International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 884-889

Received: 03-11-2017
Accepted: 04-12-2017

Ambika Verma
Ph.D Molecular Biology and Biotechnology, Department of Biotechnology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Poonam Shirkot
Professor, Department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Correspondence
Ambika Verma
Ph.D Molecular Biology and Biotechnology, Department of Biotechnology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Extracellular bacterial laccase based biodegradation of Remazol Brilliant Blue R and phytotoxicity studies

Ambika Verma and Poonam Shirkot

Abstract
Laccase producing bacteria, *Pseudomonas putida* LUA15.1 was isolated from rice rhizospheric soil samples of paddy fields of Una district, Himachal Pradesh (India). Remazol Brilliant Blue R (40 mg/l) was 90.21% decolorized within 48 hrs by *Pseudomonas putida* LUA15.1. Purification of laccase enzyme was done by using ammonium sulfate precipitation, dialysis, gel filtration chromatography and ion exchange chromatography and 94.02% decolorization of Remazol Brilliant Blue R (40 mg/l) was observed by purified laccase of *Pseudomonas putida* LUA15.1. UV-Visible absorption spectrum showed the decolorization of Remazol Brilliant Blue R and phytotoxicity studies revealed the degradation of Remazol Brilliant Blue R into non-toxic products by purified laccase of *Pseudomonas putida* LUA15.1. Seed germination, length of plumule and radicle of *Phaseolus vulgaris* were significantly affected by Remazol Brilliant Blue R than its degradative metabolites indicating less toxic nature of degradation metabolites as compared to dye. It was therefore concluded that *Pseudomonas putida* LUA15.1 and its extracellular laccase has a good potential for use in the treatment of industrial effluent containing Remazol Brilliant Blue R.

Keywords: Decolorization, *Pseudomonas putida*, remazol brilliant blue-r, phytotoxicity, *Phaseolus vulgaris*

Introduction
Due to rapid industrialization and urbanization, a lot of chemicals including dyes, pigments and aromatic molecular structural compounds were extensively used in several industrial applications such as textiles, printing, pharmaceuticals, food, toys, paper, plastic, cosmetics and led to releasing of industrial effluents containing various toxic products into the environment (Mohana *et al.,* 2008) [1], (Ashrafi *et al.,* 2013) [2], (Mirzadeh *et al.,* 2014) [3]. Effluent that release from the production process of textiles is not properly disposed, can cause grave environmental pollution, sometimes to levels that can threaten human health, livestock, wildlife, aquatic lives and collapse the entire ecosystem (Gowri *et al.,* 2014) [4]. Many of these products are problematic because of their persistence, low biodegradability and high toxicity. Thus, the wastewater must be treated before releasing into the natural environment. Among various physicochemical and biotechnological techniques, the enzymatic removal of synthetic dyes is the most preferred method due to its efficiency at high and low pollutant concentration over a wide range of pH and temperature, low energy required, minimal impact on ecosystem and less sludge production in the decolorization process (Gholami-Borujeni *et al.,* 2011) [5], (Gholami-Borujeni *et al.,* 2013) [6], (Asadgol *et al.,* 2014) [7]. Amongst the methods used in biological treatment of wastewater containing dyes, the microbial decolorization and degradation of dyes has been of considerable interest (Shah, 2014) [8], (Barathi and Arulselvi, 2015) [9]. A large variety of microorganisms are reported to be capable of decolorization of dyes (Chang and Kuo, 2000) [10], (Fu and Viraraghavan, 2002) [11], (Fournier *et al.,* 2004) [12], (Kumar *et al.,* 2005) [13], (Gupta *et al.,* 2006) [14], (Kalyani *et al.,* 2009) [15], (Chang *et al.,* 2011) [16], (Olukanni *et al.,* 2013) [17], (Saravanakumar and Kathiresan 2014) [18], (Rezaei *et al.,* 2015) [19], (Verma *et al.,* 2017) [20]. Bacterial treatment offers a cheaper and environment friendlier alternative for color removal in textile effluents (Olukanni *et al.,* 2006) [21]. The potential of microbes to degrade synthetic dyes have been linked with the production of enzymes during degradation (Olukanni *et al.,* 2013) [17], (Moturi and Singara-Charya, 2009) [22]. The three major classes of these enzymes include laccases, lignin peroxidases and manganese dependent peroxidases. Laccase has been shown to be of critical importance to the dye-degrading and

various recent studies have demonstrated the versatility of laccases as industrial biocatalyst (Olukanni *et al.*, 2013) [17], (Saravanakumar and Kathiresan 2014) [18], (Rezaei *et al.*, 2015) [19], (Verma *et al.*, 2017) [20], (Olukanni *et al.*, 2006) [21], (Moturi and Singara-Charya, 2009) [22], (Xin *et al.*, 2013) [23], (Sahasrabudhe and Pathade, 2013) [24]. In this study, the ability of laccase from *Pseudomonas putida* LUA15.1 to degrade Remazol Brilliant Blue-R was investigated and the phytotoxicity experiments were also performed *in vitro* and *in vivo* on *Phaseolus vulgaris* to evaluate the toxicity of untreated and laccase treated dye.

