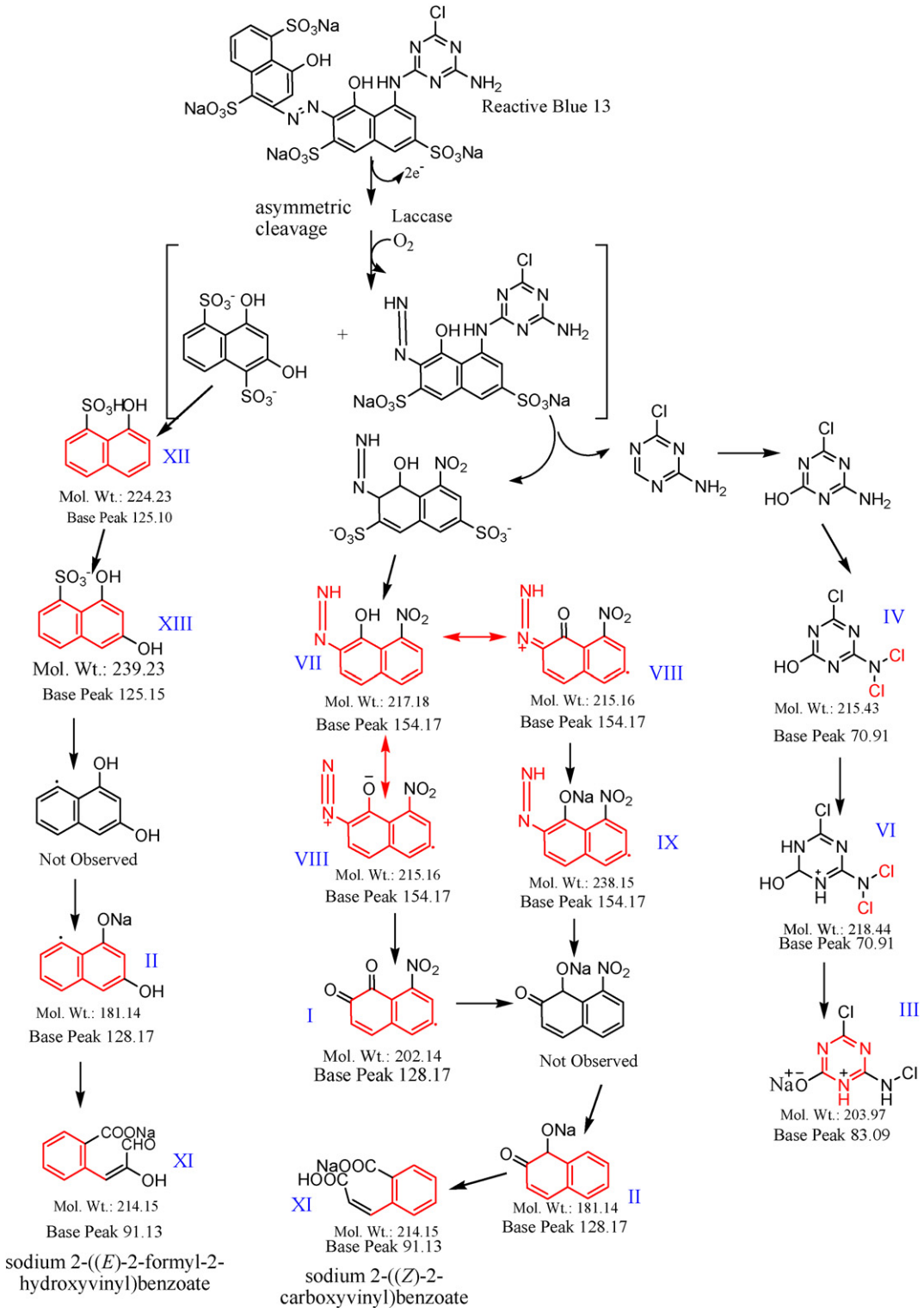


Fig. 5. Proposed pathway for biodegradation of Reactive Blue 13 by *Proteus mirabilis* LAG; structures responsible for the base peaks are in red. Blue numbering are peak numbers as eluted by the GCMS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The phytotoxicity study was performed on seeds of *Zea mays* and *Phaseolus vulgaris*, two plants commonly found in Nigeria and India. The seeds (10 each) were wetted (5 ml per day) with dye solution (1000 ppm) or metabolite solution in separate Petri dishes. Seeds wet with tap water were included as controls. Length of plumule (shoot), radical (root) and germination (%) were recorded after 7 days.

