Appl Biochem Biotechnol (2008) 151:9–19
DOI 10.1007/s12010-008-8175-6

Strategies for Enhancing Laccase Yield from *Streptomyces psammoticus* and Its Role in Mediator-based Decolorization of Azo Dyes

K. N. Niladevi · P. S. Sheejadevi · P. Prema

Received: 7 November 2007 / Accepted: 4 February 2008 /
Published online: 13 May 2008

Abstract Enhanced production of laccases from *Streptomyces psammoticus* in solid-state fermentation was carried out using two different strategies: laccase inducers and scale-up process. Laccase yield was enhanced by a wide range of aromatic inducers. The best inducer was pyrogallol, which yielded 116 U/g as compared to the control (55.4 U/g). Scale-up studies in packed bed bioreactor was performed at different aeration rates. Aeration at 1.5 vvm was identified as the optimum condition for laccase production (75.4 U/g) in the column bioreactor. The enzyme yield was enhanced further by combining the best conditions from the first two experiments. Fermentation was carried out in bioreactors in the presence of 1 mM pyrogallol, which resulted in 3.9-fold increase in laccase yield (215.6 U/g). The role of laccase in azo dye decolorization was evaluated in the presence of four different laccase mediators, at different concentrations. 1-Hydroxybenzotriazole (HOBT) proved to be the best mediator for *S. psammoticus* laccase and decolorized the azo dyes efficiently. Acid orange, Methyl orange, and Bismarck brown were decolorized at the rates of 86%, 71%, and 75% respectively, by HOBT.

Keywords Laccase · Aromatic inducers · Packed bed reactor · Laccase-mediators · Dye decolorization · *Streptomyces psammoticus*

Introduction

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belong to the family of blue multicopper oxidases, which catalyze the one-electron oxidation of four reducing-substrate molecules concomitant with the four-electron reduction of molecular oxygen to water (1). These enzymes are known to catalyze the oxidation of a wide range of substrates including phenolic compounds and aromatic amines. Laccases have been the focus of attention in the recent years due to their potential use in varying fields like pulp bleaching (2), fruit juice and

K. N. Niladevi · P. S. Sheejadevi · P. Prema (✉)
Biotechnology Division, National Institute for Interdisciplinary Science and Technology (CSIR), Trivandrum 695019, India
e-mail: prema@csrrltrd.ren.nic.in

wine stabilization (3), dye decolorization (4), and detoxification of environmental pollutants (5). Dye decolorization remains one of the widely studied applications of laccases in general. Synthetic dyes are extensively used in the textile and printing industries of which, azo dyes represent the largest group and account for about 70% of all textile dyes produced (6).

The reported redox potentials of laccases are lower than those of nonphenolic compounds, and hence they are unable to oxidize nonphenolic compounds including a wide range of synthetic dyes. However, in the presence of a suitable redox mediator, laccases are also able to oxidize nonphenolic structures (7, 8), expanding thus the range of compounds that can be oxidized by these enzymes. Redox-mediated laccase catalysis has been used in a wide range of applications including dye degradation. Claus et al. (9) have reported the ability of a laccase-plus-mediator system in the decolorization of dyes that were initially resistant to laccase.

The ever-increasing demand for this potential enzyme in the industrial sectors requires large quantities of enzyme to be produced, and hence, any attempt to increase the production of enzymes from microbial sources would be of considerable interest. The production of laccases can be considerably enhanced by the addition of inducers, which includes a wide variety of aromatic and phenolic compounds (10, 11). The use of inducers for laccase production has been widely reported from fungi (12). Another valued approach for enhanced enzyme production in solid-state fermentation (SSF) is the use of various bioreactors. It includes tray, drum, and packed bed reactors. Tray bioreactors tend to be very simple in design, with no forced aeration or mixing for the solid substrate. However, in the tray bioreactors, only thin layers can be used, to avoid overheating and to maintain aerobic conditions (13). The use of tray fermenters in large-scale production is limited as they require intensive labor and a large operational area, with high chances of contamination (14). Although the mass heat transfer, aeration, and mixing of the substrate are better in rotating drum reactors, the rotation may cause damage to the inoculum that may affect the final product yield. Column fermenters have most commonly been used in laboratory studies for the production of enzymes, organic acids, and biologically active metabolites (14). Column fermenters have been reported to be useful for product developments with efficient process controls, particularly for heat removal.

