INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0116/2024/31–6–401–409
DOI: 10.17957/IJAB/15.2157
http://www.fspublishers.org

Full Length Article

Exploring Enhanced Dopamine Activity using an Intracellular Tyrosinase of Two different *Lentinula edodes* Strains

Sikander Ali†*, Hijab Zahra†, Muhammad Usman Ahmad, Hajrah Usmani, Saba Sana, Madiha Shoukat and Atif Iqbal
Department of Microbiology, Dr. Ikram-ul-Haq Institute of Industrial Biotechnology, GC University Lahore, Pakistan
*For correspondence: dr.sikanderali@gcu.edu.pk
†Contributed equally to this work and are co-first authors
Received 02 March 2024; Accepted 05 March 2024; Published 16 April 2024

Abstract

Tyrosinase, a vital enzyme involved in the formation of melanin, is also required for the hydroxylation of L-tyrosine, which produces dopamine. The tyrosinase activity of *Lentinula edodes* (Berk), better known as shiitake mushroom, is known for its nutritional and therapeutic properties. This study focuses on the biochemical characterization of an intracellular tyrosinase extracted from two different strains of *L. edodes*, (Shii-I and Shii-II) for higher dopamine activity. Different organic solvents (acetone, 1-propanol, 1-butanol, ethanol, n-hexane and methanol) were analyzed for the pre-treatment of biomass of mushrooms, out of which, 1-butanol was found to be the most suitable. Saline water (25 mL) was selected as an extracting agent as it depicted the highest dopamine (DA) activity. Optimal level of mushroom biomass and time of incubation was found to be 2 g and 36 h, respectively. A significant increase in DA activity was obtained when saline water was supplemented with SDS (Shii-I) and chitosan (Shii-II). The highest DA activity (68 μg/mL) was observed at 60°C and incubation time of 20 min by Shii-I. After optimizing conditions more than 4-fold increase in DA activity was obtained which was highly encouraging ($P \leq 0.05$). Overall, this work emphasizes how crucial strain selection is when using *L. edodes* tyrosinase to increase dopamine activity. In order to fully utilize the potential of Shiitake mushrooms as a functional food with neuroprotective and mood-enhancing qualities, it may be advantageous to comprehend the biochemical nuances of tyrosinase variants and optimize their cultivation and processing techniques.

Keywords: Tyrosinase; Dopamine; *Lentinula edodes*; Mushrooms; L-DOPA; DA activity

Introduction

An enzyme tyrosinase (EC 1.14.18.1) shows diversity in terms of tissue distribution, cellular location and structural properties. It differs in glycosylation patterns and activation characteristics, structure and size (Zaidi *et al.* 2014a, b). Tyrosinase (tyrosine, L-DOPA: oxygen oxidoreductase; diphenol oxidase; catecholase; polyphenol oxidase – PPO) is a copper containing enzyme which with several phenolic substrates' catalyses sequential oxidation steps (Faria *et al*. 2007; Flurkey and Inlow 2017). Tyrosinase exhibit two types of catalytic activities in the presence of oxygen (Sys *et al*. 2020): first is the monophenolase activity (Garcia-Molina *et al*. 2022), in which hydroxylation of monophenols to O-diphenols occur, second is the diphenolase activity (Li *et al*. 2021), involving oxidation of diphenols to their corresponding o-quinones (Cieńska *et al.* 2016) (Fig. 1). All tyrosinases in their active sites contain a copper centre of two copper atoms. Each copper atom is coordinated by three residues of histidine. In crystalline form, these enzymes contain six residues of histidine and three imidazoles of histidine for each copper atom, in active site. Activities of tyrosinases are widely distributed from microorganisms to mammals. They are found in mushrooms, fruits, vegetables, bacteria, fungi as well as humans (Zaidi *et al.* 2014a, b).

Agaricus bisporus, *A. oryzae*, *Amanita muscaria*, *Neurospora crassa*, *Lentinula edodes*, *L. boryana* and *Pycnoporus sanguineus* are different fungi that are used for isolation of tyrosinase (Zaidi *et al.* 2014a, b). Bacterial tyrosinases have been reported in number of species such as *Bacillus thuringiensis*, *Marinomonas mediterranea*, *Pseudomonas maltophilia*, *P. putida*, *Streptomyces castaneoglobisporus*, *Symbiobacterium thermophilum* and *Verrucomicrobium spinosum* (Zaidi *et al.* 2014a, b). Most of the current development of biotechnological applications of tyrosinases is focused on mushroom tyrosinases (Faria *et al*. 2007). In 1985, first biochemical investigation of tyrosinase was carried out on *Russula nigricans*, a mushroom, whose cut flesh turns black and red when exposed to air. Similarly, the enzyme extracted from *A. bisporus*, is homologous to that of mammals, and thus renders *A. bisporus* a well-matched model to carry out studies on melanogenesis (Chang 2009). *L. edodes* commonly known as shiitake

mushroom is the second most commercially produced species of mushrooms (Cai *et al.* 2017). During post-harvest preservation, the gill of *L. edodes* fruit body turns brown. This gill browning of fruit bodies of shiitake is thought to be caused by biosynthesis of melanin catalysed by tyrosinase (Sato *et al.* 2009). Tyrosinase is responsible for generating the pigment melanin (Jimenez *et al.* 1988; Narasimhappa and Ramamurthy 2023) as described in Fig. 2.

The most important application of tyrosinase being investigated is the synthesis of L-DOPA from L-tyrosine using *Erwinia herbicola, E. coli* (Faria *et al.* 2007). Tyrosinase is also a potential pro-drug for melanoma treatment (Morrison *et al.* 1985; Jin *et al*. 2024). Several studies involving mushroom tyrosinase are being carried out for removal, detection and quantification of phenolic compounds present in water samples. This enzyme is being utilized for producing cross-linked protein networks (Faria *et al.* 2007). Herbicides are a potent hazard to human health if present in surface or ground water, thus, their presence can be detected by using biosensors based on principle of inhibition activity of tyrosinase enzyme (Wang *et al.* 2006). For tailoring meats' gelation properties, cross linking enzymes play an essential role (Cirlincione *et al*. 2023). Recently tyrosinases have been tested for chicken proteins processing (Lantto *et al.* 2006; Lantto *et al.* 2007).

