18. Mushrooms as a Source of Tyrosinase Enzyme Operable in the Production of L-Dopa

Mrunal G. Partike
Deepali R. Goyanka
Dr. Harish S. Malpani

Abstract

Tyrosinase is a key enzyme in many important biochemical reactions including production of Dopamine, deficiency of which may result in Parkinson's disease. L-DOPA, a dopamine precursor, is used in the management of Parkinson's disease. This study aimed to develop a cost-effective method for the production of L-DOPA using Tyrosinase isolated from edible mushrooms. The production media included Tyrosinase (100 mg; immobilized in gel beads), Tyrosine (2.5mM; substrate), and ascorbic acid (2.5mM). The isolated L-DOPA was characterized using thin layer chromatography. RF value of produced L-DOPA (0.73) was compared with that of the standard (0.79). Further the effect of temperature and pH on enzyme activity was studied. The optimum enzyme activity was found at 60°C at pH 8.0.

1. Introduction

Mushrooms Thallophyta contains Basidiomycota which includes mushrooms. The scientific name for mushroom is *Agaricus bisporus.* The word "Mushroom" is derived from the Latin and Greek words "fungus" and "mykes". There are various types of mushroom such as *Agaricus bisporus* (White button mushroom), *Lentinus edodes* (Shiitake mushrooms), *Pleurotus ostreatus* (Oyester mushroom).Mushroom contains nutritional value, antioxidant activity and health – beneficial properties, as well as flavor and texture properties. During Mushroom development it secretes various enzymes for all metabolic activities of the mushroom like hydrolysis, oxidation reduction and transfer etc.

Tyrosinase is ubiquitous enzyme involved in pigmentation. Tyrosinase belongs to group of types-3 copper proteins which are involved in melanin synthesis. Tyrosinases also known as polyphenoloxidase, O- diphenyl oxidase, monophenoloxidase or cresolace etc. In the presence of molecular oxygen, tyrosinase catalyzes the hydroxylation of monophenols and the oxidation of diphenols. The conversion of phenols to O-diphenols by tyrosinase is a potentially attractive

catalytic ability, and thus tyrosinase has attracted a lot of attention in terms of its biotechnology application, as the catechol products are useful as drugs or drugs synthesis, for example:- L-Dopa (Min et al., 2015).

(L-DOPA) 3, 4 dihydroxyphenyl -L-alanine is a preferred drug for Parkinson's disease. Parkinson's disease, a prevelant degenerative disorder of the central nervous system that affects the nerve cells in the brain with reduced dopamine levels. L-dopa, an analogy of L – tyrosine, which is precursor to dopamine is a drug useful in the alleviation of the effects of Parkinson's disease because it can cross the blood brain barrier whereas dopamine cannot perform this feat. The passage of L-dopa across this barrier allows for the increased presence of dopamine thus allowing for Parkinson's disease to be effectively treated.

This current study is to report extraction of the tyrosinase enzymes from Agaricus bisporus. Report for presence of Tyrosinase in various species of mushroom. In this study mushroom was selected as a source of tyrosinase for the synthesis of L – Dopa from tyrosinase enzyme. Tyrosinase extracted from the mushroom was entrapped in sodium alginate as beads using calcium chloride solvent system.The utilization of the immobilized enzyme in L-dopa production under statistically optimized conditions allows for large-scale production (Sarkar et al., 2022).

2. Material and Methods

2.1 Material

The present study was conducted in the Department of Biochemistry, Shri R.L.T College of Science, Akola from January 2024 to March 2024. Various species of mushroomssuch as *Agaricus bisporus* and *Pleurotus ostreatus* were collected from local city market.

