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Full Length Research Paper

Production of lignin peroxidase by *Ganoderma leucidum* using solid state fermentation

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The main objectives of this study were to optimize the culture conditions for the production of lignin peroxidase by *Ganoderma leucidum,* economic utilization of waste corn cobs as inducers substrate by pollution free fermentation technology and to optimize the solid state fermentation (SSF) process for lignin peroxidase (LiP) production. Growth medium employed for the culture of *G. leucidum* for the production of LiP was supplied with fermentation conditions that were optimized before selection like incubation period, inoculum size, temperature, pH, substrate to water ratio, nitrogen source, yeast extract and cane molasses. Culture was harvested on the fourth day and diluted five times with distilled water and filtrate was stored in Eppendoff tubes for enzyme assay using 310 nm wave lengths in the spectrophotometer. Lignin peroxidase production was enhanced and maximum LiP activity (2807 U/ml) was found in the growth medium after 96 h, inoculum size 3 ml, pH 4.5 and temperature 35°C with substrate to water ratio of 20 ml/5 g, yeast extract 4%, $(NH_4)_2SO_4$, 2% and cane molasses 3%. Results indicate the excellent scope of corn cobs as solid state substrate for the production of lignin peroxidase by *G. leucidum.*

Key words: Lignin peroxidase (LiP), *Ganoderma leucidum,* fermentation, corn cobs, cane molasses.

INTRODUCTION

Lignin is the most abundant natural aromatic polymer in the biosphere. Almost 20 to 30% of woody plant cell walls consist of lignin surrounding the cellulose and hemi-celluloses in the form of a matrix thus providing support and protection. Lignin is a branched, heterogeneous three-dimensional structure made up of phenylpropanoid units interlinked through a variety of bonds. Lignin is biosynthesized via a plant-peroxidase catalyzed oxidation of methoxy substituted para-hydroxycinnamyl alcohols (Schoemaker and Piontek, 1996).

Lignin makes the second most abundant group of bio-polymers in the biosphere; thus, its biodegradation is an important step of the global carbon cycle. Studies of lignin biodegradation is also of great importance for possible biotechnological applications, as lignin polymers are a major obstacle to the efficient utilization of cellulosic materials in a number of industrial processes. Naturally, lignin degradation occurs by the action of ligninolytic enzymes of white rot fungi (WRF). Most commonly used WRF in early studies was *Phanerochaete chrysosporium*. It mostly produces two specific peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP), other WRF secrete variable sets of enzymes also include laccase and versatile peroxidase (VP) (Claudia et al., 1996; Asgher et al., 2006a).

Lignin peroxidase (LiP) is a glycoprotein consisting of a single polypeptide chain, with an iron protoporphyrin prosthetic group. It has the ability to degrade lignin oxidatively via an electron transfer (ET) mechanism (Dunford, 1999; Ten and Teunissen, 2001). Lignin peroxidase is also able to catalyze the oxidation of nonphenolic, electron-rich aromatic lignin model

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Abbreviations: LiP, Lignin peroxidase; **SSF,** solid state fermentation.

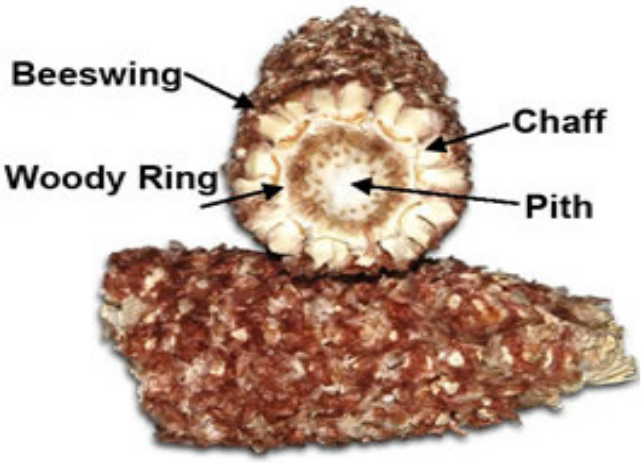

Figure 1. Structure of a corn cob.

compounds as well as the oxidation of other different classes of organic compounds with a redox potential up to 1.4 V versus normal hydrogen electrode (NHE) (Schoemaker and Piontek, 1996; Labat and Meunier, 1990; Gerini et al., 2003). Lignin peroxidase has its potential applications for processing of renewable resources in the pulp and paper industry, for enzymatic transformations of polyaromatic hydrocarbons and decolourization of dyes and coloured water from textile industries (Asgher et al., 2006b).