The use of inducers and scale-up processes are two different strategies widely practiced for enhancing laccase yield. The role of inducers in enhanced laccase production in bioreactors using a fungal strain has been reported (15). However, in such instances, it is difficult to determine the contribution of each of these two different strategies toward the enhanced laccase yield. Hence, in the present study, we studied initially the effect of inducers and the scale-up process on laccase production individually and later combined both the strategies by performing laccase production in the bioreactor, in the presence of the inducer. To the best of our knowledge, there has been no other report on enhancing laccase production from actinomycetes using these strategies. The effective use of laccase from *S. psammoticus* in mediator-based decolorization of azo dye is attempted, which is again a relatively least studied subject among actinomycetes.

Materials and Methods

Microorganism and Inoculum Preparation

Streptomyces psammoticus MTCC 7334 used for the present study is an aerobic filamentous bacterium isolated from a mangrove swamp (16). The culture was grown and maintained on starch casein agar slants and subcultured regularly. One-week-old fully

grown slants were used for inoculum preparation. The culture was aseptically transferred to the inoculum medium with the following composition (g/L): glucose—5.0, yeast extract—3.0, $(NH_4)_2SO_4$—0.1, Mg SO_4—0.1, $CaCO_3$—0.02, and 10 mL of trace elements solution that contained 0.1% ferrous sulfate, 0.09% zinc sulfate, and 0.02% manganese sulfate. The culture was allowed to grow in the above medium for 48 h and used as the inoculum.

Solid-state Fermentation

SSF in 250-mL Erlenmeyer flasks was carried out using 5 g of rice straw. Fermentation was performed under the conditions optimized earlier in our laboratory (17).

Effect of Inducers

To study the effect of inducers on laccase production, various aromatic compounds such as *p*-anisidine (*p*-methoxy aniline), gallic acid (3,4,5-trihydroxy benzoic acid), ferulic acid (4-hydroxy, 3-methoxy cinnamic acid), guiacol (2-methoxy phenol), pyrogallol (Benzene-1,2,3 triol), veratryl alcohol (3,4-dimethoxy benzyl alcohol), catechol (Benzene-1,2 diol), and vanillic acid (4-hydroxy, 3-methoxy benzoic acid) were incorporated in the fermentation medium. All the inducers were added at a concentration of 1 mM. Catechol and vanillic acid were dissolved in sterile water, while all the other aromatic inducers were dissolved in 50% alcohol. The inducers were added to the flasks just before inoculation.

SSF Bioreactor

The SSF bioreactor system typically consisted of an aerator pump, air filter unit (sterilized glass wool), air saturation unit, cylindrical glass column (22×5 cm), and an air exit unit. A nonaerated column reactor and a 250-mL flask were maintained along with the bioreactor system as a control for the experiments. The glass column was placed vertically and packed with 15 g of preinoculated rice straw. The cultural conditions optimized earlier for the flask level experiment (17) were adopted for bioreactor studies. The air from the aerator pump was passed through sterilized glass wool packed in a glass column to minimize the chances of contamination from the air. The filtered air was then passed through the air saturation unit, and the filtered moist air was finally passed through the bottom of the packed column. The fermentation was carried out for 72 h at 30±2 °C. The effect of aeration on enzyme production was studied by varying the aeration rate from 0.5 to 2.0 vvm (vessel volume per minute). To study the effect of inducers in scale-up studies, the aromatic compounds were added to the substrate prior to inoculation.

Enzyme Extraction

The fermented material was extracted with distilled water, and the contents were mixed thoroughly by keeping the flasks on a rotary shaker at 200 rpm for 1 h. The mixture was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was collected and used for the laccase assay and partial purification.

Laccase Assay

Laccase activity was measured by monitoring the oxidation of 500 μM ABTS (2,2′-azino-*bis*-[3-ethyl benzothiazoline-6-sulfonic acid]; SIGMA) buffered with 0.2 M sodium phosphate

buffer (pH 7.5) at 420 nm for 1 min (8). The reaction mixture (3 mL) contained 1 mL of culture filtrate. One unit of enzyme activity was defined as 1 μM of ABTS oxidized per min. To calculate enzyme activity, an absorption coefficient of 3.6×10^4 M^{-1} cm^{-1} was used.

Biomass Estimation

Biomass estimation was carried out by determining the *N*-acetyl glucosamine present in the cell wall (18). It was expressed as milligram of glucosamine per gram of dry fermented matter.

