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Isolation, Identification and Optimization of Tyrosinase enzyme from newly identified *Fusarium* species

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ABSTRACT

Tyrosinases (monophenol, di-phenol oxygen oxidoreductase, EC (1.14.18.1) are the copper- containing enzymes which catalyze the o-hydroxylation of monophenols and even subsequent oxidation of di-phenols to quinones. These enzymes are involved in pigmentation and are the important factors in wound mending and primary immune response. Tyrosinases are found in eukaryotic and prokaryotic microorganisms, in invertebrates, mammals and plants. *Fusarium* are included in the Nectriaceae family and represents one among the most important genus of the Hypocreales order ascribable to its impactful number of species and their practical part. In fungi, tyrosinases are basically associated with the formation and stability of spores, in defense and acridity mechanisms, and in browning and saturation. First characterized from the comestible mushroom *Agaricus bisporus* because of undesirable enzymatic browning problems during postharvest storage, tyrosinases were introduced, more recently, in several other fungi with relative insights into molecular and inheritable characteristics and into reaction mechanisms, pointing their veritably promising properties for biotechnological operations.

Key words: *Tyrosinase, Polyphenol oxidases, Melanin, Fusarium, Screening, Optimization.*

Introduction

Tyrosinases are a class of binuclear copper enzymes, and they are closely related to compounds such as catechol oxidases, which are collectively referred to as polyphenol oxidases. These enzymes are primarily found in various groups of bacteria, fungi, plants, and animals and they use phenolic compounds as substrates to produce melanin and numerous other biologically significant polyphenolic products (Halaouli *et al.*, 2005; Claus and Decker, 2006 and Marusek *et al.*, 2006). According to Fairhead and Tony-Meyer (2012), tyrosinase (EC 1.14.18.1) has two functions: it may oxidize

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diphenols to o-quinones and hydroxylate monophenols to o-diphenols, which is known as monophenolase activity. For living things to perform a variety of tasks, such as producing melanin as a protective mechanism against UV radiation, this enzyme is crucial (Claus and Decker, 2006; Halaouli *et al.*, 2006). According to Marusek *et al.* (2006), it is crucial for plants to produce phenolic polymers such tannins, lignin, and flavonoids.

Tyrosinase is helpful for biotechnological, biosensor, and biocatalysis applications because of its capacity to react with phenols (Fairhead and Thony-Meyer, 2012; Jus *et al.*, 2008; Jus *et al.*, 2009, Jus *et al.*, 2011). It can also be used in the biosynthesis of L-DOPA (L-3,4-dihydroxyphenylalanine), as a medication that is highly recommended for patients with Parkinson's disease (Seetharam and Saville, 2002; Zaidi *et al.*, 2013), to detoxify water and soil contaminated with phenolic compounds (Martorell *et al.*, 2012), and as food additives because of their cross-linking properties during food processing (Lantto *et al.*, 2007; Selinheimo *et al.*, 2007; Danial and Birshi, 2018; Zaidi *et al.*, 2014). It is also claimed that tailoring polymers occurs, such as when silk proteins are grafted onto chitosan as a result of the tyrosinase reaction (Anghileri *et al.*, 2007; Freddi *et al.*, 2006). According to Gu *et al.* (2009), immobilized tyrosinase can function as an electrochemical biosensor for a variety of phenolic chemicals. The most well-characterized tyrosinase can be obtained from *Streptomyces glaucescens* as well as fungi like *Agaricus bisporus* and *Neurospora crassa.* Tyrosinase from fungal and vertebrate sources starts the process of converting tyrosine into melanin. Since plants have a variety of phenolic substrates, tyrosinase oxidizes them, as shown in the browning pathway when plant tissues are damaged. Because the enzyme extract from *Agaricus bisporus* is quite similar to that of mammals, it can be used as a model for studying melanogenesis (Chang, 2009; Nawaz *et al.*, 2017; Elsayed and Danial, 2018).

Materials and Methods

Collection of Soil Samples

Soil samples are collected in the surroundings of the laboratory (Scientific and Industrial Research Centre, Goraguntepalya, Bengaluru. A plant is plucked in the field and the soil held on roots of that plant is collected in a polythene bag. Likewise, three samples are collected from different fields and the samples are named as Sample 1, 2 and 3. (Fig. 1). The samples are transported to the laboratory.

