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Effect of inducers and process parameters on laccase production by locally isolated marine Streptomyces lydicus from Red Sea, Egypt

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Abstract: The process parameters influencing the production of extracellular laccases by *Streptomyces lydicus* were optimized in submerged fermentation. Peanut shell is the best substrate for laccase production by this strain. The optimization studies revealed that the laccase yield was maximum at pH 7, temperature 28 ^{0}C and 150 rpm. Salinity of the medium was also observed to be influencing the enzyme production. 2 g/L of Na_2HPO_4 enhanced the enzyme production. $CuSO_4$, L-tryptophan and L-leucine proved to be the best inducers for laccase production by this strain. Mn^{2+}, Zn^{2+}, Fe^{+2}, EDTA and arbutin decreased the production, while Ag^+, sodium azide and L-cystein completely inhibited the enzyme production. Mutation induced by using ultraviolet (UV) and ethylmethanesulfonate (EMS) had an inhibitory effect on laccase production.
Keywords: culture conditions, inducers, *Streptomyces lydicus*, laccase, mutation.

Introduction

Laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) are the most extensively studied group of enzymes among oxidases. They belong to the family of blue multi-copper oxidases, which catalyze the one-electron oxidation of four reducing-substrate molecules concomitant with the four-electron reduction of molecular oxygen to water [1]. Laccase has broad substrate specificity towards aromatic compounds containing hydroxyl and amine groups. These enzymes were known to catalyze the oxidation of a wide range of phenolic compounds and aromatic amines. White rot fungi are the best-known laccase producers [2] also there exist certain bacteria [3] and actinomyctes [4] that are known to produce laccases. Laccases have recently been found from a different species of *Streptomyces* such as *Streptomyces cyaneus* [4], *Streptomyces lavendulae* [5], *Streptomyces psammoticus* [6], *Streptomyces ipomoea* [7], *Streptomyces coelicolor* and *Streptomyces cinnamomensis* [8].

Laccases have attracted increasing scientific attention in the recent years due to their application in diverse industrial sectors such as food, cosmetics [9], textile [10], paper [11], decolorize dyes [12], beverage production industries [13], and also they have a role in bioremediation of contaminated soil [14].

Laccases expression in streptomycetes is influenced by culture conditions, media

composition, pH, temperature, presence of inducers and etc.

Mutations that perform by either physical or chemical mutagens may increase the production of the secondary metabolites such as enzymes [15].

The aim of this work designs the effect of different parameters and inducers on the production of laccase from marine *Streptomyces lydicus* which isolate from Red Sea, Egypt. The effect of mutations obtained using UV light and ethylmethanesulfonate (EMS) on the enzyme production was studied.

Experimental

Microorganism and its maintenance

Streptomyces lydicus was isolated and identified from Hurghada area at Red Sea, Egypt. The culure was grown and maintained on starch nitrate agar slants [16].

Medium and Cultural conditions for submerged fermentation

Streptomyces lydicus colonies were grown in the laccase production medium [17] containing (per 500 ml of distilled water): 20g soya bean, 1g KH_2PO_4 (anhydrous), 4g Na_2HPO_4, 0.2g NaCl, 0.2g $MgSO_4$. $7H_2O$, 0.05g $CaCl_2$. $2H_2O$ and 500 ml sea water at pH 7. The cells (10^7/ml) were grown in flasks containing 50 ml medium and maintained at 28 ^{0}C under constant shaking 150 rpm for 4 days.

Laccase assay

Lacasse assay was performed with 0.5 ml of culture filtrate, 2.4 ml of 0.2M phosphate buffer (pH 6.5) were equilibrate to 30 ^{0}C and 0.01 ml of syringaldazine 0.216 mM. Reaction was initiated by addition of syringaldazine, the change in absorbance per minute was measured at 526 nm [18]. A standard laccase enzyme unit was defined as the amount of enzyme catalysing the oxidation of 1 µmol of syringaldazine to its quinine form per minute at 30 ^{0}C in 0.2 M phosphate buffer (pH 6.5) using a molar absorptivity of 65 $mM^{-1}cm^{-1}$ for the product [19].

