

## Partial purification and characterization of tyrosinase (EC 1. 14. 18 .1) extracted from *Agaricus bisporus*

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Tyrosinase from mushrooms (*Agaricus bisporus*) was partially purified and characterized. The enzyme exhibited both monophenolase and diphenolase activities that were measured spectrophotometrically using L-tyrosine and pyrogallol as substrates [1, 2]. A two-fold purification in both activities was achieved by ammonium sulfate fractionation. The monophenolase activity was 3.35 EU/ml, and the diphenolase activity was 189.3 EU/ml. Tyrosinase was relatively stable at -15°C for 44 days. The enzyme was not very heat stable, and its activity decreased when incubated at the temperatures higher than 35°C. Tyrosinase activity showed two pH optima, at 5.3 and 7.0 at 25°C when pyrogallol was used as the substrate.

Mono-, di- and triphenols were substrates for PPO. Using  $V_{max}/K_m$  as a specificity constant, pyrocatechol was the better substrate followed by pyrogallol. The kinetic parameters of the enzyme were:  $V_{max} = 78$  EU/min/ml,  $K_m = 1.4$  mM and  $K_S = 250$  mM for pyrogallol and  $V_{max} = 168$  EU/min/ml,  $K_m = 0.40$  mM and  $K_S = 270$  mM for the pyrocatechol. Of the inhibitors tested, competitive-type inhibition was observed with benzoic acid and sodium azide. A mixed-type inhibition was observed with L-cysteine and sodium fluoride.

**Keywords:** Mushroom, Tyrosinase, Extraction, Purification, Characterization.

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[2] Mayer A M, Harel E (1979) Polyphenol oxidase in plants. *Phytochemistry* **18**, 193-215.