African Journal of Biotechnology Vol. 11(27), pp. 7009-7015, 3 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3615
ISSN 1684–5315 © 2012 Academic Journals

*Full Length Research Paper*

# Improving production of laccase from novel basidiomycete with response surface methodology

**Yonghui Zhang, Shujing Sun*, Kaihui Hu and Xiaoyong Lin**

College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, People's Republic of China

Accepted 5 March, 2012

**A *Mycena purpureofusca* strain screened from six basidiomycota fungi is well characterized in submerged fermentation for its high production of laccase and very low mycelium generation. Optimization of submerged fermentation medium for laccase production by *M. purpureofusca* was carried out with two statistical methods including Plackett-Burman (P-B) and Box-Behnken (B-B) designs. Three variables (sucrose, $MgSO_4$ and $CuSO_4$) were found to affect laccase production significantly by P-B screening. B-B design with three-factor at three levers was performed to explain the combined effects of the three medium constituents. The optimum medium consisted of sucrose (4.26 g/L), yeast powder (15 g/L), $MgSO_4$ (4.83 g/L), $KH_2PO_4$ (2.7 g/L), $CuSO_4$ (5.625 mg/L) and vitamin B1 (0.1 g/L). The laccase production was increased by 1.87 folds (277.5 U/L) using this optimized medium. Furthermore, the experimental value was closed to the prediction under the optimal condition, indicating that the chosen methods were successful to determine the optimal medium components, which is the least time consuming and most effective for laccase production. The data obtained in this study may provide new insights to large-scale laccase production by *Mycena* sp.**

**Key words:** *Mycena purpureofusca*, Laccase, Medium optimization, Plackett-Burman design, Box-Behnken design

## INTRODUCTION

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are blue multicopper oxidases that catalyze the oxidation of an array of aromatic substrates concomitantly with the reduction of molecular oxygen to water (Giardina et al., 2010). The majority of laccases are often found in white-rot fungi and higher plants such as the varnish tree *Rhus vernicifera* (Morozova et al., 2007). Among these resources, glycol content of laccase from fungi is generally lower than that of laccase from plant (Liang et al., 2008). In fungi, laccases play a variety of physiological roles in their life cycle, such as lignin degradation, pathogenesis, detoxification and morphogenesis (Baldrian, 2006; Leonowicz et al., 2001;Sun et al., 2011). The laccases from fungi have many advantages, such as substrate non-specific, directly oxidizing various phenolic compounds, using molecular oxygen as the final electron acceptor instead of hydrogen peroxide, and showing a considerable level of stability in the extracellular environment (Eggert et al., 1997). Therefore, the laccases from fungi have been widely applied in biotechnology and industry, such as delignification of lingocellulosics, paper pulping/bleaching, and degradation of different recalcitrant compounds, bioremediation, sewage treatment, dye decolorization and biosensors (Osma et al., 2010; Rodriguez Couto et al., 2005; Shervedani and Amini, 2012).

Up to date, the use of laccase on a commercial scale is restrained by the low productivity in microbial fermentation. There have been a few studies on fermentation optimization of laccases by *Trametes* sp., *Botryosphaeria* sp., *Panus tigrinus* and *Pleurotus ostreatus* (Ana Flora et al., 2000; Daniele et al., 2008; Dekker et al., 2007; Galhaup and Haltrich, 2001; Liu et al., 2009; Nyanhongo

*Corresponding author. E-mail: shjsun2004@126.com. Tel: +86-591-83789492. Fax: +86-591-83789352.

**Abbreviations: RSM,** Response surface methodology; **P-B,** Plackett-Burman**; B-B,** Box-Behnken; **$R^2$,** determination coefficient.

et al., 2002; Tong et al., 2007). These strains can secret laccase abundantly, although a large amount of mycelial pellets are formed in the fermentation process, which seriously limited the utilization of laccases from these strains. It is known that culture medium is important to laccase production (Elisashvili et al., 2008). For this reason, it is useful to optimize the medium composition for the production of laccase produced by fungi. The development of an economically productive medium requires selecting carbon, nitrogen and trace element sources. There were two ways by which the problem of medium component limitations may be addressed: classical and statistical (Mao et al., 2010). Conventional optimization procedures involve altering of one variable at a time and keeping all other variables constant, which enables it to assess the impact of those particular variables on the process performance. Compared with the classical method, statistical experimental designs are useful tools for medium optimization to screen the main variables rapidly from a multivariable system. The statistical experimental designs have many advantages, including more advanced results with less process variability, closer confirmation, less development time and less overall costs. Response surface methodology (RSM) has been successfully applied in the optimization of the medium conditions of laccase product from different microorganism (Arockiasamy et al., 2008; Bhattacharya et al., 2011; Niladevi et al., 2009).

