Annuaire de l'Université de Sofia "St. Kliment Ohridski"
Faculte de Biologie
2015, volume 100, livre 4, pp. 70-75
First National Conference of Biotechnology, Sofia 2014

ISOLATION AND PURIFICATION OF TYROSINASE FROM DIFFERENT PLANT SOURCES

POLINA VELICHKOVA[1], DESISLAVA MARINKOVA[1],
SPASKA YANEVA[2*], LYUBOV YOTOVA[1]

1 – University of Chemical Technology and Metallurgy, Department of Biotechnology, Sofia, Bulgaria
2 – University of Chemical Technology and Metallurgy, Department of Bases of Chemical Technology, Sofia, Bulgaria
**Corresponding author: sp_yaneva@uctm.edu*

Keywords: enzymes, tyrosinase sources, isolation, purification

Abstract: Tyrosinases from potato (*Solanum tuberosum*), banana (*Musa acuminata*) and *Agaricus bisporus* were isolated by two methods. The obtained filtrates were purified by precipitation with ammonium sulfate and ice-cold ethanol.

The aim of this study was to compare specific activities of enzymes, laboratory isolated from different sources using industrial purified tyrosinase as a reference.
The temperature, pH optimums and kinetic parameters were determined.

INTRODUCTION

Tyrosinases (monophenol, o-diphenol: oxygen oxidoreductase, EC 1.14.18.1) belong to a larger group of proteins named type-3 copper proteins, which include the catechol oxidases from plants and the oxygen-carrier haemocyanins from mollusks and arthropods (Halaouli et al., 2006). Matoba (Matoba et al., 2006) determined the crystal structure of tyrosinase, isolated from *Streptomyces castaneoglobisporus* at a 1.4 Å resolution. Tyrosinase is a key enzyme in the biosynthesis of melanin (primary pigment in melanocytes) involved in determining the color of skin, eyes and hair in mammals (Kim et al., 2005). Studies of tyrosinases were motivated by the need to understand and prevent the enzymatic browning that occurs in the presence of air when mushrooms, fruits or vegetables are cut or bruised. This phenomenon is related to the tyrosinase activity and causes severe economic losses in the food industry. Recently, the focus has moved to the biotechnological and environmental applications of tyrosinases, and

macrofungal tyrosinases have commonly been chosen for these studies. The most important application of the tyrosinases is related to the biosynthesis of L-DOPA, detection and quantification of phenolic compounds in water samples, removal of phenolic compounds from wastewaters and production of cross-linked proteins (Otavio de Faria et al., 2007). Therefore, finding inexpensive sources of tyrosinase for immobilization on solid matrices is very important.

Tyrosinases are common for bacteria, fungi and eukaryotes (higher plants and animals). Tyrosinases were first described in several species of Streptomyces, but the enzyme has also been reported in other species such as *Rhizobium, Symbiobacterium thermophilum, Pseudomonas maltophilia, Sinorhizobium meliloti, Marinomonas mediterranea, Thermomicrobium roseum, Bacillus thuringiensis and Pseudomonas putida F6*. Tyrosinases were also found in *Neurospora crassa, Neurospora* sp., *Streptomyces glaucescens* and mushroom *Agaricus bisporus*. Recently, a unique tyrosinase with a high tyrosine-hydroxylation/dopa-oxidase ratio was discovered in *Ralstonia solanacearum*. Such enzymes have also been isolated from dog rose fruit, papaya, pear, potato leaf, tobacco, lobster, mouse and *Homo sapiens*. (Sambasiva et al., 2013).

Tyrosinases have been isolated from: potatoes (Yang and Wu, 2006), edible fungi (Yuan et al., 2005), filamentous fungi (Haghbeen et al., 2004), rotting mushrooms (Selinheimo et al., 2007), apples (Carvalho da Silva et al., 2013), bananas (Galeazzi et al.,1981), avocados (Vieira et al.,2013), Sicilian tomatoes (Brisolari et al., 2014), eggplants (Duartea et al., 2002), and quince (Spagna et al., 2005), and even from green coconut water (Pérez-Gilabert et al., 2000), (Todaro et al., 2011).