Optimization of cultural and nutritional parameters

Different cultural and nutritional parameters were examined in order to characterize their influence on laccase production. The initial pH of the medium was changed from 4 to 10 using 0.1M HCl and 0.1M NaOH to study its effect. Temperature optimization was done by incubating the culture flasks at temperatures ranging from 20 to 60 ^{0}C and agitation rate (0-200 rpm). The influence of salinity was determined by altering the level of distilled water in the medium with different proportions of seawater. Various agricultural wastes like peanut shells, wheat bran, soya bean, sugar cane bagasse, rice straw and corn cops (each 20 g/L) were also screened. Moreover, different concentrations of Na_2HPO_4 (0.5-5 g/L) and NaCl (0.05-0.3 g/L) have been examined.

Effect of metal ions and inhibitors

Different metal ions Mn^{2+} ($MnCl_2$), Zn^{2+} ($ZnCl_2$), Fe^{+2} ($FeSO_4$) , Ag^+ ($AgNO_3$) and mixture of the metal ions have been added to the culture medium at concentration equimolar to the Ca^2 concentration of the basal medium. Different inhibitors have been used such as sodium azide, EDTA (ethylenediaminetetraacetic acid), L-cystein and arbutin with different concentrations (0.08 1mM).

Effect of inducers

Different inducers were investigated for their capacity to increase laccase production such as $CuSO_4$ (1 mM) [20], ammonium tartrate (55 µM) [21], L-methionine, L-tryptophan, L-valine, L-leucine and biotin with (0.2% w/v) concentration [22]. Amino acids and vitamin were sterilized by filtration.

Effect of mutations

Physical mutagens (Ultraviolet (UV) irradiation)

An ultraviolet lamp (20 watts, Wear Goggles, U.S 100V, A.C.D.C., patented and past pend, SUN-kraft YNC. Chicago 10,111.1.) was fixed at 25cm and 254nm in a tightly closed wooden chamber. An aliquot of *Streptomyces lydicus* cells grown in complex medium for 7 days old was exposed to UV-irradiation. During the exposure time it was held in a dish 20 cm in diameter, gently agitated on a vibratory shaker. The UV mutants were isolated at different intervals (5, 10, 15 and 20 min.). After irradiation, the treated spore suspensions were protected from light for two hours.

The mutant cells were plated into complex medium [23] containing (per 500 ml of distilled water): 30g glucose, 25g tryptone, 10g $MgSO_4$. $7H_2O$, 0.6g $MnSO_4$, 10g K_2HPO_4 (anhydrous), 20g agar and 500 ml sea water, and minimal medium agar plates [24] containing (per 500 ml of distilled water): 0.5g NH_4CL, 3g Na_2HPO_4, 1.5g KH_2PO_4 (anhydrous), 0.25g NaCl, 1ml $MgSO_4$. $7H_2O$ (1M), 5ml glucose 20% (w/v), 50ml $CaCl_2$ (1M), 20g agar and 500 ml sea water. After the cultures were diluted serially into 0.85% NaCl and incubated at 28^{0}C for 7 days, the number of survivals was calculated and laccase activity was assayed [18].

Chemical mutagens {Ethylmethanesulfonate (EMS)}

Streptomyces lydicus was grown in tryptone soybean broth [25] containing (per 500 ml

of distilled water): 3g soya peptone, 2.5g glucose, 17g peptone from casein, 2.5g K_2HPO_4, 5g NaCl and 500 ml sea water, for 7 days at 28 ^{0}C. Different concentrations from 20-100 µl of EMS (1.17 g/L) were added to 2 ml cell suspension and incubated for 5, 10 and 15 minutes in a shaking incubator. Then 2 ml of a freshly made 5% (w/v) filter-sterilized solution of sodium thiosulphate was added to cell suspension to quench the EMS, then centrifugated at 3000 rpm for 5 minutes. The treated cells were suspended in 2 ml sodium phosphate buffer (50 mM) at pH 6.2. This suspension was diluted serially and the number of survivals was calculated and laccase activity was assayed [18].

