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Original Research Article

Biodegradation and detoxification of Scarlet RR dye by a newly isolated filamentous fungus, *Peyronellaea prosopidis*

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ABSTRACT

Efficient mitigation and management of environmental pollution caused by indiscriminate disposal of textile industry dyes and effluents deserves special attention. The aim of the present study was to evaluate the efficiency of *Peyronellaea prosopidis* for the decolorization, degradation and detoxification of Scarlet RR dye. Ultraviolet visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, High-Performance Liquid Chromatography and Gas Chromatography–Mass Spectrometry (GC–MS) were used in analyzing the degraded metabolites of the dye. *P. prosopidis* showed decolorization potency on Scarlet RR dye, dye mixture and textile industry dye effluent at a concentration of 10 mg L^{-1} and up to 90, 84 and 85% within 5 d. Maximum decolorization of Scarlet RR dye (10 mg L^{-1}) by *P. prosopidis* was achieved at pH 6, temperature (35 °C) and biomass dose (1 g). Furthermore, 68, 88 and 91% reduction was recorded in the biological oxygen demand, chemical oxygen demand and color intensity of the textile industry effluent, respectively, after treatment with *P. prosopidis*. The degradation mechanism mediated by enzymes revealed significant inductions in lignin peroxidase (85%), laccase (58%), and manganese peroxidase (48%) after treatment of Scarlet RR dye with *P. prosopidis*. FTIR spectra of the metabolites showed significant disappearance and shifts in peaks in comparison with controls. Metabolites obtained from the GC–MS analysis were: N-(1I^3-chlorinin-2-yl)-2-{methyl[(4-oxo-3,4-dihydroquinolin-2-yl)methyl]amino}acetamide; N-(1I^3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide; 5-({[2-(1I^3-chlorinin-2-ylamino) ethyl]amino}methyl)cyclohexa-2,4-dien-1-one and N-ethyl-1I^3-chlorinin-2-amine after degradation of Scarlet RR dye. The detoxified status of the dye metabolites was confirmed with significant growth of plumule and radicle.

1. Introduction

The textile industry worldwide has witnessed tremendous growth over the years in the use of synthetic dyes [1]. This however comes with attendant increase in pollution from wastewater disposal. Indiscriminate discharge of synthetic dyes as industrial effluents constitutes pollutant menace in our environment [2]. Pollutants indiscriminately discharged into our environment poses great threat to the survival flora and fauna in the ecosystem. Textile dyes makes significant portion of textile effluent and municipal sewages in most developing nations [3]. Dye pollutants in water bodies are highly visible which causes reduction in light penetration and in turn affects photosynthetic processes of aquatic plants [4].

Dyes in the environment are always difficult to degrade or decolorize by many known chemical and physical methods. Dyes complex chemical structure confers on them the ability to remain stable/recalcitrant to degradation in water and soil [5]. Scarlet RR, a disperse dye, is used extensively in the textile industry in the

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dyeing of polyester fibers. The physio-chemical treatment techniques are labor intensive, highly uneconomical and fraught with methodological challenges [6]. To quench the negative effect of dye pollutants, there is a need for the development and application of ecofriendly biological treatment techniques. In addition, there is an increasing research interest and drift at discovering new fungi strains capable of detoxifying and degrading dye pollutants. Biological removal of dyes provides an alternative to existing expensive and commercially or environmentally unattractive, physiochemical technologies. The biological treatment processes could be applied to vast majority of dyes or dye containing industrial effluents [7]. Microorganisms exhibit great efficiency in decolorization of dyes with different enzyme systems.

Several reports are available in literature on dye bioremediation prowess of microorganisms [8]. The dye molecules and fungal cells play significant role in the degradation mechanism of different dyes [9]. Furthermore, there is a growing interest in the use of fungal biomass in wastewater treatment technologies of treating dye polluted wastewater [10]. Recently, research has drifted from the use of bacteria biomass to fungi because bacteria isolation is arduous, requires abundant supply of nutrients with relatively low biomass yield [11]. Different fungi species have shown potency in degrading a wide range of pollutant dyes inclusive compounds through secretion of highly oxidative and non-specific ligninolytic enzymes [12,13]. The main lignin-modifying enzymes are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase [14].

Virtually, dyes from all chemically distinct groups have been found prone to fungal oxidation due to their lignin modifying systems [15]. Fungal degradation and decolorization of dyes through biosorption has been extensively reported [16] or through mineralization [17]. The biosorption is considered the most advantageous than others for the treatment of colored waters [18]. The use of relatively inexpensive media to produce extensive and large fungal hyphal systems has proven to be very effective in dye decolorization by biosorption [9]. However, there is paucity of information on the enzyme mechanisms and biochemical pathway of degradation of Scarlet RR dye by *Peyronellaea prosopidis*.

This present study focusses on the biodegradation capacity of new fungal strains as a potent means of bioremediation. Hence, in continuation of our work, this present research is aimed at studying the decolorization, biodegradation and detoxification efficiency of a new filamentous soil fungi isolate, *P. prosopidis* on a disperse dye, Scarlet RR, dye mixture and textile effluent and also assess the toxicity status of the myco-degraded products.

2. Material and methods

2.1. Dyes and chemicals

Potato dextrose broth (PDB) (Sisco Research Laboratories, Mumbai, Maharashtra, India), riboflavine-2-sulfonic acid (Kemira Chemicals, Bradford, United Kingdom), aniline-2-sulfonic acid, veratryl alcohol chloranil, and dimethylformamide (BDH chemicals, Mumbai, Maharashtra, India) were used. Congo Red, Red RBL, Rubine GFL, Scarlet RR and Scarlet GDR were obtained from Sunflag Nigeria Limited, Surulere, Lagos State, Nigeria. The dyes and other chemicals used were of high purity and analytical grade. The plants (*Triticum aestivum* and *Phaseolus mungo*) used were purchased from Rajarampuri, Kolhapur, India.