Materials and Methods

Bacterial culture

In this study, the bacterial culture used was *Pseudomonas putida* LUA15.1, which was isolated from rice rhizospheric soil samples of paddy fields of Una district, Himachal Pradesh (India).

Decolorization of Remazol Brilliant Blue-R by *Pseudomonas putida* LUA15.1

Pseudomonas putida LUA15.1 was tested for its ability to decolorize Remazol Brilliant Blue-R. To determine the effect of dye concentration on decolorization activity of *Pseudomonas putida* LUA15.1, decolorization assays with varing dye concentrations between 20-100 mg/l were carried out. A loopful of bacterial culture was inoculated in each of five 250 ml flasks containing 100 ml TY broth and incubated at 28 ºC for 24 hrs. After 24 hrs of incubation, Remazol Brilliant Blue-R was added in each flask at concentrations of 20, 40, 60, 80 and 100 mg/l and 3 ml of the culture media was withdrawn at six hour time intervals between 0 hr and 48 hrs, from each flask respectively. Aliquots were centrifuged at 5000 rpm for 15 minutes to separate the bacterial cell mass, clear supernatants were used to measure the decolorization and decrease in color intensity of Remazol Brilliant Blue-R was observed at 600 nm. Spectral analysis of the samples was performed using UV-Vis Spectrophotometer (Simadzu UV-Vis 1800, Japan). Abiotic controls (without microorganism) were always included. Dye decolourization was expressed in terms of percentage calculated according to the equation.

$$\text{Decolourisation (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

Where, A_0 is an initial absorbance of Remazol Brilliant Blue-R and A_t is final absorbance after each time intervals.

Laccase activity assay

Pseudomonas putida LUA15.1 was inoculated into the Tryptone Yeast (TY) broth and flask was kept on a rotary shaker at 150 rpm for 24-48 hrs at 28 ºC. The culture supernatant was obtained by centrifugation of overnight culture of *Pseudomonas putida* LUA15.1 at 10,000 rpm, for 10 mins at 4 ºC and used for the enzyme assay. Laccase activity was measured by monitoring the oxidation of ABTS (Faramarzi and Forootanfar) [25]. The reaction mixture was prepared by adding 0.5 ml of the enzyme solution on top of the ABTS (3 mM) substrate dissolved in 0.5 ml of 0.1 M acetate buffer (pH = 4.5) and then it was incubated at 32 °C. The oxidation of ABTS was determined by monitoring the increase in absorbance at 420 nm. And the one unit of a laccase activity was defined as the required amount of enzyme to oxidize 1 µmol of ABTS/min ($\varepsilon 420$ = 36,000 M^{-1} cm^{-1}).

Optimizations for maximum laccase production

Pseudomonas putida LUA15.1 was further investigated to study effect of different factors such as incubation temperature, pH and incubation time on laccase enzyme production. The pH range was optimized using TY medium adjusted from 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 separately and temperature range for incubation investigated varied from 20-50 ºC where as effect of different incubation times was studied for 24, 48, 72, 96, 120 and 144 hrs. In all cases optical density was monitored by using a double beam UV/VIS scanning spectrophotometer.

Purification of extracellular laccase enzyme

All steps of purification were performed using 100 mM sodium phosphate buffer, pH 6.5 at temperature of 4 ºC. For the purification of extracellular laccase enzyme, purification techniques used were ammonium sulfate precipitation, dialysis, gel filtration chromatography and ion exchange chromatography. The laccase enzyme preparations after various stages of purification were analyzed for protein concentration and enzyme activity.