3. Result and discussion

P. mirabilis is a Gram negative, facultative anaerobic bacterium. Along side, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, the organism has been reported to degrade organosilicone compounds [27]. While only few reports are available on the ability of the organism to decolorize azo dyes [28], no report exists to the best of our

Table 1
GCMS data for metabolites obtained after biodecolorization of Reactive Blue 13.

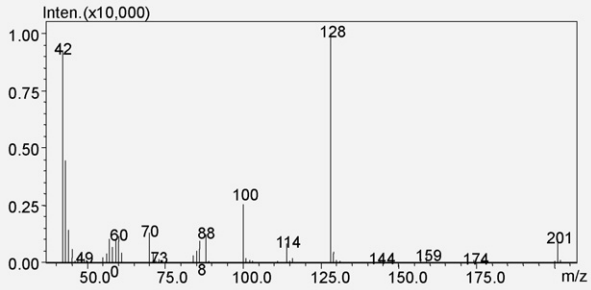
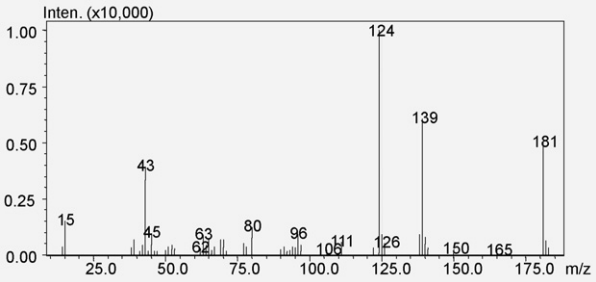
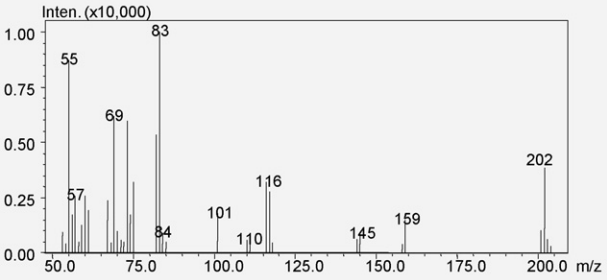
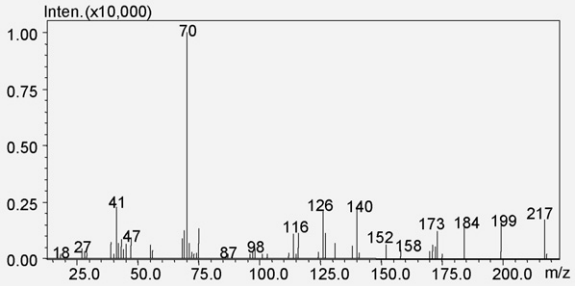
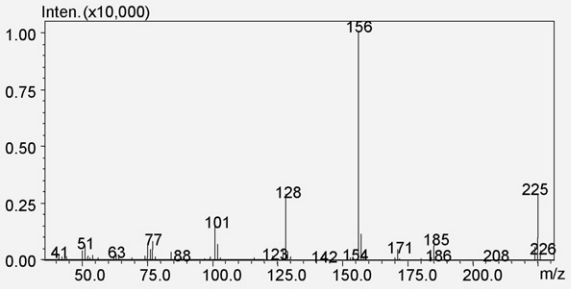
Peak no.	Description	Percentage (%)	Mass peak
I	R.T.: 20.082 min, *m*/*z* 201, base peak 128; C.N.: 8-nitronaphthalene-1,2-dione	5.03	
II	R.T.: 20.203 min; *m*/*z* 181, base peak 128; C.N.: sodium-8-hydroxylnaphtoxide	1.61	
III	R.T.: 20.627 min, *m*/*z* 202, base peak 83, C.N.: sodium-6-chloro-4-(chloroamino)-1-3-5-triazinoxide	4.29	
IV	R.T.: 21.391 min; *m*/*z* 217; base peak 70; C.N.: 6-chloro-4-(chloroamino)-1-3-5-triazin-2-ol	8.61	
V	R.T.: 21.609 min, *m*/*z* 225; base peak 156; C.N.: sodium-2-diazenyl-6,8-dihydroxylnaphthoxide	3.24	

