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A Study on Extracellular Tyrosinase Producing Actinomycetes

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Abstract- Actinomycetes isolation, characterisation, and biotechnological uses, with a focus on Streptomyces species for the synthesis of extracellular tyrosinase. Using morphological, biochemical, and molecular criteria, actinomycetes were identified from samples taken from a variety of habitats, including freshwater, soil, and marine systems. Their capacity to produce enzymes, beneficial chemicals, and most notably melanin through copper-dependent tyrosinase activity is highlighted in the paper. Enzyme yield and melanin synthesis were maximized by optimizing fermentation and physicochemical conditions. Important discoveries include the discovery of strong Streptomyces isolates with high tyrosinase activity, which have uses in environmental remediation, bioprocessing, and biosensors. Techniques including qRT-PCR, SDS-PAGE, and UV-V is spectrophotometry confirmed the genetic expression, activity, and purification of tyrosinase. This study highlights the strain-specific adaptability of actinomycetes, indicating that more screening and genetic advancement will enable them to reach their full potential in industry and medicine. The complete examination of the attached files served as the foundation for all findings and conclusions, guaranteeing the incorporation of all pertinent information.

Keywords: Actinomycetes; Streptomyces; Tyrosinase; Melanin; Bioprocessing.

I. INTRODUCTION

Actinomycetes are Gram-positive bacteria with DNA that contains a high percentage of G+C (>55%). Actinomycetes are today recognized as prokaryotic creatures however, they were once thought to be a group that lay in between bacteria and fungi. These are saprophytic bacteria that are widely distributed in water, soil, and colonizing plants. The Streptomyces genus contains several species that produce bioactive compounds, including pigments, antibiotics, and numerous extracellular enzymes. Their ability to provide tyrosine was examined to a far lower degree.

Furthermore, this group of Actinomycetes can produce and excrete dark pigments called melanin or melanin when grown on organic substrates. These pigments are thought to be helpful criteria in taxonomic research (Shivaveerakumar and Hire math 2018; Dhahbi et al., 2025).

Fig 1: Isolation of actinomycetes on Starch Casein Agar medium (Sapkota et al., 2020).

Actinomycetes are now recognized as prokaryotic creatures; however, they were once thought to be a group that lay in between bacteria and fungi. These are saprophytic bacteria that are widely distributed in water, soil, and colonizing plants. (Roes- Hill et al., 2018; Groth et al., 1999; Tiwari and Gupta. 2013; Mochon et al., 2016 and Harir 2017 and Shivaveerakumar and Hiremath 2018).

Bioactive substances including antibiotics, pigments, and several extracellular enzymes have all been investigated in relation to the Streptomyces genus. for example, cellulase, amylase, glucose isomerase, and proteases. All Streptomyces species synthesize melanin or melanin-like pigments, and tyrosinase

contributes to melanin generation. A copper-containing monooxygenase enzyme that belongs to the oxidoreductase family and is also referred to as polyphenol oxidase is tyrosinase (monophenol, L-dopa: oxygen oxidoreductase). Two distinct enzymatic processes, such as diphenolase or catalase activity and monophenolase or cresolase, are catalyzed by molecular oxygen (Gajanan et al., 2020' (Gopalakrishnan et al. 2020; Meliani et al., 2022; Nazari et al., 2022 and Roes-Hill et al., 2017).

Beijerinck investigated how actinomycetes and other bacteria produce dark colors in 1900, 1911, and 1913. When he put any of these compounds to a medium, he discovered that "A. chromogenus" formed the black pigment he named melanin from peptone but not from free tyrosine. According to him, the pigment was a byproduct of the organic nitrogen's catabolism. Tyrosinase is a copper containing metalloprotein found universally in nature, from prokaryotes to eukaryotes, including microorganisms, plants, and animals. This bifunctional monooxygenase catalyzes two key reactions: the hydroxylation of monophenols (cresolase activity) and the subsequent oxidation of the resulting o-diphenols to quinones (catecholase activity). The monophenolase activity is the initial and rate-limiting step of this process (Pretzler and Rompel 2024).