Dopamine (DA) is a major neurotransmitter belonging to monoaminergic neuroreceptor family that supports the central nervous system (Missale *et al.* 1998; Zhang *et al.* 2016). Many neurological diseases are a result of disorder in levels of dopamine (Donzella *et al*. 2022). These diseases include Parkinson's disease (Nagatsu *et al*. 2023), Alzheimer's, schizophrenia, sleeping and eating disorders (Zhang *et al.* 2016). For production of dopamine by the central nervous system, L-DOPA acts as a precursor. Due to this reason, L-DOPA is a potent drug for treating patients with Parkinson's disease (Richmond *et al.* 2023). Myocardium neurogenic injury can also be regulated by using dopamine (Ali *et al.* 2007).

Materials and Methods

The chemicals including ethylenediaminetetraacetic acid (EDTA), L-catechol, L-ascorbic acid, L-tyrosine, sodium nitrite ($NaNO_2$), sodium molybdate (Na_2MoO_4), sodium acetate ($CH_3COONa.3H_2O$), sodium hydroxide (NaOH), sodium dodecyl sulphate (SDS) and chitosan were of analytical grade and were purchased from Sigma-Aldrich (Germany).

Collection of mushrooms

Black mushroom commonly known as shiitake mushroom (*L. edodes*) was purchased from a store in dried form. The mushrooms were of two types differing in their developmental and morphological stages thus named as Shii-I and Shii-II.

Pretreatment of mushroom

The mushroom biomass was thoroughly dried and grinded by using mortar and pestle. The grinded biomass was further treated with 1-butanol. Organic solvent was added in a conical flask till mushroom was completely dipped in it and was incubated for 20 min in a shaking incubator (VS-8480, Vision Scientific, Daejeon-Si, Korea) at 160 rpm at 30°C. After incubation mushroom biomass was filtered with muslin cloth and double washed with ice cold water (4°C) to remove any residual organic solvent. The biomass was oven dried (DHG-9202, SANFA, Yangzhou, China) at 105°C for 2 h.

Enzyme harvesting and filtration

Pretreated mushroom biomass was incubated in saline water as an extractant at 37°C in a shaking incubator for 24 h. After incubation, the extractant solution was filtered to remove mushroom biomass and centrifuged (1020 D.E, Centurion Scientific, West Sussex, U.K) at 3500 rpm for 15 min to remove any residual contents of biomass (Zaidi *et al.* 2014a, b).

Reaction procedure

Reaction mixture containing L-tyrosine, as a substrate was used for the biosynthesis of DA. For biosynthesis of DA the reaction was carried out using 2.5 mg/mL L-tyrosine as a substrate. Reaction mixture consisted of 50 m*M* of acetate buffer (pH 3.5) in which 1 mL of extract, 2.5 mg/mL of L-tyrosine and 5 mg/mL of ascorbic acid were added. The reaction was carried out at 50°C in a water bath (MaxTurdy 18, Dhaihan Scientific, Wonju-si, South Korea) at 120 rpm for 60 min (Koyanagi *et al*. 2012).

Enzyme extraction

The reaction mixture was centrifuged at 3000 rpm for 20 min. The supernatant was separated in a clean falcon and pellet was discarded. Supernatant was stored at -4°C in a refrigerator (Koyanagi *et al*. 2012).

Analytical techniques

Tyrosinase assay

The method reported by Kandaswami and Vaidyanathan (1973) was used to determine the tyrosinase activity. Enzyme extract (0.5 mL), 1 mL of 50 mM phosphate buffer and 0.1 mL of 1% L-catechol was added in a test tube. This mixture was incubated for 10 min at 30°C. After completion of incubation, 0.1 mL of 1% L-ascorbic acid and 0.1 mL EDTA of 100 ppm concentration was added in the same test tubes. The contents were mixed thoroughly. Distilled water (1.2 mL) was added to raise volume to 3 mL. Absorbance was noted at a wavelength of 390 nm with spectrophotometer

(UV-1700, Shiimadzu Corp., Kyoto, Japan). The activity of tyrosinase was calculated by using the following formula:

$$\text{Tyrosinase activity (U/mg)} = \frac{\Delta\,\text{A390 nm/ min test} - \Delta\,\text{A390 nm/ min}}{\text{0.001 mg enzyme /reaction mixture}}$$

One unit of enzyme activity was equal to a ΔA_{390nm} of 0.001 per min at pH 7 (25°C) in 3 mL of reaction mixture containing reagents, L-catechol and L-ascorbic acid.

DA assay

Enzyme extract of about 1 mL was taken in a test tube. In the same test tube 1 mL of 0.5 N HCl and 1 mL of nitrite molybdate reagent was added. The contents were mixed thoroughly. After mixing, 1 mL of 1 N NaOH was added to the same tube. The volume of this mixture was raised to 5 mL by using distilled water. The absorbance was analyzed at 460 nm (A_{460}) by using spectrophotometer and the amount of DA produced was determined (Arnow 1937; Fan *et al*. 2021).

Optimization of reaction conditions

Different organic solvents (acetone, 1-propanol, 1-butanol, ethanol, n-hexane and methanol) were used for pretreatment of mushroom biomass. The effect of level of different extracting agents (distilled water, saline water, 0.1 N H_2SO_4, phosphate buffer pH 7.2, acetate buffer pH 4.8 and 0.1 N tris-HCl) on tyrosinase activity and production of DA was studied. By varying the level of mushroom biomass (0.5 g, 1, 1.5, 2, 2.5 and 3 g) and changing the time of incubation (12 h, 24, 36, 48, 60 and 72 h), the production of DA was investigated. The effect of various extractants (SDS, MOT, Chitosan, Tween-80, Triton X-100 and PEG) along with their different concentration was determined. The tyrosinase assay was later performed at different temperature (20–30°C) and DA assay was performed at one of the optimum temperatures by varying the time of incubation by an interval of 10 min (10–60 min) (El-Hadi *et al.* 2014).