Partial Purification

The supernatant collected after centrifugation was concentrated by fractionated precipitation between 30% and 60% $(NH_4)_2SO_4$ saturation. After centrifugation, the precipitate was resuspended in minimal quantity of 50 mM phosphate buffer, pH 7.0, and extensively dialyzed against the same buffer and used for dye decolorization studies.

Dyes and Mediators

Three azo dyes (Acid orange, Methyl orange, and Bismarck brown) were selected for dye decolorization studies. The mediators used were ABTS, 1-hydroxybenzotriazole (HOBT), aniline, and *para*-hydroxybenzoic acid (*p*HBA).

Dye Decolorization Studies

Stock solutions of dyes were diluted in distilled water to a final concentration of 50 ppm. The reaction mixture (1.5 mL) contained 0.5 mL dye solution, 0.5 mL enzyme (58.3 nkat), and 0.5 mL buffer (50 mM sodium phosphate buffer, pH 6.0). Decolorization was determined by monitoring the absorbance decrease at the maximum wavelength in the visible spectrum of each dye (Acid orange—480 nm, methyl orange—466 nm, and Bismarck brown—457 nm) and was expressed in terms of percentage decolorization.

For the studies on mediator-based decolorization, the mediator compounds were tested at different concentrations ranging from 0.5 to 2.0 mM each. The stock solution of HOBT was prepared in 50% dimethyl sulfoxide. The reaction mixture (2 mL) contained 0.5 mL each of the dye solution, mediator, buffer, and enzyme. In both sets of experiments, the reactions were arrested by adding 2 mM sodium azide (0.5 mL). In the controls, the enzyme was replaced with a heat-killed enzyme.

All the experiments were carried out in triplicates, and the mean values were taken. The standard deviation for the experiments was less than ±5%.

Results and Discussion

Enhanced Production of Laccase

Results of our study implied that inducers play a significant role in enhancing the production of laccase. The studies in the flask level experiments revealed that pyrogallol is the most efficient inducer for laccase production by *S. psammoticus*, followed by *para*-

anisidine. Pyrogallol enhanced the laccase production by twofold giving a yield of 116.0 U/g against the control (55.4 U/g), while 34% increase in laccase production was achieved with *para*-anisidine (74.2 U/g). Other inducers like gallic acid, catechol, veratryl alcohol, guaiacol, and ferulic acid were also found to be enhancing the laccase yield, while vanillic acid was found to be exerting a negative influence on laccase production by this strain (Fig. 1). Aromatic inducers and phenolic compounds have been widely used to elicit enhanced laccase production by different organisms (10, 11), and the nature of the compound that induces laccase production differs greatly with the species. However, it remains a general practice to select the inducers in such a way that they are either polyphenols or lignin-related structures.

The bioreactor studies indicated that laccase production by *S. psammoticus* could be enhanced considerably using a packed bed bioreactor, provided that forced aeration is supplied. The schematic representation of the packed bed bioreactor used in the present study is given in Fig. 2. The growth and enzyme yield was highest in the upper zone of the packed bed reactor, which was divided into three equal zones. Growth and enzyme production was moderate in the middle zone and lowest in the lower zone of the column, irrespective of the aeration rate provided (Table 1). This was probably due to the development of axial gradients of temperature within the packed bed column. Axial temperature gradients are impossible to avoid within packed-bed bioreactors due to the use of convective cooling with unidirectional flow of air (19). The evolution of heat, which is directly related to the metabolic activity of the microorganism, is one of the well-documented characteristics of SSF systems (20). In the present study, it was observed that forced aeration using humidified air enabled to remove heat from the substrate bed. A similar result on forced aeration has already been reported (21). Forced aeration of 1.5 vvm was the optimum for laccase production, which resulted in a yield of 75.4 U/g (upper zone), which was 36% higher as compared to the flasks (55.4 U/g). The yield was reduced when low aeration was provided, and the yield was almost negligible in the nonaerated control column. This could be attributed to the inadequate supply of oxygen in the less aerated and nonaerated columns. Oxygen is one of the major influencing factors for the growth and metabolite production by aerobic organisms like *S. psammoticus*.