Solid state fermentation (SSF) holds tremendous potential for the production of enzyme especially where the raw fermented product may be used directly as the enzyme source (Tengerdy, 1998). In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformation involving organic solvent media, mainly for bioactive compounds. This system offers numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipments, etc (Pandey, 1994).

MATERIALS AND METHODS

In order to explore the possibility of using corn cobs as substrate for the production of lignin peroxidase, a bioprocess was developed using corn cobs as lignocellulosic substrate in solid state fermentation employing *G. leucidum* as the fermentative organism.

Substrate

Corn cobs (Figure 1) were purchased from the grain market of Rawalpindi and the substrates were dried and grounded to powder form (40 mm mesh size). The substrate was then stored in plastic jar for subsequent use in a fermentation medium.

Fermentative microorganism

The pure culture of white rot fungus *G. leucidum* was acquired from the culture collection of Industrial Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan. The fungus was cultured on potato dextrose agar (PDA) slants (Table 1) for a period of 5 to 6 days at a pH 4.5 and a temperature of 30°C of the culture obtained was preserved at 4°C in the refrigerator.

Sporulation media

Kirk's basal salts medium (Table 1) having the following composition (g/L) was used for sporulation: ammonium tartarate, 0.22 g/l; KH_2PO_4, 0.2 g/l; $MgSO_4.7H_2O$, 0.005 g/l; $CaCl_2$, 0.01 g/l; thiamine, 1 mg/L; 10 ml/L of 10% (w/v) Tween-80 solution; 100 mM veratryl alcohol and 10 ml/L trace elements solution was added. Trace elements solution given in Table 2 had the composition $CuSO_4$, 0.08; H_2MnO_4, 0.05; $MnSO_4.4H_2O$, 0.07; $ZnSO_4.7H_2O$, 0.043 and $Fe_2(SO_4)_3$; 0.05 (Tien and Kirk, 1983).

Inoculum preparation

Inoculums in the form of spore suspension for the production of LiP were obtained by adding 1% (w/v) sterile glucose solution to fungal culture. The slant was gently shaken to transfer spores to the liquid medium. This spore suspension was then passed through sterile glass wool column to filter out hyphal fragments. Concentration of the eluted suspension was determined by haemocytometer counting. The conidial suspension was adjusted to get $1x10^8$ c/ml. After about 7 days of incubation at 35°C the spore counting was done. This was then stored at 4°C to be used as inoculum. The process was repeated again and again to get fresh spore suspension after every 10 to 15 days.

Solid state fermentation process

Solid state fermentation flasks (250 ml) were prepared in triplicate, each containing 5 g of corn cobs as substrate, moistened with Kirk's nutrient medium before sterilization. The pH was adjusted to 4.0 before sterilization (121°C) in an autoclave for 15 min. On gradual cooling to room temperature, 5 ml of inoculum was aseptically added to each flask in laminar air flow (Dalton PAU. 1300BN, Japan). The inoculated flasks were incubated for 10 days at 120 rpm in a temperature controlled (30°C) shaking incubator.

Sample harvesting

Triplicate flasks were harvested after every 24 h. The contents of the flasks were filtered and centrifuged at 10,000 rpm for 30 min. The supernatants were collected and subjected to LiP assay.

Optimization of process parameters

The various parameters that may influence the production of lignin peroxidase during SSF process were optimized. The classical method was adopted for optimization of fermentation parameters by varying one parameter in an experiment and to incorporate it at a standardized level before optimizing the next parameter. Different process parameters that were standardized include the effect of incubation period, inoculum size, temperature, pH, and substrate to water ratio, nitrogen sources, yeast extract and cane molasses.

Table 1. Composition of sporulation medium for* *G. leucidum.*

S/N	Ingredient	Quantity
1	Potato extract	250 g/L
2	Glucose	20 g/L
3	Agar agar	15 g/L
4	Ammonium tartarate	0. 22 g/L
5	KH_2PO_4	0. 21 g/L
6	$MgSO_4.7H_2O$	0. 05 g/L
7	$CaCl_2$	0. 01 g/L
8	Thiamine	0. 001 g/L
9	10% Tween 80	10 ml/L
10	Trace elements solution	10 ml/L
11	100mM Veratryl alcohol	10 ml/L
12	Chloramphenicol	1 cc/L

pH 4.5, temperature 35°C.