In this study, *Mycena purpureofusca* was screened out of six fungal strains according to laccase-secreting ability. Medium optimization for production of laccase by *M. purpureofusca* was reported to make it clear that fermentation factors influenced the laccase yield under statistical experimental design. Until now, there have been no reports on the medium optimization for enhancing laccase production by a fungus in the genus *Mycena*.

## MATERIALS AND METHODS

### Fungal strains

Six strains including *Pleurotus spodoleucas, M. purpureofusca*, *Pleurotus florida*, *Pleurotus abalonus*, *Tremella aurantialba* and *P. ostreatus* were obtained from Fujian General Station of Technology Popularization for Edible Fungus (Fuzhou, China) and maintained on potato dextrose agar (PDA; potato 200 g/L, glucose 20 g/L, agar 20 g/L) at 25°C with periodic transfer. Six strains were selected for further experiments based on the results obtained from previous research (Xu et al., 2011).

### Medium and culture conditions

The minimal liquid medium contained (per liter) 10 g sucrose, 10 g yeast powder, 2.7 g $KH_2PO_4$, 2 g $MgSO_4 \cdot 7H_2O$, 2.5 mg $CuSO_4 \cdot 5H_2O$, and 0.1 g vitamin $B_1$ (Sun et al., 2010). The amounts of every component, however, changes in different optimization experiments. All the experiments were carried out in triplicate and each data point is the mean of three independent scorings. First, the strains were grown on PDA plate, then five evenly mycelium mats (ca. 50 $mm^2$) from the plate were made by a sterile Pasteur pipette (5-mm-diameter) and transferred to the seed culture medium. The seed culture was grown in a 250-ml flask containing 100 ml of liquid medium at 24°C on a rotary shaker at 110 rpm for eight days. The broth was filtered through nylon mesh (100 mesh), the filtrate further was clarified by centrifugation at 8000×*g* for 15 min at 4°C, and the supernatant were retained and stored at 4°C for further experiments.

### Laccase assay

Laccase activity assay is conducted in 3 ml reaction mixtures consisting of 2.7 ml of 0.1 M sodium acetate buffer (pH 4.5), 0.2 ml of 1 mM 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) solution, and 0.1 ml culture supernatant. The reaction was monitored by measuring the change in $A_{420}$ for 3 min at 30°C. One unit of enzyme activity is defined as the amount of enzyme that oxidized 1 µM ABTS per minute. The extinction coefficient of $3.6 \times 10^4$ $mol^{-1}$ $cm^{-1}$ was used for oxidized ABTS (Niladevi et al., 2009).

### Strain selection

Laccase production of six strains was determined in two batch experiments. First, six strains were incubated in 250 ml Erlenmeyer flasks containing 100 ml of main medium at 24°C on a rotary shaker at 110 rpm, and then laccase activities were assayed every 24 h for 14 days and the peak time of activity was detected. The next step was to determine total laccase activity. Total laccase activity of every strain was calculated with the maximum activity of every strain per volume of fermentation broth. The strain with the highest laccase-secreting ability was selected for subsequent research.

### Experimental design

### Screening for the significant factors

As shown in Table 1, the Plackett-Burman (P-B) design was applied to screen the key nutrient factors for the production of laccase. Three virtual variables were introduced to reduce the effect of manipulative error. The signs -1 and +1 represented the lower and higher levels, respectively of the corresponding components. The higher levels of the components were equal to 1.5-folds of the lower levels (Table 2).