2.2. Textile industry effluent collection

The textile industry effluent was obtained from Sunflag Nigeria Limited, Surulere, Lagos State, Nigeria. The effluent was collected in airtight stoppered plastic container. In order to remove impurities, the textile effluent was later carefully filtered in the laboratory. The pH of the filtered effluent was kept at 7.0 with the addition of phosphate buffer and stored at 4 °C temperature prevent contamination till further usage.

2.3. Isolation and molecular typing of fungus

P. prosopidis stock culture was maintained on potato dextrose agar isolated previously from a textile dye wastewater contaminated site at Itoku, Abeokuta, Ogun State, Nigeria [19]. The stock culture was maintained on PDB at 4 °C. In g L^{-1}, dextrose (20), yeast extract (5) and potatoes infusion (200) were part of the PDB composition used for decolorization studies. The internal transcribed spacer region amplification of 23S rRNA, obtained after isolation using Zymo research method, was done with primer (D1/D2). Accession number KR262714 was assigned after documentation on the National Centre for Biotechnology Information (NCBI) database. The NCBI provided the BLAST used in comparing the sequence data obtained with the ones previously submitted. The fungus was grouped in the genus *Peyronellaea* with 100% homologous similarity. Evolutionary relationships from 1000 replicates of the retrieved 18 nucleotide sequences (with 510 dataset positions) was inferred through maximum likelihood method based on the Jukes-Cantor model [20,21]. Molecular Evolution Genetic Analysis software version 7 [22] was used in conducting the analysis.

2.4. Decolorization experiment and optimization of physicochemical parameters

Decolorization of Scarlet RR dye was carried out under static condition with 100 mL culture of *P. prosopidis* (KR262714) pregrown in PDB [23]. The dye (10 mg L^{-1}) was added into each 250 mL Erlenmeyer flask containing 100 mL of individual pregrown cultures of *P. prosopidis* and further incubated until decolorization was observed. Aliquots of the culture supernatant were withdrawn at regular time intervals during the process of decolorization. A 10,000 g centrifugation for 15 min was carried out after removing the suspended particles from the culture supernatant by adding equal volume of methanol. The color intensity and decolorization was monitored by measuring the change in absorbance maxima of the dye (λ_{max} of Scarlet RR 530 nm) using a UV/Vis spectrophotometer (Hitachi U-2800, Japan). The pH (3–7), temperature (15, 25, 35, 45 and 55 °C), biomass cells (0.2, 0.4, 0.5, 0.8 and 1.0 g) and initial dye concentration (10–50 mg L^{-1}) were scaled up to determine their effect on decolorization efficiency. The experiments were carried out in three sets to minimize random error and the % decolorization was calculated as follows:

$$\text{Decolorization}(\%) = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100 \quad (1)$$

2.5. Decolorization of dye mixture and textile industry effluent

Decolorization of dye mixture (containing 10 mg L^{-1} each of Scarlet RR, Red RBL, Rubine GFL, Congo Red and Scarlet GDR) and textile industry effluent was carried out in the 250 mL capacity Erlenmeyer flask containing 100 mL of the pre-grown culture of *P. prosopidis*. The textile industry effluent was pre-sterilized at 121 °C for 20 min. and 50 mg L^{-1} of the textile industry effluent and dye mixture was then added into each flask containing the pregrown culture of *P. prosopidis* and incubated at 29 °C [23]. Supernatant (2 mL) of effluent and dye mixture were withdrawn after

centrifugation at 10,000 g for 10 min. The biological oxygen demand (BOD) and chemical oxygen demand (COD) were measured using the Standard Methods [24] procedure. Decolorization was monitored using the American Dye Manufacturer's Institute (ADMI 3WL) tri stimulus filter method reported earlier [25]. Textile industry effluent physicochemical parameters were pH 7.2, COD = 990 mg L^{-1} and BOD = 310 mg L^{-1} [24]. The transmittance was taken in three sets at different wavelengths (590, 540 and 438 nm) and the ADMI value was calculated using the 'Adams-Nickerson chromatic value' formula [24]. ADMI % decolorization was evaluated as follows:

$$\text{ADMI removal ratio}(\%) = \frac{\text{Initial ADMI}\,(0\,\text{day}) - \text{Observed ADMI}(t)}{\text{Initial ADMI}} \times 100 \quad (2)$$

where, ADMI (0 day) = initial ADMI values at (0 day) and ADMI (t) = ADMI values after a particular incubation time (t). Experiments (decolorization) were carried out in triplicates and performed under similar conditions.

2.6. Metabolites analyses

This was carried out using UV–Vis spectrophotometric analysis, High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared (FTIR) Spectroscopy and Gas Chromatography–Mass Spectroscopy (GC–MS). From the reaction medium, the fungal mycelia were first collected. This was adequately followed by the extraction of the metabolites after decolorization of the dye, dye mixture and textile industry effluent. The supernatant was dried over anhydrous Na_2SO_4 after extraction with equal volume of ethyl acetate. The resulting product was then dissolved in a small quantity of HPLC grade methanol for further analyses [26].

2.6.1. FTIR analysis

The analysis was carried out using Shimadzu 8400 S FTIR spectrometer (Shimadzu, Japan) in mid infra-red region of 400–4000 cm^{-1} with 16 scan speed. Potassium bromide-KBr (95:5 ratio) was used in preparing the samples, pellets were fixed in the cabin (sample) and analyzed [26].