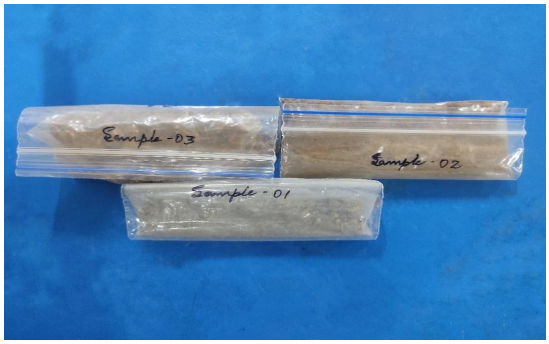


Fig. 1. Soil samples collected from the field

Isolation of microbes from soil samples

The serially diluted samples are taken to the Laminar Air Flow to maintain the aseptic conditions and 10^{-4} is selected in all the samples in order to maintain the required load of the organism uniformly. 0.1 ml of the 10^{-4} sample is taken and spread plated on the CZA media plate using a L shaped spreader. The plates are kept for the incubation at room temperature for 3 to 4 days.

Primary screening of tyrosinase producing Fungi

The preparation of two distinct media is done; tyrosine is added to the CZA media, and L-DOPA is added to the other CZA media. The colonies that have formed on the primary CZA plates (Isolation plates) are then inoculated on the screening media plates by streaking the media with the swabs and letting it sit at room temperature for three to four days (Fig. 2).

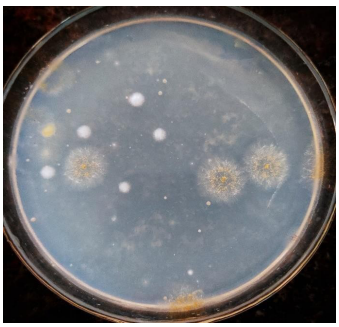


Fig. 2. Growth of fungal strains

Microscopic observation of tyrosinase producing fungi

Colonies developed on screening media plates (Fig.

Fig. 3. Growth of C01 fungal strain

3) are removed, stained under a microscope with Lactophenol Cotton Blue, and the organism contained within is identified (Fig. 4)

Optimization of culture conditions for the Production of Tyrosinase

There are certain parameters that must be met in order to grow the organism in a laboratory. Finding the appropriate parameters is therefore necessary (Danial and Al-Birsh, 2018; Barate and Sonar, 2023).

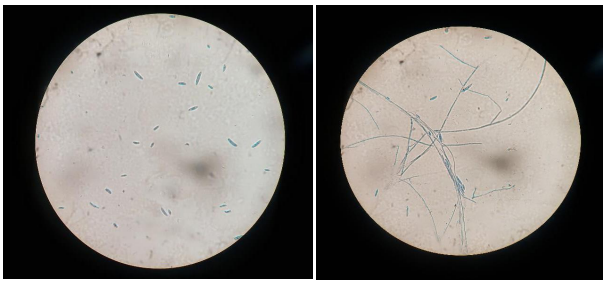
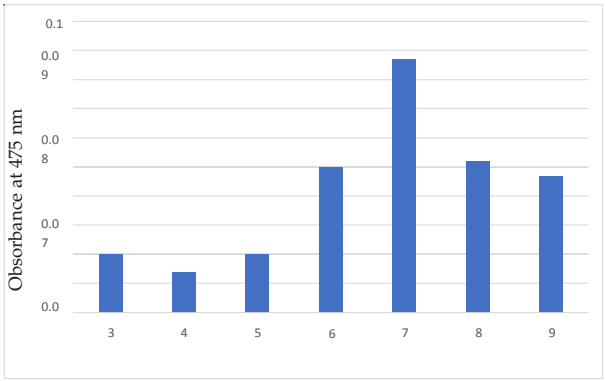


Fig. 4. Microscopic observation of *Fusarium* sp.C01

Optimization of pH

Different pH values of 3, 5, 6, 7, 8, and 9 are used to prepare Czapek Dox broth. Sterile swabs are used to introduce the organism into the broth, and the inoculation is done at room temperature. Spectrophotometric measurements are made every day at 475 nm for five days, with a 24-hour interval between measurements (Table 1). The best-growing organism from the pH optimization is chosen for additional temperature optimization (Graph 1).