### Box-Behnken design

Based on analysis of P-B and steepest ascent experiments, Box-Behnken (B-B) design was employed to establish a response surface of laccase production for further optimization of the variables' level. B-B design with three variables at three levels was used as listed in Table 3. The behavior of the system was explained by a regression equation (Equation 1), where $Y$ is the predicted response, $\beta_0$ is offset term, $\beta_i$ is linear effect, $\beta_{ii}$ is squared effect, $\beta_{ij}$ is interaction effect, and $X_i$ is dimensionless coded value of independent variables under study (Murat, 2004). Data were processed to attain Equation 1, indicating the interaction between the process variables and laccase production.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_{ji} \quad (1)$$

**Table 1.** P-B design for screening significant variables with coded values.

| Run | Variables in coded level | | | | | | | | | Y |
|---|---|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | |
| 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | 319.1 ± 5.3 |
| 2 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 318.9 ± 5.1 |
| 3 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 1 | 315.3 ± 9.9 |
| 4 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | -1 | 330.2 ± 7.3 |
| 5 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | -1 | 350.3 ± 11.4 |
| 6 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | -1 | 297.8 ± 3.1 |
| 7 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 1 | 379.1 ± 4.5 |
| 8 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | -1 | 344.6 ± 2.6 |
| 9 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 1 | 320.2 ± 8.6 |
| 10 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 1 | 303.6 ± 8.6 |
| 11 | -1 | 1 | -1 | -1 | -1 | 1 | 1 | 1 | -1 | 347.8 ± 9.1 |
| 12 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 315.9 ± 7.5 |

P-B, Plackett-Burman; Y, predicted response; X, dimensionless coded value of independent variables.

**Table 2.** Assigned concentration of variables at different levels in P-B design.

| Factor | Variables with designate | Lower lever (-1) | Higher lever (+1) |
|---|---|---|---|
| $X_1$ | Sucrose (g/L) | 10 | 15 |
| $X_2$ | yeast powder (g/L) | 10 | 15 |
| $X_3$ | Virtual 1 | | |
| $X_4$ | $MgSO_4$ (g/L) | 2 | 3 |
| $X_5$ | $KH_2PO_4$ (g/L) | 2.7 | 4 |
| $X_6$ | Virtual 2 | | |
| $X_7$ | $CuSO_4$ (mg/L) | 2.5 | 3.75 |
| $X_8$ | Vitamin B1 (g/L) | 0.1 | 0.15 |
| $X_9$ | Virtual 3 | | |

P-B, Plackett-Burman; X, dimensionless coded value of independent variables.

**Table 3.** The B-B design for the values in coded and the observed values in response.

| Run | $X_1$ Sucrose (g/L) | $X_2$ $MgSO_4$ (g/L) | $X_3$ $CuSO_4$ (mg/L) | laccase activity (U/ml) |
|---|---|---|---|---|
| 1 | 2 | 3.7 | 5.625 | 474.1 ± 9.6 |
| 2 | 2 | 6.1 | 5.625 | 511.2 ± 9.6 |
| 3 | 9 | 3.7 | 5.625 | 436.8 ± 2.9 |
| 4 | 9 | 6.1 | 5.625 | 386.1 ± 7.9 |
| 5 | 5.5 | 3.7 | 5 | 491.6 ± 15.9 |
| 6 | 5.5 | 3.7 | 6.25 | 477.5 ± 8.6 |
| 7 | 5.5 | 6.1 | 5 | 474.8 ± 2.8 |
| 8 | 5.5 | 6.1 | 6.25 | 460.5 ± 15.9 |
| 9 | 2 | 4.9 | 5 | 457.3 ± 11.4 |
| 10 | 9 | 4.9 | 5 | 447.3 ± 5.9 |
| 11 | 2 | 4.9 | 6.25 | 483.6 ± 3.5 |
| 12 | 9 | 4.9 | 6.25 | 390.2 ± 19.1 |
| 13 | 5.5 | 4.9 | 5.625 | 522.5 ± 3.3 |
| 14 | 5.5 | 4.9 | 5.625 | 517.8 ± 7.7 |
| 15 | 5.5 | 4.9 | 5.625 | 512.4 ± 8.1 |

P-B, Plackett-Burman; X, dimensionless coded value of independent variables.