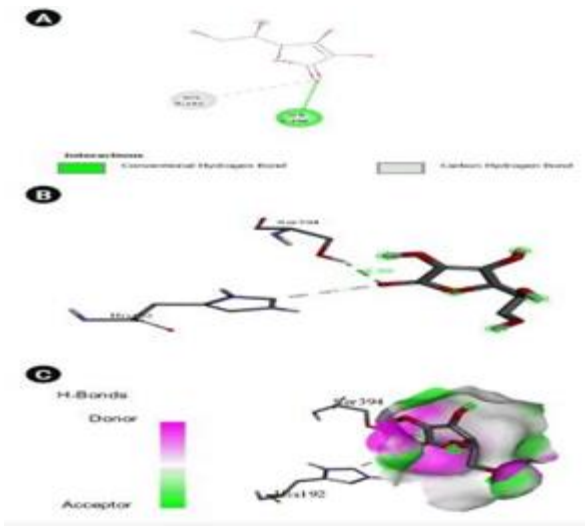

Fig 2: 2D interaction of human TYR and ascorbic acid. (B) 3D interaction of human TYR and ascorbic acid. (C) H-bonds interaction of human TYR and ascorbic acid graphical representation Because of its catalytic properties, tyrosinase has various industrial applications. It is used in food and nonfood processes, such as engineering the structure of meat products and tailoring polymers in material science, for example, grafting silk proteins onto chitosan. Traditionally, tyrosinase has been sourced from plant-based foods like tea, coffee, raisins, and cocoa, where it contributes to their unique flavors. However, the tyrosinases from fruits and vegetables are also responsible for unwanted browning reactions. (Lakkireddy et al., 2012).

Sources of Tyrosinase

Tyrosinase, an enzyme with a wide range of applications, can be sourced from various organisms, including fungi, bacteria, and plants.

- Bacterial Tyrosinase Bacterial tyrosinases, particularly those from Streptomyces species, are well-understood. This extracellular enzyme plays a key role in the production of melanin. Other bacterial sources include (Claus and Decker 2006). Rhizobium, Symbiobacterium thermophilum, Pseudomonas maltophilia, Sinorhizobium meliloti, Marinomonas mediterranea, Thermomicrobium roseum, Bacillus thuringiensi, Pseudomonas putida, Streptomyces castaneoglobisporus, Ralstonia solanacearum, Verrucomicrobium spinosum (Zaidi et al., 2014).

Properties of Various Kinds of Tyrosinase

Tyrosinases, while sharing a common function, exhibit diverse properties depending on their source. Here's a breakdown of the characteristics of mushroom, mammalian, and human tyrosinases.

Mammalian Tyrosinase

Mammalian tyrosinase is a single membrane-spanning protein with significant structural and functional diversity across species and tissues. Its catalytically active site is located within organelles called melanosomes. (Obaid et al., 2021).

Human Tyrosinase

In humans, melanin is produced in melanosomes, which are found in skin and hair melanocytes, as well as in retinal pigment epithelium cells. Melanin

biosynthesis involves 3 key tyrosinase like enzymes tyrosinase (TYR), and tyrosinase-related proteins 1 and 2 (TYRP1 and TYRP2) (Obaid et al., 2021).

II. DISTRIBUTION AND HABITAT OF ACTINOMYCETES PRODUCING TYROSINASE

Nature and habitat

Actinomycetes are primarily found in soil, plant remnants, water body silt, and the atmosphere. In the soil, they are the most prevalent organisms that create filaments that resemble threads. The distinctive "earthy" scent of just turned, healthy soil is caused by these hyphae-like fungi. A pervasive group of bacteria found in natural ecosystems worldwide, (Bhatt et al., 2011).

Terrestrial habitat

environments and are found in natural ecosystems all over the world. Many researchers have gathered actinobacteria from diverse terrestrial environments and examined their potential to produce novel medicinal chemicals. Despite the extensive research being done now, just a small percentage of the variety of cultivable actinobacteria has been identified (Tennalli et al., 2017).

Rhizosphere soil

By stimulating growth and yield, the microorganisms in the rhizosphere help plants. Plant growth-promotion (PGP) and the cycling of soil nutrients are two functions of actinomycetes, which are a significant component of rhizosphere microbial communities. PGP chemicals, lytic enzymes, and antibiotics are examples of secondary metabolites that actinomycetes creat (Sreevidya et al., 2016).

