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Screening of a Soil Tyrosianse Producing Bacterial Strain HM24 and Comparison of its Tyrosinase Activity in Different Mediums Containing Natural Sources of L-tyrosine

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ABSTRACT

Tyrosinases are essential enzymes with diverse applications, such as breaking down phenolic compounds and producing melanin. Actinobacteria are one of the most notable tyrosinase producers. This study investigated tyrosinase-producing actinobacterial strains and evaluated enzyme activity in different sources of L-tyrosine. Twenty strains were isolated from the soil of Langar, Kerman, Iran, with characteristics of actinobacteria (gram-positive filamentous or rod-shaped bacteria and powdery consistency colonies that stick firmly to agar surface). All strains were cultured in a medium with L-tyrosine to assess tyrosinase production. The best tyrosinase-producing strain was selected based on dark-colored colony formation. Also, enzyme activity was evaluated in the four mediums with different L-tyrosine sources (Tyrosine, Glucose-Yeast-Peptone (GYP), Soybean, and Peanut). On the other, the pigment produced by actinobacteria was investigated for significant properties such as Sun Protection Factor (SPF) and antioxidant activity. The results showed that the HM24 strain is the best strain for producing tyrosinase. The results showed that the HM24 strain was the best tyrosinase-producing strain, and the most enzyme activity was in the peanut medium as well as in the soybean meal substrate. According to the 16S rRNA sequencing, the HM24 strain belonged to the genus *Microbacterium* with 99.97% identity. The pigment produced by HM24 demonstrated an SPF of 134.36 and a 69% DPPH reduction, which indicated strong antioxidant activity. These findings underscore the significant potential of actinobacterial strains in various industrial applications. Considering the production of tyrosinase enzyme by strain HM24, its ability to efficiently utilize natural L-tyrosine sources, and the biological properties of the resulting pigment, this strain holds significant potential for applications in various industrial processes.

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Introduction

Tyrosinase (TYR), also known as polyphenol oxidase, is a copper-containing enzyme found in various organisms and plays a crucial role in melanogenesis (melanin production) and browning reactions in its specific environment. Tyrosinases exhibit dynamic behavior in their active site, interacting with phenolic substrate and co-substrate oxygen. They cycle through three distinct oxidation states. In the oxy state, tyrosinase binds oxygen and enables the hydroxylation of monophenols to diphenols, transitioning to the met form. The met form is responsible for oxidizing diphenols to quinones. Subsequently, the enzyme shifts to the deoxy form, which returns to the oxy form by binding to molecular oxygen. The met form serves as the enzyme's resting state, constituting up to 85% of its presence in solution (Faccio *et al*., 2012). This industrially important enzyme can be

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produced by Actinomycetes. Tyrosinase has effective applications in medical, industrial, and agricultural fields and acts as a limiting oxidase enzyme that controls melanin production; specifically, it operates directly on two pathways of melanin synthesis: 1) hydroxylation of monophenol and 2) conversion of diphenol to o-quinone, which further leads to melanin formation through subsequent reactions. Research has demonstrated that this copper-containing enzyme regulates oxidative reactions from tyrosine to melanin and other pigments in animal and plant tissues. In plants, tyrosinase is responsible for producing the brown color under conditions such as exposure to air, as observed in crops like potatoes. Notably, tyrosinase is prominently found in melanosomes of human skin melanocyte cells. The gene encoding tyrosinase (TYR) is present in both humans and actinobacteria (Pavan *et al*., 2020). This enzyme is responsible for catalyzing the hydroxylation of tyrosine into L-DOPA and then oxidizing it to o-dopaquinone. Quinone is spontaneously converted to quinone indole, and finally, this carboxylated compound spontaneously forms DOPA melanins, also known as eumelanin (Shivaveerakumar and Hiremath, 2019). Tyrosinase exhibits a multifaceted mechanism of action when acting on monophenols. Several processes occur, including the hydroxylation of monophenols to o-diphenols, subsequent oxidation to o-quinones, and the eventual transformation toward melanin. While the enzyme's mechanism is distinct, it can exhibit variations based on the specific chemical properties of the substrate. The substrates for this enzyme are 1) L-tyrosine, the biological substrate for mammalian, fungal, and bacterial tyrosinase; 2) some substrates that are unable to accumulate o-diphenol in the medium due to their susceptibility to oxidation. These substrates require the presence of hydrogen peroxide for the enzyme to exhibit activity. An example of such a substrate is hydroquinone; 3) substrates that release o-diphenol in the medium. When the enzyme oxidizes o-diphenol, it forms a stable o-quinone. As a result, no additional o-diphenol is produced in the medium. An example of such a substrate is 4-tert-butylphenol in the medium; 4) Deoxyarbutin is an example of a substrate that does not release or generate o-diphenol in the medium. Instead, it produces a stable o-quinone (García-Molina *et al*., 2022).

