Optimization of Medium Components and Cultural Variables for Enhanced Production of Tyrosinase by \textit{Funalia trogii}

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ABSTRACT

Tyrosinases are widely distributed in nature; These enzymes are known as type 3 copper proteins having a diamagnetic spin-coupled copper pair in the active centre. In this study, the objective was to produce tyrosinase enzyme efficiently from an alternative fungal source. Some cultural parameters of medium were optimized in order to maximize tyrosinase production. When the effect of the carbon and additional nitrogen sources in the cultural medium on tyrosinase production was investigated, it was observed that glucose and NaNO$_3$ was suitable. Some physiological conditions were optimized for enzyme production and it was concluded that, incubation for 7 days at 30$^\circ$C, 200 rpm of tyrosinase production medium containing $\%$ 2 and $\%$ 0.2 ratio of Glucose and NaNO$_3$ with pH 5, was suitable.

Keywords : \textit{Funalia trogii}, Optimization, Tyrosinase

1. INTRODUCTION

Tyrosinases are widely distributed in nature; they are found both in prokaryotic as well as in eukaryotic microbes, in mammals and plants. These enzymes are known as type 3 copper proteins having a diamagnetic spin-coupled copper pair in the active centre(Lerch 1983). Tyrosinases accept both mono and diphenols as substrates. The hydroxylation ability of the enzyme is also referred to cresolase or monophenolase activity (EC 1.14.18.1), and the oxidation ability to catecholase or diphenolase activity (EC 1.10.3.1). Monophenolase activity of Tyrosinases is known to be the initial rate-determining reaction(Robb 1984; Tudela et al. 1992). In tyrosinase-catalyzed reactions, molecular oxygen is used as an electron acceptor and it is reduced to water. Tyrosinases are involved in several biological functions. Presently there is an increasing interest in using tyrosinases in industrial applications. Traditionally tyrosinases have been exploited in plant-derived food products, e.g. tea, coffee, raisins and cocoa, where they produce distinct organoleptic properties(Seo, Sharma et al. 2003). However, in fruits and vegetables, tyrosinases are also related to undesired browning reactions(Martinez and Whitaker 1995) where upon, methods for controlling tyrosinase activity are constantly searched in the food industry. It has been shown that tyrosinase catalyze oxidation
reaction of phenolic compounds, which are highly toxic and hazardous for environment. Thus, it plays an important role in phenol removal from wastewaters. However, despite the using tyrosinase facilitates the phenol removal from wastewaters as an alternative method, the cost of mushroom tyrosinase is currently very high. This work reports tyrosinase production by *Funalia trogii*. Experiments also described the optimization of cultural conditions for the enhanced production of tyrosinase in 100 mL cotton plugged shake flasks.

2. MATERIALS AND METHODS

2.1 Microbial Strain

*Funalia trogii*, obtained from fungal stock of Biotechnology section of Hacettepe University (Ankara, Turkey), was used for production of tyrosinase.

2.2 Culture Conditions for Tyrosinase Production

Stock cultures were maintained on potato dextrose agar at 4 ºC. The Vogel medium (Horowitz 1970) was used with some modification for growth and enzyme production, and contained (as g L⁻¹):

- Na₂HPO₄: 15, MgSO₄·7H₂O: 1.0, CaCl₂·H₂O: 0.5, with %2 glucose as carbon source.
- After sterilization, a trace element solution at 0.1% concentration was added. The trace element solution contained (as g L⁻¹):
  - ZnSO₄·7H₂O: 0.25, Fe(NO₃)₂(SO₄)₂·6H₂O: 0.5, CuSO₄·5H₂O: 0.125, MnSO₄·H₂O: 0.025, H₃BO₃: 0.025, H₃P₂O₁₀·H₂O: 0.025. For growth 100 mL medium was inoculated with 1 mL of mycelium suspension.

2.3 Enzyme Activity Assay

Tyrosinase activity was measured by modifying the method described by Sun (Sun 1996), using 50 mM sodium phosphate buffer (pH 7). The culture filtrate was centrifuged at 7200 rpm for 15 min and used as the enzyme sample. 0.3 mL of L-DOPA solution (in 50 MM, pH 7 sodium phosphate buffer) and 3 mL enzyme sample were incubated for 15 min at 28 ºC. The amount of dopachrome formation was measured spectrophotometrically (SHIMADZU® UV-1700 PharmaSpec) at 475 nm (εdopachrome=3400 M⁻¹ cm⁻¹).

3. RESULTS AND DISCUSSION

3.1 The Effect of Initial pH on Tyrosinase Production

Figure 1 shows the tyrosinase activities of cultures with different initial pH values. The maximum tyrosinase production was observed at initial pH of the basal medium of 5. Initial pH is the parameter which depends on both the microorganism and the product. Reported optimal initial pH values for tyrosinase synthesized produced by fungi were similar to our results (Ikehata and Nicell 2000).
3.2 Effects of Temperature on Tyrosinase Production

To establish the impact of growth temperature, tyrosinase activity were determined at different growth temperatures ranging from 25 to 40°C while other circumstances were stable. Maximum tyrosinase production occurred at fermentation temperature of 30°C (Figure 2). 30°C is also known as the optimal incubation temperature for fungi. At higher temperature, the requirement of maintenance energy for the cell was high which was attributed to thermal denaturation of other enzymes of the metabolic pathway or tyrosinase and hence the production was minimum. Maximum activity in fungi was obtained around neutral pH of the medium and temperature 30°C. Similar observations were reported for the production of tyrosinase by various fungi (Raju 1993).

