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Degradation and Detoxification of Disperse Dye Scarlet RR by *Galactomyces geotrichum* MTCC 1360

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Galactomyces geotrichum **MTCC 1360 degraded the Scarlet RR (100 mg/l) dye within 18 h, under shaking conditions (150 rpm) in malt yeast medium. The optimum pH and the temperature for decolorization were pH 12 and 50°C, respectively. Enzymatic studies revealed an induction of the enzymes, including flavin reductase during the initial stage and lignin peroxidase after complete decolorization of the dye. Decolorization of the dye was induced by the addition of $CaCO_3$ to the medium. EDTA had an inhibitory effect on the dye decolorization along with the laccase activity. The metabolites formed after complete decolorization were analyzed by UV-VIS, HPLC, and FTIR. The GC/MS identification of 3 H quinazolin-4-one, 2-ethylamino-acetamide, 1-chloro-4-nitro-benzene, *N*-(4-chloro-phenyl)-hydroxylamine, and 4-chloro-pheny-lamine as the final metabolites corroborated with the degradation of Scarlet RR. The phytotoxicity study revealed the nontoxic nature of the final metabolites. A possible degradation pathway is suggested to understand the mechanism used by *G. geotrichum* and thereby aiding development of technologies for the application of this organism to the cleaning-up of aquatic and terrestrial environments.**

Keywords: Decolorization, *Galactomyces geotrichum*, lignin peroxidase, detoxification, phytotoxicity, bioremediation

Synthetic textile dyes are one of the most serious pollutants that contaminate steadily higher amounts of wastewater as industrial effluents [2]. The dyes are highly recalcitrant owing to their chemical structure. They are often toxic, or transform into a toxic product when released into the environment [7]. One of the major problems with textile effluents is that they have a toxic effect on the germination rates and biomass of many plant species that play important ecological functions [16]. Conventional wastewater treatments are not efficient to remove recalcitrant dyestuffs from effluents. The physical and chemical methods for removing dyes are not suitable, owing to their high cost and low efficiency [22]. Thus, biotechnological approaches have been attracting interest for the textile industry [2]. Yeasts have attractive features for dye decolorization, as they grow faster than most filamentous fungi and have the ability to resist unfavorable environments, when compared with bacteria and filamentous fungi. To date, the use of yeast strains in dye wastewater treatment has been limited [14].

In previous studies, we found that the yeast *Galactomyces geotrichum* MTCC 1360 could effectively decolorize various industrial dyes, including the disperse dye Scarlet RR. Disperse dyes are widely used in the textile industry, as they are the only dyes that can be used for dyeing polyester fibers [5, 25]. Accordingly, the present study explored the ability of *G. geotrichum* to degrade and detoxify the dispersive dye Scarlet RR.

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Materials and Methods

Microorganism and Culture Conditions

Galactomyces geotrichum MTCC 1360 was obtained from the Microbial Type Culture Collection, India. A pure culture was maintained on malt yeast agar slants at 4°C. The composition of the malt yeast medium was (g/l) malt extract 3, yeast extract 3, peptone 5, and glucose 10 for the decolorization studies.

Chemicals

The tartaric acid, *n*-propanol, 3'3'-diaminobenzidine tetrahydrate, and riboflavin were purchased from Sisco Research Laboratories, India. The chemicals used were of the highest purity available and of an analytical grade. The Scarlet RR dye was procured from the Manpasand Textile Company, Ichalkaranji, India.

Decolorization of Scarlet RR

The *G. geotrichum* was grown for 24 h at 30°C in 250-ml Erlenmeyer flasks containing 100 ml of the malt yeast medium. After 24 h of growth, the malt yeast medium was supplemented with 100 mg/l Scarlet RR (SRR) and incubated at 50°C (pH 12, 150 rpm) in an orbital shaking incubator. An aliquot (3 ml) of the culture medium was withdrawn at different time intervals, centrifuged (4,000 rpm, 10 min), and the supernatant separated. Methanol (2 ml) was added to the cell pellet to extract the bound dye, followed by centrifugation (4,000 rpm, 10 min). The decolorization was monitored by measuring the absorbance of the culture supernatant mixed with the methanol extract at 510 nm. The time required for the complete decolorization of SRR was noted. The change in pH was also studied during the decolorization.

The percent decolorization and average decolorization rate were measured at different time intervals [8, 20]. All the decolorization experiments were performed in three sets. Abiotic controls (without microorganisms) were always included.