Table 1 (*Continued*)

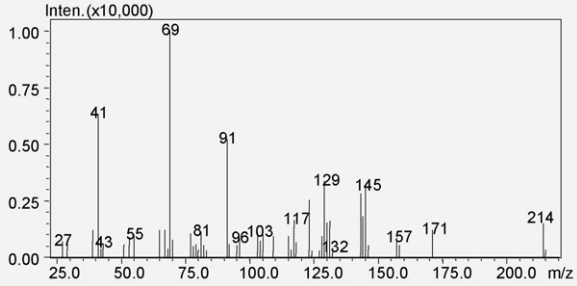
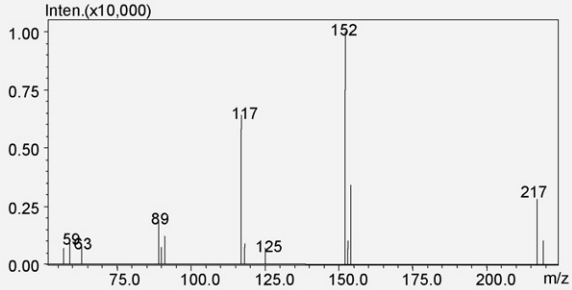
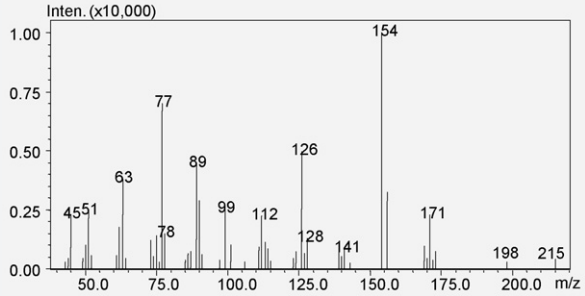
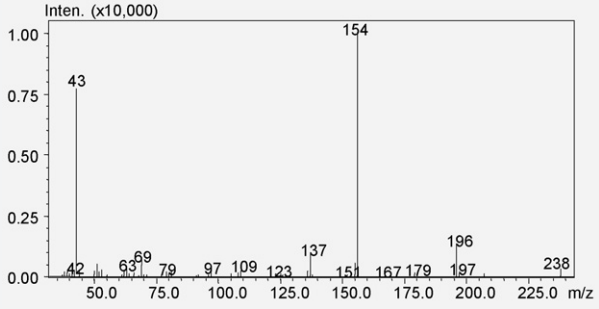
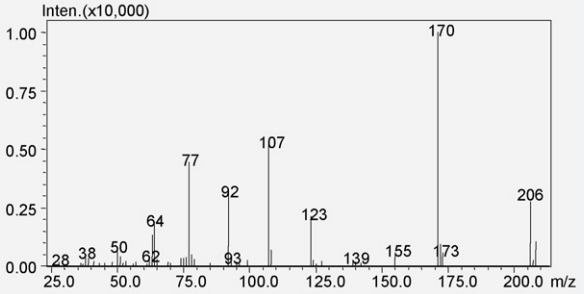
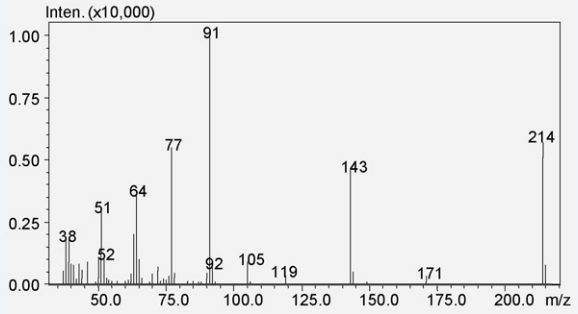
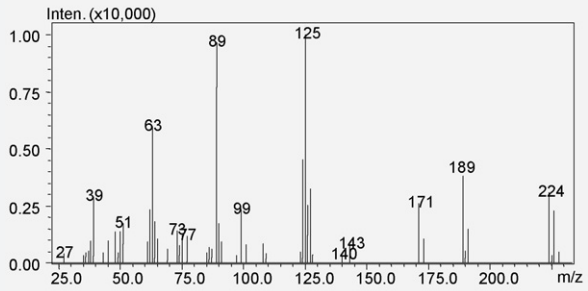
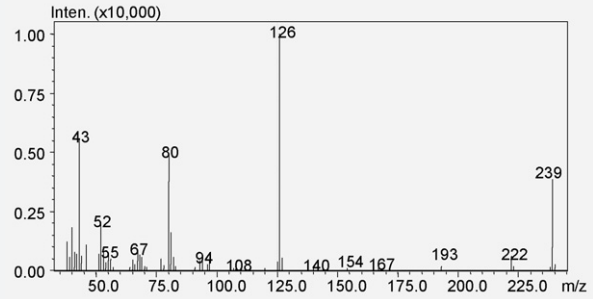
Peak no.	Description	Percentage (%)	Mass peak
VI	R.T.: 21.712 min; *m*/*z* 214; base peak 70; C.N.: 6-chloro-4-(chloroamino)-1-2-dihydro-1-3-5-triazin-2-ol	3.46	
VII	R.T.: 22.606 min; *m*/*z* 217; base peak 152; C.N.: 2-diazenyl-8-nitronaphthalen-1-ol	9.55	
VIII	R.T.: 22.820 min; *m*/*z* 215; base peak 154; C.N.: 2-diazonium-8-nitronaphthoxide	32.24	
IX	R.T.: 22.920 min, *m*/*z* 238; base peak 154; C.N.: sodium-2-diazonium-8-nitronaphthoxide	9.03	
X	R.T.: 25.486 min; *m*/*z* 206; base peak 170; C.N.: 8-nitronaphthalene-1,2-diol	3.46	