**Table 4.** Screening fungal strains with laccase activity in liquid state fermentation.

| Strains | Genus | Peak time (day) | Maximum activity (U/ml) | Volume (ml) | Total activity (U) | Productivity (U/day) |
|---|---|---|---|---|---|---|
| *P. spodoleucas* | *Pleurotus* | 11 | 378.9 ± 3.1 | 52.5 ± 2.6 | 19893.26 | 1808.5 |
| *M. purpureofusca* | *Mycena* | 10 | 277.5 ± 5.7 | 82.3 ± 2.4 | 22838.25 | 2283.8 |
| *P. florida* | *Pleurotus* | 11 | 364.7 ± 7.3 | 59.7. ± 3.5 | 21520.92 | 1979.7 |
| *P. abalonus* | *Pleurotus* | 12 | 219.7 ± 2.3 | 84.2 ± 0.8 | 18458.14 | 1541.8 |
| *T. aurantialba* | *Tremella* | 14* | 187.2 ± 2.4 | 74.5 ± 5.1 | 13855.70 | 996.4* |
| *P. ostreatus* | *Pleurotus* | 11 | 370.5 ± 3.7 | 56.5 ± 4.2 | 20938.06 | 1903.5 |

*The peak of laccase yield did not occur after 14 days of cultivation.

**Table 5.** Analysis of variance for P-B factorial model.

| Source | Degrees of freedom | Sum of square | Mean square | F-value | Pr > F |
|---|---|---|---|---|---|
| Model | 9 | 5727.305 | 636.3672 | 15.19741 | 0.0632 |
| Sucrose | 1 | 883.589 | 883.589 | 21.10143 | 0.0443 |
| Yeast powder | 1 | 476.9632 | 476.9632 | 11.3906 | 0.0777 |
| Virtual 1 | 1 | 71.35344 | 71.35344 | 1.704027 | 0.3217 |
| $MgSO_4$ | 1 | 1723.39 | 1723.39 | 41.15715 | 0.0234 |
| $KH_2PO_4$ | 1 | 519.9227 | 519.9227 | 12.41654 | 0.072 |
| Virtual 2 | 1 | 17.83836 | 17.83836 | 0.426007 | 0.581 |
| $CuSO_4$ | 1 | 1781.579 | 1781.579 | 42.54677 | 0.0227 |
| ABTS | 1 | 175.794 | 175.794 | 4.198225 | 0.177 |
| Virtual 3 | 1 | 76.87558 | 76.87558 | 1.835904 | 0.3082 |
| Error | 2 | 83.74682 | 41.87341 | | |
| Total | 11 | 5811.052 | | | |

P-B, Plackett-Burman.

In the regression equation, the test variable were coded according to Equation 2, where $X_i$ is the independent variable coded value, $U_i$ is the independent variable value, $U_i^0$ is the independent variable real value on the center point and is the step change value (Murat, 2004).

$$X_i = \frac{U_i - U_i^0}{\Delta U_i} \quad (2)$$

Statistical analysis of the experimental results and generation of response surfaces were performed by SAS 9.2 (SAS institute inc., Cary, NC, USA.).

## RESULTS

### Strain selection

According to Table 4, the laccase activities in extracellular culture fluids of six strains reached the peak at different time. Laccase activity of *M. purpureofusca* reached its peak on the 10th day and dropped slowly. Total laccase production by *M. purpureofusca* reached 22838.25 U after 10-days cultivation and the highest productivity was 2283.8 U/day. The peak in *T. aurantialba* strain did not occur until 14th day. These results show that the strains have different laccase-secreting abilities. The strains of *P. spodoleucas* and *P. ostreatus* showed relatively higher laccase activities. Moreover *P. spodoleucas* and *P. ostreatus* generated more mycelia than other strains, which resulted in the decrease of the broth volume after centrifugation at 11th day, then further weakened laccase accumulation. *M. purpureofusca* had best potential of biotechnology for lacccase production in submerged fermentation based on the above research. Therefore, *M. purpureofusca* was used in the following research to optimize laccase fermentation medium by RSM.

### Screening for the significant factors

To check the adequacy of the model, statistical analysis was carried out using the Fisher's test for analysis of variance (ANOVA) according to the results of P-B experiment (Table 1). As shown in Table 5, sucrose, $MgSO_4$ and $CuSO_4$ were found to be the main variables significantly influencing laccase production according to

the regression coefficient and P > F value. $CuSO_4$ concentration with P > F value of 0.0227 was found to be the most important variable followed by $MgSO_4$ (P > F = 0.0234) and sucrose (P > F = 0.0443). The first-order polynomial equation for the predicted response *Y* of laccase yield was given by Equation 3. $X_1$ (namely sucrose) has a regression coefficient value of -8.58, which indicated sucrose with high concentration had repression effect on laccase production by *M. purpureofusca*. The concentration of sucrose was gradually decreased to approach the central region in the next steepest ascent experiment.