Effect of Physicochemical Parameters

The decolorization of SRR by *G. geotrichum* was studied at different temperatures (5, 30, and 50°C) at pH 12, and under various pH conditions (pH 3, 5, 7, 9, and 12) at 50°C. The effect of the inoculum size (2, 4, and 6 g) and various dye concentrations (200, 300, 400, and 500 mg/l) was studied at 50°C (pH 12).

Preparation of Cell-Free Extract

The *G. geotrichum* was grown in the malt yeast medium at 30°C for 24 h and the biomass collected by filtration through Whatmann filter paper No 1. The mycelium was then suspended in a 0.1 mM sodium phosphate buffer (pH 7.4) and sonicated (Sonics-Vibracell ultrasonic processor) based on a 60 amplitude output at a temperature below 4°C with 12 strokes of 30 sec each at 1-min intervals. The resulting extract was then used as an enzyme source.

Enzyme Assays

The lignin peroxidase (LiP) and laccase activities were assayed in both the cell-free extract and the culture supernatant. The LiP activity was determined by monitoring the formation of propanaldehyde at 300 nm in a 2.5-ml reaction mixture containing 100 mM *n*-propanol, 250 mM tartaric acid, and 10 mM H_2O_2 [21]. The laccase activity was determined by measuring an increase in the optical density at 410 nm in a 2-ml reaction mixture containing 5 mM 3'3'-diaminobenzidine tetrahydrate in a 0.1 M acetate buffer (pH 4.8) [3]. All the enzyme assays were also carried out with reference blanks containing all the components except an enzyme. All the enzyme assays were run in triplicate and the average rates calculated. One unit of enzyme activity was defined as the change in the absorbance unit per minute per milligram of protein.

The NADH-dichlorophenol indophenol (NADH-DCIP) reductase activity was determined using the procedure reported earlier by Salokhe and Govindwar [18]. The riboflavin reductase NAD(P)H: flavin oxidoreductase was measured spectrophotometrically by monitoring the decrease in absorbance at 340 nm. The cell-free extract was added to a solution (final volume, 1 ml) containing 100 mM of Tris-HCl (pH 7.5), 25 mM of NADPH, and 0.003 U/l of riboflavin. The reaction rates were then calculated using a molar extinction coefficient of 6.3 mM^{-1}/cm^{-1} [17].

Aminopyrine *N*-demethylase (AND) activity was assayed according to the procedure of Salokhe and Govindwar [18]. Aniline hydroxylase (ANH) activity was measured using a procedure reported earlier [4].

Effect of $CaCO_3$ and EDTA on Dye Decolorization

Different concentrations (10–100 mg/l) of calcium carbonate ($CaCO_3$) were added to the malt medium during the growth of *G. geotrichum* to induce decolorization. Ethylenediaminetetraacetic acid (EDTA) was also added (at various concentrations, 0.010–0.050 mM, at 50°C, pH 12) to the medium after 24 h of growth of *G. geotrichum* along with the dye to study its effect on the decolorization performance. The LiP and laccase activities were also measured.

Extraction and Analysis of Metabolites

The decolorization was qualitatively analyzed using a UV-visible spectrophotometer (Hitachi U-2800), and the biodegradation was monitored using high performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FTIR). Identification of the metabolites was carried out by gas chromatography-mass spectroscopy (GC/MS). The culture broth was centrifuged at 13,000 rpm for 30 min after complete decolorization of the dye. An equal volume of ethyl acetate was used to extract the metabolites from the clear supernatant. The extracts were then dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary evaporator.

The biodegraded SRR was characterized by an HPLC analysis, which was carried out on a C_{18} column (Waters model No. 2690, symmetry of 4.6×250 mm) with methanol as the mobile phase at a flow rate of 0.75 ml/min, and using a UV detector at 510 nm (Perkin Elmer, Spectrum One). FTIR analysis was performed in the mid-IR region of 400–4,000 cm^{-1} with a 16 scan speed. The samples were mixed with spectroscopically pure KBr in a ratio of 5:95, the pellets fixed in a sample holder, and the analyses performed. The GC/MS analysis of the metabolites was carried out using a Shimadzu 2010 MS Engine, equipped with an integrated gas chromatograph and HP1 column (60 m long, 0.25 mm id, nonpolar). Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 280°C, while the oven conditions were 80°C for 2 min, followed by an increase to 200°C based on a rate of 10°C/min, followed by a further increase to 280°C based on a rate of 20°C/min. The compounds were identified on the basis of their mass spectra and using the National Institute of Standards and Technology (NIST) library.