Results and discussion

Research efforts have been directed mainly towards examination the effect of physical and nutritional parameters on the enzyme production. Results of pH optimization studies **(Table 1)** indicated that pH 7 was the optimum for laccase production (0.472 U/ml). The enzyme production was found to be increasing gradually with the increase in initial pH, reached the maximum at 7 and then decreased at higher pH values such as pH 9 (0.005 U/ml). The pH of the culture medium is critical for the growth and enzyme production. The culture pH strongly affects many enzymatic processes and transport of various components across the cell membrane [26]. The optimum pH of laccase production, as reported in many streptomycetes, falls between 7-7.5 [20].

The optimum temperature for laccase production (0.468 U/ml) was observed at 28 **^{0}C (Table 2).** Considerable enzyme yield (0.441 U/ml)was obtained also at 30 ^{0}C, while the production reduced as the temperature increased. Temperature is another critical parameter that must be controlled and varied from microorganism to other. The mechanism of temperature on enzyme production is not well understood [27]. However, some studies showed that a link existed between enzyme synthesis and energy metabolism in bacilli, which was controlled by temperature and oxygen uptake [28]. The obtained results are in agreement with previous reports for laccase production from streptomycetes [6 and 8].

Table (1). Effect of pH on laccase production from *Streptomyces lydicus*

pH values	Laccase activity (U/ml)
4	0
5	0.152
6	0.347
7	0.472
8	0.243
9	0.005
10	0

Table (2). Effect of incubation temperature on laccase production from *Streptomyces lydicus*

Temperature (˚C)	Laccase activity (U/ml)
20	0
25	0.227
28	0.468
30	0.441
35	0.325
40	0.115
45	0.067
50	0.049
55	0.004
60	0

Table (3). Effect of agitation rate on laccase production from *Streptomyces lydicus*

Agitation rate (rpm)	Laccase activity (U/ml)
0	0.064
50	0.146
100	0.300
150	0.466
200	0.437

Table (4). Effect of salinity on laccase production from *Streptomyces lydicus*

Percentage of sea water (%)	Laccase activity (U/ml)
0	0.119
25	0.356
50	0.473
75	0.282
100	0.064

The agitation rate influenced the enzyme production in such a way that the yield increased from static culture (0.064 U/ml) till 100 rpm (0.30 U/ml) as shown in **Table (3).** Abrupt increase in laccase activity was observed at 150 and 200 rpm (0.466 and 0.437 U/ml) respectively. The enhancement in enzyme production at higher agitation rate was due to the better aeration in the well-agitated flasks that was essential for the growth and enzyme production by *Streptomyces lydicus* which is aerobic organism. The slightly drop in enzyme yield at 200 rpm could be attributed to the possible damage that may occur in the filamentous structure of the organisms and thus hindering the enzyme production. High production of laccase was noticed by *Streptomyces cinnamomensis* with 150 rpm [8] and by *Streptomyces* sp. 200 rpm [17 and 20].

Salinity was a significant parameter influencing the enzyme production. Maximum laccase yield (0.473 U/ml) was obtained when 50% of distilled water in the production medium was replaced with equal volume of seawater (**Table 4).** The enzyme production was almost reduced at 75% and rapidly disappeared at 100% salinity. *Streptomyces lydicus* which used in the present study was marine isolate and hence maintaining a moderate level of salinity in the production medium was essential for enzyme production by this organism. The enzyme production was influenced by some of the elements present in the seawater [20].