2.6.2. GC–MS analysis

This was done using a Shimadzu QP 2010 GC–MS Engine (Shimadzu, Japan) by modifying the procedure reported earlier [27]. The ionization voltage was 70 eV. GC was conducted in the temperature programming mode with a Restek column (0.25 mm id, 60 m long, nonpolar; XTI-5). The initial column temperature was 80 °C for 2 min, then increased linearly at 10 °C min^{-1} to 280 °C, and held for 7 min. The temperature of the injection port was 280 °C and the GC–MS interface was maintained at 290 °C. Helium was used as carrier gas with a flow rate of 1.0 mL min^{-1}. Degradation products were identified by comparison of retention time and fragmentation pattern, as well as with mass spectra in the NIST spectral library support stored in the GC–MS solution software (version 1.10 beta, Shimadzu).

2.6.3. HPLC analysis

Filtration of the metabolites extracted ab initio was done using membrane filter (0.22 μm pore size) and analyzed HPLC engine fully equipped with Waters™ 2690, UV–visible detector and C18 hypersil column (4.6 mm × 250 mm) with a mobile phase of methanol (80%) and deionized water (20%) at a flow rate of 1 mL min^{-1} for 10 min. An aliquot of 20 μL was injected and analyzed by UV–Vis detector at wavelength of 460 nm.

2.7. Enzyme activities during decolorization

A reaction mixture of 2 mL containing 10% 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) in 0.1 M acetate buffer (pH 4.9) was used in determining the laccase activity with the increase in optical density measured at 420 nm [5]. The oxidative conversion of veratryl alcohol to veratryl aldehyde by H_2O_2 was employed in the determination of LiP activity. Increase in absorbance (at 310 nm) was then monitored after oxidation [23,28]. Relative changes in absorbance (unit min^{-1} mg of

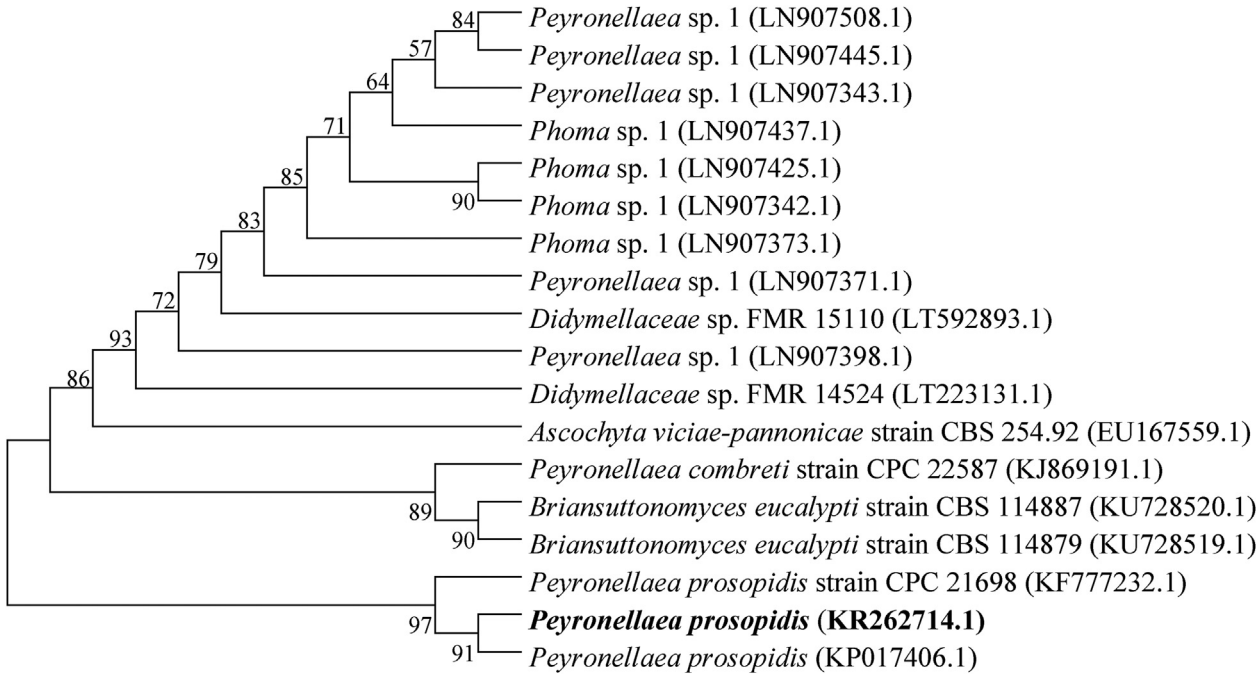


Fig. 1. Molecular phylogenetic analysis of *Peyronellaea prosopidis*. The numbers at the nodes indicate the levels of bootstrap support based on maximum-likelihood method.

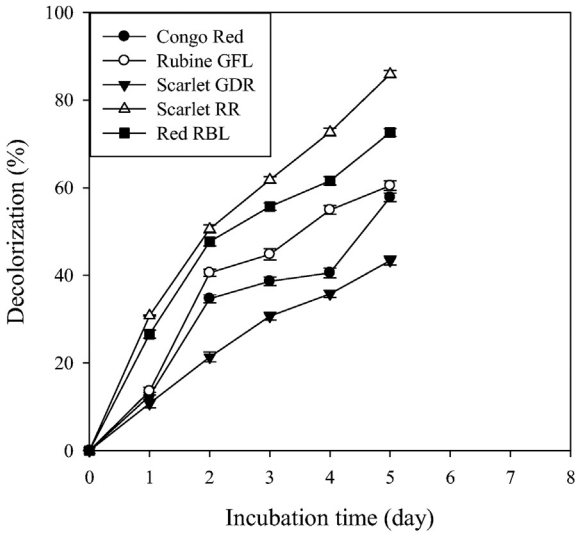


Fig. 2. Decolorization efficiency of *Peyronellaea prosopidis* on different dyes (at pH 7, temp. 30 °C, conc. 10 mg L^{-1}, biomass dose 1.0 g).

protein^{-1}) were used in expressing the average rates of a unit of enzyme activity (in triplicates). Phenol red (10 mg mL^{-1}) was used in the determination MnP activity. The MnP activity was evaluated through subtraction of phenol red (10 mg mL^{-1}) values obtained in the presence and absence of manganese ions in a 1 mL reaction mixture containing lactate (25 mM), $MnSO_4$ (0.1 mM), bovine serum albumin (1 mg mL^{-1}), phenol red (0.1 mg mL^{-1}), and culture (0.5 mL) in sodium succinate (20 mM) buffer. The protein content was determined using the method of Lowry et al. [28] with bovine serum albumin as the standard.