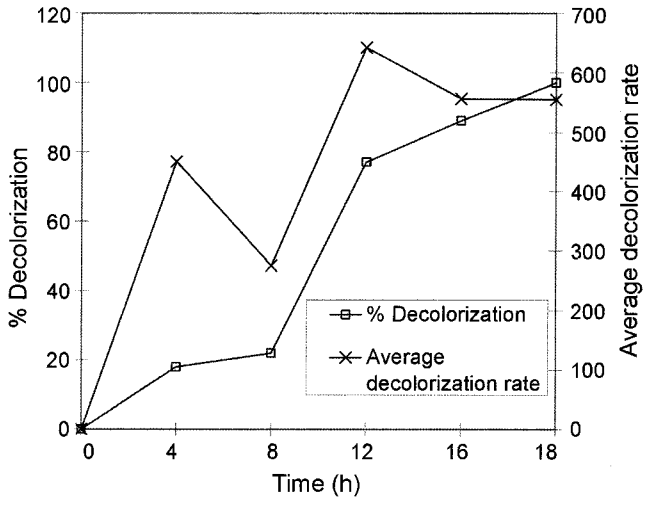

Fig. 1. Percent decolorization (□) and average decolorization rate (×) of Scarlet RR by *Galactomyces geotrichum* MTCC 1360 in malt yeast medium.

Table 1. Activities of dye-degrading enzymes at different time intervals and in control *Galactomyces geotrichum* MTCC 1360 cells.

Enzymes	Control	6 h	12 h	18 h
Lignin peroxidase[a]	0.009±0.001	0.030±0.013	0.078±0.013**	0.154±0.012***
Laccase[a]	0.058±0.010	0.037±0.006	0.044±0.008	0.067±0.006
NADH-DCIP reductase[b]	582.66±69.10	466.33±20.57	420.33±20.57	571.16±40.02
AND[c]	12.190±0.310	6.120±0.500**	6.280±1.810*	6.576±0.296*
ANH[d]	2.250±0.25	1.130±0.20	1.220±0.30	1.523±0.24
Riboflavin reductase[e]	4.36±0.02	8.83±0.58***	4.23±0.34	4.96±0.40

[a]Units/min/mg protein.
[b]μg DCIP reduced/min/mg protein.
[c]nmole formaldehyde liberated/mg protein/min.
[d]nmole *p*-amino phenol liberated/mg protein/min.
[e]μg riboflavin reduced/mg/min.
Values are mean of three experiments±SEM. Significantly different from the control (uninduced cells) at $*P<0.05$, $**P<0.01$, and $***P<0.001$ by one-way ANOVA with the Tukey-Kramer multiple comparisons test.

Phytotoxicity

This test was performed to assess the toxicity of the untreated and treated samples at a concentration of 4,000 ppm. The tests were carried out according to Parshetti *et al.* [15] on two kinds of seeds commonly used in Indian agriculture: *Triticum aestivum* and *Sorghum bicolor.*

Statistical Analysis

The data were analyzed by a one-way analysis of variance (ANOVA) using a Tukey-Kramer multiple comparison test.

RESULTS

Decolorization of Scarlet RR

The *G. geotrichum* produced 100% decolorization of SRR (100 mg/l) after 18 h of incubation. The average rate of decolorization was 555.55 μg/h after 18 h; however, it was higher after 12 h (Fig. 1). There was change in pH from 12 to 5. The decolorization was not due to change in the pH, as there was no change in the dye absorbance at different pHs (5–12).

Effect of Physicochemical Parameters

The degradation of SRR by *G. geotrichum* was found to be temperature sensitive. The degradation increased with an increase in temperature. The optimum temperature was found to be 50°C, which took 18 h for complete degradation. Scarlet RR was decolorized by 22% and 60% after 48 h of incubation with *G. geotrichum* MTCC 1360 at 5 and 60°C, respectively, whereas it took 18 h for 80% decolorization at 30°C. The decolorization of SRR at different pHs showed a decrease in the time required with an increase in pH. The time required at pH 3, 5, 7, 9, and 12