Table 2. Composition of trace element solution.

S/N	Trace element	Quantity (g/L)
1	$CuSO_4$	0.08
2	H_2MoM_4	0.05
3	$MnSO_4.4H_2O$	0.07
4	$ZnSO_4.7H_2O$	0.043
5	$Fe_2(SO_4)_3$	0.05

Incubation period

For the optimization of incubation period, the culture was grown in a set of fifteen flasks. All flasks were autoclaved before inoculation with the spore suspension (Table 1). All flasks were incubated at 35°C. Triplicate flasks were harvested after every 24 h, and culture supernatants were subjected to LiP assay (Kirk and Tien, 1988).

Inoculum size

To investigate the effect of inoculum size on the production of lignin peroxidase, four different levels of inoculum (2, 3, 4 and 5 ml) were tested in triplicate and compared with a control without inoculum. The culture was harvested on the fourth day of incubation.

Incubation temperature

In order to determine the most suitable incubation temperature for the efficient production of lignin peroxidase, the media was adjusted to pH 4.5. After autoclaving, all flasks were inoculated and incubated at varying temperatures (20, 25, 30, 35 and 40°C) for four days. The samples were removed after four days of incubation and were diluted five times to measure the enzyme activity (Kirk and Tien, 1988).

pH

Triplicate flasks containing 5 g of corncobs were moistened with 20 ml of Kirk's medium of varying pH (4.5, 5.0, 5.5, 6.0, 6.5 and 7.0). Prior to inoculation, all flasks were autoclaved and then were incubated in an incubator at 35°C. After four days of inoculation, samples were harvested; the contents of the flasks were diluted five times with distilled water and were analyzed to determine the lignin peroxidase activity.

Substrate to water ratio

For the optimization of substrate to water ratio, 5 g substrate was moistened with varying volumes (10, 15, 20 and 25) of Kirk's medium. The flasks were autoclaved and then incubated at 35°C. After four days of incubation (optimum incubation period) samples were removed and assayed for lignin peroxidase (Kirk and Tien, 1988).

Nitrogen source

This experiment was conducted for the selection of best nitrogen source for the production of lignin peroxidase. Five different nitrogen sources, that is, $(NH_4)_2SO_4$, NH_4NO_3, $NH_4H_2PO_4$, Urea and Peptone were tested in uniform concentrations of 2 g in the growth medium with pre-optimized parameters. Triplicate flasks were inoculated and incubated for 96 h in orbital shaker at 35°C. The culture was harvested on the fourth day of incubation (Kirk and Tien, 1988).

Yeast extract

Five different concentrations of yeast extract were used in the presence of optimum conditions of temperature, pH, nitrogen source, inoculum size, incubation period and substrate to water ratio. Flasks were inoculated, incubated for 96 h and harvested after four days to determine the enzyme activity.

Cane molasses

Varying concentrations of cane molasses were used as additional carbon and mineral source to increase the fermentation rate. Five different concentrations of sterilized cane molasses (1, 2, 3, 4 and 5%) were added aseptically into the pre-optimized fermentation medium. The culture was harvested on the fourth day of incubation; diluted five times with distilled water and assayed for lignin peroxidase.

Analytical method

Enzyme assay

Lignin peroxidase was assayed by monitoring the oxidation of veratryl alcohol at 310 nm (Kirk and Tien, 1988). The reaction mixture (2 ml) containing 4 mM veratryl alcohol in 10 mM sodium tartarate buffer (pH 3) was incubated with 100 µl of the culture fluid at 30°C. The reaction was initiated with addition of suitable amount of 0.2 mM H_2O_2. The blank contained buffer for comparison with veratryl alcohol.

Enzyme activity

One unit of lignin peroxidase activity was defined as the amount of enzyme catalyzing the formation of 1 µ/mol of veratraldehyde per

Table 3. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with varying incubation periods.