2.8. Toxicity studies

It is instructive to note that exposure of agricultural crops to dye and dye effluents disposed of indiscriminately may lead to distortion in plant physiological processes. In order to assess the toxicity of dye Scarlet RR, dye mixture and textile industry effluent and their metabolites formed after decolorization by *P. prosopidis*, two kinds of commonly cultivated crops: *T. aestivum* (monocot) and *P. mungo* (dicot) as described by Rane et al. [5] were used. About 5 mL each of 1000 ppm Scarlet RR dye, dye mixture and textile industry effluent and their degraded metabolites (1000 ppm) was used for the assay. Twenty (20) healthy seeds of each crop were separately sowed in petri plates containing little quantity of distilled water laced with tissue paper. Simultaneously the control sets involved watering the seeds with distilled water (daily 5 mL) at ambient temperature. Germination (%) and the shoot length (plumule) and root length (radicle) were recorded after 7 d. Germination (%) was evaluated as follows:

$$\text{Germination}(\%) = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sowed}} \times 100 \qquad (3)$$

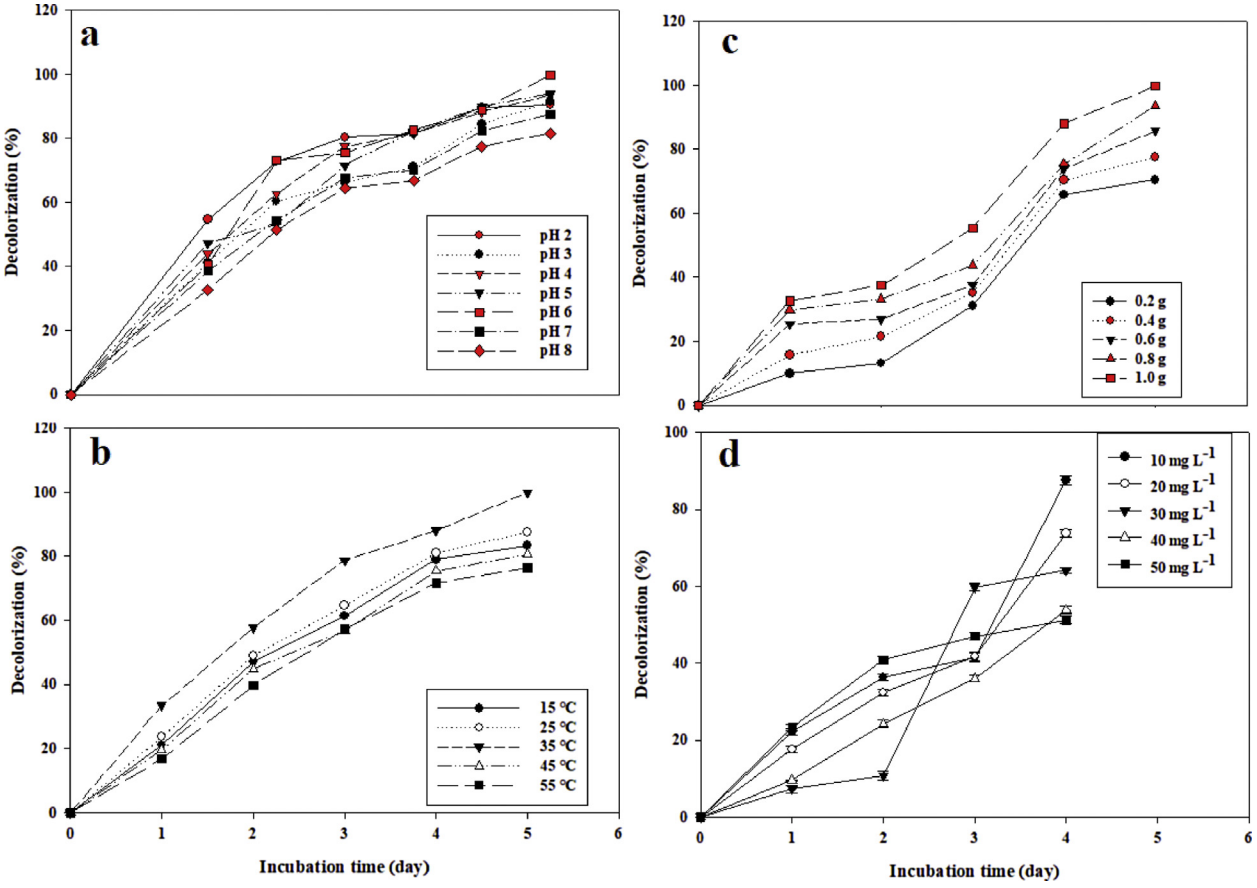


Fig. 3. (a) Influence of pH, (b) temperature (°C), (c) biomass (g), (d) initial dye concentration (mg L^{-1}) on percentage decolorization of Scarlet RR dye by *Peyronellaea prosopidis*.

2.9. Statistical analysis

One-way analysis of variance with Tukey–Kramer multiple comparison tests was used for the statistical analysis [19].