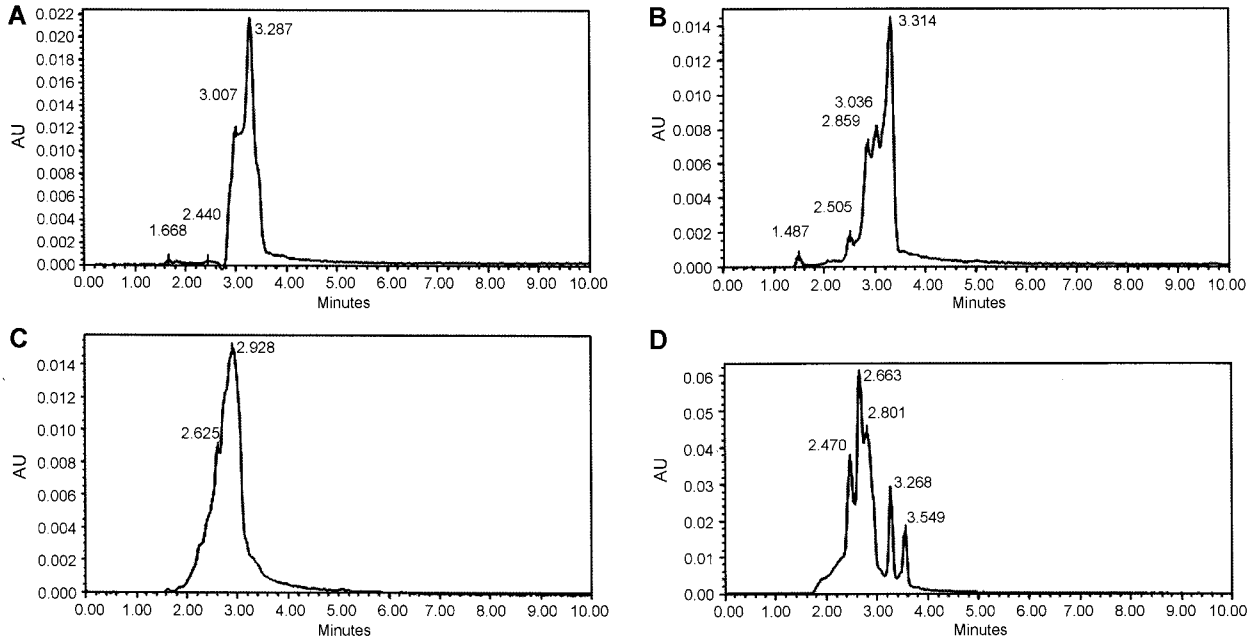

Fig. 2. HPLC chromatograms of (**A**) control Scarlet RR, (**B**) metabolites of Scarlet RR after 6 h, (**C**) metabolites of Scarlet RR after 12 h, and (**D**) metabolites of Scarlet RR after 18 h.

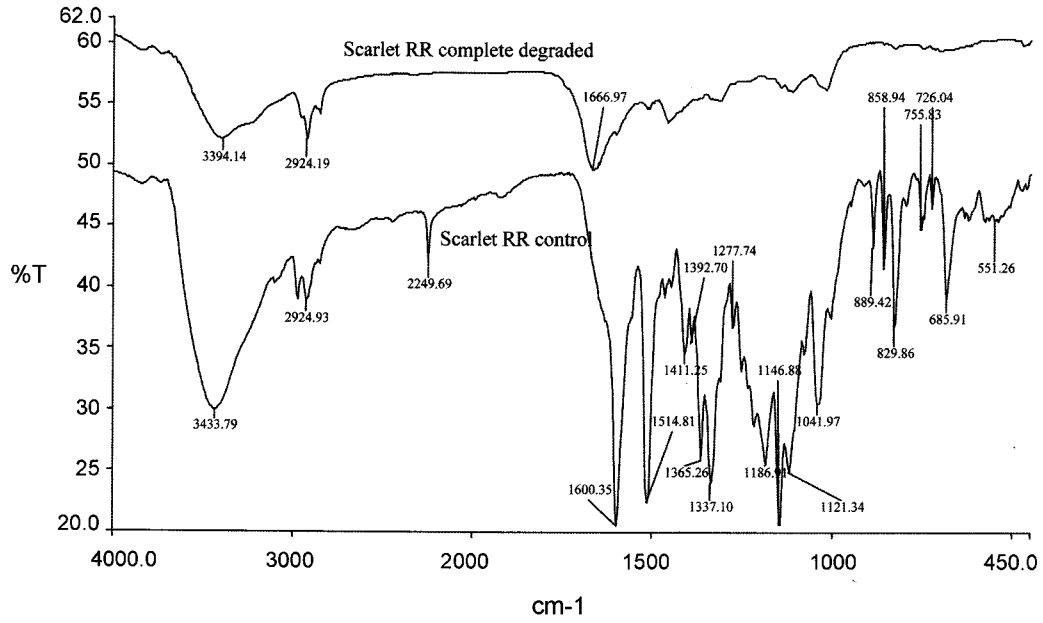

Fig. 3. FTIR spectroscopy of (a) control SRR and (b) metabolites formed after complete decolorization at 18 h (50°C at pH 12).