Decolourization of Remazol Brilliant Blue-R by laccase enzyme

In this study, it was investigated that whether the crude and purified laccase enzyme from *Pseudomonas putida* LUA15.1 can be used in the degradation of Remazol Brilliant Blue-R used in industry or not. For this purpose, Remazol Brilliant Blue-R was solublized in distilled water to the final concentration of 40 mg/l. The reaction mixture (6.0 ml) contained 2.0 ml acetate buffer (pH: 4.6), 2.0 ml of dye solution and 2.0 ml of laccase enzyme preparations followed by incubation at 37 ºC for 0-48 hrs. Control sample was run in parallel without addition of laccase enzyme. The decolorization percentage was determined spectrophotometrically as the relative decrease of absorbance at each maximal absorbance wavelength of the dyes.

In vitro phytotoxicity study

The effect of Remazol Brilliant Blue-R and its degradative metabolites on germination and early seedling growth of *Phaseolus vulgaris* was evaluated under *in vitro* conditions. The original dye solution and dye solutions degraded with crude and purified laccase enzyme preparations, were used for phytotoxicity studies. Seeds of *Phaseolus vulgaris* were sterilized first followed by dipping five seeds each in original dye solution, dye treated with crude enzyme preparation, dye treated with purified laccase enzyme preparation and in distilled water for about 4 hrs before transferring to the surface of the paper in petri dish. The seeds were germinated separately in sterile petri dishes, layered with sterile filter paper. The phytotoxicity study was carried out at room temperature in relation to *Phaseolus vulgaris* seeds (5 seeds per plate) by watering with respective solutions separately. Seeds germinated in water irrigated petri dish were used as a control. Length of plumule (shoot), radicle (root) and germination (%) were recorded for period of one week.

In vivo phytotoxicity study

The effect of Remazol Brilliant Blue-R and its degradative metabolites on germination and early seedling growth of *Phaseolus vulgaris* was also evaluated under *in vivo* conditions. Similarly, the original dye solution and dye solutions degraded with crude and purified laccase enzyme preparations, were used for phytotoxicity studies. Five seeds

of *Phaseolus vulgaris* were sown per pot at equidistance, at a uniform depth of 5 times diameter of the seed. The phytotoxicity study was carried out in relation to *Phaseolus vulgaris* seeds (5 seeds per pot) by watering with respective solutions separately *viz.*, original dye solution, dye treated with crude laccase enzyme preparation, dye treated with purified laccase enzyme preparation and distilled water. Seeds germinated in water irrigated pot were used as a control. Length of plumule (shoot), radicle (root) and germination (%) were recorded for one month.

Results

Decolourization of Remazol Brilliant Blue-R by *Pseudomonas putida* LUA15.1

The laccase producing bacterial isolate *Pseudomonas putida* LUA15.1 was tested for its ability to decolorize Remazol Brilliant Blue-R and it was found that this bacterial isolate was able to degrade this dye effectively (Figure-1). The strain demonstrated 90.21% decolorization of Remazol Brilliant Blue-R at 40 mg/l concentration, within 48 hrs (Figure-2). Absorbance values decreased from 1.84 at 0 h to 0.71 at 24 hrs and further 0.18 at 48 hrs and significant decolourization of 61.41% was observed after 24 hrs and 90.21% decolorization of Remazol Brilliant Blue-R was observed after 48 hrs (Figure-2, 3). Thus, it have been depicted that bacterial isolate *Pseudomonas putida* LUA15.1 can degrade textile dyes successfully.

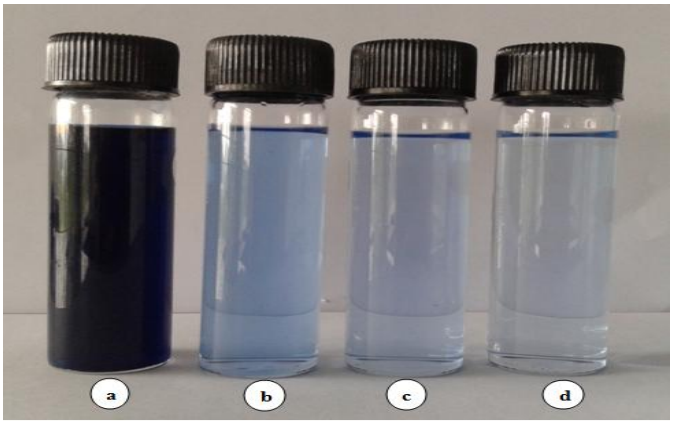

Fig 1: Remazol Brilliant Blue-R dye degradation a) Dye b) Dye + *Pseudomonas putida* LUA15.1 c) Dye + Crude laccase enzyme preparation d) Dye + purified laccase enzyme preparation respectively from left to right