Among the different agricultural wastes screened for laccase production, peanut shell was the most suitable substrate for enzyme production (0.654 U/ml) by *Streptomyces lydicus* in submerged fermentation (**Table 5).** Wheat bran and soya bean were also identified as promising substrates for laccase production (0.558 and 0.402 U/ml) respectively, while the production in other substrates rice straw, and corn cops were very low. Considerable production of enzyme (0.391 U/ml) was observed with sugar cane bagasse. The presence of significant amount of polyphenols in peanut shell could very well substantiate the enhanced production of laccase, which is a polyphenol oxidase [29]. The use of wheat bran for the production of laccases has been reported widely from fungal as well as actinomycetes strains **[**30]. Arias et al [17] use soya flour for laccase production by *Streptomyces cyaneus*. Recent researches [8] reported that wheat bran and rice straw were identified as promising substrates for laccase production by *Streptomyces psammoticus* and *Streptomyces cinnamomensis* respectively, while the production of laccase by sugarcane bagasse was very low.

Concentrations of Na_2HPO_4 were used to check their effect on laccase production (**Table 6).** The maximum production of laccase was noticed at 2 g/L (0.727 U/ml). Considerable enzyme yield (0.7 U/ml) was obtained also at 2.5 g/L. The laccase activity decreased by increasing the concentration of disodium hydrogen phosphate until a complete inhibition at 5 g/L. It was reported that the absence of mineral sources of phosphorus in the medium causes a substantial drop in the activity and a decrease in the intensity of growth of the culture, which is due not only to the significance of phosphorous as an element of nutrition, but also to the buffering of solutions of its salts [26].

Table (5). Effect of agricultural wastes on laccase production from *Streptomyces lydicus*

Agricultural wastes	Laccase activity (U/ml)
Peanut shell	0.654
Wheat bran	0.558
Soya bean	0.402
Sugar cane bagasse	0.391
Rice straw	0.102
Corn cops	0.045

Table (6). Effect of Na_2HPO_4 concentrations on laccase production from *Streptomyces lydicus*

Concentration of Na_2HPO_4 (g/L)	Laccase activity (U/ml)
0.5	0.222
1.0	0.427
1.5	0.576
2.0	0.727
2.5	0.700
3.0	0.664
3.5	0.657
4.0	0.650
4.5	0.383
5.0	0

Table (7). Effect of sodium chloride concentrations on laccase production from *Streptomyces lydicus*

Concentration of NaCl (g/L)	Laccase activity (U/ml)
None	0.067
0.05	0.155
0.10	0.304
0.15	0.571
0.20	0.721
0.25	0.532
0.30	0.369

The effect of concentrations of sodium chloride on the production of laccase is shown in **Table (7).** As the concentration of NaCl increased the activity increased until reached the maximum production (0.721 U/ml) at 0.2 g/L and the activity decreased by increasing the concentrations. The obtained result was agreed with different researchers [31 and 32] where they indicated that the excess of chlorine ion was the cause of high levels of reduced production of laccase.

The effect of metal ions and inhibitors on laccase production were shown in **Table (8).** The laccase production reaching the maximum production with supplementation of Ca^{2+} (0.730 U/ml) and decreased with Fe^{+2} **(**0.49 U/ml), Mn^{+2} (0.429 U/ml) and Zn^{+2} (0.370 U/ml). Ag^{+} ion and mixture of metal ions inhibit laccase production. It was mentioned that the requirement for specific metal ions depends on the source of enzyme [33]. Laccases can be inhibited by metals such as Fe^{+2}, Mn^{+2}, Zn^{+2} and Ag^{+} [34 and 35]. Ions such as iron may interrupt the electron transport system of laccase and substrate conversion [36].

Table (8). Effect of different metal ions on laccase production from *Streptomyces lydicus*

Metal ions	Laccase activity (U/ml)
None	0.125
Ca^{+2}	0.730
Fe^{+2}	0.490
Mn^{+2}	0.429
Zn^{+2}	0.370
Ag^{+}	0
Mixture of metal ions	0

Table (9). Effect of different inhibitors on laccase production from *Streptomyces lydicus*

Concentrations (mM)	Residual activity of lacaase (%)			
	Sodium azide	EDTA	L-cystein	Arbutin
0.08	0	100	12	100
0.1	0	93	5	88
0.2	0	91	0	80
0.4	0	87	0	71
0.6	0	83	0	66
0.8	0	80	0	61
1	0	75	0	54