was 192, 144, 34, 34, and 18 h, respectively. Furthermore, increase in the mycelium concentration decreased the time period required for decolorization, whereas increasing the dye concentration increased the time required for decolorization.

Enzyme Activities During Decolorization

A significant induction (1,611%) of LiP was observed during the decolorization of SRR. Riboflavin reductase was also induced by 103% during the initial stage (6 h of incubation), and then decreased to the control level. The laccase and DCIP reductase remained same as that in the control, yet there was a decrease in AND and ANH (Table 1).

Effect of $CaCO_3$ and EDTA on Dye Decolorization

The LiP and laccase enzymes play a crucial role in the dye decolorization, as evidenced by the increased level of LiP during the decolorization of SRR. Among the various concentrations of $CaCO_3$ investigated (10–100 mg/l), a 0.050 mg/l concentration produced a decrease in the time required for decolorization from 18 h to 11 h. However, further increase in the $CaCO_3$ concentration had no significant effect on the decolorization.

Among different concentrations of EDTA investigated (0.010–0.050 mM), a 0.050 mM concentration produced a complete inhibition of laccase activity and decolorization of dye, although the LiP activity remained the same as that in the control cells (data not shown).

Analysis of Decolorization Products

The spectrophotometric analysis of the culture supernatant at 510 nm showed a complete decolorization of SRR. The HPLC analysis showed the presence of new peaks at

Table 2. Mass spectral data, retention times, and proposed identities of metabolites formed after degradation of Scarlet RR by *Galactomyces geotrichum* MTCC 1360.

Sl. No.[a]	R_t (min) and M_w (*m/z*)	Relative abundances in mass spectrum: *m/z* (% relative intensity)	Proposed compound
1	19.267,146	52 (20), 63 (50), 70 (90), 90 (100), 99 (30), 125 (40), 142 (80), 156 (10), 172 (80)	3H-quinazolin-4-one
2	28.250,103[M+1]	65 (10), 70 (60), 91 (50), 103 (2), 125 (100), 126 (5), 153 (60), 154 (2), 244 (40)	2-Ethylamino-acetamide
3	19.808,157	51 (5), 55 (40), 69 (60) ,83 (100), 84 (10), 111 (90), 112 (10), 126 (10), 154 (70)	1-Chloro-4-nitrobenzene
4	18.133,143	50 (10), 65 (90), 80 (30), 92 (40), 108 (70), 122 (5), 138 (100)	*N*-(4-Chloro-phenyl)-hydroxylamine
5	24.775,128[M+1]	55 (20), 68 (20), 86 (70), 98 (2), 112 (5), 140 (20), 155 (2), 170 (100), 183 (2)	4-Chlorophenylamine

different retention times in the samples after 6, 12, and 18 h, during degradation, when compared with the control, representing the breakdown of the SRR into various compounds (Fig. 2). The FTIR analysis (Fig. 3) of the control dye revealed the presence of aromatic amines at 1,337 cm^{-1}, a benzene ring at 755–829 cm^{-1}, halides (C-Cl) at 685 cm^{-1}, CH_3 vibrations at 1,392 cm^{-1}, and C-N vibrations at 1,121 cm^{-1}. In contrast, the FTIR analysis of the dye metabolites recorded the presence of amines as N-H stretching at 3,394 cm^{-1}, CH_2 as C-H stretching at 2,924 cm^{-1}, and the formation of cross conjugates possessing C=O at 1,666 cm^{-1}. Thus, the appearance of new peaks and the absence of the major peaks representing the bonding interactions in the dye supported biodegradation of the dye.