2.2 Methods

2.2.1 Tyrosinase extraction and assay: Various species of fresh mushroom were homogenized in cold phosphate buffer of pH 7.5, using a pre-chilled mortal & pestle. The homogenate was filtered using a sterile cheese cloth and then further centrifuged at 3000x g for 30 min. The supernatant was removed and cold phosphate buffer was added in to the precipitate. This was centrifuged at 3000x g for 30 min. The supernatant collected served as the source of the enzyme. Tyrosinase activity in the supernatant was determined using L-DOPA as the substrate. Presence of tyrosinase was determined by change in color from colourless to yellow.

2.2.2 Tyrosinase immobilization: Tyrosinase was immobilized using sodium alginate. About 15ml of tyrosinase enzyme was mixed with 0.3 g of sodium alginate and heated with continuous stirring for 3 min. The enzyme-alginate solution was dropped into 1% CaCl2 solution using a sterile syringe to form beads.The beads were stored at 15°C and used in the production of L-DOPA.

2.3 L-DOPA production using the immobilized tyrosinase

A medium containing 2.5mM L-Ascorbic Acid, 2.5mM L- Tyrosine and 100mg an immobilized tyrosinase bead was prepared in a flask. The flask was placed on a rotary shaker at 200 revolution/min. Samples were withdrawn every 2h interval and checked for the production of L-DOPA.

2.4 Chromatographic analysis of L-DOPA

A glass plate pre-coated with silica gel was used for TLC.The solvent obtained from flask, standard L-DOPA solution was spotted on dry TLC plate. The plate was placed in the mobile phase (water: acetic acid: n -propanol: butanol) after the run, the plate was dried, sprayed with 1% ninhydrin solution in acetone. Analysis of dried chromatographic plates was done by calculating the Rf value.

2.5 Effect of pH and temperature

The activity of tyrosinase was evaluated at different pH values ranging between 5-11. Buffers used were acetate buffers (pH 5.0), phosphate buffer (pH 6.0-8.0), carbonate buffer (pH 9.0-11). Optimum pH for enzyme activity was observed.The activity of tyrosinase was evaluated at different temperature values ranging between 40-80°C.Optimum temperature for enzyme activity was observed by observing O.D at 520nm.

3.0 Results and Discussion

3.1 Result

Tyrosinase enzyme was detected using different types of mushrooms which were homogenized in phosphate buffer with standard L-DOPA as a substrate. This confirmed the presence of tyrosinase, which was responsible for the yellow color. Tyrosinase was entrapped in sodium alginate organic matrix beads and then dropped in CaCl2 solution. For the production of L-DOPA immobilized tyrosinase was agitated in rotary shaker along with tyrosine: ascorbic acid (5 : 1).

Two distinct spots corresponding to the standard (L-DOPA) and unknown (L-DOPA) were observed after spotting the reaction product from the batch process on thin layer chromatographic plates. The Rf values were determined as 0.79 and 0.73 for the standard (L-DOPA) and unknown (L-DOPA), respectively. The two spots on the lane A and B were observed respectively on the thin layer chromatogram.

Tyrosinase enzyme showed maximum activity at pH 8.0 and at temperature 60° C.