Table 1 (*Continued*)

Peak no.	Description	Percentage (%)	Mass peak
XI	R.T.: 26.744 min; *m*/*z* 214; base peak 91; C.N.: sodium-2(2-formyl-2-hydroxyvinyl) benzoate or sodium-2(2-carboxyvinyl) benzoate	1.58	
XII	R.T.: 27.855 min, *m*/*z* 224; base peak 125; C.N.: 8-hydroxylnaphthalene-1-sulphonic acid	5.79	
XIII	28.411 min; *m*/*z* 239, base peak 125; C.N.: 6,8-dihydroxylnaphthalene-1-sulphonic acid	12.10	

R.T.: retention time; C.N.: compound name.

knowledge on its ability to mineralize such dyes. However, decolorization of distillery spent waste water by a *P. mirabilis* has been reported by Mohana et al. [29].

3.1. Identification of the organism

The result of 16S rDNA of this strain showed that the organism is *P. mirabilis* (Fig. 1). The complete sequence of the 16S rDNA of the organism has been deposited to GenBank with accession number GU945541.

3.2. Decolorization experiments

Shaking condition seemed to increase the lag phase of microbial growth from about 1 h (under anoxic condition) to 3 h (Fig. 2). This resulted in poor decolorization of the dye (22.67 ± 2.6%) under shaking condition. Despise the prolonged lag phase experienced by the organism in shaking condition; cell growth was higher than static condition at the end of the 5 h incubation period. The organism however showed 87.91 ± 0.6% decolorization within 5 h, under static anoxic condition. The results of the research depicted the versatility of degradation by this strain of *P. mirabilis*. The ability of the organism to decolorize 100 mg/l RB 13 within 5 h is commendable. Kalme et al. [30] has suggested competition between azo dyes and oxygen for reduced electron carriers under aerobic condition as the reason for decreased decolorization at shaking condition.