Graph 1. Optimization of pH

Optimization of temperature

After making the Czapek Dox broth, adjust the pH to 7. Using a sterile swab, the organism is inoculated

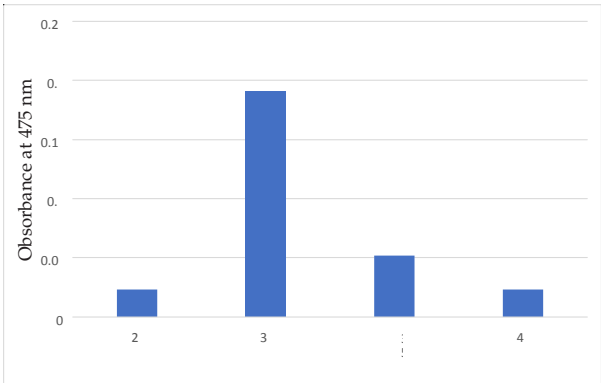
Table 1. Optimization of pH

pH	Enzyme (ml)	Substrate L-DOPA (ml)	Incubate for 15 minutes at room temperature	Phosphate buffer (ml)	OD at 475 nm
3	0.5	0.5		3.0	0.020
4	0.5	0.5		3.0	0.014
5	0.5	0.5		3.0	0.020
6	0.5	0.5		3.0	0.050
7	0.5	0.5		3.0	0.087
8	0.5	0.5		3.0	0.052
9	0.5	0.5		3.0	0.047

Table 2. Optimization of temperature

Temperature (°C)	Enzyme (ml)	Substrate L-DOPA (ml)	Incubate for 15 minutes at room temperature	Phosphate Buffer (ml)	OD at 475 nm
25	0.5	0.5		3.0	0.023
30	0.5	0.5		3.0	0.191
35	0.5	0.5		3.0	0.052
40	0.5	0.5		3.0	0.023

and then incubated at various temperatures. 25 to 30 to 35 to 40 degrees Celsius are involved. Spectrophotometric measurements are made every day at 475 nm for five days, with a 24-hour interval between measurements (Table 2). The best-growing organism from the temperature optimization is chosen for additional inoculum size optimization (Graph 2).



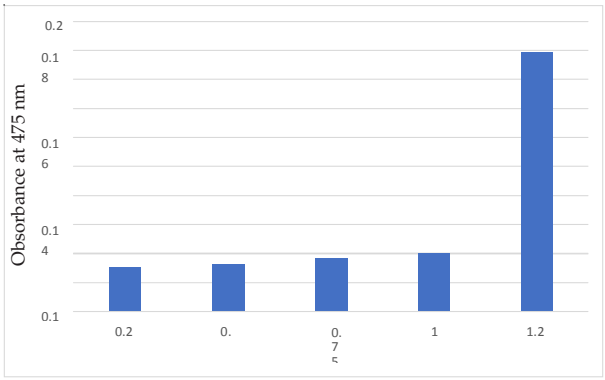
Graph 2. Optimization of temperature

Optimization of inoculum size

The pH is adjusted to 7, the Czapek Dox broth is made, and the incubation temperature is set to 30 °C. A change in inoculum size occurs. Namely, 0.25, 0.5, 0.75, 1.0, and 1.25 milliliters (Table 3). Spectrophotometric measurements are made every day at 475 nm for five days, with a 24-hour interval between measurements (Graph 3).

Results and Discussion

Three samples are taken from the study to be examined in order to identify soil strains that produce tyrosinase. The organism is cultivated on Czapek Dox agar medium. The stain used was cotton blue lacto phenol stain. The staining method allowed for the identification of the C 01 strain as *Fusarium* sp., and it was found that this strain had the highest activity out of the three. The organism is designed to grow as much as possible in order to achieve the best possible growth for the enzyme. To get the maximum amount of enzyme growth, conditions such as pH, temperature, and inoculum size are optimized to the highest degree. To prevent contamination, every experiment is carried out in an aseptic manner. The experiments are carried out using LAF.