Results

Pre-treatment of mushroom biomass with different organic solvents

The two strains of *L. edodes* or shiitake mushroom (Shii-I and Shii-II) were pretreated with organic solvents (acetone, 1-propanol, 1-butanol, ethanol, n-hexane and methanol) to study their effects on the DA activity as shown in Fig. 3. Shii-I and Shii-II pretreated with 1-butanol exhibited maximum DA activity of 17 and 15 μg/mL respectively. Both the mushrooms had tyrosinase activity of 18 and 16 U/mg respectively with 1-butanol. However, there was an exception for the highest tyrosinase activity of 23 U/mg with Shii-I using n-hexane. The lowest DA activity for Shii-I (10 μg/mL) and Shii-II (3 μg/mL) was shown by acetone and methanol.

Evaluation of different extracting agents

The biomass pretreated with 1-butanol was further assessed with various extracting agents (distilled water, saline water, 0.1N H_2SO_4, acetate buffer (pH 4), phosphate buffer (pH 7.2) and 0.1 N Tris-HCl). The DA activity of 25 μg/mL by Shii-I and of 20 μg/mL by Shii-II, using saline water was exhibited to be the highest as shown in Fig. 4a. The tyrosinase activity corresponding to the highest DA activity for both the mushrooms was 23 and 18 U/mg, respectively. The least DA activity by Shii-I was depicted by 0.1 N H_2SO_4 (3 μg/mL) and in case of Shii-II (5 μg/mL) it was depicted with acetate buffer, pH 4.8. Saline water was observed to be the optimal extracting agent. Nitrogen source is important for active metabolism of mushrooms, peptone being a nitrogen source is present in saline water making it efficient medium for DA activity.

Once saline water was optimized as an effective extractant, its levels were varied *i.e*., 12.5, 25, 37.5, 50, 62.5 and 75 mL. The Fig. 4b demonstrates the DA activity for Shii-I and Shii-II, as comparison. The maximum DA activity by Shii-I (28 μg/mL) and Shii-II (22 μg/mL) was found at 25 mL. The enzyme activity at 25 mL of saline water by Shii-I and Shii-II was 26 and 17 U/mg, respectively. The increase in level of saline water from 37.5 to 75 mL showed a gradual decline in DA activity having minimum activity of 6 and 2 U/mg, respectively at 75 mL for both mushrooms.

Effect of different levels of mushroom biomass

The amount of mushroom biomass was varied with a difference of 0.5 g that is 0.5, 1, 1.5, 2, 2.5 and 3 g. The DA activity was observed for both the mushroom as given in Fig. 5. The maximum DA activity of 35 μg/mL (Shii-I) and 28 μg/mL (Shii-II) was observed at 2 g along with increased tyrosinase activity. The DA activity was increased gradually from 12 to 35 μg/mL with Shii-I and from 13 to 28 μg/mL with Shii-II as biomass was increased from 0.5 to 2 g. The lowest DA activity was observed at 3 g. Thus, 2 g of mushroom biomass was optimal for DA activity which was highly encouraging ($P \leq 0.05$).

Effect of time of incubation

The DA activity was observed with a difference of 12 h in incubation time as given in Fig. 6. Time was increased from 12, 24, 36, 48, 60 until 72 h. The maximum DA activity of 43 μg/mL and 40 U/mg of tyrosinase activity was shown by Shii-I after incubation time of 36 h. In case of Shii-II, again maximum DA activity of 29 μg/mL was observed at 36 h with 32 U/mg of tyrosinase activity. The DA activity increased gradually from 12 to 36 h, however, further increase in time of incubation lowered the DA activity. At 72 h, DA activity of Shii-I and Shii-II was 11 and 7 µg/mL, respectively. The 36 h time of incubation was revealed to be

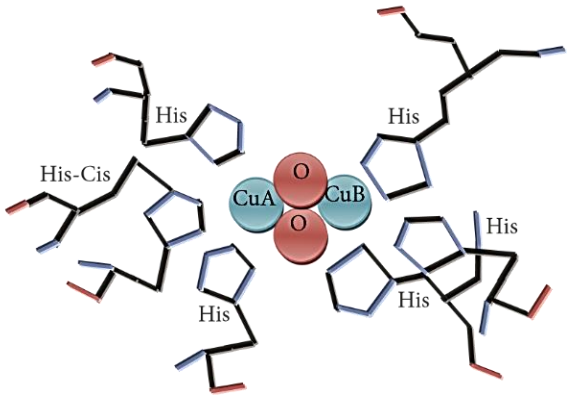


Fig. 1: Structure of oxy-form of tyrosinase

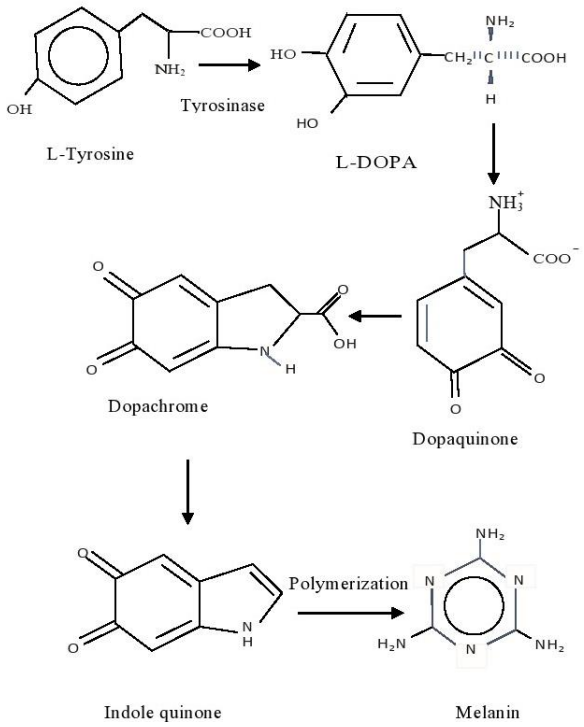
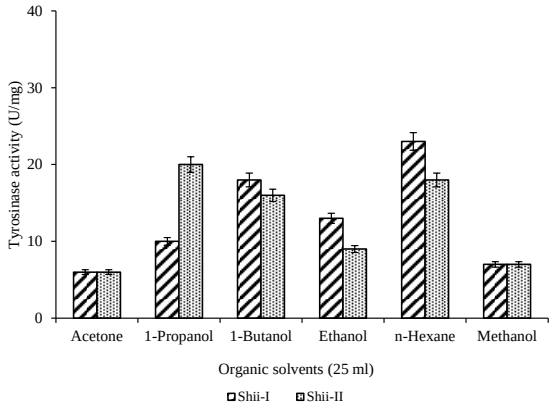


Fig. 2: Melanin biosynthetic pathway

optimal. Copeland (2023) described that if substrate concentration was kept constant throughout the reaction with increase in incubation period, after sometime substrate will become inadequate, thus, causing a decrease in nutrients for microbes to survive. Microorganisms reached the decline phase of their growth causing a reduction in enzyme formation.