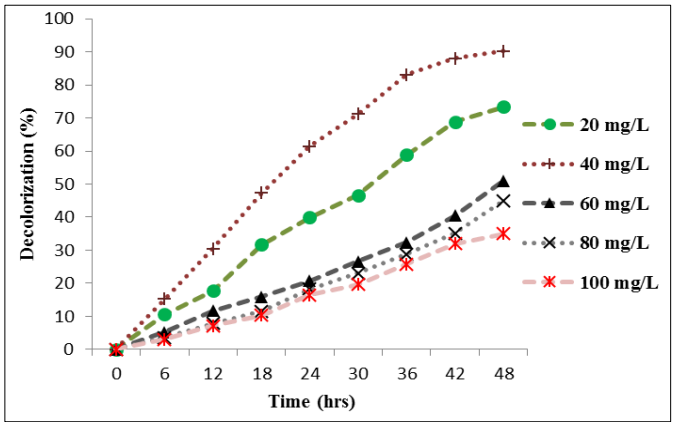

Fig 2: Decolorization of various concentrations of Remazol Brilliant Blue-R by *Pseudomonas putida* LUA 15.1

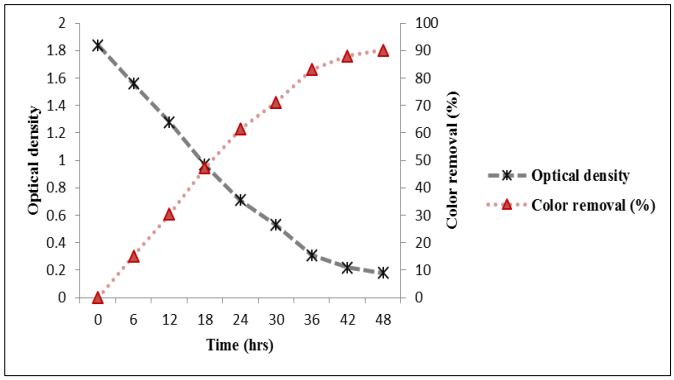

Fig 3: Decolorization of Remazol Brilliant Blue-R by *Pseudomonas putida* LUA 15.1

Optimization of culture conditions for maximum laccase production

The isolate exhibited maximum growth OD of 1.51 at wavelength of 540 nm after 96 hrs, where as maximum extracellular activity of 58.10 U/l was observed after 24 hrs of incubation period. Maximum growth OD of 1.52 and maximum extracellular activity of 58.15 U/l was observed at pH: 7.0. The selected bacteria showed a maximum growth OD of 1.51 and maximum extracellular activity of 58.18 U/l at an incubation temperature of 28 ºC. All these optimal conditions were used for Remazol Brilliant Blue-R degradation by *Pseudomonas putida* LUA 15.1 and observed 90.21% Remazol Brilliant Blue-R degradation after 48hrs.

Purification of laccase enzyme from *Pseudomonas putida* LUA15.1

As per our previous data (Verma *et al.*, 2017) [20], the ammonium sulphate fraction after the overnight dialysis of 50-90% ammonium sulphate cut exhibits the highest rate of precipitation and purified the enzyme 20.56 times with a 68.17% yield and was applied on Sephadex G-100 column equilibrated with sodium phosphate buffer (pH 6.5), purified the enzyme 28.30 times with a 32.69% yield. And in the last step of purification, it was found that laccase fractions were eluted with 1.0 M NaCl gradient which further purified laccase with 48.49 times purification and 10.08% yield.

Decolourization of Remazol Brilliant Blue-R by laccase enzyme preparations

Both crude and purified laccase enzyme extracts were also investigated for Remazol Brilliant Blue-R decolourization ability. Two ml of each laccase enzyme preparation was used in the decolourization study. It was found that 63.58% of decolourization of Remazol Brilliant Blue-R was observed after 24 hrs of incubation and then after 48 hours of incubation, 92.39% of decolourization was observed with crude laccase enzyme (Figure-1, 4). Similarly, 66.30% of decolourization was measured after 24 hrs of incubation and it decolourized up to 94.02% after 48 hrs of incubation with purified laccase enzyme preparation (Figure-1, 5). Thus it was observed that crude as well as purified enzyme preparation also have ability to effectively degrade the textile dyes.