The GC/MS analysis revealed the formation of various metabolites after the decolorization of SRR. Therefore, a pathway has been proposed for the degradation of SRR by *G. geotrichum* MTCC 1360 (Fig. 4). The asymmetric cleavage of SRR by peroxidase [6] resulted in reactive products, which then underwent demethylation, and a reduction reaction to produce stable intermediates. Two intermediates, 2-[ethyl-(4 oxo-3,4-dihydro-quinazolin-2-yl methyl)-amino]-acetamide and 1-chloro-4-nitro-benzene were formed after the asymmetric cleavage of SRR. The reduction of the latter compound then gave 1-chloro-4-nitroso-benzene, which was reduced again to form N-(4-chloro-phenyl)-hydroxylamine, and finally reduced to give the metabolite 4-chloro-phenylamine. Meanwhile, the 2-[ethyl-(4 oxo-3,4-dihydro-quinazolin-2-yl methyl)-amino]-acetamide was cleaved again by peroxidase (asymmetrically) to give 2-methyl-3 H quinazoline 4-one and 2-ethylamino-acetamide. The demethylation of 2-methyl-3 H quinazoline 4-one gave 3 H quinazolin-4-one. The FTIR spectrum of the degraded dye showed a peak at 2,924 cm^{-1}, representing C-H stretching as CH_2, which also supported the biodegradation pathway by the formation of 2-ethylamino-acetamide. The intermediates observed in the GC/MS after the degradation of Scarlet RR are shown in Table 2, and the others mentioned in the pathway were rationalized as necessary intermediates during the biodegradation.

Phytotoxicity

The SRR (4,000 ppm) inhibited the germination of *Sorghum bicolor* and *Triticum aestivum* by 80% and 100%, respectively, when compared with the control. However, the metabolites of SRR did not inhibit any of the test seeds, indicating the detoxification of SRR as regards the germination of *Sorghum bicolor* and *Triticum aestivum*.

DISCUSSION

This study demonstrated the ability of *G. geotrichum* MTCC 1360 to degrade SRR (100 mg/l) completely within 18 h at an optimum temperature of 50°C. In contrast, the consortium RVM 11.1 has been reported to decolorize Reactive Violet 5 at 30°C, and further increase in the temperature to 50°C had an adverse affect on the decolorization [11]. *Klebsiella pneumoniae* RS-1 and *Alcaligenes liquefaciens* S-1 produced no decolorization of methyl red at 45°C [23]. The current pH studies also indicated that the rate of decolorization increased at higher pH (8, 10, and 13), whereas Mali *et al.* [10] previously found that a pH from 6–8 was optimum for the decolorization of triphenyl methane dyes and azo dyes by *Pseudomonas* sp. A pH from 7–8.5 has been reported as the optimum for the decolorization of Reactive Violet 5 [11]. Similar to the present findings, Sani