The individual experiments on inducers and the bioreactor resulted in enhanced laccase production by *S. psammoticus*. Hence, we combined both the strategies with a view to further enhancing the laccase yield. The aeration of the reactor was set at 1.5 vvm, and the

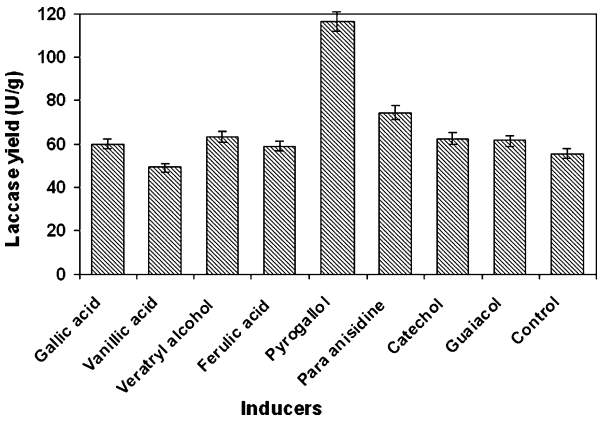

Fig. 1 Laccase production in flask level experiment in the presence of aromatic inducers. The culture conditions for SSF were particle size, 500–1,000 μm; initial moisture content 65%; and incubation temperature 32 °C. Rice straw was used as the substrate

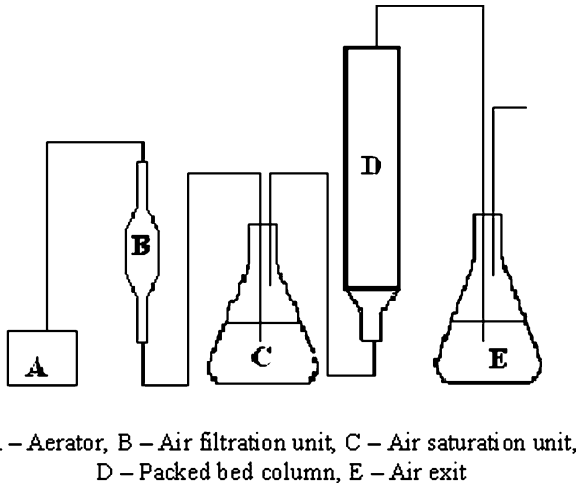

Fig. 2 Schematic diagram of the packed bed bioreactor set up used for laccase production in SSF

best laccase inducer, pyrogallol, was added to the substrate at a concentration of 1 mM. The result of the time course of laccase production in a packed bed bioreactor in the presence of pyrogallol is given in Fig. 3. The results indicated that there was no change in the hour of maximum laccase production in the reactor. The maximum laccase yield was obtained at 48 h, similar to the result observed in the flask-level experiments. The maximum laccase yield obtained in the flask-level experiments, in the presence of pyrogallol, was 116 U/g. The highest yield obtained in the bioreactor, in the absence of any inducer, was 75.4 U/g. The results of the present study showed that the use of inducers is a better strategy for enhancing laccase yield from *S. psammoticus*, as compared to the forcefully aerated bioreactor. However, a 3.9-fold increase in laccase yield (215.6 U/g) was obtained by combining both the strategies, which has undoubtedly proved that a combination of these two different strategies is a promising method for enhancing laccase yield by this strain.

Table 1 Laccase and glucosamine yields at three equally divided zones of the non-aerated and differentially aerated packed bed column reactor.

Aeration (vvm)[a]	Laccase yield (U/g)[b]			Glucosamine (mg/g)[c]		
	Upper zone	Middle zone	Lower zone	Upper zone	Middle zone	Lower zone
0.5	59.4	42.3	39.5	22.1	15.4	10.7
1.0	67.7	47.2	42.0	29.4	18.3	14.0
1.5	75.4	56.0	49.5	33.8	21.9	16.2
2.0	70.2	58.1	52.0	31.2	25.0	12.6
Control (without aeration)	9.3	4.9	1.8	5.1	2.2	0.9
Flask	55.4			28.3		

Values obtained with flask level experiment are given for comparison

[a] Vessel volume per minute

[b,c] Values presented are the mean of three independent experiments, and the standard deviation for the experiments was less than ±5%