The effect of different inhibitors on the production of laccase was illustrated in **Table (9).** Concentration of 0.08 mM of sodium azide inhibit laccase production. Concentration of 0.2 mM of L-cystein inhibited laccase production. They may inhibit the production by binding to type 2 or 3 copper resulting in the interruption of internal electron transfer. The same results were confirmed by many authors [5 and 37]. Maximum residual activity (100%) was indicated with EDTA and arbutin at 0.08 concentration. In case of EDTA the residual activity ranged from (93 to 75%) at concentrations of 0.1-1 mM, while in case of arbutin the residual activity ranged from 88 to 54%. Laccase was found to be metal ion dependent in view of their sensitivity to metal chelating such as EDTA, where EDTA chelates metals such as copper and thus decreased laccase activity [38].

The results of the various studied inducers (**Table 10)** indicated that enhanced laccase production observed in the presence of $CuSO_4$, L-tryptophan, L-leucine and L-methionine (1.625, 1.2, 0.995, 0.87 U/ml) respectively. L-valine, biotin and ammonium tartrate did not stimulate the laccase production. It was reported that copper is a laccase cofactor which presents four cupric ions each associated with one single polypeptide chain [2], but it also has been proved that this element may play an important role in laccase genes regulation at transcription level [21]. Copper sulphate was to be a promising inducer for laccase production by *Streptomyces psammoticus* [20]. It was proved that the maximum laccase production was stimulated in the presence of L-trypyophan, L-leucine and L-methionine [22].

The obtained data clearly showed that the survival percentages were decreased gradually from (100 to 5.55%) by increasing UV exposure period as seen in **Table (11).** Moreover, results showed that all UV exposure times didn't increase the production of laccase but made a complete inhibition. In case of EMS all the concentrations used, survivals of *Streptomyces lydicus* were gradually decreased by increasing the exposure time of EMS as seen in **Fig. (1),** while the laccase production completely inhibited. It was proved that *Streptomyces* species had mutagenic DNA repair mechanism [39]. In case of UV irradiation the incomplete loss of the mcr^{+} gene product or a second error-prone repair pathway reduced the mutation. Moreover, in case of EMS the efficient de-ethylation of O^{6}-ethylguanine might minimize mutagenesis by direct mispairing. Thus, residual noncoding base lesions induced by EMS might constitute premutagenic lesions requiring error prone repair for realization [40]. Since *Streptomyces* have genomes about three times the size of the *Escherichia coli* genome, they may have evolved error surveillance mechanisms more sophisticated than those found in the enteric bacteria [41]. In addition, EMS which has been considered to

be a direct acting mutagen in bacteria may induce lesions that block normal replication and that must be repaired before normal replication resumes [42].

Table (10). Effect of different inducers on laccase production from *Streptomyces lydicus*