Aquatic habitat

Fresh water habitat

Freshwater lakes are full of actinomycetes. They thrived at 60°C and are also discovered in sewage. Numerous organisms from the watery environments of the Actinoplanes, Micromonospora, Rhodococcus, Streptomyces, and endospore-forming Thermoactinomycetes genera have been isolated. It is likely that the majority of these actinomycetes are washed in from the land and gathered in freshwater environments. (Batti et al., 2016).

Marine habitat

Their presence on algae floating on the sea's surface, or the fact that water samples were collected close to docks, actinomycetes were thought to exist in marine environments. Since there are no discernible morphological or biochemical changes between the marine and terrestrial isolates, actinomycetes may have evolved on land but have acclimated to the marine water's salinity level (Bhat et al., 2017).

Marine organisms

Marine species have rather different secondary metabolites and live in quite different habitats than terrestrial ones. The water is home to a vast and extensive symbiotic connection between marine creatures and microbes. Crops and marine animals including sponges, sea squirts, corals, worms, and algae are home to a diverse variety of symbiotic microbes. (Srinivasa et al., 2021)

Extreme environment

Actinomycetes can also be found in harsh environments. Around mineral springs, alkaline soils (pH 10–12) are home to alkalophilic actinomycetes (the predominant genera are Streptomyces and Nocardiopsis). Bogoriella caseilytica, a new genus and species of alkalophilic actinomycetes, was isolated from a soda lake soil with a pH of 10. Discovered the halophilic actinomycete Saccharomonospora halophila from marsh soil, which grows best at 10% NaCl (Batti et al., 2016).

III. DIVERSITY OF MARINE ACTINOMYCETES IN INDIA

Isolated by New actinomycetes have also been isolated from marine creatures, according to reports. Rhodococcus, Micromonospora, and Streptomyces are the most commonly reported. These isolates are members of several rare genera, including Actinoplanes, Actinobispora, Actinopolyspora, Actinokineospora, Saccaropolyspora, Streptosporangium, Actinosynnema, Catellospora, Micromonospora, Strptoverticillium,

Micromonospora, Dactylosporangium, Actinomadura, and Kitasatospora, in addition to Streptomyces. Known compounds have become rediscoverable as a result of the separation and utilisation of actinomycetes from typical marine milieu. Different actinomycetes from harsh settings, however, offer a greater chance of finding novel compounds with promising properties that may be marketed. Physio- chemical settings that differ significantly from the ideal conditions that aid in life are referred to as extreme environments (Batti et al., 2016; Sarkar et al., 2022).

IV. TYROSINASE REACTION OF ACTINOMYCETES

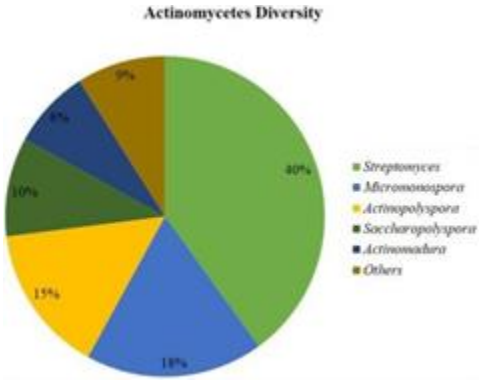

Fig: 3. Reports on different genera of actinomycetes reported from India in the past decade (2008–2017) (Sarkar and Suthindhiran 2022; Sarkar et al., 2022).

Beijerinck investigated how actinomycetes and other bacteria produce dark colors. When one or both of these compounds were given to a medium, he discovered that "A.chromogenus" generated the black pigment he named melanin from peptone but not from free tyrosine. He believed the organic nitrogen to be the catabolic source of the pigment. Tyrosine generated a strong black hue, which he discovered when he separated many vibrios from sewage, Many other microorganisms, including yeasts (like Monilia nigra) and bacteria (like Azotobacter chroococcum, Bacillus aterrimus, and Bacillus niger), are also known to produce brown or black pigments. These findings align with previous observations by Conn and Waksman on similar actinomycetes species (Meehl 1938) (Chaudhary et al., 2013). (Adegboye et al., 2018; Subhash & Kulkarni. 2015)

Bioproduction of enzyme

To produce the enzyme in larger quantities, the researchers grew pre cultures of the two strains in MPPM. However, they used different conditions for each strain: S. polyantibioticus was grown at a pH of 5.5 and incubated for 48 hours at 22°C, shaking at 160 rpm.S. pharetrae was grown at a pH of 6.5 and incubated for 48 hours at 30°C, shaking at 160 rpm.(Shivaveerakumar et al., 2013).