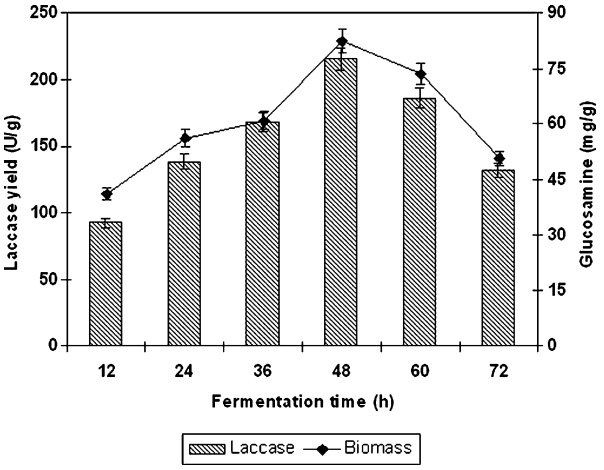

Fig. 3 Time course of laccase production in packed bed bioreactor in the presence of 1 mM pyrogallol. The culture conditions for SSF were particle size, 500–1,000 μm; initial moisture content, 65%; and incubation temperature, 32 °C. Rice straw was used as the substrate

Dye Decolorization Studies

Decolorization of the selected azo dyes by *S. psammoticus* laccase was attempted, and the results of the same are given in Fig. 4. The enzyme was found to exert only limited decolorization ability toward the azo dyes. The result was in compliance with the earlier reports that the azo dyes are recalcitrant to decolorization or could be decolorized only to a limited extent (22). Among the three dyes tested, acid orange showed more decolorization as compared to methyl orange and Bismarck brown. Acid orange exhibited 16.2% decolorization, while the rate of decolorization of methyl orange and Bismarck brown were 11.2% and 10.2%, respectively. It was observed that the reaction between the dyes and the enzyme was rapid and no further increase in decolorization was observed with an increase in time. The variation in the rate of decolorization of the different azo dyes by *S. psammoticus* laccase can be attributed to the difference in their structures. It has been well established that the individual dye structures and redox potentials influence the decolorization rates (23).

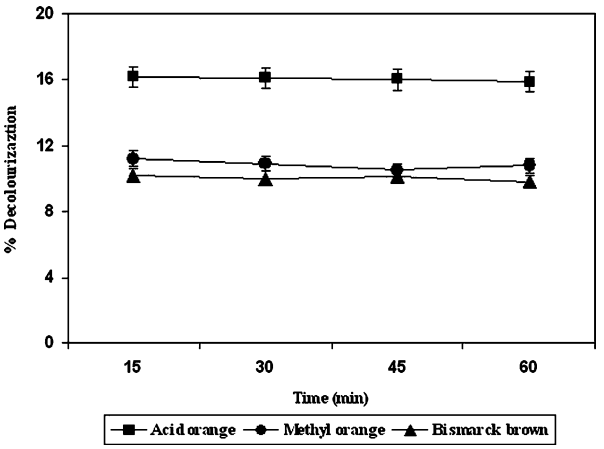

Fig. 4 Decolorization of acid orange, methyl orange, and Bismarck brown by *S. psammoticus* laccase in the absence of mediators, over different incubation times. The reaction mixture (1.5 mL) contained 0.5 mL each of the dye solution, buffer, and enzyme (58.3 nkat). The reaction time was 30 min

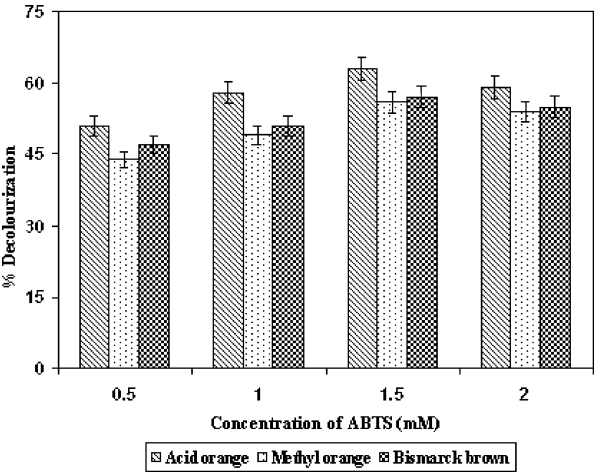

Fig. 5 Decolorization of acid orange, methyl orange, and Bismarck brown by *S. psammoticus* laccase at different concentrations of ABTS. The reaction mixture (2 mL) contained 0.5 mL each of the dye solution, mediator, buffer, and enzyme (58.3 nkat). The reaction time was 30 min