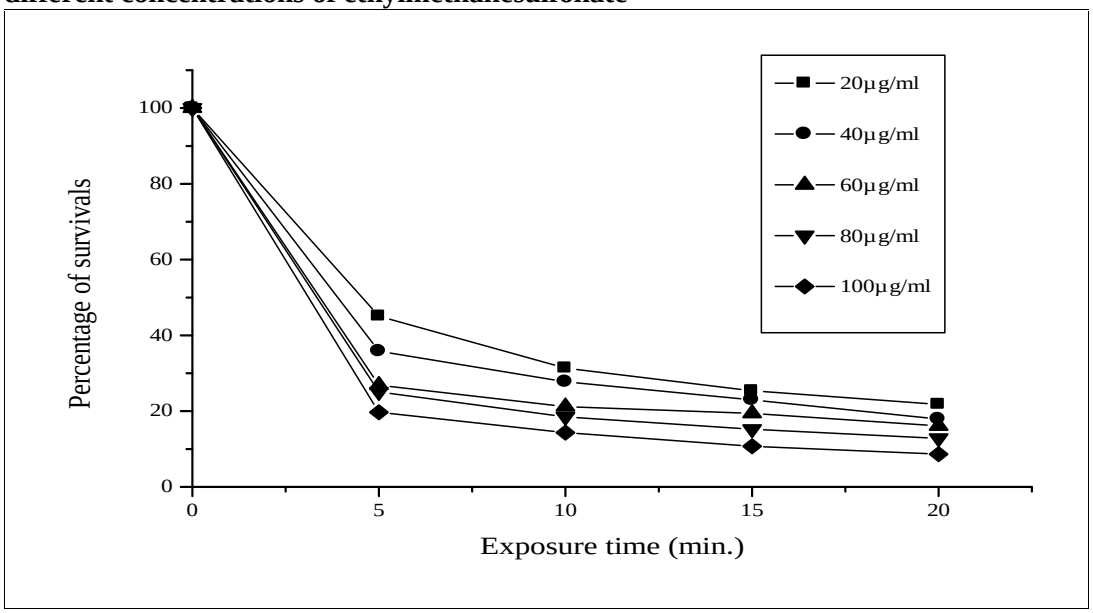
Inducers	Laccase activity (U/ml)	Residual activity of laccase (%)
Control	0.701	100
$CuSO_4$	1.625	231.81
L-Tryptophan	1.200	171.18
L-Leucine	0.995	141.94
L-Methionine	0.870	124.10
L-Valine	0.632	90.15
Biotin	0.497	70.89
Ammonium tartrate	0.255	36.37

Table (11). Effect of ultraviolet irradiation on survivals of *Streptomyces lydicus* and laccase production

UV exposure time (min)	No of colonies *CFU/ml (p^o)	percentage of survivals (%)	Log p / p^o	Laccase activity (U/ml)
0	1.44×10^7	100	0	1.693
5	5×10^6	34.72	0.459	0
10	2.2×10^6	15.27	0.815	0
15	1.3×10^6	9.02	1.044	0
20	8×10^5	5.55	1.255	0

CFU = Colony forming unit, P= Surviving population after UV exposure, P^o= Initial population

Fig. (1). Effect of exposure time on laccase production by *Streptomyces lydicus* at different concentrations of ethylmethanesulfonate



Conclusions

The optimization of various cultural and nutritional parameters for the production of laccase by *Streptomyces lydicus* showed that the enzyme production by this isolate is governed by parameters such as salinity of the production medium. The present study had also explored the potential of peanut shell to be a valuable substrate for laccase production. $CuSO_4$ which used for laccase induction have been successfully applied to enhance laccase yield from this strain. The mutations obtained by using UV light and ethylmetanesulfonate (EMS) showed inhibited laccase production. In future, this strain seems to be prospective for further biotechnological applications.