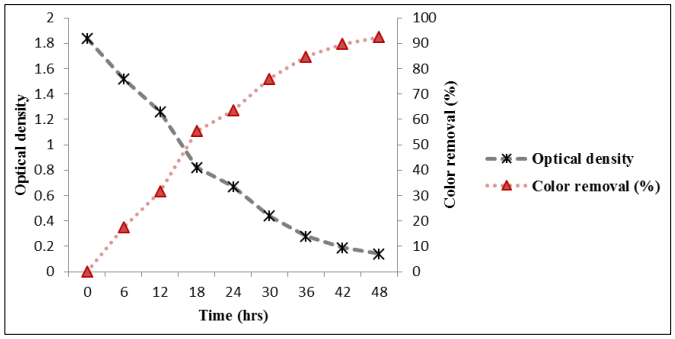

Fig 4: Decolorization of Remazol Brilliant Blue-R by crude laccase enzyme of *Pseudomonas putida* LUA 15.1

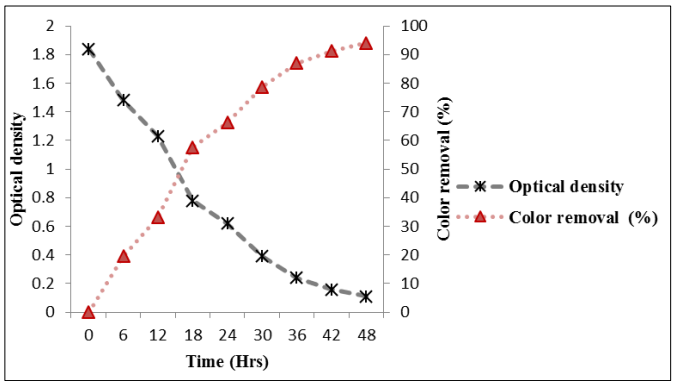

Fig 5: Decolorization of Remazol Brilliant Blue-R by purified laccase enzyme of *Pseudomonas putida* LUA 15.1

In vitro and *in vivo* phytotoxicity studies

Seed germination and plant growth bioassay are the most common technique used to evaluate the phytotoxicity. Thus, it was of primary aim to assess the phytotoxicity of the dye and its metabolites after degradation by crude laccase and purified laccase enzyme preparations. Forty percent germination of seeds of *Phaseolus vulgaris* was observed *in vitro* with Remazol Brilliant Blue-R treatment and eighty percent germination of seeds of *Phaseolus vulgaris* was observed *in vitro* with degraded metabolites of Remazol Brilliant Blue-R by crude laccase as compared to 100% germination of seeds of *Phaseolus vulgaris* was observed *invitro* with degraded metabolites of Remazol Brilliant Blue-R by purified laccase and 100% germination of seeds of *Phaseolus vulgaris* was observed *in vitro* with plain water (Figure-6, Table-1). The length of plumule and radicle were significantly affected by Remazol Brilliant Blue-R than its degradative metabolites (Table-1), indicating less toxic nature of degradation metabolites as compared to dye. Germination percentage of seeds (*Phaseolus vulgaris*) in pots irrigated with dye and its degradation metabolites by crude and purified laccase were compared with water control and found to be 40%, 80%, 100% and 100% respectively, indicating toxic nature of the dye (Figure-7, Table-1) also showed similar toxicity of Remazol Brilliant Blue-R with severely affected plumule and radicle growth of *Phaseolus vulgaris*. Toxicity of Remazol Brilliant Blue-R on *Phaseolus vulgaris* was summarized in the table-1.

Table 1: Phytotoxicity study of Remazol Brilliant Blue-R and its degradation metabolites on seed germination and growth of *Phaseolus vulgaris* under *in vitro* and *in vivo* conditions

Parameter studied	Phaseolus vulgaris (In vitro)			
	Water	**Remazol Brilliant Blue-R**	**Remazol Brilliant Blue-R treated with crude laccase enzyme**	**Remazol Brilliant Blue-R treated with partially purified laccase enzyme**
Germination (%)	100	40	80	100
Plumule (cm)	5.7	2.4	3.16	4.52
Radicle(cm)	5.9	3.9	4.08	5.64
Parameter studied	Phaseolus vulgariss (In vivo)			
	Water	Remazol Brilliant Blue-R	Remazol Brilliant Blue-R treated with crude laccase enzyme	Remazol Brilliant Blue-R treated with partially purified laccase enzyme
Germination (%)	100	20	100	100
Plumule (cm)	39.4	3.1	30.6	39.3
Radicle(cm)	6.8	1.5	5.5	6.6

Mentioned values in the table are mean of all germinated seeds in three sets.