3. Results and discussion

3.1. Identification of isolate

When compared with previous submission on the NCBI database, *P. prosopidis* molecular sequence data exhibited 100% homologous identity with the first 10 nucleotide sequences available. *P. prosopidis* was subsequently deposited in the database with accession number KR262714. The phylogenetic tree revealed that *P. prosopidis* has an evolutionary lineage and linkage with the 18 nucleotides sequences retrieved from the NCBI database (Fig. 1).

3.2. Screening for dye decolorization and optimization of physicochemical parameters

The percentage reduction in the ADMI values for dye removal was 84 and 85% for dye mixture and textile dye effluent, respectively. The percentages decolorization recorded over the 5 days experimental incubation period were 90, 73, 60, 58, and 43% for the dyes; Scarlet RR, Red RBL, Rubine GFL, Congo Red and Scarlet GDR, respectively (Fig. 2). These results showed that Scarlet RR was most decolorized dye while Scarlet GDR was least decolorized during the screening. Further studies carried out to determine the effect of pH

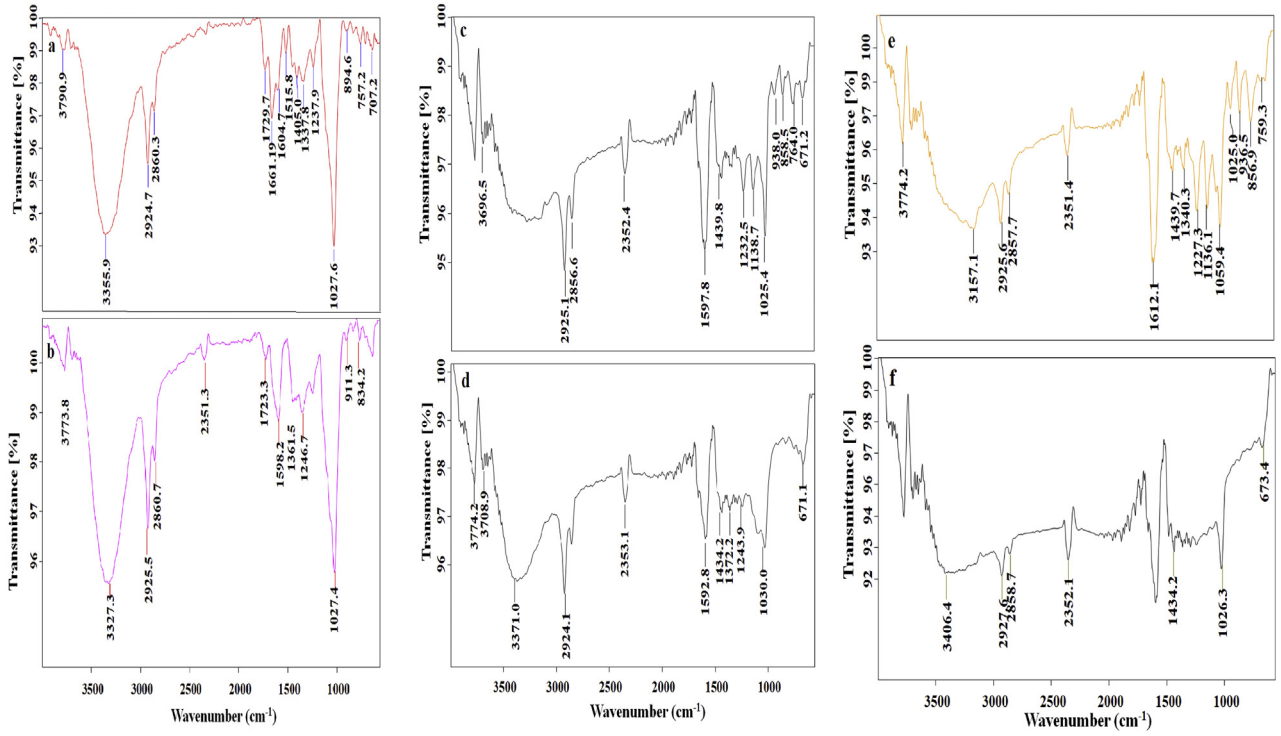
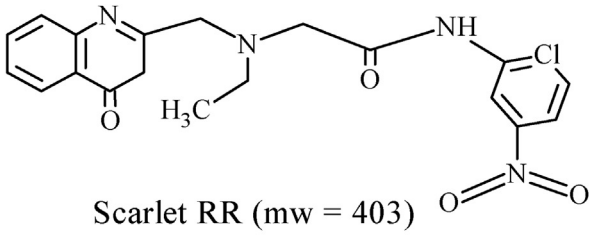


Fig. 4. FTIR spectra of (a) Untreated Scarlet RR, (b) degraded product of Scarlet RR by *Peyronellaea prosopidis*, (c) Untreated dye mixture, (d) degraded product of dye mixture by *Peyronellaea prosopidis*, (e) Untreated textile effluent, (f) degraded product of textile effluent by *Peyronellaea prosopidis*.

Table 1
FTIR analysis of Scarlet RR dye, dye mixture and textile effluent before and after treatment with *Peyronellaea prosopidis*.