Effect of different surfactants and their concentrations

Different surfactants (tween-80, SDS, triton X-100, PEG, chitosan and MOT) were added in saline water (0.1 % w/v) to investigate the changes in DA activity. The Fig. 7a reveals that both mushrooms showed optimum DA activity



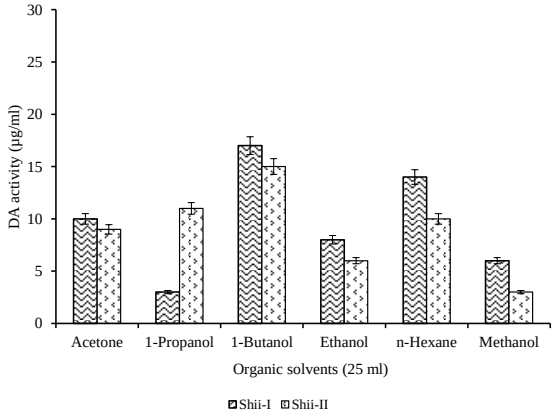


Fig. 3: Pre-treatment of mushroom biomass with different organic solvents for higher tyrosinase production and subsequent dopamine activity. Time of incubation 24 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

with different surfactants. It was observed that with Shii-I, maximum DA activity of 58 µg/mL and 60 U/mg of tyrosinase activity was found when saline water was supplemented with SDS. The maximum DA activity of 50 μg/mL and 47 U/mg of tyrosinase activity was exhibited by Shii-II with chitosan. The minimal DA activity was exhibited with saline water containing tween-80, 15 μg/mL by Shii-I and even lower by Shii-II (11 μg/mL). Thus, SDS was optimized for Shii-I and chitosan for Shii-II.

The effect of different concentrations of SDS (2.5, 3, 3.5, 4, 4.5 and 5 m*M*) on DA activity was investigated for Shii-I. The data is represented in Fig. 7b. The maximum DA activity (64 μg/mL) was exhibited by SDS at concentration of 4.5 m*M*. The DA activity increased from 2.5 to 4.5 m*M*, 31 to 64 μg/mL, respectively. However, DA activity was minimum at 5 mM SDS, showing a sharp decline to 39 μg/mL. The effect of different concentrations of chitosan (0.2, 0.4, 0.6, 0.8, 1 and 1.2 m*M*) on DA activity is shown in Fig. 8. Saline water supplemented with 1 m*M* of chitosan in case of Shii-II had maximum DA activity of 59 μg/mL and 57 U/mg of tyrosinase activity. The lowest DA activity, 38

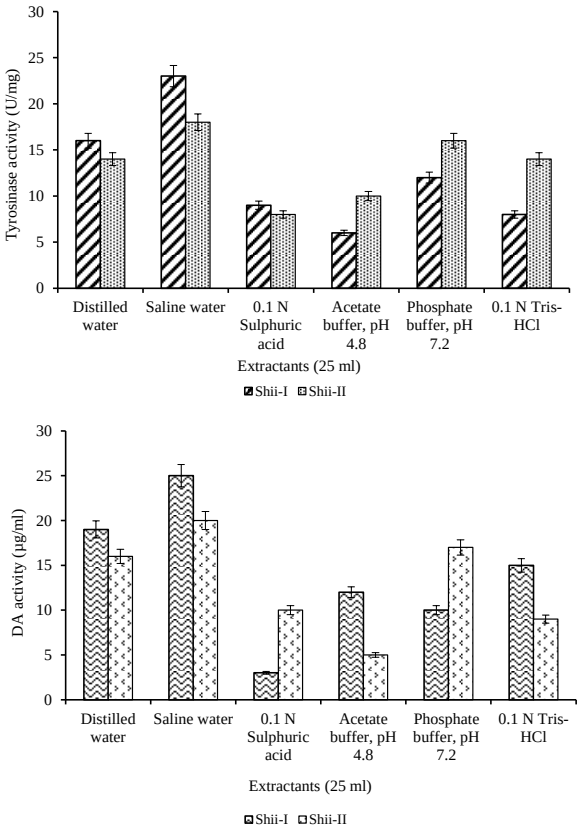


Fig. 4a: Evaluation of different extracting agents for higher tyrosinase production and subsequent dopamine activity. *Pretreatment 1-butanol, Time of incubation 24 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1h.Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

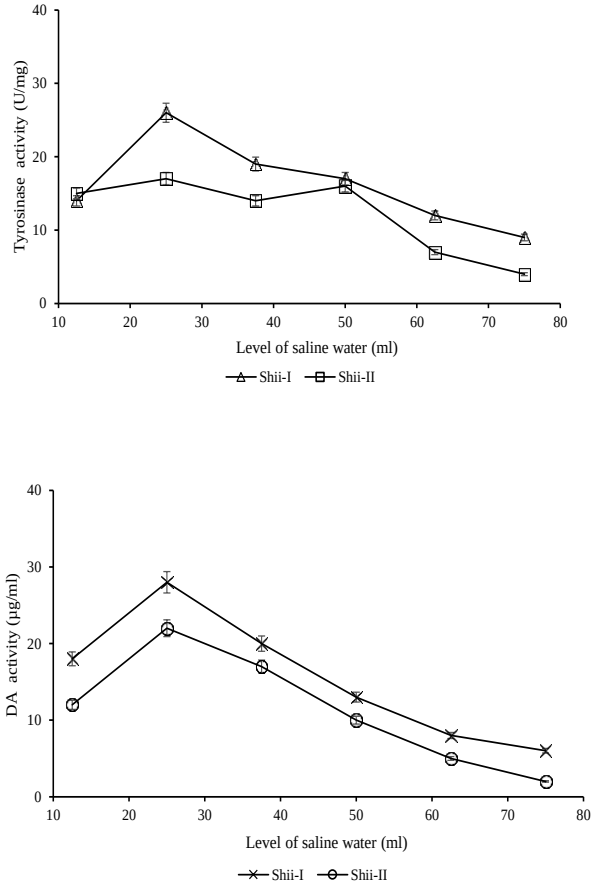


Fig. 4b: Effect of different level of saline water on higher tyrosinase production and subsequent dopamine activity. *Pretreatment 1-butanol, Saline water pH 7, Time of incubation 24 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

μg/mL was at 0.2 m*M*. An increasing trend was perceived from 0.2 m*M* till 1 m*M*, whereas, further increase in chitosan concentration showed a noticeable decline in DA activity.