3.3. Effect of pH and temperature on decolorization of RB13

The strain showed high decolorization activities under optimum neutral pH and wide range of temperature (20–40 °C). The organ-

Table 2
Enzyme activities in control and induced (24 h decolorization) state.

Enzyme assays		Control	Induced
Azoreductase (NADH)[a]	Extracellular	ND	ND
	Intracellular	ND	ND
Azoreductase (NADPH)[a]	Extracellular	ND	0.05 ± 0.02
	Intracellular	ND	0.09 ± 0.02
Laccase[a]	Extracellular	0.07 ± 0.06	0.06 ± 0.03
	Intracellular	2.50 ± 0.40	2.17 ± 0.11
Veratryl alcohol oxidase[a]	Extracellular	0.20 ± 0.07	0.10 ± 0.01
	Intracellular	2.19 ± 0.27	3.20 ± 0.55
Lignin peroxidase[a]	Extracellular	ND	ND
	Intracellular	ND	ND
Tyrosinase[a]	Extracellular	ND	0.03 ± 0.02
	Intracellular	0.08 ± 0.05	ND

ND: not in detectable range. Values are mean of triplicate readings ± SEM.
[a] Values show change in absorbance units min^{-1} mg $protein^{-1}$.

Table 3
Phytotoxicity study of Reactive Blue 13 and its degradation product.

Parameters	*Zea mays*			*Phaseolus vulgaris*		
	Water	Reactive Blue 13[a]	Extracted metabolite[a]	Water	Reactive Blue13[a]	Extracted metabolite[a]
Germination (%)	100	70	90	100	90	90
Plumule (cm)	8.15 ± 3.12	3.62 ± 1.25*	7.26 ± 2.50	6.56 ± 0.39	3.30 ± 0.60*	4.96 ± 1.09
Radicle (cm)	7.45 ± 2.59	2.43 ± 0.68*	6.69 ± 1.97	4.74 ± 0.79	5.70 ± 1.21	4.92 ± 0.97

[a] 1000 ppm concentration.
* Significantly different from the control (seeds germinated in water) at $P < 0.05$, using one-way analysis of variance (ANOVA) with Turkey–Kramer comparison test.

ism showed optimal pH of 7 and temperature of 35 °C (Fig. 3). This is not strange to bacteria [19]; has reported a strain of *P. mirabilis* with optimal pH and temperature of between 6.5–7.5 and 30–35 °C respectively. The ability of this strain to decolorize the textile reactive azo dye under a broad range of pH and temperature suggested that this strain of *P. mirabilis* could be useful in practical dyeing of textile wastewater.

3.4. Biodegradation studies

An obvious difference was noticed between the FTIR spectra of Reactive Blue 13 and its metabolites (Fig. 4). The spectrum of the control dye showed specific peaks in the fingerprint region for substituted aromatic compounds (617, 704 and 834 cm^{-1}). The S=O stretching vibration was noticed as a sharp peak at 1047 cm^{-1}. The –N=N– stretching vibrations form peaks around 1623–1739, while those at 3453 cm^{-1} were those of the stretching of secondary amino (–NH–) group. Virtually all the peaks in the FTIR spectrum of the Reactive Blue 13 (control) were found to be distorted in that of the 24 h metabolite. Worth noting is the appearance of the peak at 1458 cm^{-1} for –N=O stretching and the removal of the 617 cm^{-1} peak. The mineralization of aromatic rings was supported by the removal of the aromatic C–H bending (617 cm^{-1}) peak. While, the distortion of those at 704 and 834 cm^{-1} in the FTIR spectra of the metabolite suggested the removal of the aromatic rings of the dye. Similarly, changes in the peaks around 1500 and 1650 cm^{-1} have been reported as the reduction of azo bond [31].