Microbial Melanin Production

Microorganisms like bacteria and fungi produce melanin, a valuable natural pigment. This microbial source is particularly appealing because its production isn't limited by seasons and is both cost effective and environmentally friendly.

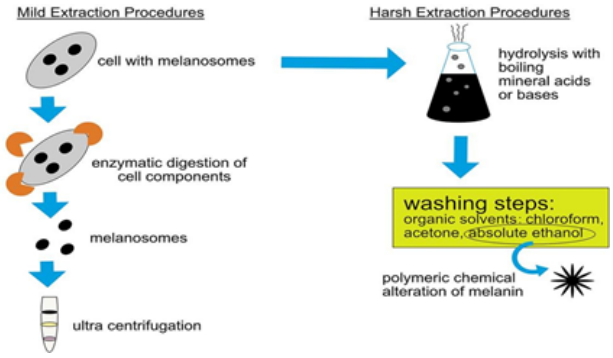

Fig. 4. Mild and harsh melanin extraction steps currently used to obtain melanin from living cells (Tran-Ly et al., 2020; Yardman-Frank and Fisher 2021; Davis and Callender 2010; Dereure 2001; Mahrous et al., 2025)

Physicochemical Properties

Solubility is a key initial characteristic used to identify melanin. It is soluble in solvents like dimethyl sulfoxide (DMSO), alkaline water (pH > 8), and phosphate buffered saline (pH 7.2), but largely insoluble in water and many other solvents. (Wibowo et al., 2022; Qiu et al., 2023). Characterization of NMR Spectroscopy (Duff et al., 1988). High-Performance Liquid Chromatography (HPLC) (Omar et al., 2025).

V. OPTIMIZING MICROBIAL MELANIN PRODUCTION

The provided table summarizes various studies focused on optimizing the production of melanin from different microorganisms. Researchers have explored a range of factors, including the type of microorganism, the specific type of melanin produced, and the conditions under which it's synthesized. The studies show that both bacteria and fungi can be used to produce melanin. For instance, bacteria like Streptomyces kathirae SC-1 and demonstrated high melanin yields of 13.7 g/L and 7.6 g/L, respectively. Many of these studies focused on the effects of adding specific compounds to the growth medium.

A common strategy was to add tyrosine or L-DOPA (3,4-dihydroxyphenylalanine), which are precursors to melanin. Metal ions, such as copper (Cu), iron (Fe), and magnesium (Mg), were also frequently added to enhance production. Additionally, a variety of substrates were used, including simple sugars like glucose, complex components like yeast extract and peptone, and even fruit waste extract. These findings highlight that tailoring the growth conditions and nutrient availability is crucial for maximizing melanin production from different microbial sources. (Tran-Ly et al., 2020).

Tyrosinase Assay and Identification

To quantify the tyrosinase activity, a reaction mixture containing phosphate buffer, L- tyrosine solution, crude enzyme (from the culture supernatant), and water was prepared. The mixture was oxygenated, and the absorbance was measured at 280 nm using a UV-Vis spectrophotometer. The enzyme activity was then calculated using a specific formula. One unit of enzyme activity was defined as the amount that causes an increase in absorbance of 0.01 per minute (Dobara et al., 2019).

Primary and Secondary Screening for Tyrosinase

The primary screening for tyrosinase-producing Streptomyces was performed using skimmed milk plates. The plates, with a pH between 6.5 and 7.2, were incubated at 30°C for 2-3 days. A clear zone around a colony indicated a positive result (+), while no clear zone indicated a negative result (-). The isolates that showed a positive result were then subjected to further secondary (Subhash and Kulkarni 2015).