Fig 6: Germination of seeds of *Phaseolus vulgaris* under *in vitro* conditions irrigated with a) Remazol Brilliant Blue-R dye b) Remazol Brilliant Blue-R dye treated with crude laccase enzyme c) Remazol Brilliant Blue-R treated with purified laccase enzyme d) water control, respectively from left to right

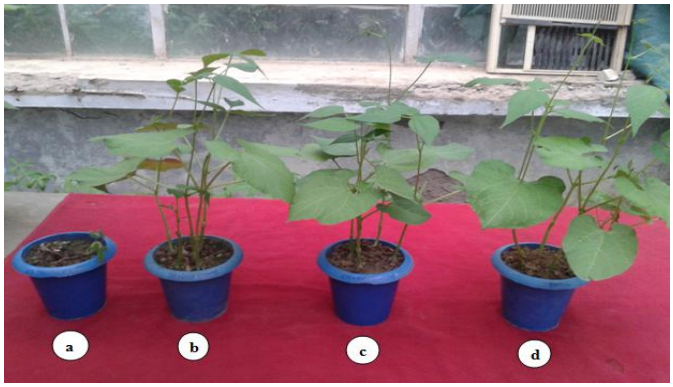

Fig 7: Germination and growth of *Phaseolus vulgaris* under *in vivo* conditions irrigated with a) Remazol Brilliant Blue-R dye b) Remazol Brilliant Blue-R dye treated with crude laccase enzyme c) Remazol Brilliant Blue-R dye treated with purified laccase enzyme d) water control, respectively from left to right.

Discussion

In the present study, the laccase producing bacterial isolate *Pseudomonas putida* LUA15.1 was tested for its ability to decolorize Remazol Brilliant Blue-R and it was found that this bacterial isolate was able to degrade this dye effectively. It was also observed that decolorization efficiency increased with increasing concentration of Remazol Brilliant Blue-R up to 40 mg/l and over 90.21% decolorization was observed at 48 hours at 40 mg/l of Remazol Brilliant Blue-R concentration. The initial increase in decolorization as the concentration increased may be due to induction of enzymes involved in decolorization (Olukanni *et al.,* 2013) [17]. It has been shown that dyes that act as inducers of enzyme production in a culture medium are in turn decolorized by the enzymes, and the highest inducer is decolorized highest (D'Souza *et al.,* 2006) [26]. When the concentration was increased to 100 mg/l however, decolorization efficiency dropped to 35.05%, indicating that Remazol Brilliant Blue-R may be toxic to the organism at the higher concentration levels. It has been shown that rate of decolorization was decreased with increasing concentration of dyes and also inhibits the growth of bacterium, which indicates toxicity at higher dye concentration (Olukanni *et al.,* 2013) [17], (Parshetti *et al.,* 2006) [27], (Lal and Srivastava, 2011) [28] while Cha *et al*. (2001) [29] and Youssef *et al.* (2008) [30] have also reported similar observation for inhibition of fungal growth at higher concentration of dyes. The isolate exhibited maximum growth after 96 hrs, where as maximum extracellular activity was observed after 24 hrs of incubation period. Maximum growth and maximum extracellular activity was observed at pH: 7.0. The selected bacteria showed a maximum growth and maximum extracellular activity at an incubation temperature of 28 ºC. All these optimal conditions were used for Remazol Brilliant Blue-R degradation by *Pseudomonas putida* LUA 15.1 and observed 90.21% Remazol Brilliant Blue-R degradation after 48hrs. Saravana kumar and Kathiresan, had also achieved 89% degradation of dye under optimal conditions of temperature 30 ºC, pH of 5.8 and incubation period of 10 days (Saravanakumar and Kathiresan 2014) [18]. It was found that laccase enzyme was purified with 48.49 times purification and 10.08% yield (Verma *et al.,* 2017) [20]. Kumar *et al*. (2014) [31] developed simple cost effective and scalable purification procedure to purify extracellular laccase from *P. ostreatus* and obtained 161% yield with 27.8 fold purity, which has been found to have an enormous potential to degrade textile dyes. Both crude and purified laccase enzyme extracts were investigated for Remazol Brilliant Blue-R decolourization ability and it was observed that crude as well as purified enzyme preparation have ability to effectively degrade the textile dyes. Decolourization of remazol brilliant blue R have been reported by enzymatic extract and submerged cultures of a newly isolated *Pleurotus ostreatus* MR3 (Moturi and Singara-Charya, 2009) [22]. Remazol Brilliant Blue-R dye decolourization by laccase produced by *Pleurotus Sajor-caju* via solid-state fermentation has also been reported (Ang *et al.,* 2014) [32]. Toxicity in terms of germination and growth of seeds dipped or irrigated with purified laccase enzyme treatment was less than partially purified laccase enzyme and native dye compound**.** Parshetti *et al*. (2006) [27] also showed the germination of *T. aestivum* was less with dye treatment compared to its degradation products and plain water. Kumar *et al*. (2014) [31] reported 100% seed germination with water whereas seed germination has been found to be inhibited when seeds were treated with dyes. In previous studies, Verma *et al.* (2017) [20] reported the decolorization of malachite green using purified laccase of *Pseudomonas putida* LUA15.1 with respect to phytotoxicity studies and it has been found that seed germination, length of plumule and radicle of *Phaseolus aureus* were significantly affected by malachite green than its degradative metabolites after laccase treatments.