$$Y = 328.5602 - 8.580933*X_1 + 6.304517*X_2 + 2.438467*X_3 + 11.98398*X_4 - 6.582317*X_5 + 1.219233*X_6 + 12.18462*X_7 - 3.827467*X_8 - 2.53333*X_9 \quad (3)$$

After the main variables were identified, a steepest ascent experiment was carried out to investigate the central point of these variables values for subsequent response surface design. The initial concentrations of sucrose, $MgSO_4$ and $CuSO_4$ were 12.5, 2.5 g/L and 3.125 mg/L, respectively, which were the central point of their level in P-B design. The concentration of other three insignificant variables was as follows: yeast powder 15 g/L, $KH_2PO_4$ 2.7 g/L and vitamin B1 0.1 g/L, according to their positive or negative effect to laccase production which can be identified by their regression coefficient in P-B experiment. The highest laccase activity of 511.1 U/L was achieved when the concentration of sucrose, $MgSO_4$, $CuSO_4$ were at 5.5, 4.9 g/L and 5.63 mg/L, respectively. The corresponding concentration levels of sucrose, $MgSO_4$ and $CuSO_4$ were -2.8, 4.8 and 4, respectively. These results suggest that the configuration could be considered as a fine central point for experimental design of response surface in further optimization.

## Box-Behnken design

A Box-Behnken experiment was employed to determine the second-order polynomial equation including term of interaction between the selected experimental variables obtained by previous research. The step sizes of the variables involved in the experiment were double size as the corresponding size of the steepest ascent design. The other three insignificant variables were used in the same concentration in steepest ascent experiment. The experimental conditions and results of Box-Behnken experiment are summarized in Table 3. By multiple regression analysis, the second-order polynomial equation to explain the model for laccase production is given below:

$$Y = 517.5785 - 33.21446*X_1 - 5.937975*X_2 - 7.379062*X_3 - 48.51201*X_1*X_1 - 21.9462*X_1*X_2 - 20.86973*X_1*X_3 - 17.01644*X_2*X_2 - 0.06945*X_2*X_3 - 24.48231*X_3*X_3 \quad (4)$$

Experimental data were processed using SAS 9.2, including ANOVA to check the statistical significance of this regression model (Table 6). The quality of the fit of this model was expressed by the coefficient of determination ($R^2$) in the same program. The results of ANOVA indicated that the model of the equation for laccase yield was significant at the 1% level. The $R^2$ value of 97.82% and the adjusted $R^2$ value of 93.90% showed that the response surface model was highly reliable. Also the lack of fit (Pr > F = 0.1415), which is not significant at 5% level proved that except for the considered factors, there was no factor influencing this prediction model significantly.

The three dimensional response surface plots are given in Figure 1 by SAS 9.2 analysis. Each Figure presented the effect of two variables on the production, while other variable was held at zero level. Figure 1 shows the response surface for the variation in the production of laccase. There is a strong interaction effect of sucrose/$MgSO_4$ (Figure 1A) and sucrose/$CuSO_4$ (Figure 1C), while there is no significant interaction effect between $CuSO_4$ and $MgSO_4$ (Figure 1B). At the same time, the values of laccase activity for different concentrations of the variables could also be predicted. The maximal laccase yield (523.3 U/L) by *M. purpureofusca* was modeled using response surface regression analysis at the following medium components: 4.26 g/L sucrose, 4.83 g/L $MgSO_4$ and 5.625 mg/L $CuSO_4$. In order to verify the reliability of this prediction model, the experiment was performed with the optimized medium and the maximal laccase production was found to be 519.3 U/L, which was in close agreement with the model prediction.

## DISCUSSION

In recent years, laccase has received extensive attention for its industrial application such as environmental pollution control, textile industry, biosensors, food industry and organic synthesis et al (Tukayi et al., 2011). In general, it is time consuming to cultivate the fruiting bodies of fungi for obtaining laccase. Therefore, submerged cultivation of fungi for efficient laccase production is looked at as a promising alternative to fruiting body cultivation. In this study, among six fungal strains, *M. purpureofusca* shows the highest laccase-secreting ability and low mycelia generation, which is its advantages in submerged fermentation. Although *P. spodoleucas* and *P. ostreatus* represented better laccase activities than *M. purpureofusca*, the growing mycelia nearly covered the entire flask after 14 days, which resulted in the decrease oxygen transfer, and then inhibited laccase accumulation. At the same time, a large amount of mycelial pellets reduced the effective working volume of the fermentation broth containing laccase, which further limited the application laccase. Unlike these two strains, the growth of *M. purpureofusca* was very