The low activity of laccase toward the selected azo dyes led us to explore the possibilities of using different mediators to achieve better decolorization of the dyes. The efficiency of the laccase–mediator system in dye decolorization is well documented in the literature (9, 24). Four different laccase mediators, viz., ABTS, HOBT, aniline, and *p*HBA, were used for the study, and each of the mediator was tested at four different concentrations to identify the optimum level required for dye decolorization. Figure 5 shows the decolorization rate (percent decolorization) of the dyes at different concentrations of ABTS over an incubation time of 30 min. Maximum decolorization of the dyes was observed at 1.5 mM of ABTS. It was observed that at a higher concentration of ABTS (2 mM), the decolorization of all the dyes was reduced. Increasing the incubation time up to 24 h resulted in an increase in the absorbance of reaction mixture (data not shown). The increase in absorbance was due to the darkening of reaction mixture over a prolonged incubation period, and the color change was more distinct at higher concentration of ABTS. The formation of purple color in a solution of laccase and ABTS in a buffer, after a long storage, has been reported (25). In the present study, the color change was rather intense than the

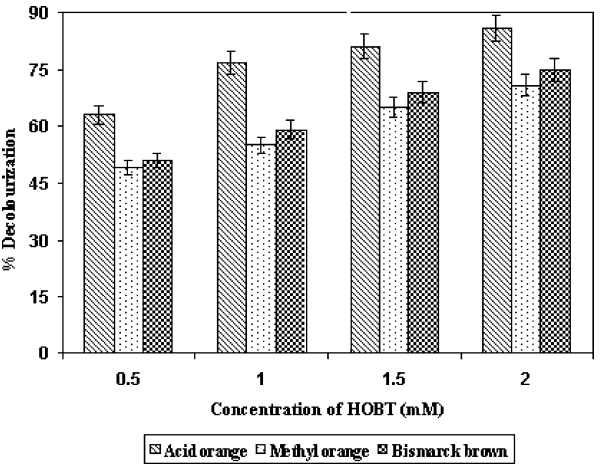

Fig. 6 Decolorization of acid orange, methyl orange, and Bismarck brown by *S. psammoticus* laccase at different concentrations of HOBT. The reaction mixture (2 mL) contained 0.5 mL each of the dye solution, mediator, buffer, and enzyme (58.3 nkat). The reaction time was 30 min

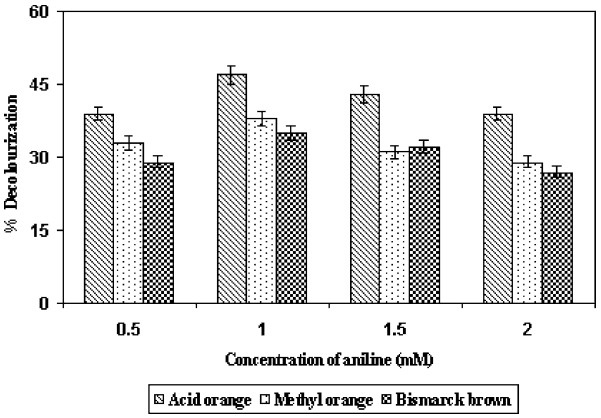

Fig. 7 Decolorization of acid orange, methyl orange, and Bismarck brown by *S. psammoticus* laccase at different concentrations of aniline. The reaction mixture (2 mL) contained 0.5 mL each of the dye solution, mediator, buffer, and enzyme (58.3 nkat). The reaction time was 30 min

purple color reported earlier, and the probable reason for this may be the auto-oxidation of excess ABTS present in the reaction mixture. However, more detailed study is required to explain the formation of dark color in the reaction mixture.

HOBT is one of the most intensively researched mediators, which is oxidized to its nitroxide radical by laccase (26, 27). The decolorization of dyes by laccase in the presence of HOBT is given in Fig. 6. Unlike that of ABTS, the maximum decolorization was observed at 2 mM of HOBT. The results of the present study indicated that HOBT was a better laccase–mediator system than ABTS, for the laccase from *S. psammoticus.* Considerable enhancement in the decolorization of the dyes was observed when the laccase–HOBT system was used. A decolorization rate of 86% of acid orange was attained with this system. The rate of decolorization for methyl orange and Bismarck brown was 71% and 75%, respectively. The improved degradation of the azo dye, methyl orange, by the laccase–HOBT system has been reported (28). In the present study, it was observed that the laccase–HOBT system could decolorize acid orange and Bismarck brown more efficiently than methyl orange. The current results on methyl orange decolorization could be explained on the basis of the earlier observation that there exists a remarkable difference in the decolorization efficiency of laccases from different sources toward a particular dye (23).