Scarlet RR (Control) Peak Bond (cm^{-1})	Scarlet RR (Treated) Peak Bond (cm^{-1})	Dye mixture (Control) Peak Bond (cm^{-1})	Dye mixture (Treated) Peak Bond (cm^{-1})	Textile effluent (Control) Peak Bond (cm^{-1})	Textile effluent (Treated) Peak Bond (cm^{-1})
3709.0 N–H Str.	3773.8 N–H Str.	3696.5 N–H Str.	3774.2 N–H Str.	3774.3 N–H Str.	3406.4 N–H Str.
3355.9 O–H Str.	3327.3 O–H Def.	2925.1 C–H Str.	3708.9 N–H Str.	3157.1 O–H Str.	2927.6 C–H Str.
2924.7 C–H Str.	2925.5 C–H Str.	2856.6 C–H Str.	3371.9 O–H Str.	2925.6 C–H Str.	2858.7 C–H Str.
2860.7 C–H Str.	2860.7 C–H Str.	2352.4 C=C Str.	2924.1 C–H Str.	2857.7 C–H Str.	2352.1 C=C Str.
1729.7 N=N Str.	2351.3 C=C Str.	1597.8 C=C Str.	2353.1 C=C Str	2351.4 C=C Str.	1434.2 C–N Def.
1661.1 N=N Str.	1723.3 N=N Vibr.	1439.8 C–N Str.	1592.8 C=C Str.	1612.1 N–N Str.	1026.3 C–H Str.
1604.7 N=N Str.	1598.2 N=N Str.	1232.5 C–H Str.	1434.2 C–N Def.	1439.7 N–O Str.	673.4 C–Cl Str.
1515.8 N=N Str.	1361.5 C–OH Str.	1138.7 C–H Str.	1372.2 C–OH Str.	1340.3 N–O Str.	
1405.0 S=O Str.	1246.7 S=O Def.	1025.4 C–H Str.	1243.9 C–H Str.	1227.3 S=O Str.	
1337.8 NO_2 Str.	1027.4 C–H Str.	938.0 C–OH Str.	1030.0 C–H Str.	1136.1 S=O Str.	
1237.9 C–H Str.	911.3 C–H Str.	858.5 C–OH Str	671.1 C–Cl Str.	1059.4 C–H Str.	
1027.6 C–H Str.	834.2 C–H Str.	764.0 N–O Str.		1025.0 C–OH Str.	
894.6 C–H Str.		671.2 N–O Str.		936.5 C–OH Str.	
757.2 N–O Str.				856.9 C–OH Str.	
707.2 C–Cl Str.				759.3 N–O Str.	

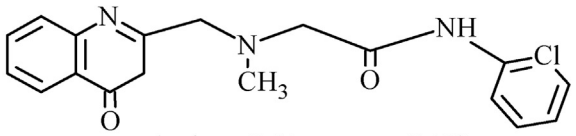
Def. – Deformation; Str. – Stretching; Vibr. – Vibration.



Scarlet RR (mw = 403)

Asymmetric cleavage by lignin peroxidase (LiP)

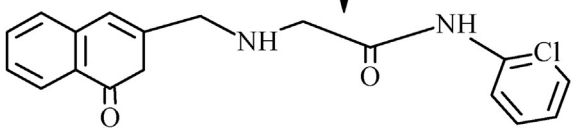
Demethylation and denitrification



(m/z = 341, mw = 343)

N-(1l^3-chlorinin-2-yl)-2-{methyl[(4-oxo-3,4-dihydroquinolin-2-yl)methyl]amino}acetamide

Demethylation

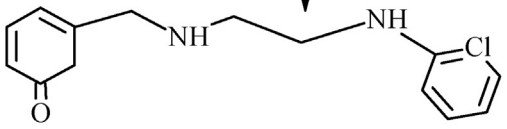


(m/z = 325, mw = 328)

N-(1l^3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide

Laccase

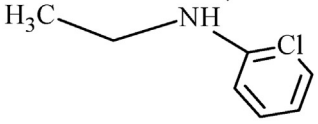
Ring cleavage and reduction



(m/z = 267, mw = 264)

5-({[2-(1l^3-chlorinin-2-ylamino)ethyl]amino}methyl)cyclohexa-2,4-dien-1-one

Ring cleavage and reduction



(m/z = 147, mw = 144)

N-ethyl-1l^3-chlorinin-2-amine

Fig. 5. Proposed schematic pathway for degradation of Scarlet RR by *Peyronellaea prosopidis*.

(Fig. 3a) revealed that 99% decolorization efficiency was attained at the pH 6. Generally filamentous grows optimally at an acidic pH range (4–6) as also reported by Kumari and Abraham [29]. The influence of temperature showed that optimal decolorization (99%) rate took place at 35 °C (Fig. 3b). Most filamentous fungi grow optimally at temperature range of 25–35 °C [30], hence its efficient influence on the decolorization rate. The decolorization potency of fungi increases with increasing temperature but declines at much higher temperatures (45–55 °C). Biomass dose scale up (optimization) studies revealed decolorization (99%) of Scarlet RR dye when 1 g cell biomass of the fungus was added to the set up (Fig. 3c). Chen and Ting [31] have earlier reported the advantages of using 1 g of fungal biomass in dye decolorization studies. The effect of initial dye concentration (10, 20, 30, 40 and 50 mg L^{-1}) on decolorization of Scarlet RR by *P. prosopidis* revealed the removal of 88, 74, 64, 54 and 51% respectively. These results showed significant decrease in percentage decolorization with increasing concentrations of Scarlet RR dye by *P. prosopidis* (Fig. 3d). Significant reduction in BOD (68%), COD (88%) and color intensity (91%) was achieved on treatment of the textile industry effluent by the fungus. Ali and El-Mohamedy [32] affirmed that maximum decolorization efficiency is highly favored at low concentrations. Furthermore, maximum (100%) decolorization efficiency was recorded at low initial concentrations (10–50 mg L^{-1}) while at concentrations above 100 mg L^{-1}, less decolorization efficiencies (< 100%) was achieved [33]. The rate of molecular exchange between the dye and the absorbent is controlled by the initial dye concentration [34].