References

1. Piontek K., Antorini M. and Choinowski T., Crystal structure of a laccase from the fungus *Trametes versicolor* at 1.90-Å resolution containing a full complement of coppers, J. Biol. Chem., 2002, 277, 37663-37669.
2. Thurston C. F., The structure and function of fungal laccases, Microbiol., 1994, 140, 19-26.
3. Martins L. O., Soares C. M., Pereira M. M., Teixeira M., Costa T., Jones G. H. and Henriques A. O., Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the *Bacillus subtilis* endospore coat, J. Biol. Chem., 2002, 277, 18849-18859.
4. Berrocal M. M., Rodriguez J., Ball A. S., Perez-Leblic M. I. and Arias M. E., Solubilization and mineralization of [^{14}C] lignocellulose from wheat straw by *Streptomyces cyaneus* CECT 3335 during growth in solid state fermentation, Appl. Microbiol. Biotechnol., 1997, 48, 379- 384.
5. Suzuki T., Endo K., Ito M., Tsujibo H., Miyamoto K. and Inamori Y. A., Thermostable laccase from *Streptomyces lavendulae* REN-7: purification, characterization, nucleotide sequence and expression, Biosc. Biotechnol. Biochem., 2003, 67, 2167-2175.
6. Niladevi K. N., Rajeev K., Sukumaran K., Jacob N., Anisha G. S. and Prema P., Optimization of laccase production from a novel strain *Streptomyces psammoticus* using response surface methodology, Microbiol. Res., 2009, 164, 105-113.
7. Molina-Guijarro M., Pérez J., Muñoz-Dorado J., Guillén F., Moya R., Hernández M. and Arias M., Detoxification of azo dyes by a novel pH-versatile, salt-resistant laccase from *Streptomyces ipomoea*, Int. Microbiol., 2009, 12, 13-21.
8. Jing D. and Wang J., Controlling the simultaneous production of laccase and lignin peroxidase from *Streptomyces cinnamomensis* by medium formulation, Biotechnol. Biofu., 2012, 5, 9-15.
9. Roriz M. S., Osma J. F., Teixeira J. A. and Couto S. R., Application of response surface methodological approach to optimise reactive black 5 decolouration by crude laccase from *Trametes pubescens*, J. Hazar. Mater., 2009, 169, 691-696.
10. Devi V., Inbathamizh L., Ponnu T., Premalatha S. and Divya M., Dye Decolorization using fungal laccase, Bull. Environ. Pharmacol. Life Sci., 2012, 1, 67-71.
11. Virk A., Sharama P. and Capalash N., Use of Laccase in pulp and paper industry, Biotechnol. Prog., 2012, 28, 21-32.
12. Blánquez P., Casas N. and Font X., Mechanism of textile metal dye biotransformation by *Trametes versicolor*, Water Res., 2004, 38, 2166-2172.
13. Neifar M., Ghorbel R., Kamoun A., Baklouti S., Mokni A., Jaouani A. and Chaabouni S., Effective clarification of pomegranate juice using laccase treatment obtanied by response surface methodology followed by ultrafiltration, J. Food Proc. Eng., 2011, 34, 1199- 1219.
14. Bastos A. C. and Magan N., *Trametes versicolor*: potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions, Int. Biodeter. Biodegrad., 2009, 63, 389-394.
15. Kamal F., Samadi N., Assadi M. M., Moazami N. and Fazeli M., Mutagenesis of *Leuconostoc mesenteroides* and selection of dextransucrase hyperproducing strains, Daru., 2001, 9, 18-23.
16. Waksman S. A., The actinomycetes. Classification, identification and descriptions of genera and species, The Williams and Wilkins Co., Baltimore., 1961, 1, 1-363.
17. Arias M. E., Arenas M., Rodrı´guez J., Soliveri J., Ball A. S. and Hernandez M., Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from *Streptomyces cyaneus* CECT 3335, Appl. Environ. Microbiol., 2003, 69, 1953-1958.
18. Petroski J., Peczynska-Czoch W. and Rosazza J., Analysis, production, and isolation of an

extracellular laccase from *Polypours anceps*, Appl. Environ. Microbiol., 1980, 40, 1003-1006.