The efficiency of aniline and *p*HBA to act as laccase mediators was also investigated in the study. Both these compounds enhanced the decolorization of dyes in an effective way as

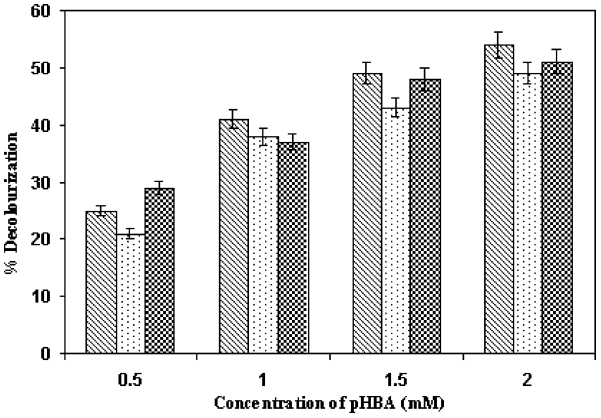

Fig. 8 Decolorization of acid orange, methyl orange, and Bismarck brown by *S. psammoticus* laccase at different concentrations of *p*HBA. The reaction mixture (2 mL) contained 0.5 mL each of the dye solution, mediator, buffer, and enzyme (58.3 nkat). The reaction time was 30 min

compared to the nonmediated system, although the efficiency of these compounds was less than that of ABTS and HOBT. The rate of decolorization achieved with the laccase–aniline system and laccase–*p*HBA system was given in Figs. 7 and 8, respectively. Figure 7 shows that 1 mM concentration of aniline resulted in better decolorization of dyes, while the concentration of *p*HBA required for maximum decolorization was 2 mM. *p*HBA proved to be a better mediator than aniline as it was evident from the decolorization rates achieved (Fig. 8). The results indicated that nature of the mediator plays a significant role in the decolorization of dyes by laccase.

The overall picture of azo dye decolorization in the presence and absence of mediators indicated that the decolorization ability of *S. psammoticus* laccase could be improved significantly with the use of mediators. A fivefold increase in the decolorization of acid orange was obtained in the presence of the best mediator (HOBT; Fig. 6) as compared to the nonmediated system (Fig. 4). Similarly, a sixfold increase in the decolorization of methyl orange and a sevenfold increase in the decolorization of Bismarck brown were attained with HOBT (Fig. 6) as compared to that obtained in the absence of the mediator (Fig. 4). Despite the nature of the mediator used, acid orange was decolorized more efficiently, and the same pattern was observed even in the absence of the mediator. This indicated that the structure of dye is an important factor that determines the rate of decolorization. The influence of structure on azo dye degradation with fungal laccase–mediator systems has already been established (29). The dyes used in the present study have remarkable differences in their structure. The dyes acid orange and methyl orange are sulfonated monoazo dyes, but acid orange had a hydroxyl substitution at the ortho position relative to the azo linkage. Bismarck brown was a diazo dye. The degradation of various substituted azo dyes by the peroxidases produced by *Streptomyces* sp has been suggested (30). However, the role of *Streptomyces* laccases in azo dye decolorization has not been cited in the literature, and it adds to the novelty of the present work.

Conclusion

The use of inducers and SSF bioreactors for laccase production from fungal strains has been well documented. However, similar reports from actinomycetes are relatively rare. Considering the industrial applications of laccases, any attempt to improve the production of this versatile enzyme would be appreciable. The present study has successfully evaluated the enhancement of laccase production from a novel mangrove isolate, *S. psammoticus*, using a wide range of inducers and a scale-up process. The application of the enzyme in azo dye decolorization in the presence of mediators yielded promising results and suggested that the enzyme could play a significant role in the treatment of dye-containing effluents.

Acknowledgments The authors are grateful to the Council of Scientific and Industrial Research, Government of India, for the Research Fellowship given to K.N. Niladevi.

References

1. Piontek, K., Antorini, M., & Choinowski, T. (2002). *Journal of Biological Chemistry*, *277*, 37663–37669.
2. Arias, M. E., Arenas, M., Rodriguez, J., Soliveri, J., Ball, A. S., & Hernandez, M. (2003). *Applied and Environmental Microbiology*, *69*, 1953–1958.
3. Minussi, R. C., Pastore, G. M., & Durany, N. (2002). *Trends in Food Science and Technology*, *13*, 205–216.