Effect of different temperatures on tyrosinase activity

The effect of different incubation temperatures (20, 40 and 60ºC) on tyrosinase activity was studied as depicted in Fig. 8. From 20 to 60ºC, the tyrosinase activity increased from 30 to 66 U/mg, respectively for Shii-I. However, Shii-II exhibited maximum tyrosinase activity (60 U/mg) at 40ºC followed by a sharp decline at 60ºC. This decline is because of denaturation of tyrosinase at high temperature and it is unable to convert L-tyrosine to L-DOPA. The temperature optimized for tyrosinase activity of Shii-I and Shii-II mushrooms was 60 and 40ºC, respectively.

Effect of different time of incubation on DA activity

The effect of time of incubation of reaction mixture on the DA activity was determined. The incubation time was varied from 10 to 60 min with a difference of 10 min at each interval as presented by data in Fig. 9. Maximum DA activity of 68 μg/mL was exhibited by Shi-I at 20 min whereas at 40 min Shi-II displayed maximum DA activity of 62 μg/mL. The DA activity for both mushrooms increased from 10 min onward, but increase was more gradual for Shii-II. From 30 to 60 min, the DA activity of Shii-I decreased from 56 to 25 μg/mL, respectively. The incubation time optimized for both strains was 20 and 40 min, respectively.

Discussion

Shiitake mushrooms are second most cultivated species. In current study cultivation and processing parameters were optimized for enhanced dopamine activity. Pre-treatment of mushroom biomass with different organic solvents was

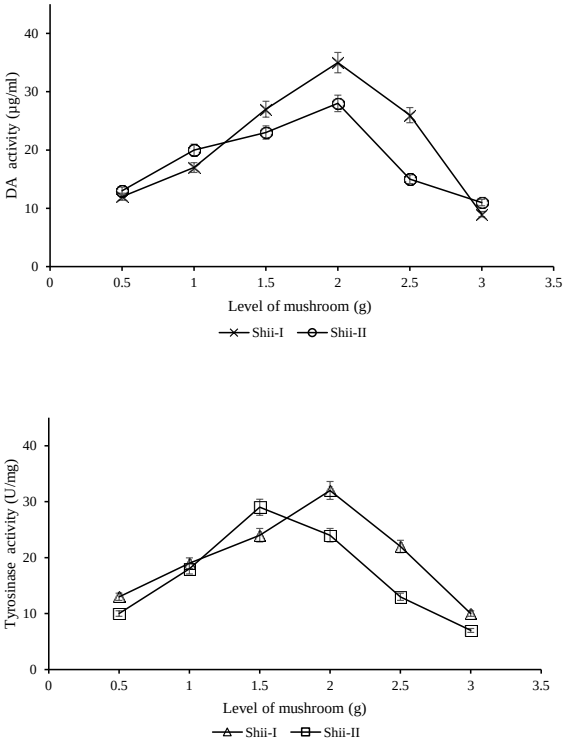


Fig. 5: Varying levels of mushroom biomass for higher tyrosinase production and subsequent dopamine activity. *Pretreatment 1-butanol, Saline water (pH 7) 25 mL, Time of incubation 24 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

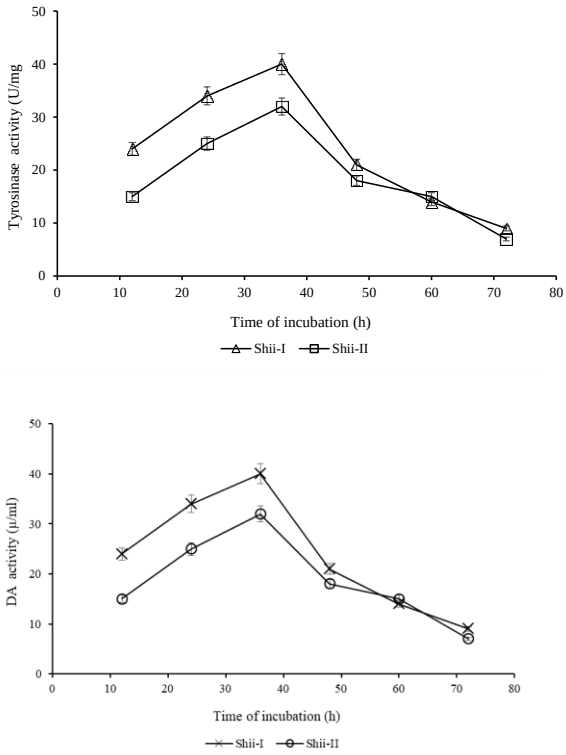


Fig. 6: Effect of time incubation on tyrosinase production and subsequent dopamine activity. *Pretreatment 1-butanol, Biomass 2 g, Saline water (pH 7) 25 mL, Time of incubation 24 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