Optimization of Tyrosinase enzyme:

The process for producing tyrosinase involves several key steps, starting with the development of a microbial inoculum. The inoculum for fermentation is prepared by introducing a suitable microbial isolate into a sterile peptone yeast extract iron broth. This mixture is incubated at 30°C on a rotating shaker at 100 rpm for 3 to 5 days, until the spore concentration reaches approximately 1.4×106 spores/ml. The tyrosinase fermentation itself is conducted in a sterile basal fermentation medium. A 2% (v/v) portion of the 24-hour-old inoculum is added to the medium, which is then incubated at 30°C for up to 144 hours on a 100 rpm rotating shaker. Tyrosinase activity is measured every 24 hours to monitor production.

To maximize tyrosinase production, the fermentation parameters are optimized. This involves using a "one factor at a time" method, where one variable is adjusted while all others remain constant. Both physicochemical factors (such as pH, temperature, and agitation speed) and nutritional factors (carbon, nitrogen, and mineral sources) are tested. After determining the optimal conditions, tyrosinase is produced using the refined set of parameters. This includes a fresh medium with optimal concentrations of 0.4% glucose (carbon source), 0.6% yeast extract (nitrogen source), and 0.02% CuSO4 (mineral source), at a pH of 8.5. The medium is inoculated with 2% of the 24-hour-old inoculum and incubated at the optimal temperature of 35°C on a rotary shaker at 150 rpm for up to 144 hours. Tyrosinase activity is recorded every 24 hours, and the resulting fermented broth is then ready for further purification (Mali & Gare 2020) In an investigation of tyrosinase activity, researchers used both free and immobilized tyrosinase enzymes to study melanin production.

They first created a standard curve by measuring the absorbance of known melanin concentrations at 470 nm. Then, they tested how different conditions

affected melanin production by measuring the absorbance of the enzyme at 470 nm under the following conditions (El-Aziz et al., 2023).

Effect of pH

To find the ideal pH for tyrosinase to oxidize L-DOPA, a variety of buffers were used to create a range of pH conditions. Specifically, the researchers used:Sodium acetate buffers for a pH range of 3.0 to 5.5, Potassium phosphate buffers for a pH range of 6.0 to 7. Tris-HCl buffers for a pH range of 8.0 to 10.0

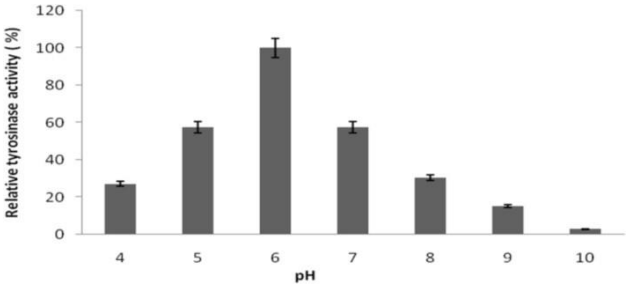

Fig: 7. Effect of pH on the tyrosinase activity

Effect of Temperature

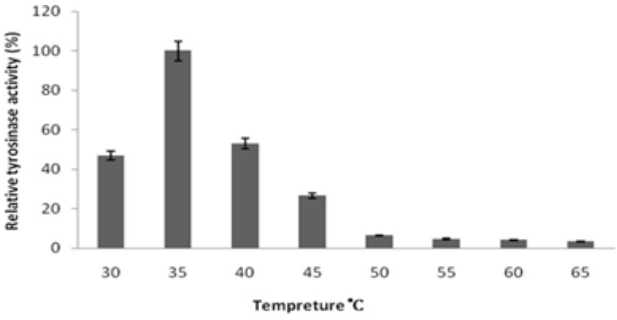

Fig: 8. Effect of pH on the tyrosinase activity

To see how temperature influences the enzyme's activity, the researchers measured L- DOPA oxidation at various temperatures from 5°C to 70°C. The tyrosinase and L-DOPA were incubated together in a temperature-controlled system at 30°C, 40°C, 50°C, 60°C, and 700C. (Harir, et al., 2018; El-Aziz et al., 2024).