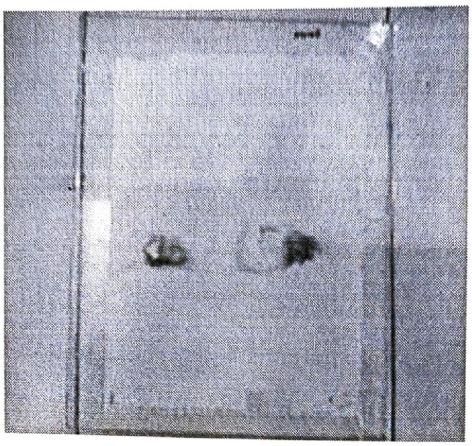
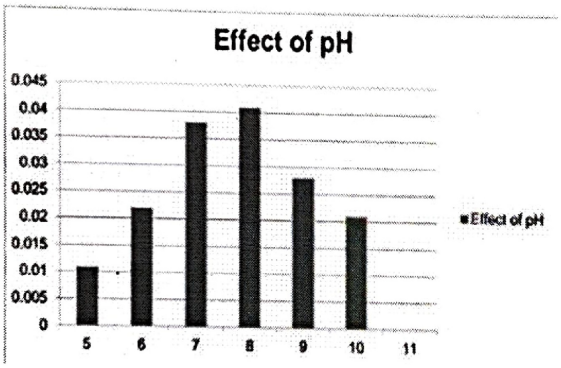
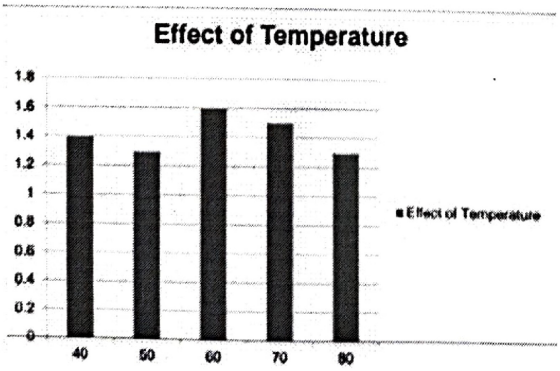


Figure showing chromatographic analysis of L-DOPA through TLC



From the above graph the optimum activity of tyrosinase were shown at pH -8.0



From the above graph the optimum temperature was shown at 60° C.

3.2 Discussion

In the present study, two different mushroom varieties were used as sources to extract tyrosinase enzyme. The species which were used as a source of extraction were Button Mushroom (*Agaricus Bisporus*) and Oyster Mushroom (*Pleurotus ostreatus*). The findings showed that using the phosphate buffer method is more successful for isolating tyrosinase which produce yellow color. Using sodium alginate beads to immobilize tyrosinase an affordable method for entrapping enzyme. An immobilized enzyme in employed in a batch reactor for the synthesis of L-dopa. Thin Layer Chromatography has been used to measure the efficiency of L-DOPA production. The Rf were determine as 0.73 and 0.79 for the unknown L-dopa solution and known tyrosine solution.The sample showed maximum activity at pH 8.0, which was considered as 100%. Tyrosinase has been a subject of intense investigation in the past two decades because of its increasing use (Illesamni & Adewale., 2020). The present study evaluated the pharmaceutical potential of the tyrosinase in mushroom. The characterized tyrosinase showed very high similarities compared to human tyrosinase. Tyrosinase was present in the peels of banana with a specific activity of 7.9 +- 0.2 U/mg. (Ademakinwa & Agunbiade., 2022). Extracted tyrosinase activity in the supernatant was determined using L-dopa as the substrate as described by (Illesanmi & Adewale., 2022).

The enzyme- agar solution was quickly dropped into a chilled tyrosinase: chloroform solution using a sterile syringe to form the beads (Illesanmi & Adewale., 2020). The beads were used in the production of L-dopa. HPLC analysis confirms the formation of L-dopa during the reaction (Ademakinwa & Agunbiade., 2022)The activity of tyrosinase was extracted by taking various buffers citrate phosphate (3.0-5.0), potassium phosphate (6.0 -7.0), Tris HCL (8.0-9.0), Glycine NaOH (9.0-10) by maintaing temperature ranging from 35 to 65° C. The optimum activity of sample showed at 7.0 (Zaidi et al., 2014).

4.0 Conclusion

In this study, extraction, detection, immobilizations, production was done using mushroom. The enzyme was extracted from two distinct mushroom varieties. The presence of tyrosinase in the mushroom was established. The isolation of tyrosinase was more efficient with phosphate buffer. The immobilization of the tyrosinase in sodium alginate beads offers a cheap and readily available technique for the entrapment of the enzyme. The immobilized enzyme was able to produce more L-dopa in the batch reactor even at equimolar concentration. The optimum

L-dopa productivity in a batch process occurs when the ascorbic acid: tyrosine ratio was 5:1. Productivity of L-dopa was analyzed by thin layer chromatography. Effect of various pH and temperature was done on tyrosinase enzyme extracted from mushroom. At pH 8.0 tyrosinase enzyme shows maximum activity.