Graph 3. Optimization of Inoculum size

Throughout all of the experiments, the same strain is employed. The process of submerged fermentation, in which all of the nutrients are dissolved in water and the medium is in liquid phase, is used to produce enzymes. In order to attain the highest possible growth of the enzyme, it is allowed to grow for five to six days while receiving all the necessary media supplements and environmental factors like temperature and pH. Ammonium sulphate is added in three intervals over the course of ten hours while the magnetic stirrer is running at 300 rpm to achieve purification. After that, the crude enzyme is subjected to dialysis for a full day in order to rid it of any contaminants. Following the acquisition of the pure enzyme, its stability, pH, and temperature are examined to determine its effects. A spectrophotometer is used to monitor the substrate reaction at 475 nm. The enzyme's optimal activity is mentioned. It concludes that it was successful to produce the tyro-

Table 3. Optimization of Inoculumsize

Inoculum size (ml)	Enzyme (ml)	Substrate L-DOPA (ml)	Incubate for 15 minutes at room temperature	Phosphate Buffer (ml)	OD at 475 nm
0.25	0.5	0.5		3.0	0.031
0.50	0.5	0.5		3.0	0.033
0.75	0.5	0.5		3.0	0.037
1.0	0.5	0.5		3.0	0.040
1.25	0.5	0.5		3.0	0.179

sinase enzyme from *Fusarium* sp. C-01.

Conclusion

From the study, 3 samples are taken for the examination to isolate tyrosinase producing strains from the soil. The C 01 strain was identified as *Fusarium* sp. from the staining technique and is designated as *Fusarium* sp C 01 showed the highest activity among the 3 strains. The organism is optimized for the maximum growth of it to attain optimal growth of the enzyme. Conditions like, pH, Temperature and Inoculum size are optimized at the finest level to get maximum enzyme growth. It concludes that, producing the tyrosinase enzyme from *Fusarium* sp. C 01 was effective. It was observed that further detail studies on tyrosinase enzyme from the isolates would have potential in melanin production and its application in future.

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Conflict of Interest- None

References

Anghileri, A., Lantto, R., Kruus, K., Arosio, C. and Freddi, G. 2007. Tyrosinase-catalyzed grafting of sericin peptides onto chitosan and production of protein polysaccharide bioconjugates. *Journal of Biotechnology*. 127(3): 508-519.

Barate, D.L. and Sonar, N.R. 2023. Isolation of tyrosinase producing bacteria from rhizosphere soil samples. *International Journal for Multidisciplinary Research*. 5(3): 1-9.

Chang, T.S. 2009. An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences.* 10(6): 2440-2475.

Claus, H. and Decker, H. 2006. Bacterial tyrosinases. *Systematic and Applied Microbiology.* 29: 3-14.

Danial, N.E. and Al-Birshi, W.M. 2018. Optimization of medium composition for increased production of tyrosinase enzyme in recombinant *Bacillus megaterium*. *Journal of pharmaceutical Biology and Chemical Sciences*. 9(1): 480- 486.

Elsayed, E.A. and Danial, E.N. 2018. Isolation, identification and medium optimization for tyrosinase production by a newly isolated *Bacillus subtilis* NA2 strain. *Journal of Applied Pharmaceutical Science.* 8(9): 093-101.

Fairhead, M. and Thony-Meyer, L. 2012. Bacterial tyrosinases: Old enzymes with new relevance to biotechnology. *National Biotechnology*. 15: 29(2): 183-191. doi: 10.1016/j.nbt.2011.05.007.

Fairhead, M. and Thony-Meyer, L. 2012. Bacterial Tyrosinases: old enzymes with new relevance to biotechnology. *New Biotechnology.* 29 (2): 183-199.

Freddi, G., Anghileri, A., Sampio, S., Buchert, J., Monti, P. and Taddei, P. 2006. Tyrosinase-catalyzed modification of *Bombax mori* silk fibroin: Grafting of chitosan under heterogenous reaction conditions. *Journal of Biotechnology.* 125 (2): 281-294.