Replication	Treatment					
	Control	T_1	T_2	T_3	T_4	T_5
	0 h	24 h	48 h	72 h	96 h	120 h
A	0	547	404	648	843	503
B	0	575	322	418	858	1077
C	0	565	375	514	851	823
Mean	0^c	562.33^b	367^b	526.6^b	850.6^a	801^a

Means with similar superscript in rows are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

minute under the assay conditions. Enzyme activity was determined by the following formula:

$C = A/Єl$

Where, C is the concentration of enzyme; A is the absorbance of enzyme solution; Є is the Epsilon and l is the length of cuvette (cm)

Statistical analysis

All the experiments were run in triplicate flasks. The data values in the tables were presented as mean ± Standard error (S.E). All the data obtained was subjected to statistical analysis by using Analysis of Variance (ANOVA) under completely randomized design (CRD) and treatment means were compared by using Duncan's Multiple Range (DMR) test (Steel et al., 1997).

RESULTS AND DISCUSSION

Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or the other. Current developments in biotechnology are yielding new applications for enzymes. Lignin peroxidase is an enzyme which is potentially useful for processing of renewable resources in the pulp and paper industry such as ligniocellulosics, as well as for enzymatic transformations of polyaromatic hydrocarbons. Lignin peroxidase (LiP) also catalyzes decolourization of colored water released from textile industries containing different dyes present in it. Different parameters were optimized for maximum production of lignin peroxidase by *G. leucidum* in SSF.

Effect of incubation period

For the optimization of incubation period, the culture was grown in a set of fifteen flasks. A set of three flasks was harvested after every 24 h, diluted five times and was analyzed to determine the enzyme activity. Activities of lignin peroxidase produced by *G. leucidum* with varying incubation periods are given in Table 3. Highest enzymatic activity was recorded at 96 h (850.6 U/ml) followed by 120 h (801 U/ml) and 24 h (562.33 U/ml). The results reveal that there was significant (P<0.05) difference among different times of incubation (h) (Table 3). The duration of incubation for different substrates in SSF is different and may range from 3 to 10 days depending on the type of white rot fungi. So our results are in significant accordance with international standards.

Effect of inoculum size

Four different levels of inoculum were used in triplicate and compared with a control receiving no inoculum. The culture was harvested after 96 h. Activity of lignin peroxidase by *G. leucidum* with varying inoculum size is given in Table 4. The significantly highest enzymatic activity (793.3 U/ml) was recorded at a concentration of 3 ml followed by 5 ml (662.3 U/ml) and 4 ml (627.3 U/ml) inoculum respectively. The results reveal that there was highly significant (P<0.05) difference among different treatments for different concentrations of inoculum size (Table 4). The inoculum used is basically spore suspension of the particular fungus being used. The inoculum size is affected by the density of spores in the suspension. The reported inoculum size is variable due to density of spores in the suspension. The appropriate concentration of the spores (10^6/ml) has been reported by Vahabzadeh et al. *(*2004).

Effect of incubation temperatures

Lignin peroxidase production by *G. leucidum was* carried out at varying temperatures. Results given in the Table 5 indicate that maximum enzyme production was obtained at 35°C (686.6 U/ml) followed by 40°C (612 U/ml) and 20°C (506 U/ml). The optimum temperature for various WRF isolates has been found to vary between 25 and 37°C (Tekere et al., 2001). Analysis of variance of the data revealed that there was significant (P<0.05) difference among different incubation temperatures.

Table 4. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with varying inoculum size.

Replication	Treatment				
	Control	T_1	T_2	T_3	T_4
	0	2%	3%	4%	5%
A	0.0	648	843	507	691
B	0.0	644	858	700	686
C	0.0	589	679	675	610
Mean	0.0^{c}	627^{b}	793.3^{b}	627.3^{a}	662.3^{b}

Means with similar superscript in rows are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance = 0.05.

Table 5. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with varying incubation temperatures.

Replication	Treatment				
	T_1	T_2	T_3	T_4	T_5
	20°C	25°C	30°C	35°C (control)	40°C
A	438	473	374	691	752
B	588	387	412	686	459
C	492	405	391	683	625
Mean	506^{bc}	421.6^{cd}	392.3^{cd}	686.6^{a}	612^{ab}

Means with similar superscript in rows are non-significant (P>0.05) with each other. Duncan's multiple range test with level of significance 0.05.

Table 6. Activity of lignin peroxidase (U/ml) production by *G. leucidum* on varying pH.

Replication	Treatment						
	T_1	T_2 (control)	T_3	T_4	T_5	T_6	T_7
	pH 4	pH 4.5	pH 5	pH 5.5	pH 6	pH 6.5	pH 7
A	499	797	173	375	304	349	362
B	440	469	304	461	387	349	382
C	475	525	240	405	351	349	373
Mean	471.3^{a}	597^{b}	239^{bc}	413.6^{c}	347.3^{ab}	349^{d}	372.3^{bc}

Means with similar superscript in rows are non-significant (P>0.01) with each other. Duncan's multiple range test with level of significance = 0.05.