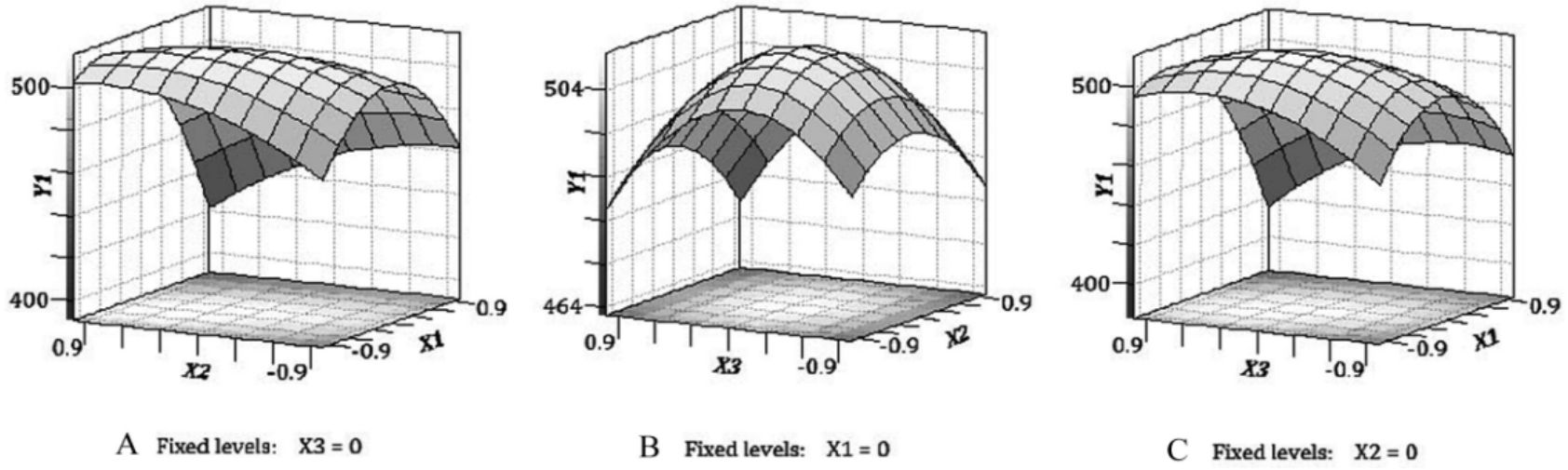


**Figure 1.** A, Effect of sucrose and $MgSO_4$ concentration on the laccase production by *M. purpureofusca*. Another variable is maintained at zero level. B, Effect of $MgSO_4$ and $CuSO_4$ concentration on the laccase production by *M. purpureofusca*. Another variable is maintained at zero level. C, Effect of sucrose and $CuSO_4$ concentration on the laccase production by *M. purpureofusca*. Another variable is maintained at zero level. X1, Sucrose; X2, $MgSO_4$; X3, $CuSO_4$; Y1, laccase yield.

slow, and even slower than their early stages of growth. Therefore, this advantage makes *M. purpureofusca* an excellent resource for large-scale laccase production. At the same time, its culture period is relatively short and its culture process can be optimized to achieve a high laccase production.

In fermentation process optimization, the optimization of fermentation medium is greatly important to enhance laccase production. Using statistical tools like P-B and RSM designs, the variables that played important role in enzyme production were determined and adjusted to an optimized composition. According to P-B experiment, sucrose, $MgSO_4$ and $CuSO_4$ were determined to be the most important variables in laccase production of *M. purpureofusca*. The data obtained in P-B experiment show that sucrose has negative effect and yeast powder reflect positive effect on laccase production. This means that the fermentation medium with a low ratio of carbon to nitrogen can increase laccase yield by *M. purpureofusca*, which may be due to the following facts. First, laccase production of *M. purpureofusca* was triggered when the carbon source was depleted in short time. Second, the largest increase in activity and highest abundance of *lac1Pt* transcripts was observed when glucose had been almost depleted (Daniele et al., 2008), and third, the expression of the main laccase gene (*lap2*) was repressed when glucose concentration exceeds about 1 g/L (Ronne 1995). These results were different from the previous reports by Eggert et al. (1996) and Kaal et al. (1995). They found that a high ratio of carbon to nitrogen can stimulate laccase production. Of course, our research is also in agreement with previous results that $Cu^{2+}$ was an extremely important metal ion and could induce laccase secretion (Galhaup and Haltrich, 2001; Shutova et al., 2008). This role of $Cu^{2+}$ may be due to its induction effect on transcription of the laccase gene (Galhaup et al., 2002). Another reason is that $Cu^{2+}$ may be buried at the catalytic center and maintains the stability of the laccase.