evaluated. 1-butanol was revealed to be the optimum organic solvent for shiitake mushrooms pretreatment. The study revealing that cultures exposed to organic solvents show a significant increase in dopamine synthesis was investigated, hypothesizing, that the rate of dopamine synthesis may increase on exposure to organic solvents without affecting dopamine receptors as reported by Edling *et al.* (1997). Evaluation of different extracting agents was carried out in current study. El-Hadi *et al.* (2014) reported that peptone was a good nitrogen source for tyrosinase production from *A. horta* comparable to current results. The decrease in DA activity was due to increase in dilution of saline water as reported by El-Hadi *et al.* (2014). Thus, 25 mL of saline water was found to be the optimal extracting agent. As far as effect of different levels of mushroom biomass is concerned the increase in biomass other than the optimal limit caused an inhibition of enzyme and subsequent reduction of DA activity as described by Copeland (2023). Effect of time of incubation was an evaluating parameter in this study. This study is strengthened by Copeland (2023). Who described that if substrate concentration was kept constant throughout the reaction with increase in incubation period, after some time substrate will become inadequate, thus, causing a decrease in nutrients for microbes to survive. Microorganisms reached the decline phase of their growth causing a reduction in enzyme formation. Effect of different surfactants and their concentrations was observed and comparable with Yang *et al.* (2007). He reported that different surfactants *i.e*., aerosol OT, Brij 52 and CTAB, act differently on the activity of tyrosinase. When AOT was added, the mushroom tyrosinase activity increased with increase in concentration of AOT. Brij-52 and AOT both showed a positive response by displaying the ability to activate the enzyme while CTAB sharply inhibited the tyrosinase activity. Some other studies also reported the effect of different temperatures on tyrosinase activity. Koyanagi *et al.* (2012) reported that the stability of tyrosinase obtained from *Pseudomonas putida* for DA production from L-DOPA was at temperatures less than 45ºC. The maximum tyrosinase activity of *Lentinula boryana* was at 40°C as reported by Zaidi *et al.* (2014a, b). Halaouli *et al.* (2006) exhibited 30°C to be the optimum incubation temperature for tyrosinase production by *P. sanguineus* CBS 614.73. As far as effect of different time of

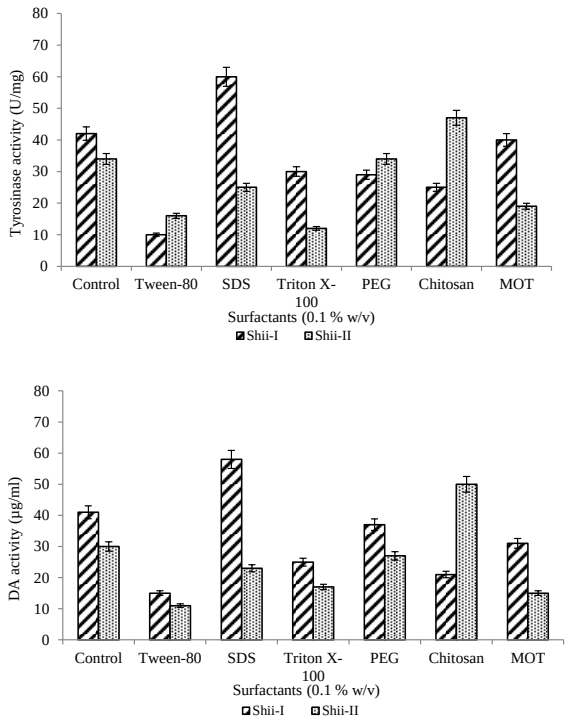


Fig. 7a: Effect of different surfactants on tyrosinase production and subsequent dopamine activity. *Pretreatment 1-butanol, Biomass 2 g, Saline water (pH 7) 25 mL, Time of incubation 36 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

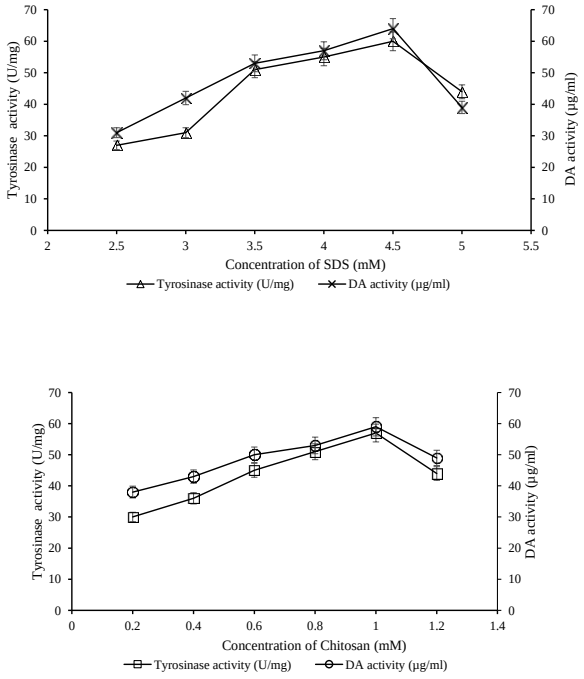


Fig. 7b: Effect of different concentrations of surfactants on tyrosinase production and dopamine activity. *Pretreatment 1-butanol, Biomass 2 g, Saline water (pH 7) 25 mL, Time of incubation 36 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h, Surfactant SDS/chitosan. Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

incubation on DA activity, Ali and Nawaz (2016) reported that DA activity was lowest at the start of reaction. As incubation period was increased from 50 to 70 min there was a two-fold increase in DA activity. However, when the time of incubation was increased above 70 min, the activity of DA declined effectively. Pre-treatment of mushroom biomass with different organic solvents revealed that 1-butanol was the optimum organic solvent for shiitake mushrooms pretreatment. The study revealing that cultures exposed to organic solvents show a significant increase in dopamine synthesis was investigated, hypothesizing, that the rate of dopamine synthesis may increase on exposure to organic solvents without affecting dopamine receptors as reported by Edling *et al.* (1997). Evaluation of different extracting agents was evaluated. El-Hadi *et al.* (2014) reported that peptone was a good nitrogen source for tyrosinase production from *A. horta* comparable to current results. The decrease in DA activity was due to increase in dilution of saline water as reported by El-Hadi *et al.* (2014). Thus, 25 mL of saline water was found to be the optimal extracting agent. Effect of different levels of mushroom biomass revealed that the increase in biomass other than the optimal limit caused an inhibition of enzyme and subsequent reduction of DA activity as described by Copeland (2023). Copeland (2023) described that if substrate concentration was kept constant throughout the reaction with increase in incubation period, after sometime substrate will become inadequate, thus, causing a decrease in nutrients for microbes to survive. Microorganisms reached the decline phase of their growth causing a reduction in enzyme formation. Yang *et al.* (2007) reported that different surfactants *i.e*., aerosol OT, Brij 52 and CTAB, act differently on the activity of tyrosinase. When AOT was added, the mushroom tyrosinase activity increased with increase in concentration of AOT. Brij-52 and AOT both showed a positive response by displaying the ability to activate the enzyme, while CTAB sharply inhibited the tyrosinase activity. Effect of different temperatures on tyrosinase activity was a part od current study and reported by others too. Koyanagi *et al.* (2012) reported that the stability of tyrosinase obtained from *Pseudomonas putida* for DA production from L-DOPA was at temperatures less than 45ºC. The maximum tyrosinase activity of *Lentinula boryana* was at 40°C as reported by Zaidi *et al.* (2014a, b). Halaouli *et al.* (2006) exhibited 30°C to be the optimum incubation temperature for tyrosinase production by *P. sanguineus* CBS 614.73. Ali and Nawaz (2016) reported that DA activity was lowest at the start of