Suitable temperature range for different white rot fungi ranges from 30 to 35°C (Tekere et al., 2001).

Effect of pH

Production of lignin peroxidase by *G. leucidum* with varying levels of pH is given in Table 6. Significant enzymatic activity (597 U/ml) was recorded at pH 4.5 followed by pH 4 (471.3 U/ml) and pH 5.5 (413.6 U/ml). The results (Table 6) reveal that there was significant (P<0.01) difference among different treatments for levels of pH (Swamy and Ramsay, 1999; Asghar et al., 2006 b).

According to results, the highest enzymatic activity was achieved at 4.5 pH.

Effect of substrate to water ratio

For optimizing substrate to water ratio, the cultures were assigned following substrate to water ratio (10, 15, 20 and 25) prior to inoculation. The inoculated flasks were incubated at 35°C. Significantly higher enzymatic activity (Table 7) was recorded from the medium containing 20% substrate to water ratio (570.6 U/ml) as compared to 10% (481.3 U/ml) and 25% (454 U/ml). Statistical analysis of the data revealed (Table 7) that there was highly significant (P<0.01) difference among different treatments for substrate to water ratio. SSF processes are distinct from submerged fermentation (SmF) culturing, since microbial growth and product formation occurs at or near the surface of the solid substrate particle having low

Table 7. Activity of lignin peroxidase (U/ml) production by *G. Leucidum* with varying substrate to water ratio.

Replication	Treatment				
	Control	T_1	T_2	T_3	T_4
	5	10	15	20	25
A	353	499	503	575	420
B	353	440	418	547	473
C	353	505	405	590	469
Mean	353^c	481.3^b	302.8^a	570.6^b	454^b

Means with similar superscript in rows are non-significant (P>0.01) with each other. Duncan's multiple range test with level of significance = 0.05.

Table 8. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with varying nitrogen source.

Replication	Treatment					
	Control	T_1	T_2	T_3	T_4	T_5
	Blank	NH_4NO_3	$(NH_4)_2SO_4$	$(NH_4)H_2PO_4$	Peptone	Urea
A	459	1705	2332	1928	1865	1967
B	459	1877	1652	1457	1604	1700
C	459	1795	2006	1564	1746	1825
Mean	459^b	1792.3^a	1996.6^a	1649.6^a	1738.3^a	1830.6^a

Means with similar superscript in rows are non-significant (P>0.01) with each other. Duncan's multiple range test with level of significance = 0.05.

moisture contents. Thus, it is crucial to provide optimized water content, and control the water activity (a_w) of the fermenting substrate for; the availability of water in lower or higher concentrations affects microbial activity adversely.

Effect of varying nitrogen sources

Five different nitrogen sources were tested in triplicate at the same concentration. The medium containing no additional nitrogen source was used as control. The fermentation process was carried out for 96 h (optimum incubation period) with 5 g of corn cobs as substrate at a pH of 4.5 and a temperature of 35°C. Results reveal (Table 8) that *G. leucidum* utilized $(NH_4)_2SO_4$ as best additional nitrogen source and showed better lignin peroxidase production as compared to other nitrogen containing compounds. Highest enzymatic activity was recorded in the medium containing $(NH_4)_2SO_4$ (1996.6 U/ml) followed by Urea (1830.6 U/ml) and NH_4NO_3 (1792.3 U/ml). Statistical analysis (Table 8) of the data revealed that there was highly significant (P<0.01) difference among different treatments for different nitrogen sources. Effect of urea and ammonium nitrate, each as a source of nitrogen was studied. Urea as an organic source of nitrogen was not effective and hence did not enhanced the ligninolytic enzyme production, similarly, ammonium nitrate neither decreased nor increased the ligninolytic enzyme activity (Vahabzadeh et al., 2004).