19. Bauer R. and Rupe C., Use of syringaldazine in a photometric method for estimating free chlorine in water, Anal. Chem., 1971, 43, 421-425.
20. Niladevi K. and Prema P., Effect of inducers and process parameters on laccase production by *Streptomyces psammoticus* and its application in dye decolourization, Biores. Technol., 2008, 99, 4583-4589.
21. Cavallazzi J., Kasuya C. and Soares M., Screening of inducers for laccase production by *Lentinula edodes* in liquid medium, Braz. J. Microbiol., 2005, 36, 383-387.
22. Dhawan S. and Kuhad R., Effect of amino acids and vitamins on laccase production by the bird's nest fungus *Cyathus bulleri*, Bioresour. Technol., 2002, 84, 35-38.
23. Kim J. H., Yoo S. J., Oh D, K., Kweon Y, G., Park D.W., Lee C. H. and Gil G. H., Selection of *Streptoccous equi* mutant and optimization of culture condition of high molecular weight hyaluronic acid, Enz. Microbiol. Technol., 1996, 19, 440-445.
24. Jeftrey M. B., Guy A. C. and Eve A. Z., Preparation of culture media. In: Biotechnology a laboratory source, Academic press, Inc., San Diego, California, 1990, 33-40.
25. Stonesifer J., Matsushima P. and Baltz R. H., High frequency conjugal transfer of tylosin genes and amplifiable DNA in *Streptomyces fradiae*, Mol. Gen. Genet., 1986, 202, 348-355.
26. Moon S. H. and Parulekar S. J., A., Parametric study of protease production in batch and fed-batch cultures of *Bacillus firmus*, Biotechnol. Bioeng., 1991, 37, 467-483.
27. Chaloupka J., Temperature as a factor regulating the synthesis of microbial enzymes, Microbiol. Sci., 1985, 2, 86-90.
28. Frankena J., Koningstein G. M., Van Verseveld H. W. and Stouthamer A. H., Effect of different limitations in chemostat cultures on growth and production of exocellular protease by *Bacillus licheniformis*, Appl. Microbiol. Biotechnol., 1986, 24, 106-112.
29. Win M., Abdul-Hamid A., Baharini B., Anwar R., Sabui M. and Par-Dek M. S., Phenolytic compound and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour, Pak. J. Bot., 2011, 43, 1635-1642.
30. Rodriguez Couto S. and Sanroman M. A., Application of solid state fermentation to ligninolytic enzyme production, J. Biochem. Eng., 2005, 22, 211-219.
31. Ullrich R., Huong L., Dung N. and Hofrichter M., Laccase from the medicinal mushroom *Agaricus blazei*: production, purification and characterization, Appl. Microbiol. Biotechnol., 2005, 67, 357-363.
32. Li L., Wenkui D., Peng Y., Jian Z. and Yinbo Q., Decolorisation of synthetic dyes by crude laccase from *Rigidoporus lignosus* W1, J. Chem. Technol. Biotechnol., 2009, 84, 399-404.
33. Kumar C. G. and Takagi H., Microbial alkaline proteases: From a bioindusterial viewpoint, Biotechnol. Adv., 1999, 17, 561-594.
34. Zavarzina A. G., Leontievsky A. A., Golovleva L. A. and Trofimov S. Y., Biotransformation of soil humic acids by blue laccase of *Panus tigrinus* 8/18: An *in vitro* study, Soil Biol. Biochem., 2004, 36, 359-69.
35. Couto S. Sanromán M. A. and Gübitz G. M., Influence of redox mediators and metal ions on synthetic acid dye decolourization by crude laccase from *Trametes hirsute*, Chemosph., 2005, 58, 417-422.
36. Kim Y. and Nicell J. A., Impact of reaction conditions on the laccase catalyzed conversion of bisphenol A, Bioresour. Technol., 2006, 97, 1431-42.
37. Desai S. S. and Netyanand C., Microbial laccases and their applications: a review, Asi. J. Biotechnol., 2011, 3, 98-201.
38. Paterson R., Meona S., Zainal Abidina M. and Limab N., Prospects for inhibition of lignin degrading enzymes to control *Ganoderma* white rot of oil palm, Curr. Enz. Inhibit., 2008, 4, 172-179.
39. Sonesifer J. and Baltz R., Mutagenic DNA repair in *Streptomyces*, Proc. Natl. Acad. Sci., 1985, 82, 1180-1183.
40. Drake J. W. and Baltz R. H., The biochemistry of mutagenesis, Annu. Rev. Biochem., 1976, 45, 11-37.
41. Drake J. W., Scott D., Bridges B. A. and Sobels F. H., Fundamental mutagenic mechanisms and their significance for environmental mutagenesis, Gene Toxicol., 1977, 43-55.
42. Ishii Y. and Kondo S., Comparative analysis of deletion and base change mutabilities of *Escherichia coli* B strains differing in DNA repair capacity (wild type, uvrA-, polA-, recA-) by various mutagens, Mutat. Res., 1975, 27, 27-44.

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