Actinobacteria are one of the most diverse phyla of bacteria. Their genomes have a high G+C content and are abundant in both terrestrial and aquatic environments. Additionally, many of these bacteria are capable of forming spores and often exhibit a filamentous structure. Furthermore, while some can grow anaerobically, most genera respire aerobically. Actinobacteria exhibit a diverse spectrum in soil environments, constituting approximately 10-50% of the total microbial population in soil. With their filamentous, rod-shaped morphology, ability to form spores, and production of mycelium-like aerial hyphae, actinobacteria are easily distinguished from other bacteria. These diverse and beneficial characteristics make them unique rhizosphere microorganisms that can be used to enhance plant growth and manage diseases. Actinobacteria can also produce significant amounts of phytohormones, further aiding plant growth. In addition, actinobacteria play an important role in solving essential mineral compounds such as phosphates, zinc, and potassium in nutrient-poor soils, contributing to plant growth (Behera and Das, 2023).

Actinobacteria are important generators of natural products for discovery and producing various drugs. To date, more than half of the clinically relevant antibiotics have been produced by the genus *Streptomyces sp*. Some rare actinobacteria, distinct from *Streptomyces*, have garnered attention due to their unique physiological features. These rare strains are found in harsh environments such as arid and aquatic habitats (Liao *et al*., 2019). Actinobacteria, with other soil inhabitants such as pathogenic and symbiotic microbes, constitute approximately 20% of the microbial population in spring, over 30% in autumn, and 13% in winter. These bacteria include genera like *Streptomyces*, *Arthrobacter*, *Actinomyces*, *Corynebacterium*, *Micrococcus*, *Frankia*, and several others. The secondary metabolites of the actinobacteria play a crucial role in economically and biotechnologically relevant microbial communities. Among the 23,000 biologically active metabolites identified from microorganisms, approximately 10,000 are

produced by actinobacteria. In the actinobacterial group, the genus Streptomyces alone generates around 7,600 bioactive compounds. These metabolites exhibit diverse properties, including drug resistance suppression and other pharmaceutical benefits. In addition to antibiotics, Streptomyces also produces fermentative compounds, siderophores, and several other enzymes. These play essential roles in breaking down organic materials such as polysaccharides, cellulose, starch, chitin, organic acids, proteins, amino acids, and lipids. Beyond their medical significance, actinobacteria contribute significantly to agriculture and mineral recycling in ecosystems, mainly through the decomposition of plant biomass and the leaching of mineral substances into soil (Hazarika and Thakur, 2020; Martín *et al*., 2021). Motivated by the paramount significance of the enzyme tyrosinase in industrial applications, particularly its role in the remediation of industrial wastewater contaminated with hazardous phenolic compounds and its potential for melanin pigment or precursor production, this study aimed to investigate the isolation of tyrosinase-producing actinobacterial strains, identify a superior strain, and subsequently evaluate its tyrosinase activity level.

Materials and Methods

Sampling and culture of bacteria

A soil sample was collected from a depth of 15 cm in the Langer Mahan region of Kerman, Iran, and transported to the laboratory in sterile containers. One gram of aliquots of each sample was serially diluted in 9 mL of sterile distilled water to achieve a 1:10 dilution series. The isolation of actinobacteria was then performed using the spread plate technique on Gause agar medium containing (Starch 20 mg/ml; Agar 20 mg/ml; KNO3 1 mg/ml; NaCl 0.5 mg/ml; K_2HPO_4 0.5mg/ml; $MgSO_4$ 0.5 mg/ml; $FeSO_4$ 0.1 mg/ml; and distilled water) and the plates were incubated in 30ºC for three to seven days. Upon observing bacterial growth and colony morphology, the actinobacterial strains were cultured on a Gause medium using the same method as before. The strains confirmed based on morphology and Gram staining were then stored in glycerol stocks at -20ºC (Ait Assou *et al*., 2023; Mohammadi *et al*., 2022).

Screening of tyrosinase-producing bacteria

The isolated actinobacteria were cultured in a tyrosine agar medium (ISP7) and incubated at 30ºC for three days. After that, the colonies that produced dark brown pigment on the culture medium were selected and recultured on the tyrosine agar medium (ISP7) to confirm their characteristics. Finally, the strain that produced a darker brown pigment was chosen as the final selection (El-Naggar and El-Ewasy, 2017; Eskandari and Etemadifar, 2021).