MATERIALS AND METHODS

Reagents

Tyrosinase (E.C. 1.14.18.1), 1715 units/mg solid isolated from mushrooms, was supplied by Sigma-Aldrich; potato *Solanum tuberosum*, edible mushroom *Agaricus bisporus* and banana *Musa acuminate*, used as source materials of tyrosinase, were purchased from a local supermarket in Sofia, Bulgaria; L-tyrosine was purchased from Fluka.

Isolation of tyrosinase from different sources

Tyrosinase from potato (*Solanum tuberosum*), banana (*Musa acuminata*) and edible mushrooms (*Agaricus bisporus*) was laboratory isolated by two methods. Either 50 mM K-phosphate buffer, pH=7.0 or 50 mM Tris-HCl buffer, pH=5.8 were used. In both methods, 100 g of the sources of tyrosinase has been blended with 120 ml of the respective buffer, and the resulting solution was filtered through a Buchner funnel.

Purification of tyrosinase from different sources

Isolated tyrosinase from potato (*Solanum tuberosum*), banana (*Musa acuminata*) and edible mushrooms (*Agaricus bisporus*) was purified by precipitation with ammonium sulfate and ice-cold ethanol.

Determination of enzyme activity and protein content

Tyrosinase activity was determined with L-tyrosine as substrate at λ= 280 nm according to the Worthington manual Decker (1977). The reaction mixture containing tyrosinase was aerated with Vortex, BOECO. The activity of tyrosinase was measured by spectrophotometric method.

One unit of tyrosinase activity was defined as the enzyme quantity which converts 1 μmol of L-tyrosine for 1 min at 25°C and pH = 6.5.

The total protein content was determined by the Lowry et al (1951).

pH and temperature optimum

pH optimum of industrial isolated and purified tyrosinase was determined at pH= 5.8 to 8.5 values. The temperature optimum was determined in the range of 30°C to 50°C.

Determination of K_m and V_{max}

Kinetic parameters of two types of tyrosinase were tested against L-tyrosine. The Michaelis - Menten constant (K_m) and V_{max} values of tyrosinase were obtained from the Hanes plots.

RESULTS AND DISCUSSION

In the present study, different sources of tyrosinase - mushrooms, bananas and potatoes were monitored. The kinetic parameters, pH and temperature optimum were determined and compared for both types of tyrosinase.

Catalytic properties of isolated tyrosinase

The enzyme was isolated from three different sources: mushrooms, bananas and potatoes. The results showed that the Tris-HCl buffer-based method is more effective than the phosphate buffer-based for isolation of tyrosinase. The catalytic properties of the isolated tyrosinase from different sources are shown in Table 1.

Table 1. Catalytic properties of isolated tyrosinase from different sources.

Source	**Protein content [mg/ml]**	**Specific activity [U/mg]**
Banana	1.37	0.15
Potato	1.01	0.099
Mushroom	1.58	3.8

Comparison between industrial purified and isolated mushroom tyrosinase

The catalytic properties of two types of tyrosinase from mushroom are shown in Table 2.

Table 2. Catalytic properties of industrial isolated and purified tyrosinase laboratory isolated tyrosinase from mushrooms.

Type enzyme	Protein content [mg/ml]	Specific activity [U/mg]	pH optimum	Temp. optimum [°C]	K_m [M]	V_{max} [M/min]
Industrial isolated and purified tyrosinase	0.43	686.05	7.5	40	$16.69.10^{-4}$	$13.2.10^{-4}$
Laboratory isolated tyrosinase	12.2	6.80	7.0	38	$12.59.10^{-4}$	$2.75.10^{-4}$

The pH optimum of the industrial isolated tyrosinase is 7.5 and the laboratory isolated – 7.0 (Figure 1, A and B).