The 3D response surface curve (Figure 1) determines optimum condition of each component for maximum response and their interaction effect when other variable was fixed at zero level. The convex response surfaces implied that a maximum *Y* value was predicted by this model which could also be demonstrated by the negative quadratic coefficients in Equation 3 (Liu et al., 2010). The interactions between the variables can be inferred from the shapes of response surface plots (Yu et al., 2008). The validation experiment for the response surface model was carried out using optimized fermentation medium consisting of 4.26 g/L sucrose, 15 g/L yeast powder, 4.83 g/L $MgSO_4$, 2.7 g/L $KH_2PO_4$, 5.625 mg/L $CuSO_4$ and

0.1 g/L vitamin B1. The laccase yield reached 519.3 U/L, which was very close to the predicted production of 523.3 U/L with the optimized fermentation medium. This result therefore confirms the precision of statistical and theoretic effectiveness of the model. Laccase production with non-optimized medium was 277.5 U/L under the same culture condition. The highest laccase yield was increased by 1.87 times after medium optimization. The data obtained in this study may provide new insights to large-scale laccase production by *M. purpureofusca* or *Mycena* genus. At the same time, this study also demonstrates that RSM is effective for the medium optimization in the submerged cultivation of higher fungi.

## ACKNOWLEDGEMENTS

This work was financially supported by the Key Project of Fujian Province of China (No.2010N0004) and General Program of the Provincial Education Department of Fujian Province of China (No. JA11079).

### REFERENCES

Ana Flora DV, Aneli MB, Robert FHD, Ieda SS, Maria Inês R (2000). Optimization of laccase production by *Botryosphaeria* sp. in the presence of veratryl alcohol by the response-surface method. Process Biochem. 35: 1131-1138.

Arockiasamy S, Krishnan IP, Anandakrishnan N, Seenivasan S, Sambath A, Venkatasubramani JP (2008). Enhanced production of laccase from *Coriolus versicolor* NCIM 996 by nutrient optimization using response surface methodology. Appl. Biochem. Biotechnol. 151: 371-379.

Baldrian P (2006). Fungal laccases- occurrence and properties. FEMS Microbiol. Rev. 30: 215-242.

Bhattacharya SS, Garlapati VK, Banerjee R (2011). Optimization of laccase production using response surface methodology coupled with differential evolution. New Biotechnol. 28: 31-39.

Daniele Q, Mario C, Ermanno F, Alessandro DA (2008). Response surface methodology study of laccase production in *Panus tigrinus* liquid cultures. Biochem. Eng. J. 39: 236-245.

Dekker RF, Barbosa AM, Giese EC, Godoy SD, Covizzi LG (2007). Influence of nutrients on enhancing laccase production by *Botryosphaeria rhodina* MAMB-05. Int. Microbiol. 10: 177-185.

Eggert C, Temp U, Eriksson KE (1996). The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. Appl. Environ. Microbiol. 62: 1151-1158.

Eggert C, Temp U, Eriksson KE (1997). Laccase is essential for lignin degradation by the white-rot fungus *Pycnoporus cinnabarinus*. FEBS Lett. 407: 89-92.

Elisashvili V, Penninckx M, Kachlishvili E, Tsiklauri N, Metreveli E, Kharziani T, Kvesitadze G (2008). *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresour. Technol. 99: 457-462.

Galhaup C, Goller S, Peterbauer CK, Strauss J, Haltrich D (2002). Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. Microbiology, 148: 2159-2169.

Galhaup C, Haltrich D (2001). Enhanced formation of laccase activity by the white-rot fungus *Trametes pubescens* in the presence of copper. Appl. Microbiol. Biotechnol. 56: 225-232.

Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G (2010). Laccases: a never-ending story. Cell Mol. Life Sci. 67: 369-385.