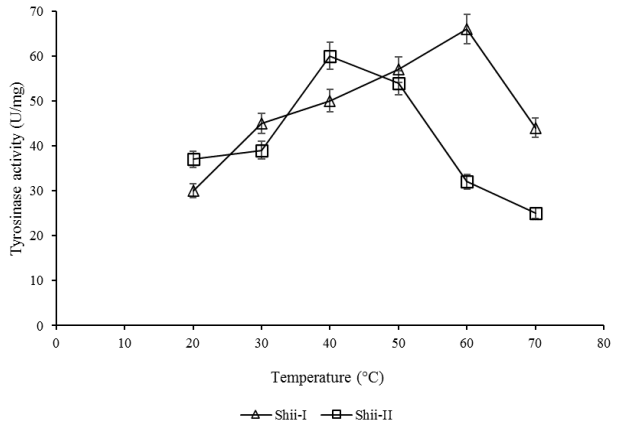


Fig. 8: Effect of different temperatures on tyrosinase activity. *Pretreatment 1-butanol, Biomass 2 g, Saline water (pH 7) 25 mL, Time of incubation 36 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h, Surfactant SDS (Shi-I) Chitosan (Shi-II). Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

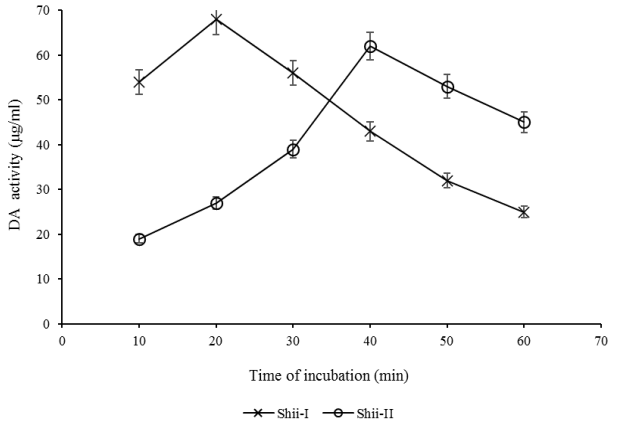


Fig. 9: Effect of different time of incubation on DA activity. *Pretreatment 1-butanol, Biomass 2 g, Saline water (pH 7) 25 mL, Time of incubation 36 h, Temperature 37°C, Reaction temperature 50°C, Reaction time 1 h, Surfactant SDS (Shi-I) Chitosan (Shi-II), Tyrosinase assay 60°C (Shi-I) 40°C (Shi-II).Y-error bars indicate the values of standard deviation (± SD) from the sum mean at a level of 5%. The values in each set differ significantly at $P \leq 0.05$

reaction. As incubation period was increased from 50 to 70 min there was a two-fold increase in DA activity. However, when the time of incubation was increased above 70 min, the activity of DA declined effectively.

Conclusion

In the present study, two strains of *L. edodes*, commonly known as shiitake mushroom were used as a potential source of enzyme tyrosinase for enhanced activity of dopamine (DA). Prior to the optimization of some significant parameters, the mushroom types *i.e*., Shii-I and Shii-II exhibited 17 and 15 μg/mL of DA, respectively in the reaction mixture. The optimization of harvesting and reaction conditions particularly pretreatment of mushroom biomass by 1-butanol, SDS & chitosan as surfactants and 36 h of incubation improved the DA activity by 4-fold. The maximal DA activity was found to be 68 μg/mL during the course of study. Overall, the results both in terms of enzyme and DA activity of shii-I and shii-II mushrooms are highly encouraging ($P \leq 0.05$), thus indicating the viability of the process used.

Acknowledgements

The authors highly acknowledge Higher Education Commission of Pakistan and GC University Lahore to provide necessary facilities for conduct of this research work.

Author Contributions

SA and MUA planned the experiments, HZ and HU conducted the experiments. SA, MUA and SS supervised the experimental work. MS and AI facilitated in data analysis. HZ and SA managed the article write up. SS and MUA helped in article formatting.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

References

Ali S, W Nawaz (2016). Optimization of nutritional requirements for dopamine synthesis by calcium alginate entrapped mutant strain of *Aspergillus oryzae* EMS-6. *Nat Prod Res* 31:281–288

Ali S, JL Shultz, IU Haq (2007). High performance microbiological transformation of L-tyrosine to L-dopa by *Yarrowia lipolytica* NRRL-143. *BMC Biotechnol* 7:50

Arnow LE (1937). Colorimetric determination of the components of L-3, 4 dihydroxyphenylalanine-tyrosine mixture. *J Biol Chem* 118:531–537

Cai Y, Y Gong, W Liu, Y Hu, L Chen, L Yan, Y Zhou, Y Bian (2017). Comparative secretomic analysis of lignocellulose degradation by *Lentinula edodes* grown on microcrystalline cellulose, lignosulfonate and glucose. *J Proteom* 163:92–101

Cateni F, ML Gargano, G Procida, G Venturella, F Cirlincione, V Ferraro (2022). Mycochemicals in wild and cultivated mushrooms: Nutrition and health. *Phytochem Rev* 21:339–383

Chang TS (2009). An updated review of tyrosinase inhibitors. *Intl J Mol Sci* 10:2440–2475

Cieńska M, K Labus, M Lewanczuk, T Kozlecki, J Liesiene, J Bryjak (2016). Effective L-tyrosine hydroxylation by native and immobilized tyrosinase. *PLoS One* 11:e0164213

Copeland RA (2023). *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 3rd edn. John Wiley & Sons Publishing, New York, USA

Donzella S, A Colacicco, L Nespoli, ML Contente (2022). Mimicking natural metabolisms: Cell-free flow preparation of dopamine. *Chem Biol Chem* 23:e202200462