3.3 Effects of Rotation Speed (rpm)

In order to determine the relationship between rotation speed and enzyme production, cultures incubated at different conditions as static and the altered rpm values to understand the effect of the rotation speed. In static cultures, biomass was formed at the surface, therefore contact of nutrients and oxygen was low. So that the agitated cultures produced more enzyme than the static cultures as it is expected (figure 3). Agitated conditions are more favorable for tyrosinase production than static conditions. expriments were varied in the range of 100-200 rpm in literature (Chevalier, de Rigal et al. 1999).

3.4 Effect of Alternative Nitrogen Sources on Enzyme Production

The nitrogen source as well as its concentration in the cultivation medium greatly affects the biosynthesis of tyrosinase. For this purpose, pepton, yeast extract, ammonium sulphate, ammonium nitrate and sodium nitrate were tested. As shown in figure 4, it was found that sodium nitrate was suitable for enzyme production. Enhanced production of tyrosinase was reached at % 0,2 (g/L) concentration of NaNO₃ (Fig. 5). Maximum enzyme production was obtained when sodium nitrate was used as nitrogen source on 7th day of incubation. Although use of ammonium nitrate gave high specific activity, enzyme production was about 65% of the activity produced with the use of sodium nitrate. In literature, best nitrogen sources differ from one fungus to another. Yeast extract, peptone, urea, (NH₄)₂SO₄, and NaNO₃ are the commonly used nitrogen sources. Tyrosinase production is triggered by nitrogen depletion (P Keyser 1978) but some nitrogen sources do not affect the enzyme activity (Leatham 1983).

3.5 Influence of Carbon Sources on Tyrosinase Synthesis

Relationship between various carbon sources (glucose, sucrose, maltose, xylose, lactose, fructose, raffinose and starch) and production of tyrosinase is shown in figure 6. When glucose was used as a carbon source in the mycelium production medium, the tyrosinase activity increased constitutively and enhanced production of tyrosinase was reached at % 2 (g/L) concentration of glucose (Fig. 7). Minimum production was obtained with raffinose. This study is in good agreement with that of
Raju (1993) who also found glucose to be the best carbon source to provoke maximum production of mycelia and tyrosinases. In other enzyme systems, disaccharides or higher molecular weight substrates have been found to be the best supporters of the enzymes production (Galhaup 2002). It can be concluded that tyrosinase is a constitutive enzyme, which was further altered with respect to production of tyrosinases in the presence of glucose.

4. CONCLUSION AND RECOMMENDATIONS

The aim of the study was to produce tyrosinase from a white-rot fungi, *Fanalito trogii*, and optimization the culture of enzyme synthesis. The highest tyrosinase activity, 104.5 U/ml was measured in a medium containing 0.2 % NaNO₃, 2 % Glucose at pH 5.0, 30°C and 200rpm. The enzyme obeys Michealis-Menten kinetics and the Km and Vmax values were calculated as 0.3 mg L-Dopa/ml, respectively. The optimum temperature and pH of the enzyme were determined as 30°C and pH 5.0, respectively. In conclusion, *F. trogii* produces tyrosinase in a shorter fermentation period in comparison with other fungi. Production in shorter time provides advantage by decreasing the cost of fermentations in several applications. In the future, tyrosinase production by *F. trogii* could be increased by applying different genetic manipulations. Enzyme can be immobilized for the preparation of PPO electrode and membrane bioreactors. Characterization of the enzyme could be studied in more detail, amino acid sequence and three dimensional structure of the enzyme can be identified by sequencing and NMR techniques.

5. Acknowledgment

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6. References


**Figure 1. The Effect of Initial pH on Tyrosinase Production**

*Experiments was performed at 30 °C and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.*

![Figure 1](image1)

**Figure 2. Effects of Temperature on Tyrosinase Production**

*Experiments was performed at 25, 30, 35 and 40 °C and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.*

![Figure 2](image2)
Figure 3. Effects of Rotation Speed (rpm)
Experiments were performed at 30 °C, 100, 150, 200 and 250 rpm, 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.
Figure 4. Effect of Alternative Nitrogen Sources on Enzyme Production
Experiments were performed at 30 °C, 200 rpm and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.

![Graph showing the effect of alternative nitrogen sources on enzyme production.](image)

Figure 5. Concentration of NaNO₃
Experiments were performed at 30 °C, 200 rpm and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.

![Graph showing the concentration of NaNO₃ and enzyme activity.](image)
Figure 6. Influence of Carbon Sources on Tyrosinase Synthesis

Experiments were performed at 30 °C, 200 rpm and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.

Figure 7. Concentration of Glucose

Experiments were performed at 30 °C, 200 rpm and 7 days incubation. Values are average of three studies, defines the standard deviation bars in shapes.