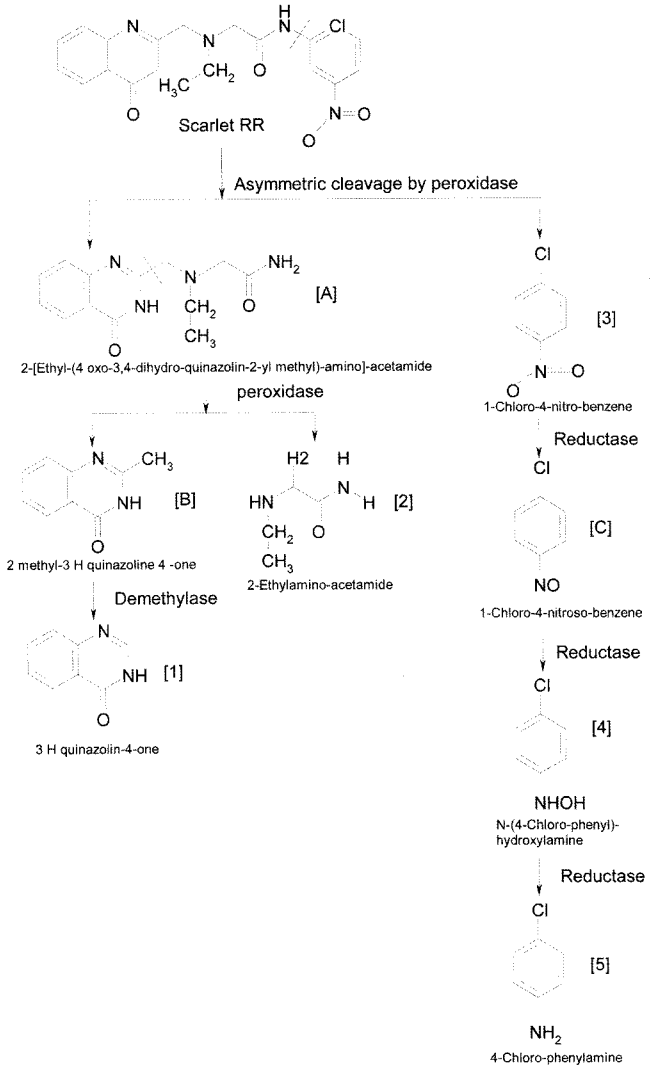

Fig. 4. Proposed pathway for the degradation of Scarlet RR by *Galactomyces geotrichum* MTCC 1360.
The compounds with Arabic numbers were identified in the reaction mixture, and details of their GC/MS analyses are shown in Table 2. The compounds represented by the alphabet were not identified in the reaction mixture; however, their existence was rationalized as necessary intermediates for the final products.

and Banerjee [19] also observed a decrease in the time required for decolorization when increasing the mycelium concentration.

The high LiP activity in *G. geotrichum* MTCC 1360 indicated that this enzyme may have an important role in the degradation of SRR. The decrease in AND and ANH activities might be due to the inhibitory effect of the dye on these enzymes. Macedo *et al.* [9] previously reported an increase in the level of LiP activity with 0.5% and 5.0% concentrations of $CaCO_3$; however, the present results showed the same levels of these enzymes in the cells as observed after 12 h, in the absence of $CaCO_3$. In the study by Macedo *et al.* [9], they found that the increase in LiP activity was due to a decrease in the pellet size, and a similar mechanism may have occurred with *G. geotrichum* MTCC 1360. EDTA is a common inhibitor of LiP and laccase [24]. Thus, similar to the current results, Nagai *et al.* [12] observed a slight inhibition of laccase activity in the edible mushroom *Lentinula edodes* due to EDTA (1 mM), whereas a 30% laccase inhibition was observed in *Peniophora* with a 0.5 mM concentration of EDTA [13].

The GC/MS showed the formation of 3 H quinazolin-4-one by demethylation, indicating the role of laccase, which agrees well with the results previously reported by Abadulla *et al.* [1]. Finally, the reduced phytotoxicity of the metabolites produced by the action of *G. geotrichum* MTCC 1360, enables this microorganism to be used in the biological treatment of industrial effluents containing the disperse dye SRR.