Conclusion

The strain *Pseudomonas putida* LUA15.1 and its extracellular laccase, decolorize and degrade Remazol Brilliant Blue-R successfully. The obtained results reveal that the use of *Pseudomonas putida* LUA15.1 and its laccase has a huge potential to degrade Remazol Brilliant Blue-R. Seed germination, length of plumule and radicle of *Phaseolus vulgaris* were significantly affected by Remazol Brilliant Blue-R than its degradative metabolites indicating less toxic nature of degradation metabolites as compared to dye. It was therefore concluded that *Pseudomonas putida* LUA15.1 and its extracellular laccase has a good potential for use in the treatment of industrial effluent containing Remazol Brilliant Blue-R. So this strain and enzyme can be used for treating textile wastewaters and for water recycling.

Aknowledgement

The author acknowledges the financial assistance received from Department of Biotechnology at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh to carry out the whole part of this research work.

References

1. Mohana S, Shrivastava S, Divehi J, Medawar D. Response surface methodolgy for optimization of medium for decolorization of textile dye Direct black 22 by a novel bacterial consortium. Biores. Technol. 2008; l99:562-569.
2. Ashrafi SD, Rezaei S, Forootanfar H, Mahvi AH, Faramarzi MA. The enzymatic decolorization and detoxification of synthetic dyes by the laccase from a soil-isolated ascomycete, *Paraconiothyrium variabile*. Int. Biodeter. Biodegr. 2013; 85:173-181.
3. Mirzadeh SS, Khezri SM, Rezaei S, Forootanfar H, Mahvi AH, Faramarzi MA. Decolorization of two synthetic dyes using the purified laccase of *Paraconiothyrium variabile* immobilized on porous silica beads. J Environ. Health Sci. Eng. 2014; 12:9-15.
4. Gowri RS, Vijayaraghavan R, Meenambigai P. Microbial degradation of reactive dyes- A Review. Int. J. Curr. Microbiol. App. Sci. 2014; 3:421-426.
5. Gholami-Borujeni F, Mahvi AH, Nasseri S, Faramarzi MA, Nabizadeh R, Alimohammadi M. Enzymatic treatment and detoxification of acid orange 7 from textile wastewater. Appl. Biochem. Biotechnol. 2011; 165:1274-1284.
6. Gholami-Borujeni F, Faramarzi MA, Nejatzadeh-Barandozi F, Mahvi AH. Oxidative degradation and detoxification of textile azo dye by horseradish peroxidase enzyme. Fresenius Environ. Bull. 2013; 22:739-744.
7. Asadgol Z, Forootanfar H, Rezaei S, Mahvi AH, Faramarzi MA. Removal of phenol and bisphenol-A catalyzed by laccase in aqueous solution. J Environ. Health Sci. Eng. 2014; 12:93-98.
8. Shah K. Biodegradation of azo dye compounds. Int. Res. J Biochem. Biotech. 2014; 1:5-13.