VI. ENZYME PURIFICATION

a. **Binding:** The cell lysate was mixed with the Tyr-Si-CMC-MNPs for two hours at 25°C. The tyrosinase enzyme attached to the nanoparticles through an interaction with the tyrosine ligand on their surface.

b. Separation: An external magnetic field was used to pull the nanoparticles—now bound to the tyrosinase out of the solution, leaving behind other proteins.

c. Washing: The magnetic nanoparticles were washed with a phosphate buffer to remove any proteins that were not specifically bound.

d. Elution: To release the tyrosinase, an elution buffer containing l-tyrosinamide was used.

The l-tyrosinamide competed with the tyrosine residues on the nanoparticles, causing the enzyme to detach. (Quino et al., 2025). To begin the purification process, the culture broth was lyophilized after 48 hours. A crude enzyme extract was then prepared by adding 100 mL of 50 mM phosphate buffer at a pH of 7 to the lyophilized broth. Next, proteins were precipitated by adding ammonium sulfate to achieve a saturation of 35-80%. The precipitated proteins were then collected and resuspended in 30 mL of the same phosphate buffer. This protein solution underwent dialysis for 24 hours. Finally, the enzyme was further purified using two chromatography columns: first, an anion exchange column (DEAE-Sepharose), followed by a gel filtration column Quino et al., 2025).

SDS-PAGE Analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to analyze the protein samples. The process involved:

e. Sample Preparation: A loading buffer was added to the protein samples, which were then diluted to a concentration of 15 µg per lane.

f. Electrophoresis: The proteins were separated in an electrophoresis unit at 120V for 2.5 hours using a separating gel and a stacking gel.

g. Visualization: After separation, the gel was stained with Coomassie brilliant blue G-250 for one hour to visualize the protein bands, then destained with distilled water. (El-Aziz et al., 2023).

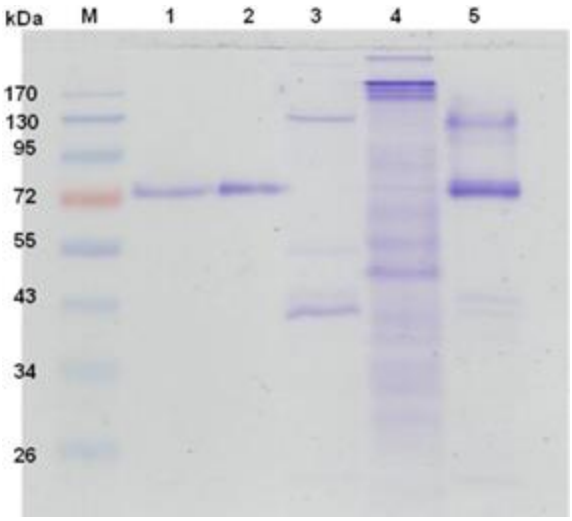

Fig. 7 SDS-PAGE of tyrosinase produced by the transformant A412-3 of A. rouxii. Numbers in the gel lanes indicate the fractions obtained in each purification step. M) Molecular weight marker (PageRuler™ prestained Protein Ladder). 1 and 2) Fraction 6 from anionic exchange column; 3) Fraction 23 from filtration gel column; 4) Fraction 14 from anionic exchange column; 5) Commercial tyrosi nase from anionic exchange column

Western blotting:

To perform a Western blot analysis, B16 cells were grown in 6-well plates and treated with varying concentrations of undecylprodigiosin. The next day, the cells were washed with cold PBS, and proteins were extracted using a specific RIPA buffer and protease inhibitors. After centrifugation, the protein content of the collected supernatant was measured using a Pierce BCA Protein Assay Kit. Equal amounts of protein were then separated by size using 10% SDS-PAGE.