Gu, B.X., Xu, C.X., Zhu, G.P., Liu, S.Q., Chen, L.Y. and Li, X.S. 2009. Tyrosinase immobilization on ZnO Nanorods for phenols detection. *Journal of Physical Chemistry B.* 113(1): 377-381.

Halaouli, S., Asther, M. and Kruss, K. 2005. Characterization of a new tyrosinase from *Pycnoporus* species with high potential for food technological applications. *Journal of Applied Microbiology.* 98(2) : 332–343.

Halaouli, S., Asther, M., Sigoillot, J. C., Hamdi, M. and Lomascolo, A. 2006. Fungal tyrosinases: new prospects in molecular characteristics, bioengineering and biotechnological applications. *Journal of Applied Microbiology*. 100(2): 219-232.

Jus, S., Kokol, V. and Guebitz, G. M. 2008. Tyrosinase-catalyzed coupling of functional molecules onto protein fibers. *Enzyme Microb Technol*. 42(7). 535-542.

Jus, S., Kokol, V. and Guebitz, G.M. 2009. Tyrosinase-catalyzed coating of wool fibers with different protein-based biomaterials. *Journal of Biomaterial Science, Polymer.* 20(2): 253-269.

Jus, S., Stachel, I., Schloegl, W., Pretzler, M., Friess, W., Meyer, M., Birner-Gruenberger, R. and Guebitz, G.M. 2011. Cross-linking of collagen with laccases and tyrosinases. *Materials Science and Engineering C-Materials for Biological Applications.* 31(5): 1068-1077.

Lantto, R., Puolanne, E., Kruss, K., Buchert, J. and Autio, K. 2007. Tyrosinase aided protein cross linking: Effects on gel formation of chicken breast myofibrils and texture and water holding of chicken breast meat homogenate gels. *Journal of Agricultural and Food Chemistry*. 55(4): 1248-1255.

Martorell, M.M., Pajot, H.F., Rovati, J.I. and Figueroa, L.I.C. 2012. Optimization of culture medium composition for manganese peroxide and tyrosinase produce during reactive black 5 decolorization by the yeast *Trichosporon kiyoshidainum*. *Yeast.* 29 (3-4): 137-144.

Marusek, C.M., Trobaugh, N.M., Flurkey, W.H. and Inlow, J.K. 2006. Comparative analysis of polyphenol oxidase from plant and fungal species. *Journal of Inorganic Biochemistry.* 100: 108-123.

Nawaz, A., Shafi, T., Khaliq, A., Mukhtar, H. and Haq, I. 2017. Tyrosinase: Sources, structure and applica-

tion. *International Journal of Biotechnology and Bioengineering*. 3 (5): 142-148.

Seetharam, G. and Saville B.A. 2002. L-DOPA production from tyrosinase immobilized on zeolite. *Enzyme and Microbial Technology*. 31(6): 747-753.

Selinheimo, E., Autio, K., Krijus, K. and Buchert, J. 2007. Elucidating the mechanism of laccase and tyrosinase in wheat bread making. *Journal of Agricultural and Food Chemistry*. 55(15). 6357-6365.

Zaidi, K.U., Mani, A., Ali, S.A. and Ali, A.S. 2013. Evaluation of Tyrosinase producing endophytic fungi from Calotropis gigantea, *Azadirachta indica*, *Ocimum tenuiflorum* and Lantana camara. *Annual Review & Research in Biology*. 3(4): 389-339.

Zaidi, K.U., Ali, A.S., Ali, S.A. and Naaz, I. 2014. Microbial tyrosinase: promising enzymes for pharmaceutical, food bioprocessing, and environmental industry. *Biochemistry Research International*. 2014: Article ID 854687, 16 pages.

Zaidi, Kamal Uddin, Ali, Ayesha S. and Ali, Sharique A. 2014. Purification and Characterization of Melanogenic Enzyme Tyrosinase from Button Mushroom. *Enzyme Research*. 1-6. doi:10.1155/2014/120739

Zaidi, Kamal Uddin, Ali, Ayesha S., Ali, Sharique A. and Naaz, Ishrat, 2014. Microbial Tyrosinases: Promising Enzymes for Pharmaceutical, Food Bioprocessing, and Environmental Industry. *Biochemistry Research International*. 1-16. doi:10.1155/2014/854687.