This indicated that mushroom can be a prosperous source of tyrosinase and mass production of L- dopa.

5.0 References

- Ademakinwa, A.N., & Agunbiade, M. (2022). Banana peel tyrosinase immobilized in agar-agar organic matrix "beads" for L-Dopa production in improvised reactors: A Box- Behnken response methodology based approach. Research Square,
- Ademakinwa, A.N., & Agunbiade, M. (2022). Banana peel waste as a source of tyrosinase useful in the production of L-Dopa. Sustainable Chemistry and Pharmacy, 30(100853).
- Arnow, L.E., (1937). Colorimetric determination of the components of 3, 4-dihydroxyphenylalanine tyrosine mixtures.
- Banana Link (2020). All about bananas. https://www.bananalink org .ul/all-about-banans.
- Fernando, W.G. (2012). Plants: An International Scientific Open Access Journal to Publish All Facts of –Plants, their functions and interactions with the environment and other living, plants. 1(1), 1-5
- Gandhi, K.R., & Saadabadi, A. (2024) Levodopa: (L-DOPA). Treasure Island (FL): Starpearls.
- Haghbeen, K., etal. (2004). Purification of Tyrosinase from edible mushroom. Franian Journal of Biotechnology, 2(3).
- Harhvard, T.H. CHAN, The Nutritions Source https:www.hsph.harvard.edu/ nutritionsourcce/food. features /bananas.
- Hidaka, T. (1976). Reevalutation study of mushroom tyrosinase – tyrosine metabolites by means of Gas chromatography & mass spectromertry. The Kurume medical journal, 23(1)49-56.
- Hikal, W.M., etal. (2022). Banana peels: A Waste Treasure for Human being. Hindawi, 2022(7616452)

- Ilesanmi,O.S., & Adewale, I.O. (2020). Physiochemical properties of free and immobilized tyrsinase from different species of yam. Biotechnology reports, 27
- Kumar, K.P., etal. (2012). Traditional and medicinal uses of banana. Journal of pharmacognosy and phytochemistry, 1(3), 51-63.
- Kurpejovic, E., etal (2021). Tyrosinase –based production of L-Dopa by corynebacterium glutamicum. Biotechnological Products and Process engineering, 105, 9103-9111.
- Min, K., etal. (2015). Overview on the biotechnological production of L-dopa. Appl microbial biotechnology, 99(2), 575-84.
- Munoz – Munoz, J.L., etal. (2020). Study of tyrosinase and dopa enantiomers as tyrosinase substrates initiates L- and D- melanogenesis pathways. Biotechnology and Applied biochemistry, 68(4), 823-831.
- Ovallath, S., & Sultana, B. (2017). Levodopa: History and therapeutic application. Annals of Indain Academy of Neurology, 2093), 185-189.
- Robinson, R.K. (2015). Enzymes: principles and biotechnological applications. Essays biochem, 59, 75.
- Sarkar, T., etal. (2022). Role of tyrosinase extraction from banana (Musa acuminate). International journal of research of research publication and reviews, 3(1), 1300-1303.
- Sawhney, S.K., & Munjal, N. (2002). Stability and properties of mushroom tyrosinase entrapped in alginate, polyacrylamide and gelatin gels. Enzyme and Microbial Technology. 30(5), 613-619
- Sindhu, J.S., & Zafar, T. (2018). Bioactive compounds in banana fruits and their health benefits. Food quality and safety, 2(40, 183-188.
- Wani, B.A., etal. (2010). Nutritional and medicinal importance of mushroom. Journal of medicinal plants research, 4(24), 2598-2604.
- Zaidi, K.U., etal. (2014). Purification and characterization of Melanogenic enzyme tyrosinase from button mushroom. Hindawi Publicalishing Corporation Enzyme Research, 2014(120739).