The proteins were then transferred from the gel to a nitrocellulose membrane. The membrane was blocked to prevent nonspecific antibody binding, then incubated overnight at 4◦C with primary antibodies. Following this, the membrane was incubated with secondary antibodies and treated with a Clarity™ Western ECL substrate to produce a chemiluminescent signal. The resulting images of the protein bands were captured using an iBright™ CL750 Imaging System. Actin was used as a loading control to ensure that equal amounts of protein were loaded into each lane (Shi et al., 2023).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

To measure the expression of genes related to melanogenesis, B16 cells were grown and treated with different amounts of undecylprodigiosin for 24 hours. After treatment, the cells were washed, and their total RNA was isolated using the TRIzol reagent. This RNA was then converted into complementary DNA (cDNA) using the GoScript Reverse Transcription System. The relative amount of the target cDNA was then measured with a BioRad CFX Connect Optics Module and Green Supermix. To ensure accuracy, all results were standardized against the expression level of the housekeeping gene, β-actin. To prepare for a genetic study, the bacterial strains were grown in a liquid medium called tryptic soy broth (TSB) at 30 °C for three days. After cultivation, the cells were harvested, washed, and freeze- dried (Lee et al., 2024; Yardman-Frank and Fisher 202 and Fairhead and Meyer 2010).

VII. APPLICATIONS OF TYROSINASES ENZYME

Tyrosinases, particularly the commercially available mushroom enzyme, have a wide range of applications, many of which are also considered feasible for bacterial tyrosinases due to their similar reactivity. These applications leverage the enzyme's ability to oxidize both small phenolic molecules and the tyrosine side chains in proteins. The versatility of tyrosinases has led to several patent applications for enzymes from various sources, including V. spinosum, R. etli, S. antibioticus, and Pseudomonas sp. The enzyme can be used in itsisolated form, or in some cases, natural tyrosinase-producing bacterial strains can be employed for processes like bioremediation (Irfan et al., 2024).

Molecular Biology

- **Gene expression reporter:** The chromogenic reaction catalyzed by tyrosinase from

S. glaucescens has been used as a tool to monitor gene expression.

- **Strain detection:** Tyrosinase from R. etli has been used to detect bacterial strains that produce L-tyrosine.

Biosynthesis and Medical Applications

- **Production of L-DOPA:** Tyrosinases are used in the production of L-DOPA, a crucial drug for Parkinson's disease treatment. While chemical synthesis is the current industrial standard, enzyme-based processes using free or immobilized tyrosinase are being explored.
- **Synthesis of other compounds:** Tyrosinases can produce substituted catechols, which are important intermediates for pharmaceuticals, plastics, and antioxidants. They can also be used to synthesize natural compounds with estrogenic activity (e.g., coumestan) and the antioxidant hydroxytyrosol.
- **Medical treatments:** The enzyme is used to produce melanins for therapeutic purposes, such as natural antibacterial agents for wound treatment. It has also been studied for its potential role in neurological diseases and for measuring cholesterol levels in skin tests (Felix. 2025).

Bioremediation of Wastewaters

- **Removal of pollutants:** Bacterial and mushroom tyrosinases are effective in detoxifying wastewaters by removing phenolic and substituted phenolic contaminants. This can be done using tyrosinase-producing strains or the immobilized enzyme.
- **Decolorization:** Tyrosinases can degrade colored dye molecules from textile industry effluents, offering a potential solution for wastewater decolorization.
- **Reduction of COD:** The enzyme can be used to reduce chemical oxygen demand (COD) in wastewater.

Material Science and Engineering

- **Cross linking and functionalization:** The cross linking activity of tyrosinase is used to functionalize materials like chitosan. It can create covalent protein-polysaccharide bioconjugates, which modify material properties such as particle size, thermal stability, and adhesiveness.
- **Adhesives:** Tyrosinases, particularly mushroom tyrosinase, can be used to produce adhesives from polyphenolic proteins or by reacting with dopamine in the presence of chitosan.
- **Textile treatment:** The enzyme can be used to functionalize wool fibers and silk fibroin with other proteins (e.g., collagen, elastin) to impart bactericidal and fungicidal properties. (Li et al., 2023)

Food and Feed Applications

- **Food processing:** Tyrosinase is used in tea production due to its melanogenic reaction. Its cross-linking activity can modify the texture and structure of food, such as dairy products, meat, and bread.
- **Enhancing food properties:** The enzyme improves the firmness of gels made from milk and sodium caseinate and enhances the hardness of dough. It can also create a protein network in low-meat chicken breast gels.
- **Animal feed:** In animal feed, mushroom tyrosinase can increase the bioavailability of iron.