3.3. Analysis of the metabolites

3.3.1. FTIR spectroscopy

Changes in the FTIR spectra of Scarlet RR dye, dye mixture and textile industry effluent along with their biotic controls showed their transformation into different metabolites (Fig. 4, Table 1). In all, 15 peaks of the untreated Scarlet RR dye was reduced to 13 peaks after treatment, likewise the 12 peaks of the untreated dye mixture was reduced to 11 peaks after treatment while 15 peaks in the spectrum of untreated textile industry effluent was reduced to 6 peaks after treatment. The changes in bonds and reduction of peaks (Fig. 4, Table 1) in the FTIR spectra obtained before and after the treatment of Scarlet RR dye, dye mixture and the textile effluent also corroborated and supported their transformation to different products which revealed the myco-decolorization efficiency of *P. prosopidis*.

3.3.2. GC–MS analysis

The analysis was carried out to determine the identities (chemical) of the metabolites of Scarlet RR dye produced after degradation. Furthermore, it was ultimately used in predicting a metabolic pathway of degradation of Scarlet RR dye occasioned by the induction of enzymes by *P. prosopidis*. Scarlet RR dye undergoes asymmetric cleavage by the action of LiP through demethylation and denitrification to form *N*-(1l^3-chlorinin-2-yl)-2-{methyl [(4-oxo-3,4-dihydroquinolin-2-yl)methyl]amino}acetamide (m/z = 341, mw = 343) (Fig. 5, Table SM1). *N*-(1l^3-chlorinin-2-yl)-2-{methyl [(4-oxo-3,4-dihydroquinolin-2-yl)methyl]amino}acetamide undergoes demethylation to form *N*-(1l^3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide (m/z = 325, mw = 328) (Fig. 5). *N*-(1l^3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide undergoes oxidative cleavage by the action laccase to produce 5-({[2-(1l^3-chlorinin-2-ylamino)ethyl]amino}methyl)cyclohexa-2,4-dien-1-one (m/z = 267, mw = 264) which further undergoes ring

Table 2
Enzyme activities (U min^{-1} mg $protein^{-1}$) of *Peyronellaea prosopidis* at day 0 and 5 on exposure to Scarlet RR dye[a].

Enzyme	Control	Test
Lignin peroxidase[a]	65.0 ± 19.5	120.1 ± 21.3**
Laccase[a]	7.0 ± 0.0	11.1 ± 0.0*
Manganese peroxidase[a]	42.6 ± 4.9	63.3 ± 7.1**

Values[a] are presented as mean of three experiments ± Standard Error of Means (S.E.M.) Significantly different from control (day 0) and test (day 5) at *P < 0.05 and **P < 0.01 by one-way ANOVA with Tukey–Kramer comparison test.

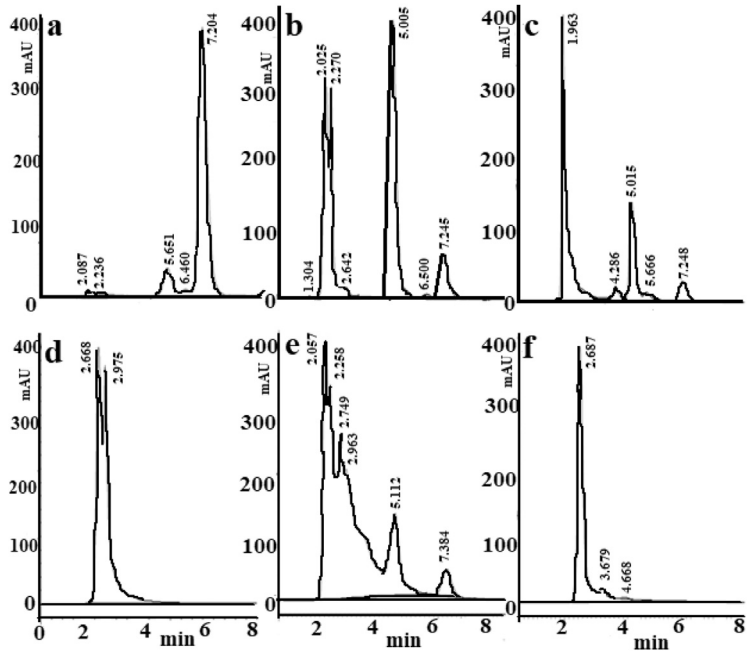


Fig. 6. HPLC chromatograms [a] Scarlet RR, [b] metabolites of Scarlet RR, [c] untreated dye mixture, [d] treated dye mixture, [e] untreated textile effluent and [f] treated textile effluent after treatment after 5 d of incubation.

Table 3
Phytotoxicity study of Scarlet RR dye, mixture of dyes and textile dye effluent and their degraded products after 5 days of treatment using *Peyronellaea prosopidis*.

Parameters studied	Water	Scarlet RR dye (Control)	Scarlet RR dye (metabolites)	Dye mixture (Control)	Dye mixture (Treated)	Textile effluent (Control)	Textile effluent (Treated)
Phaseolus mungo (*dicot*)							
Germination (%)	100	50	100	50	80	60	90
Plumule (cm)	10.31 ± 0.02	4.99 ± 0.06*	10.21 ± 0.08**	3.87 ± 0.10*	6.75 ± 0.30**	5.32 ± 0.40*	7.32 ± 0.10**
Radicle (cm)	4.62 ± 0.07	3.0 ± 0.04*	3.68 ± 0.02**	3.54 ± 0.30*	3.78 ± 0.04**	2.18 ± 0.03*	3.13 ± 0.07**
Triticum aestivum (*monocot*)							
Germination (%)	100	60	90	50	90	50	90
Plumule (cm)	5.34 ± 0.017	2.75 ± 0.02*	4.62 ± 0.05**	2.46 ± 0.03*	4.62 ± 0.40**	2.75 ± 0.08*	4.62 ± 0.01**
Radicle (cm)	5.58 ± 0.03	2.25 ± 0.08*	3.52 ± 0.10**	2.19 ± 0.20*	3.65 ± 0.05**	2.07 ± 0.02*	3.50 ± 0.04**

