

Stability of Tyrosinase Enzyme from *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 stable tyrosinase enzyme efficiently and determine stability of enzyme from an alternative fungal source, *Funalia trogii*. Temperature and pH stabilities of the crude extract of enzyme were studied and it was concluded that crude extract of tyrosinase was stable at 73 °C. pH stability of tyrosinase enzyme was among 3,5-7 ranges.

Keywords: *Funalia trogii*, stability, 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 [1]. 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 [2,3]. 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 [4]. However, in fruits and vegetables, tyrosinases are also related to undesired browning reactions [5] 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 to alternative method, the cost of mushroom tyrosinase is currently very high. Tyrosinase (polyphenol oxidase, EC 1.14.18.1) was investigated as an alternative to peroxidase enzymes for the catalytic removal of phenolic compounds from wastewaters. The maximum catalytic activity was observed at pH 7; however, significant activity was observed at pHs ranging from 5 to 8. Tyrosinase was unstable under acidic conditions and at elevated temperatures. In this study The effect of pH on tyrosinase activity was measured by varying the pH buffer and to determine the effect of temperature on enzyme activity measured by pre incubating enzyme source at different temperatures. This study reports stability of tyrosinase

enzyme which was synthesized from *Funalia trogii* under optimized conditions [6].

2. Materials and Methods

2.1. Culture Conditions for Tyrosinase Production

Tyrosinase was synthesized using the optimized medium of enzyme production. Stock cultures were maintained on potato dextrose agar at 4 °C. The Vogel medium was used with some modification for growth and enzyme production, and contained (as g L⁻¹) Na-citrat: 15, KH₂PO₄: 25, MgSO₄·7 H₂O: 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) ZnSO₄·7 H₂O: 2.5, Fe (NH₄)₂(SO₄)₂·6H₂O: 0.5, CuSO₄·5 H₂O: 0.125, MnSO₄·1H₂O: 0.025, H₃BO₃: 0.025, H₃P(MO₃O₁₀): 0.025. For growth 100 mL medium was inoculated with 1 mL of mycelium suspension [6].

2.2. Enzyme Activity Assay

Tyrosinase activity was measured by modifying the method described by Sun [7], using 50 mM sodium-phosphate buffer (pH 7) as relative activity. 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 °C. The amount of dopachrome formation was measured spectrophotometrically (SHIMADZU®UV-1700 PharmaSpec) at 475 nm (ε_{dopachrome} = 3400 M⁻¹ cm⁻¹).

3. Results and Discussion

3.1. The Effect of Incubation Temperature on Tyrosinase Activity

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. 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. In order to determine the stability of the enzyme at different temperatures, crude enzyme was pre-incubated for 1 hour at temperatures ranging from 20-75 °C and enzyme relative activities were measured. Enzyme activity was maintained at temperatures between 20-60 °C and at 70 °C was found to be decreased (Figure 1). The most important factors of industrial enzymes is preferable to show high activity in many different conditions, as well as temperature. In this study, high temperature resistance of tyrosinase was found. In a study on tyrosinase from *Agaricus bisporus* it was reported lower stability of enzyme at high temperature [8]. In another study on this enzyme, it is reported that tyrosinase is thermostable [9].

3.2. The Effect of Incubation pH on Tyrosinase Activity

In order to determine the durability of tyrosinase activity at different pH values, crude enzyme was preincubated 1 hour at different pH values and enzyme activity was measured. It was found that activity of enzyme was not changed at pH values between 3,5 and 5. Activity was reduced at pH=6 about %20 of main activity and there was no activity below pH 3,5 (Figure 2). In a study on this issue, it is reported that tyrosinase enzyme is not resistant to pH 6 and above [8]. Neutralizing the basic groups on the enzymes, may lead to resistance of enzyme at low values of pH [10].

4. Conclusion and Recommendations

The aim of the study was to produce stable tyrosinase from a white-rot fungi, *Funalia trogii*. 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 [6]. The enzyme obeys Michealis-Menten kinetics and the Km and Vmax values were calculated as 0.3mg L-Dopa/ml, respectively. The optimum temperature and pH of the enzyme were determined as 30 °C and pH 5.0, respectively while activity of tyrosinase was stable at 73 °C and between 3.5-7 pH. In conclusion, *F. trogii* produces very stable 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.

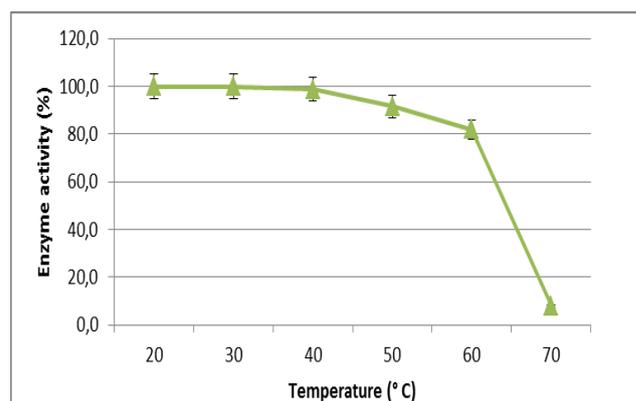


Figure 1. Effect of temperature on Tyrosinase activity
Values are average of three studies, defines the standard deviation bars in shapes

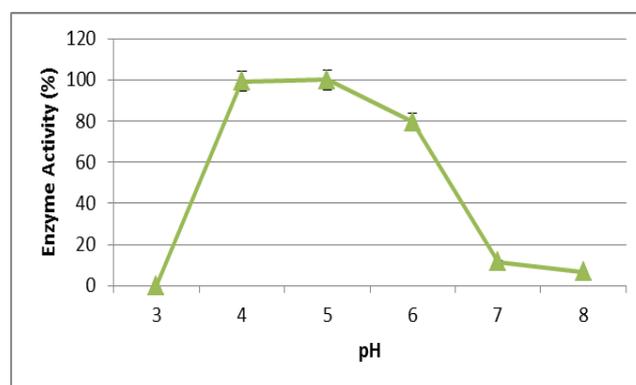


Figure 2. Effect of pH on Tyrosinase activity
Values are average of three studies, defines the standard deviation bars in shapes

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