Field	Mode of action
Production of dyes	1. Oxidation of an aromatic phenolic compound by tyrosinase and production of coloured compounds
Cosmetic applications	2. Self-tanning agent
Biosensors	1. As single enzymes 2. In a coupled assay to detect catechol-producing compounds, e.g. hormones, salicylate
Biosynthesis and medical applications	1. Production of L-DOPA 2. Production of substituted catechols 3. Production of food additives 4. Production of estrogenic compounds 5. Production of melanins for therapeutic uses 6. Treatment of neurological diseases 7. Production of mosquito repellants 8. Assay of cholesterol on the skin
Bioremediation of wastewaters	1. Removal of phenolic and substituted phenolic contaminants 2. Reduction of COD 3. Decolourisation (degradation of dyes)
Materials	1. Production of polyphenolic polymers 2. Modifications of materials 3. Production of adhesives 4. Film production 5. Treatment of textiles
Food applications	1. Production of tea 2. Production of dairy products 3. Cross-linking of meat proteins
Others	1. As a reporter protein for counting microorganisms 2. Identification of bacteria producing L-tyrosine

Table: 4. Representative applications of tyrosinase in different fields.

- **Dyes and Cosmetics Dyeing and coloring:** Tyrosinase is used to synthesize dyes and coloring solutions. It has been proposed for dyeing animal fibers and for use in hair coloring compositions. Cosmetics: The enzyme is also suggested as a self-tanning agent.
- **Biosensors Detection of phenolic compounds:** Tyrosinases from various sources have been used to develop biosensors that can detect even minute amounts of phenolic compounds in samples Food quality control: A tyrosinase-based biosensor has been developed to quantify toxic cyanogenic glycosides in fruits. The enzyme is also used to determine the phenolic content of juices, tea, and jams (Gopal et al., 2025)
- **Cell Culture:** Tyrosinase has been used in cell culture to promote the growth of nerve cells. This involves stamping the enzyme onto plastic surfaces, which leads to the formation of thin melanin films. These melanin films have a bacteriostatic effect, which can help prevent bacterial contamination in the culture.
- **Enzyme Immobilization:** To enhance its stability and reusability, tyrosinase is often immobilized. This process is crucial for converting L-tyrosine to L-DOPA. The enzyme can be entrapped in various materials, including natural polymers, modified polystyrene, or adsorbed onto surfaces like nylon zeolite, glass beads, fuller's earth, and chitin activated with hexamethylenediamine.
- **Material Modification and Grafting:** Tyrosinase's cross-linking ability is utilized to modify polymers. For instance, silk proteins have been grafted onto chitosan using tyrosinase to tailor the properties of the resulting polymers. Similarly, L-DOPA has been successfully grafted onto wool fiber proteins. The enzyme is also used for biocatalytic grafting of phenolic groups or proteins onto chitosan.
- **Hydrogels and Tissue Engineering:** The enzyme is instrumental in the creation of hydrogels for use as skin substitutes, matrices for drug delivery, and in tissue engineering applications (Li et al., 2023; Badali et al., 2021).

- **Synthesis of Bioactive Compounds:** Immobilized tyrosinase has been used for the efficient and selective synthesis of bioactive catechols under mild, environmentally friendly conditions. These biocatalysts have been characterized for their morphology and reusability. Tyrosinase and tyrosinase supported by layer-by- layer methods are also employed in the synthesis of lipophilic catechols. These derivatives have shown significant antiviral and antioxidant activity, suggesting a new inhibition mechanism based on their redox and lipophilic properties

VII. CONCLUSION

Actinomycetes, particularly Streptomyces, have proven to be promising and sustainable sources of extracellular tyrosinase. Their ability to produce stable enzymes capable of functioning under various environmental conditions makes them highly valuable for biotechnology. The extracellular nature of these tyrosinases simplifies downstream processing and supports large-scale industrial applications. Potential uses include L-DOPA production for Parkinson's disease, phenolic compound degradation in wastewater treatment, biosensor development, and organic synthesis. Strain-specific adaptability highlights the need for further screening, optimization, and improvement to enhance activity and discover novel properties. Continued research into strain improvement, enzyme immobilization, and detailed characterization will maximize their industrial and environmental benefits. Overall, Streptomyces-derived tyrosinases hold strong potential to address future pharmaceutical, industrial, and ecological challenges.

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