References

1. Abadulla, E., T. Tzanov, S. Costa, K. H Robra, A. Cavaco-Paulo, and G. M. Gubitz. 2000. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Appl. Environ. Microbiol.* **66:** 3357–3362.
2. Eichlerova, I., L. Homolka, and F. Nerud. 2006. Evaluation of synthetic dye decolorization capacity in *Ischnoderma resinosum*. *J. Ind. Microbiol. Biotechnol.* **33:** 759–766.
3. Flurkey, W. H., B. Ratcliff, L. Lopez, J. Kunglin, and R. M. Dawley. 1995. Differentiation of fungal tyrosinases and laccase using selective inhibitors and substrates, pp. 805–814. *In* C. Y. Lee and J. R. Whitaker (eds.), *Enzymatic Browning and its Prevention*. American Chemical Society, Washington DC.
4. Govindwar, S. and R. Dalvi. 1991. Stimulatory and inhibitory effect of dimethyl sulfoxide on microsomal aniline hydroxylase activity. *Toxicol. Lett.* **55:** 317–323.
5. Jadhav, S. U., S. D. Kalme, and S. P. Govindwar. 2007. Biodegradation of methyl red by *Galactomyces geotrichum* MTCC 1360. *Inter. Biodeter. Biodegrad.* (doi: 10.1016/j.ibiod.2007.12.010).
6. Kalme, S. D., G. K Parshetti, S. U. Jadhav, and S. P. Govindwar. 2007. Biodegradation of benzidine based dye direct Blue-6 by *Pseudomonas desmolyticum* NCIM 2112. *Bioresour. Technol.* **98:** 1405–1410.
7. Kandelbauer, A., A. Erlacher, A. Cavaco-Paulo, and G. Guebitz. 2004. Laccase-catalyzed decolorization of the synthetic azo dye Diamond Black PV 200 and of some structurally related derivatives. *Biocat. Biotrans.* **22:** 331–339.
8. Khehra, M. S., H. S. Saini, D. K. Sharma, B. S. Chadha, and S. S. Chimni. 2005. Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dye. *Water Res.* **39:** 5135–5141.
9. Macedo, J. M. B., L. M. F. Gottschalk, and E. P. S. Bon. 1999. Calcium carbonate mediates higher lignin peroxidase activity in the culture supernatant of *Streptomyces viridosporus* T7A. *Braz. J. Chem. Eng.* [online] **16:** 163–169.
10. Mali, P. L., M. M. Mahajan, D. P. Patil, and M. V. Kulkarni. 1999. Biodecolorizations of membranes of triphenylmethane and azo groups of dyes. *J. Sci. Ind. Res.* **59:** 221–224.
11. Moosvi, S., H. Keharia, and D. Madamwar. 2005. Decolourization of textile dye reactive violet 5 by a newly isolated bacterial consortium RVM 11.1. *World J. Microbiol. Biotechnol.* **21:** 667–672.
12. Nagai, M., T. Sato, H. Watanabe, K. Saito, M. Kawata, and H. Enei. 2002. Purification and characterization of an extracellular laccase from the edible mushroom *Lentinula edodes*, and decolorization of chemically different dyes. *Appl. Microbiol. Biotechnol.* **60:** 327–335.
13. Niku-Paavola, M. L., R. Fagerström, K. Kruus, and L. Viikari. 2004. Thermostable laccases produced by a white-rot fungus from *Peniophora* species. *Enz. Microbiol. Technol.* **35:** 100–102.
14. Pajot, H. F., L. I. C. deFigueroa, and J. I. Farina. 2007. Dye-decolorizing activity in isolated yeasts from the ecoregion of Las Yungas (Tucum'an, Argentina). *Enz. Microbiol. Technol.* **40:** 1503–1511.
15. Parshetti, G., S. Kalme, G. Saratale, and S. Govindwar. 2006. Biodegradation of malachite green by *Kocuria rosea* MTCC 1532. *Acta Chim. Slov.* **53:** 492–498.
16. Pourbabaee, A. A., F. Malekzadeh, M. N. Sarbolouki, and F. Najafi. 2006. Aerobic decolorization and detoxification of a disperse dye in textile effluent by a new isolate of *Bacillus* sp. *Biotechnol. Bioeng.* **93:** 631–635.
17. Russ, R., J. Rau, and A. Stolz. 2000. The function of cytoplasm flavin reductases in the reduction of azo dyes by bacteria. *Appl. Environ. Microbiol.* **66:** 1429–1434.
18. Salokhe, M. D. and S. P. Govindwar. 1999. Effect of carbon source on the biotransformation enzymes in *Serratia marcescens*. *World J. Microbiol. Biotechnol.* **15:** 229–232.
19. Sani, R. and U. Banerjee. 2003. Decolorization of triphenylmethane dyes and textile and dye stuff effluent by *Kuruthia* sp. *Enz. Microbiol. Technol.* **24:** 433–437.
20. Saratale, G. D., S. D. Kalme, and S. P. Govindwar. 2006. Decolourization of textile dyes by *Aspergillus ochraceus*. *Ind. J. Biotechnol.* **5:** 407–410.
21. Shanmugam, V., M. Kumari, and K. D. Yadav. 1999. *n*-Propanol as a substrate for assaying the lignin peroxidase activity of *Phanerochaete chrysosporium*. *Ind. J. Biochem. Biophys.* **36:** 39–43.
22. Shrivastava, R., V. Christian, and B. R. M. Vyas. 2005. Enzymatic decolorization of sulfonpthalein dyes. *Enz. Microbiol. Technol.* **36:** 333–337.
23. Wong, P. and P. Yuen. 1998. Decolorization and biodegradation of *N,N*-dimethyl-*p*-phenylenediamine by *Klebsiella pneumoniae*

RS-13 and *Acinitobacter liquifacienss*-1. *J. Appl. Microbiol.* **85:** 79–87.

24. Xiao, Y. Z., X. M. Tu, J. Wang, M. Zhang, Q. Cheng, W. Y. Zeng, and Y. Y. Shi. 2003. Purification, molecular characterization and reactivity with aromatic compounds of a laccase from basidiomycete *Trametes* sp. strain AH28-2. *Appl. Microbiol. Biotechnol.* **60:** 700–707.
25. Xueheng, Z. and I. R. Hardin. 2007. HPLC and spectrophotometric analysis of biodegradation of azo dyes by *Pleurotus ostreatus*. *Dyes Pigments* **73:** 322–325.