GCMS analysis showed the conversion of Reactive Blue 13 to various products most of which are substituted naphthalene and 1-3-5-triazine (Fig. 5). Worth noting is the 26.744 min peak with base peak of 91.10 identified as either sodium-2(2-formyl-2-hydroxyvinyl) benzoate or sodium-2(2-carboxyvinyl) benzoate. The results of the GCMS revealed among other peaks, the presence of sodium-2(2-formyl-2-hydroxyvinyl) benzoate, with a tropylium cation (m/z 91) as its base peak; tropylium cation is a major peak of substituted benzene fragmentation during mass spectrometry [32], this suggested the opening of the naphthalene rings of RB13 by the organism (Table 1). The proposed pathway also accounts for the high percentage (32.24%) of 2-diazonium-8-nitronaphthoxide (peak VIII); this is because of its resonance with 2-diazenyl-8-nitronaphthalen-1-ol (peak VII). We also observed that if inorganic elements (such as Na^+ and Cl^-) are accounted for in GCMS interpretations, particularly those involving extracts, important peaks would not be ignored. Summarily, the results of the FTIR and GCMS analyses clearly showed that the dye was actually degraded.

3.5. Enzyme assay

To ensure the mechanism of decolorization and biodegradation are not physiochemical, azoreductase, laccase, veratryl alcohol oxidase, lignin peroxidase and tyrosinase activities were assayed.

The result of the enzyme assay (Table 2) showed that there was no lignin peroxidase activity in both control and induced state. The tyrosinase activity appeared to shift from intracellular in the absence of dye to extracellular in the dye induced condition. The activity of NADH-dependent azoreductase was not detected in both control and induced conditions; small activities of NADPH-dependent azoreductase were however recorded in induced condition. The presence of laccase and veratryl alcohol oxidase activities in both control and induced experimental conditions suggested that the enzymes are not induced. The presence of an induced intracellular NADPH-dependent azoreductase suggested desulphonation of the azo dye RB13 prior to transport across the cell membrane, since sulphonated compounds cannot cross the membrane [33]. NADPH-dependent azoreductase has been reported in *Staphylococcus aureus* [34]; and some azoreductase are reported to require NADPH instead of NADH. It appears that azoreductase contributes just little to decolorization when the activities of laccase and veratryl alcohol oxidase are put into consideration. The presence of laccase and veratryl alcohol oxidase activities in both extracellular and intracellular fluids suggested the non-specific reduction of azo bond as shown in the proposed pathway (Fig. 5). This proposition was buttressed by the decolorization of dyes, when dyes were added to cell free culture (supernatant) of the bacterium (data not shown). The possibility of more than one mechanisms of azo bonds reduction in this bacterium is therefore not overruled. Laccase is known to attack aromatic rings via two steps electron transfer in the presence of molecular oxygen [35], while veratryl alcohol oxidase has been implicated in the prevention of polymerization of reactive rings produced by laccase thereby making laccase products susceptible to ring cleavage [36].

3.6. Phytotoxicity studies

The results of the phytotoxicity studies showed no significant effect on the % germination of the Reactive Blue 13 solution (1000 mg/l) wetted seeds; but it showed significant effect on the length of plumule and radicals. The germination percentage of both *Z. mays* and *P. vulgaris* seeds were less with RB13 treatment when compared to treatment with its degradation products and water. The RB13 treatments affected the length of plumule and radical significantly, while there were no significant effects with the degradation products (Table 3). The non-inhibition of germination with viable plumule and radical formation in the toxicity experiments of Reactive Blue 13 (at 1000 ppm) suggested that the dye was not only decolorized but also detoxified. This may be due to removal of aromatic amines by the organism.

4. Conclusions

In conclusion, the results of this research suggested that the *P. mirabilis* strain did not only decolorize the sulphonated azo dye RB 13 but also mineralize it. An enzymatic mechanism of decolorization and biodegradation was also inferred. This suggested that the organism may be useful for eco-friendly treatment of effluents and that an effective single phase anoxic bioremediation of textile effluent is possible, if catabolically versatile organisms like this strain of *P. mirabilis* are used.

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