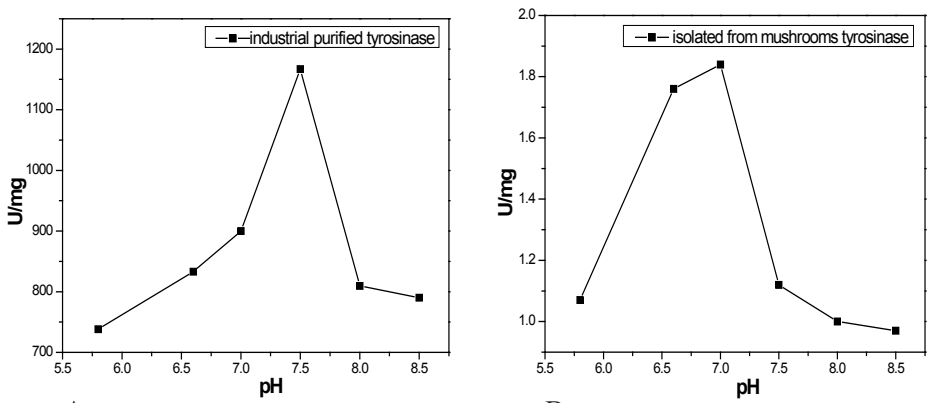

Figure 1. Profile of pH optimum of industrial isolated and laboratory isolated mushroom tyrosinase.

The temperature optimum of laboratory isolated tyrosinase is 38°C, whereas of the industrial isolated tyrosinase is 40°C.(Figure 2, A and B).

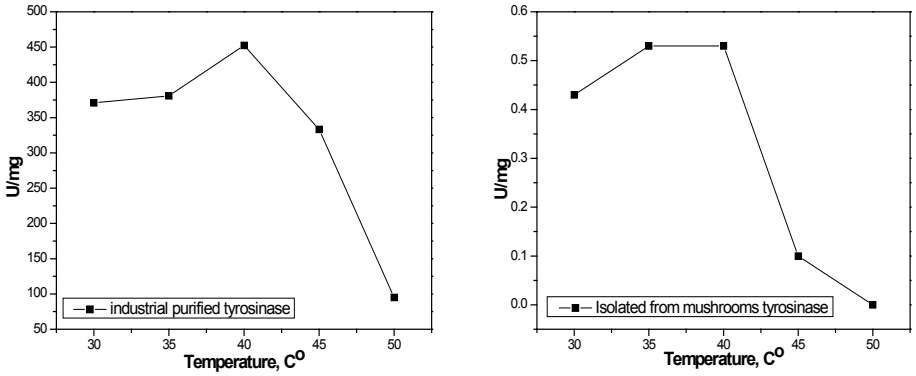

Figure 2. Profile of temperature optimum of industrial isolated and laboratory isolated mushroom tyrosinase.

CONCLUSIONS

Tyrosinase was isolated from three different sources: mushrooms, bananas and potatoes by two methods. The results showed that the most effective source of tyrosinase is *Agaricus bisporus* mushrooms. The isolation of tyrosinase with Tris-HCl buffer was more effective than with phosphate buffer. pH and temperature optimum results showed similar profiles of industrial purified and laboratory isolated tyrosinase.

Acknowledgements: The present work is supported by project N: 11254/2014 Scientific Research Sector, University of Chemical Technology and Metallurgy, Sofia, Bulgaria.