9. Barathi S, Arulselvi PI. Decolorization, Degradation and Toxicological analysis of textile dye effluent by using novel techniques- Review. Int. J Sci. Res. Man. 2015; 3:2118-2136.
10. Chang JS, Kuo TS. Kinetics of bacterial decolorization of azo dye with *Escherichia coli* NO3. Bioresource Technol. 2000; 75:107-114.
11. Fu Y, Viraraghavan T. Dye biosorption sites in *Aspergillus niger*. Bioresource Technol. 2002; 82:139-145.
12. Fournier D, Halasz A, Thiboutot S, Ampleman G, Dominic M, Hawari J. Biodegradation of octahydro- 1, 3, 5, 7- tetranitro-1, 3, 5, 7-tetrazocine (HMX) by *Phnerochaete chrysosporium*, New insight into the degradation pathway. Env. Sci. Technol. 2004; 38:4130-4137.
13. Kumar KV, Sivanesan S, Ramamurthi V. Adsorption of malachite green onto *Pithophora* sp., fresh water algae: equilibrium and kinetic modeling. Process Biochem. 2005; 40:2865-2871.
14. Gupta VK, Rastogi A, Saini VK, Jain N. Biosorption of copper (II) from aqueous solutions by *Spirogyra* species. J Col. Int. Sci. 2006; 296:59-67.
15. Kalyani DC, Telke AA, Dhanve RS, Jadhav JP. Eco-friendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. J Hazard Mater. 2009; 163:735-741.
16. Chang JS, Chou C, Chen SY. Decolorization of azo dyes with immobilized *Pseudomonas luteola.* Process Biochem. 2011; 36:757-763.
17. Olukanni OD, Adenopo A, Awotula AO, Osuntoki AA, Biodegradation of Malachite Green by Extracellular Laccase Producing *Bacillus thuringiensis* RUN1. J Basic Appl. Sci. 2013; 9:543-549.
18. Saravanakumar K, Kathiresan K. Bioremoval of synthetic dye malachite green by marine *Trichoderma* sp. Springer Plus. 2014; 3:1-12.
19. Rezaei S, Tahmasbi H, Mogharabi M, Ameri A, Forootanfar H, Khoshayand MR, *et al*. Laccase-catalyzed decolorization and detoxification of Acid Blue 92: statistical optimization, microtoxicity, kinetics, and energetic. J. Env. Health Sci. Eng. 2015; DOI 10.1186/s40201-015-0183-1.
20. Verma A, Dhiman K, Shirkot P. Biodegradation of malachite green by extracellular bacterial laccase and its phytotoxicity studies. Int. J Pure App. Biosci. 2017; 5(2):913-922.
21. Olukanni OD, Osuntoki AA, Gbenle GO. Textile effluent biodegradation potentials of textile effluent-adapted and non adapted bacteria. Afr. J Biotech. 2006; 5:1980-1984.
22. Moturi B, Singara-Charya MA. Decolorization of crystal violet and malachite green by Fungi. Sci. World J. 2009; 4:28-33.
23. Xin F, Sun Y, Hu S, Cheong K, Geng A. Decolourization of remazol brilliant blue R by enzymatic extract and submerged cultures of a newly isolated *Pleurotus ostreatus* MR3. Afr. J Biotech. 2013; 12:5778-5785.
24. Sahasrabudhe M, Pathade G. Biodegradation of azo dye C.I. Reactive Orange 16 by an actinobacterium *Georgenia* sp. CC-NMPT-T3. Int. J Adv. Res. 2013; 1:91-99.
25. Faramarzi MA, Forootanfar H. Biosynthesis and characterization of gold nanoparticles produced by laccase from *Paraconiothyrium variabile*. Coll. Surf. B. Bioint. 2011; 87:23-27.
26. D'Souza DT, Tiwari R, Shah AK, Chandralata R. Enhanced production of laccase by a marine fungus during the treatment of colored effluents and synthetic dyes. Enzyme Microb. Technol. 2006; 38:504-511.
27. Parshetti G, Kalme S, Saratale G, Govindwar S. Biodegradation of malachite green by *Kocuria rosea* MTCC 1532. Acta Chim. Slov. 2006; 53:492-498.
28. Lal N, Srivastava AK. Decolorization of malachite green by newly isolated *Bacillus Strain* MTCC-3330. Arch. Environ. Sci. 2011; 5:71-76.
29. Cha C, Doerge DR, Cerniglia CE. Biotransformation of malachite green by the Fungus *Cunninghamella elegans.* Appl. Environ. Microbiol. 2001; 67:4358-4360.
30. Youssef AS, El-Sherif MF, El-assar SA. Studies on the decolorization of malachite green by local isolate *Acremonium kiliense.* Biotechnol. 2008; 7:213-223.
31. Kumar VV, Sathyaselvabala V, Premkumar MP, Vidyadevi T, Sivanesan S. Biochemical characterization of three phase partitioned laccase and its application in decolorization and degradation of synthetic dyes. J. Mol. Cat. B: Enzy. 2014; 74:63-72.
32. Ang TN, Ngoh GC, Chua ASM, Ismail I. Remazol Brilliant Blue R Dye Decolourization by Laccase Produced by *Pleurotus Sajor-caju* via Solid-State Fermentation. Proceeding the Regional Conference on Chemical Engineering, Yogyakarta, 2014.