4. Abadulla, E., Tzanov, T., Costa, S., Robra, K., Cavaco-Paulo, A., & Guebitz, G. M. (2000). *Applied and Environmental Microbiology*, *66*, 3357–3362.
5. Mayer, A. M., & Staples, R. C. (2002). *Phytochemistry*, *60*, 551–565.
6. Soares, G. M. B., Amorim, M. T. P., Hrdina, R., & Costa-Ferreira, M. (2002). *Process Biochemistry*, *37*, 581–587.
7. Call, H. P., & Mucke, I. (1997). *Journal of Biotechnology*, *53*, 163–202.
8. Bourbonnais, R., & Paice, M. G. (1990). *Applied Microbiology and Biotechnology*, *36*, 823–827.
9. Claus, H., Faber, G., & Konig, H. (2002). *Applied Microbiology and Biotechnology*, *59*, 672–678.
10. Leonowicz, A., Cho, N. S., Luterek, J., Wilkolazka, A., Wotjas-Wasilewska, M., Matuszewska, A., et al. (2001). *Journal of Basic Microbiology*, *41*, 185–227.
11. De Souza, C. G. M., Tychanowicz, G. K., De Souza, D. F., & Peralta, R. M. (2004). *Journal of Basic Microbiology*, *44*, 129–136.
12. Revankar, M. S., & Lele, S. S. (2006). *Process Biochemistry*, *41*, 581–588.
13. Tunga, R., Banerjee, R., & Bhattacharyya, B. C. (1999). *Bioprocess Engineering*, *21*, 107–112.
14. Pandey, A., Soccol, C. R., Rodriguez-Leon, J., & Nigam, P. (Eds.) (2001). . New Delhi, India: Asiatech.
15. Meza, J. C., Lomascolo, A., Casalot, L., Sigoillot, J., & Auria, R. (2005). *Process Biochemistry*, *40*, 3365–3371.
16. Niladevi, K. N., & Prema, P. (2005). *Actinomycetologica*, *19*, 40–47.
17. Niladevi, K. N., Sukumaran, R. K., & Prema, P. (2007). *Journal of Industrial Microbiology and Biotechnology*, *34*, 665–674.
18. Sakurai, Y., Lee, T. H., & Shiota, H. (1977). *Agricultural and Biological Chemistry*, *41*, 619–624.
19. Ashley, V. M., Mitchell, D. A., & Howes, T. (1999). *Biochemical Engineering Journal*, *3*, 141–150.
20. Trilli, A. (1986). In Industrial Microbiology and Biotechnology. In A. L. Demain, & N. A. Solomon (Eds.) (pp. 227–307). Washington, DC: American Society of Microbiology.
21. Lonsane, B. K., Castenada, S. G., Raimbault, M., Roussos, S., Gonzalez, V. G., Ghildyal, N. P., et al. (1992). *Process Biochemistry*, *27*, 259–73.
22. Chivukula, M., & Renganathan, V. (1995). *Applied and Environmental Microbiology*, *61*, 4374–4377.
23. Nyanhongo, G. S., Gomes, J., Guebitz, G. M., Zvauya, R., Read, J., & Steiner, W. (2002). *Water Research*, *36*, 1449–1456.
24. Rodrıguez Couto, S., Sanroman, M., & Guebitz, G. M. (2005). *Chemosphere*, *58*, 417–422.
25. Solis-Oba, M., Ugalde-Saldıvar, V. M., Gonzalez, I., & Viniegra-Gonzalez, G. (2005). *Journal of Electroanalytical Chemistry*, *579*, 59–66.
26. Bourbonnais, R., Leech, D., & Paice, M. G. (1998). *Biochim Biophys Acta*, *1379*, 381–390.
27. Potthast, A., Rosenau, T., Kosma, P., & Fischer, K. (1999). In Proceedings of the 10th International Symposium on Wood and Pulping Chemistry, vol. 1, Yokohama, Japan, pp. 596–601.
28. Zille, A., Munteanu, F., Guebitz, G. M., & Cavaco-Paulo, A. (2005). *Journal of Molecular Catalysis B, Enzymatic*, *33*, 23–28.
29. Almansa, E., Kandelbauera, A., Pereira, L., Cavaco-paulo, A., & Guebitz, G. M. (2004). *Biocatalysis and Biotransformation*, *22*, 315–324.
30. Pasti-Grigsby, M. B., Paszczynski, A., Goszczynski, S., Crawford, D. L., & Crawford, R. L. (1992). *Applied and Environmental Microbiology*, *58*, 3605–3613.