REFERENCES

1. Brisolari A. and Gonçalves D., 2014, Immobilization of tyrosinase from avocado crude extract in polypyrrole films for inhibitive detection of benzoic acid, *Chemosensors*, 2 (3), 182-192.
2. Carvalho da Silva S., Wisniewski C., Luccas P. O., Schmidt de Magalhães C., 2013, Enzyme from banana (Musa sp.) extraction procedures for sensitive adrenaline biosensor construction, *American Journal of Analytical Chemistry*, 4, 293-300.
3. Decker A. L., 1977, Worthington Enzyme Manual, Polyphenol oxidase, Worthington Biochemical Corporation, 39-40.
4. Duartea A. C. P., Coelhob M. A. Z. and Leite S. G. F., 2002, Identification of peroxidase and tyrosinase in green coconut water, *Ciencia y Tecnologia Alimentaria*, 3, (5), 266-270.
5. Galeazzi M. M., Sgarbieri V. C. and Constantinides S. M., 1981, Isolation, purification and physicochemical characterization of polyphenoloxidases (PPO)

from a dwarf variety of banana (Musa cavendishii, L), *Journal of Food Science*, 46, (1),150–155.
6. Haghbeen K., Jazii F. R., Karkhane A. A. and Borojerdi Sh. Sh., 2004, Purification of tyrosinase from edible mushroom, *Iranian journal of biotechnology*, 2, (3),189-194.
7. Halaouli S., Asther M., Sigoillot J. C., Hamdi M., Lomascolo A., 2006, Fungal tyrosinases: new prospects in molecular characteristics, bioengeering and biotechnological applications, *Journal of Applied Microbiology*, 100, 219-232.
8. Kim Y. J., Uyama H., 2005, Tyrosinase inhibitors form natural and synthetic sources: structure, inhibition mechanism and perspective, *Cell. Mol. Life Sci.*, 62, 1707-1723.
9. Lowry O.H, Rosebrough NJ, Farr AL, Randall R.J., 1951 *J. Biol. Chem.*, 193, 265–75.
10. Matoba Y., Kumagai T., Yamamoto A., Yoshitsu H., Sugiyama M., 2006 Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis., *J. Biol. Chem.*, 281, 8981-8986.
11. Otavio de Faria R., Moure V. R., Lopes de Almeida Amazonas M. A., 2007,Krieger N. and Mitchell D. A., The biotechnological potential of mushroom tyrosinases – review, *Food Technol. Biotechnol.*,45 (3), 287–294.
12. Pérez-Gilabert M. and Carmona F. G., 2000, Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase, *J. Agric. Food Chem.*, 48 (3), 695–700.
13. Sambasiva Rao K. R. S., Tripathy N. K., Srinivasa Rao D., Prakasham R.S., 2013, Production, characterization, catalytic and inhibitory activities of tyrosinase, Research, *Journal of Biotechnology*, 8 187-99.
14. Selinheimo E., NiEidhin D., Steffensen C., Nielsen J., Lomascolo A., Halaouli S., Record E., O'Beirne D., Buchert J., Kruus K., 2007,Comparison of the characteristics of fungal and plant tyrosinases, *Journal of Biotechnology*, 130, 4471–480.
15. Spagna G., Barbagallo R. N., Chisari M. and Branca F., 2005, Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content, *J. Agric. Food Chem.*, 53 (6) 2032–2038.
16. Todaro A., Cavallaro R., Argento S., Branca F. and Spagna G., 2011, Study and characterization of polyphenol oxidase from eggplant (Solanum melongena L.), *J. Agric. Food Chem.*, 59 (20), 11244–11248.
17. Vieira N. C. S., Ferreira R. A., Valquiria da Cruz R., Guimarães F. E. G., Alvaro de Queiroz A. A., 2013, Self-assembled films containing crude extract of avocado as a source of tyrosinase for monophenol detection, *Materials Science and Engineering: C*, 33, (7), 3899–3902.
18. Yang Zh. and Wu F., 2006, Catalytic properties of tyrosinase from potato and edible fungi, *Biotechnology*, 5 (3), 344-348.
19. Yuan H., Ke-wu L., Dong Y., Xin L., Xin-he H., Cheng-kang T., Hua J., Wen-juan J., 2005, Some properties of potato tyrosinase, *Chemical Research and Application*, 1, 22-27.