Edling C, B Hellman, B Arvidson, J Andersson, P Hatrvig, A Lilja, S Valind, B Langstorm (1997). Do organic solvents induce changes in the dopaminergic system? Positron emission tomography studies of occupationally exposed subjects. *Intl Arch Occup Environ Health* 70:180–186

El-Hadi AA, S Abu El-Nour, A Hammad, Z Kamel, M Anwar (2014). Optimization of cultural and nutritional conditions for carboxymethylcellulase production by *Aspergillus hortai*. *J Radiat Res Appl Sci* 7:23–28

Fan YF, SX Zhu, FB Hou, DF Zhao, QS Pan, YW Xiang, XK Qian, GB Ge, P Wang (2021). Spectrophotometric assays for sensing tyrosinase activity and their applications. *Biosensors* 11:290

Faria ROD, VR Moure, MALA Amazonas, N Krieger, DA Mitchell (2007). The biotechnological potential of mushroom tyrosinases. *Food Technol Biotechnol* 45:287–294

Flurkey WH, JK Inlow (2017). Use of mushroom tyrosinase to introduce michaelis-menten enzyme kinetics to biochemistry students. *Biochem Mol Biol Educ* 45:270–276

Garcia-Molina P, F Garcia-Molina, JA Teruel-Puche, JN Rodriguez-Lopez, F García-Canovas, JL Munoz-Munoz (2022). Considerations about the kinetic mechanism of tyrosinase in its action on monophenols: A review. *Mol Catal* 518:112072

Halaouli S, M Asther, JC Sigoillot, M Hamdi, A Lomascolo (2006). Fungal tyrosinases: New prospects in molecular characteristics, bioengineering and biotechnological applications. *J Appl Microbiol* 100:219–232

Jimenez M, K Kameyama, WL Maloy, Y Tomita, VJ Hearing (1998). Mammalian tyrosinase: Biosynthesis, processing, and modulation by melanocyte-stimulating hormone (melanogenesis/melanoma/ melanotropin/ pigmentation/ monophenol anonooxygenase). *Proc Nat Acad Sci 85*:3830–3834

Jin W, SJ Stehbens, RT Barnard, MA Blaskovich, ZM Ziora (2024). Dysregulation of tyrosinase activity: A potential link between skin disorders and neurodegeneration. *J Pharm Pharmacol* 76:13–22

Kandaswami C, CS Vaidyanathan (1973). Enzymatic assay of tyrosinase catechol oxidase activity. *J Biol Chem* 248:4035–4039

Koyanagi T, A Nakagawa, H Sakurama, K Yamamoto, N Sakurai, Y Takagi, H Minami, T Katayama, H Kumagai (2012). Eukaryotic-type aromatic amino acid decarboxylase from the root colonizer *Pseudomonas putida* is highly specific for 3,4-dihydroxyphenyl-L-alanine, an allelochemical in the rhizosphere. *Microbiol* 158:2965–2974

Lantto R, E Puolanne, K Kruus, J Buchert, K Autio (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. *J Agric Food Chem* 55:1248–1255

Lantto R, P Plathin, M Niemist, J Buchert, K Autio (2006). Effects of transglutaminase, tyrosinase and freeze-dried apple pomace powder on gel forming and structure of pork meat. *Food Sci Technol* 39:1117–1124

Li T, N Zhang, S Yan, S Jiang, H Yin (2021). A novel tyrosinase from *Armillaria ostoyae* with comparable monophenolase and diphenolase activities suffers substrate inhibition. *Appl Environ Microbiol* 87:e00275-21

Missale C, SR Nash, SW Robinson, M Jaber, MG Caron (1998). Dopamine receptors: From structure to function. *Amer J Physiol* 78:189–224

Morrison ME, MJ Yagi, G Cohen (1985). *In vitro* studies of 2,4-dihydroxyphenylalanine, a prodrug targeted against malignant melanoma cells. 1985. *Proc Natl Acad Sci* 82:2960–2964

Nagatsu T, A Nakashima, H Watanabe, S Ito, K Wakamatsu, FA Zucca, JM Dijkstra (2023). The role of tyrosine hydroxylase as a key player in neuromelanin synthesis and the association of neuromelanin with Parkinson's disease. *J Neur Transm* 130:611–625

Narasimhappa P, PC Ramamurthy (2023). Bio-mimicking the melanogenesis pathway using tyrosinase for dopamine sensing. *Electrochim Acta* 469:143187

Richmond AM, KE Lyons, R Pahwa (2023). Safety review of current pharmacotherapies for levodopa-treated patients with Parkinson's disease. *Expert Opin Drug Saf* 22:563–579

Sato T, K Kanda, K Okawa, M Takahashi, H Watanabe, T Hirano, K Yaegashi, Y Sakamoto, H Uchimiya (2009). The tyrosinase-encoding gene of *Lentinula edodes*, Letyr, is abundantly expressed in the gills of the fruit-body during post-harvest preservation. *Biosci Biotechnol Biochem* 73:1042–1047

Sys M, M Oblukova, V Kolivoska, R Sokolova, L Korecka, T Mikysek (2020). Catalytic properties of variously immobilized mushroom tyrosinase: A kinetic study for future development of biomimetic amperometric biosensors. *J Electroanal Chem* 864:114066

Wang X, L Chen, S Xia, Z Zhu, J Zhao, JM Chovelon, NJ Renaul (2006). Tyrosinase biosensor based on interdigitated electrodes for herbicides determination. *Intl J Electrochem Sci* 1:55–61

Yang Z, J Deng, LF Chen (2007). Effect of ionic and non-ionic surfactants on the activity and stability of mushroom tyrosinase. *J Mol Catal B Enzym* 47:79–85

Zaidi KU, AS Ali, SA Ali, I Naaz (2014a). Microbial tyrosinases: Promising enzymes for pharmaceutical, food bioprocessing, and environmental industry. *Biochem Res Intl* 14:854687

Zaidi KU, SA Ayesha, A Sharique (2014b). Purification and characterization of melanogenic enzyme tyrosinase from button mushroom. *Enzyme Res* 14:120739

Zhang Y, S Qi, Z Liu, Y Shi, W Yue, C Yi (2016). Rapid determination of dopamine in human plasma using a gold nanoparticle-based dual-mode sensing system. *Mater Sci Eng C* 61:207–213