Values[a] are presented as mean of three experiments ± S.E.M. Root and shoot lengths of fifty (50) plants grown in Scarlet RR dye, mixture of dyes and textile dye effluent respectively are significantly different from that of plants grown in distilled water by *P < 0.001. Root and shoot lengths of fifty (50) plants grown in the degraded Scarlet RR dye, mixture of dyes and textile dye effluent respectively are also significantly different from that of plants grown in untreated Scarlet RR dye, mixture of dyes and textile dye effluent by **P < 0.001.

cleavage and reduction to form *N*-ethyl-1l[3]-chlorinin-2-amine (m/z = 147, mw = 144) (Fig. 5, Table SM1). Asymmetric and oxidative cleavage of dye molecules is attributed to the action of laccase and LiP, respectively [27,35]. The differences in enzyme induction influence the degradation mechanism of Scarlet RR dye by *P. prosopidis*. This further confirmed the capacity of *P. prosopidis* in degrading Scarlet RR dye into different non-toxic metabolites.

3.3.3. HPLC analysis

A disappearance of the broad peak was observed (Fig. 6b) on treatment of Scarlet RR dye (control) with *P. prosopidis* (Fig. 6a) at a retention time of 7.204 min. This resulted in the formation of new peaks at retention times of 1.3, 2.0, 2.3, 2.8, 5.0, 6.5 and 7.3 min. Peaks at retention times of 2.0, 4.3, 5.0, 5.7 and 7.3 min were observed in the dye mixture (control) (Fig. 6c) which were reduced to two peaks after treatment at retention times of 2.7 and 3.0 min (Fig. 6d). Conversely, the relative peaks at retention times of 2.1, 2.3, 2.8, 3.0, 5.1 and 7.4 min (Fig. 6e) observed in the textile industry effluent (control) have disappeared/reduced with the formation of new peaks in the treated textile industry effluent at retention times of 2.7, 3.7 and 4.7 min. The relative and comprehensive reduction of dye as a result of the formation and further mineralization of their reduction products suggested degradation of Scarlet RR dye; a metabolically triggered phenomenon linked with certain fungal oxidases [36]. The HPLC spectra (Fig. 6) differences observed in the untreated and treated samples of Scarlet RR dye, the mixture of dyes and the textile effluent confirms myco-degradation.

3.4. Enzyme analysis

Efficient secretion of extracellular enzyme confers decolorization capacity and potency on fungi. Therefore enzymes activities were carried out during scarlet RR dye removal process by *P. prosopidis* and compared with their controls (Table 2). Induction of LiP enzyme was the highest recorded while MnP was least induced [37]. Direct involvement of fungal ligninolytic enzymes is required in the mineralization of dyes [38]. Enzyme-mediated degradation and biosorption are the basic mechanism deployed by some species of microbes in the decolorization of textile dyes [39,40]. In this study, it was observed that LiP accounted for the initial asymmetric cleavage, demethylation and denitrification of Scarlet RR dye while laccase was responsible for the final breakdown into *N*-ethyl-1l[3]-chlorinin-2-amine (Fig. 5).

3.5. Toxicity studies

The toxic nature of the dye and its metabolites after treatment with *P. prosopidis* were determined using commonly cultivated crops; a monocotyledonous plant (*T. aestivum*) and dicotyledonous plant (*P. mungo*). These crops were used to assess the chemo-toxic state of Scarlet RR dye, dye mixture and textile industry effluent and their extracted metabolites. The study revealed that there was an inhibition of percentage germination in assays containing 1000 ppm of the dye Scarlet RR for both *P. mungo* and *T. aestivum* by 50 and 60%, respectively (Table 3). In the same vein, the inhibition of germination by the dye mixture on *P. mungo* and *T. aestivum* was 50 and 50% respectively. On subjecting the seeds of *P. mungo* and *T. aestivum* to textile industry effluent, 60 and 50% were recorded as inhibition of percentage germination, respectively. Furthermore, for the seeds of *P. mungo* and *T. aestivum* exposing to treated Scarlet RR dye, dye mixture and textile industry effluent, 100 and 90% germination, 80 and 90% germination and 90 and 90% germination were recorded, respectively. The enhanced and significant growth of the plumule and radicle suggests reduced toxicity of metabolites formed (Table 3). The improved germination of common plants after degradation of dye by microorganism has been reported by Dawkar et al. [41]. This study suggests that the dye, dye mixture and textile industry effluent were toxic to both plants, while the metabolites formed after myco-degradation were less toxic, which signifies the detoxification of dye, dye mixture and textile industry effluent by *P. prosopidis*. These results further qualify *P. prosopidis* as a good choice and candidate in the bioremediation of textile dye in terms of decolorization and detoxification.

4. Conclusions

P. prosopidis efficiently decolorized Scarlet RR dye, dye mixture and textile effluent. Furthermore, 68 and 88% BOD and COD reduction of the textile industry effluent were achieved. The enzymatic studies indicate the role played by LiP, MnP and laccase. Thus, the present study confirmed the myco-decolorization efficiency of Scarlet RR dye, textile dye effluent and dye mixture by *P. prosopidis*. The GC–MS analysis revealed the formation of different intermediate products. An enzymatic metabolic degradation pathway was also proposed. This study confirmed the potency of *P. prosopidis* as an inexpensive and eco-friendly candidate for bio-decolorization of dye, dye mixture and dye effluent. Further research to explore the myco-remediation efficiency of different fungi strains like *P. prosopidis* in solid state fermentation systems is on-going.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.serj.2018.03.001.

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