Kaal EEJ, Field JA, Joyce TW (1995). Increasing ligninolytic enzyme activities in several white rot basidiomycetes by nitrogen sufficient media. Bioresour. Technol. 53: 133-139.

Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001). Fungal laccase: properties and activity on lignin. J. Basic Microbiol. 41: 185-227.

Liang S, Zhou DM, Feng YR (2008). Research progress and application prospect of laccase from white rot fungus. J. Anhui Agric. Sci. 36: 1317-1319.

Liu BB, Yang MH, Qi BK, Chen XR, Su ZG, Wan YH (2010). Optimizing L-(+)-lactic acid production by thermophile *Lactobacillus plantarum* As.1.3 using alternative nitrogen sources with response surface method. Biochem. Eng. J. 52: 212-219.

Liu LH, Lin ZW, Zheng T, Lin L, Zheng CQ, Lin ZX, Wang SH, Wang ZH (2009). Fermentation optimization and characterization of the laccase from *Pleurotus ostreatus* strain 10969. Enzyme Microb. Technol. 44: 426-433.

Mao XZ, Liang XT, Wang S, Dong W, Xing YL, Wang HL, Guo LZ, Wei DZ (2010). Enhanced production of intracellular dextran dextrinase from Gluconobacter oxydans using statistical experimental methods. Afr. J. Biotechnol. 9: 1180-1189.

Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI (2007). "Blue" laccases. Biochemistry. Mosc. 72: 1136-1150.

Murat E (2004). Optimization of medium composition for actinorhodin production by *Streptomyces coelicolor* A3(2) with response surface methodology. Process Biochem. 39: 1057-1062.

Niladevi KN, Sukumaran RK, Jacob N, Anisha GS, Prema P (2009). Optimization of laccase production from a novel strain-*Streptomyces psammoticus* using response surface methodology. Res. Microbiol. 164: 105-113.

Nyanhongo GS, Gomes J, Gubitz G, Zvauya R, Read JS, Steiner W (2002). Production of laccase by a newly isolated strain of *Trametes modesta*. Bioresour. Technol. 84: 259-263.

Osma JF, Toca-Herrera JL, Rodriguez-Couto S (2010). Transformation pathway of Remazol Brilliant Blue R by immobilised laccase. Bioresour. Technol. 101: 8509-8514.

Rodriguez Couto S, Sanroman M, Gubitz GM (2005). Influence of redox mediators and metal ions on synthetic acid dye decolourization by crude laccase from *Trametes hirsuta*. Chemosphere, 58: 417-422.

Ronne H (1995). Glucose repression in fungi. Trends Genet 11: 12-17.

Shervedani RK, Amini A (2012). Direct electrochemistry of dopamine on gold-*Agaricus bisporus* laccase enzyme electrode: Characterization and quantitative detection. Bioelectrochemistry, 84: 25-31.

Shutova VV, Revin VV, Makushina YA (2008). The effect of copper ions on the production of laccase by the fungus *Lentinus* (Panus) *tigrinus*. Prikl. Biokhim. Mikrobiol. 44: 683-687.

Sun SJ, Liu JZ, Hu KH, Zhu HX (2011). The level of secreted laccase activity in the edible fungi and their growing cycles are closely related. Curr. Microbiol. 62: 871-875.

Sun W, Xia CY, Cai HH, Liu XM (2010). Purification and properties of laccase produced by *Coriolus hirsutus* in sol id state fermentation. J. Food Sci. Biotechnol. 29: 748-754.

Tong P, Hong Y, Xiao Y, Zhang M, Tu X, Cui T (2007). High production of laccase by a new basidiomycete, *Trametes* sp. Biotechnol. Lett. 29: 295-301.

Tukayi K, Gibson SN, Georg MG, Stephanie B (2011). Potential applications of laccase-mediated coupling and grafting reactions: A review. Enzyme Microb. Technol. 48: 195-208.

Xu JZ, Hu KH, J. SS, L. ZJ, Xiao YM, Lin YH (2011). The comparison and analysis of the producing ability of the ligninolytic enzyme system in different types of edible fungi. Acta. Agric. Univ. Jiangxiensis, 33: 375-380.

Yu L, Lei T, Ren XD, Pei XL, Feng Y (2008). Response surface optimization of L-(+)-lactic acid production using corn steep liquor as an alternative nitrogen source by *Lactobacillus rhamnosus* CGMCC 1466. Biochem. Eng. J. 39: 496-502.