Effect of yeast extract

The effect of supplementation of growth media with varying concentrations of yeast extract was also investigated. Results of addition of 1, 2, 3, 4 and 5% yeast extracts respectively on the production of lignin peroxidase are shown in Table 9. Results indicate that the addition of yeast extract into the fermentation medium enhanced the production of lignin peroxidase by *G. leucidum* and 4% of yeast extract resulted in maximum activity of lignin peroxidase (1491.6 U/ml). Further nitrogen addition resulted in gradual decrease in enzyme production. Statistical analysis (Table 9) of the data revealed that there was significant (P<0.05) difference among different treatments for varying concentrations of yeast extract. Contrary to Moreira et al. 1999, this difference in the optimum amounts of yeast extract may be due to the fact that culture conditions vary with the requirements of different organisms in growth media of different substrates (Moreira et al., 1999). Yeast extract promotes the growth of the microorganisms because it

Table 9. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with varying concentrations of yeast extract.

Replication	Treatment					
	Control	T_1	T_2	T_3	T_4	T_5
	Blank	1%	2%	3%	4%	5%
A	1284	1005	1466	1119	1412	1258
B	1284	1214	1506	1252	1638	1287
C	1284	1,109	1400	1180	1425	1172
Mean	1284^{b}	1109^{c}	1457.3^{a}	1183.6^{bc}	1491.6^{a}	1239^{bc}

Mean with similar superscript in row are non-significant (P>0.05) with each other. Duncan's Multiple Range Test with level of Significance = 0.05.

Table 10. Activity of lignin peroxidase (U/ml) production by *G. leucidum* with cane molasses.

Replication	Treatment					
	Control	T_1	T_2	T_3	T_4	T_5
	0	1%	2%	3%	4%	5%
A	1962	3971	2796	2887	2906	2752
B	1962	2554	3084	2910	3086	2785
C	1962	3265	2955	2903	3005	2770
Mean	1962^{b}	3263.3^{a}	2945^{a}	2900^{a}	2999^{a}	2769^{a}

Means with similar superscript in rows are non-significant (P>0.05) with each other. Duncan's Multiple Range Test with level of Significance = 0.05.

contains a mixture of B complex vitamins and some essential amino acids. In their active forms B complex vitamins act as co enzyme with various enzymes involved in energy and general metabolism of fungus and other microbes (Moo Young et al., 1985).

Effect of cane molasses

Sterilized cane molasses were added aseptically as additional energy and carbon source in culture media to increase the production of lignin peroxidase by *G. leucidum*. It was observed that with the addition of cane molasses the production of LiP increased. Molasses is a mixture of soluble sugars; it also contains some minerals and some heavy metal ions (Moo-Young, 1985).

Supplementation of culture media with molasses provides additional energy in the form of soluble sugars thus; promote the growth of *G. leucidum*. Beyond a certain level, the production of amylase decreases by increasing the level of soluble sugars leading to catabolic repression. The repressive effect of increased concentration of soluble sugars and increase in enzyme production as a result of the addition of soluble sugar was also reported in other studies (Enayati, 1999; Carlson et al., 1996; Krishna and Chandrasekaran, 1996; Uchiyama et al., 1997). Significantly, higher enzymatic activity (Table 10) was recorded at 1 ml (3263.3 U/ml) followed by 4 ml (2999 U/ml) and 2 ml (2945 U/ml). Analysis of variance of the data revealed significant ($P<0.05$) difference among the varying concentrations of cane molasses regarding the production of lignin peroxidase for different treatments. Our results are in agreement with other studies (Moo-Young, 1985; Enayati, 1999; Carlson et al., 1996; Krishna and Chandrasekaran, 1996; Uchiyama et al., 1997).

Effect of all parameters optimized

The effects of optimum conditions were investigated for the production of lignin peroxidase by *G. leucidum*. Lignin peroxidase production was increased by the addition of yeast extract, nitrogen source, cane molasses, while maximum lignin peroxidase activity (2807 U/ml) was found in the growth medium with 96 h, inoculum size 10%, pH 4.5, and temperature 35°C, substrate to water ratio of 20 ml/5 g, yeast extract 4%, $(NH_4)_2SO_4$, 2% and cane molasses, 3%. Results indicate the excellent scope of corn cobs as solid state substrate for the production of lignin peroxidase by *G. leucidum* (Table 11).

The results were statistically analyzed using ANOVA under CRD. All parameters for each condition were replicated thrice. Culture was harvested after 96 h and

Table 11. Activity of lignin peroxidase (U/ml) by *G. leucidum* with optimized parameters.

Replication	A	B	C	Mean
	2538	2930	2955	2807

filtrate was stored in Eppendoff tubes for enzyme assay using 310nm wave lengths in the spectrophotometer.

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