Molecular identification of a novel strain

The bacterial DNA was extracted using the phenol-chloroform method, and gel electrophoresis was performed to assess the success of the DNA extraction using 1% agarose gel. The novel strain was identified by analyzing the 16S rRNA gene sequences. To achieve this, after DNA extraction, a PCR reaction was performed using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3'), then the amplified products were electrophoresed on 1% agarose gel. Consequently, the sequence was determined using Sanger sequencing by MacroGen South Korea (Lee *et al*., 2003; Mohammadi *et al*., 2022). The obtained sequence was compared with sequences in the NCBI database using the BLAST algorithm, and the phylogenic tree was designed by MEGAX using the neighbor-joining method with a bootstrap test of 500 replicates of phylogeny (Irshad *et al*., 2023).

Producing tyrosinase in different substrates

Novel bacterial colonies were inoculated into a 30 mL liquid ISP7 (Tyrosine broth) and synthetic culture mediums: soybean medium (Soybean meal extract 5 g; peptone 2 g; and distilled water 1 L), GYP medium (Yeast extract 5 g; Peptone 2 g; Glycerol 1ml; and distilled water 1L), and peanut medium (Peanut 5 g; peptone 2 g; and distilled water 1L) at a constant pH 6.6. The inoculated culture medium was incubated for seven days at 30°C in a shaker incubator at 100 rpm. Subsequently, 1 ml of each sample was transferred to microtubes to evaluate

tyrosinase enzyme activity (El-Naggar and El-Ewasy, 2017).

Evaluation of tyrosinase enzyme activity

The four mediums (Tyrosine broth, GYP, Soybean medium, and Peanut medium) containing grown bacteria were centrifuged at 5000 rpm for 5 mins. After 10 rounds of ultrasonic treatment, the pellets were placed on dry ice. To measure the activity of the enzyme tyrosinase, 0.1 ml of the ultrasonically treated bacteria, 1 ml of 0.5 M phosphate buffer, 0.9 ml of reagent water, and 1 ml of 0.001 M tyrosine solution were transferred to the test tube and kept on ice for 4 to 5 mins to allow necessary reactions. Then, the optical absorption of each tube was measured by five repetitions at 280 nanometers using a varian cary 50 spectrophotometer and was included in the equation 1: Unit of $\text{enzyme}_{(U/ml)} = [(\Delta A_{280nm} \div \text{min Test}) - (\Delta A_{280nm} \div \text{min Blank})] \div [(0{:}\ 001) \times (0{:}1)]$ that described by Raval *et al.*, 2012.

Extraction of pigments

The liquid culture of strain HM24 in tyrosine broth (ISP7) was used for pigment extraction using a modified method by (Zhu *et al.*, 2020).

SPF of pigment

About 100 mg of the resulting pigment was dissolved in 1 L of ethanol, and its UV absorption spectrum was measured at a wavelength of 280-400 nm and its SPF level was investigated according to equation 2: $SPF = CF \times \lambda \times I_{[\lambda]} \times \sum Abs_{[\lambda]}$; in this equation, which were reported by Eskandari and Etemadifar, 2021; Zarkogianni and Nikolaidis, 2016, λ= Wavelength (280-400nm), CF= Correction factor (=10), I= Solar intensity spectrum (= 1), Abs = Absorbance (OD).

Antioxidant activity of pigment

Antioxidant activity of pigment measured via DPPH assay. In the first step, the pigment and vitamin C (a positive control) concentrations of 20, 10, 5, and 2.5 mg/l were prepared in ethanol. Then, 3ml DPPH solution (0.1Mm) was added. The mixture was incubated at 37 °C for 30 minutes. The optical absorption of samples was measured at 516 nm. Results were calculated using equation 3: $\%\text{DPPH inhibition} = [(A_{control} - A_{test}) \div A_{control}] \times 100$. As reported by Sharmila *et al.*, 2020, $A_{control}$ and A_{test} are the absorbances of DPPH as control and pigment, respectively

Results

Sampling and culture of bacteria

Among the 70 bacteria isolated from the soil sample, 20 were tentatively identified as actinobacteria based on their Gram-staining characteristics (positive or variable), mycelial morphology, and the nature of their colonies (powdery, rough, and chalky, which data not shown).

Screening of tyrosinase-producing bacteria

Out of 20 actinobacterial isolates, two had dark brown colonies, which is a good sign of maximum consumption of L-tyrosine on tyrosine Agar (ISP7) through the activity of the tyrosinase enzyme (Fig.1). After culturing with three repetitions on ISP7, the isolate that produced darker colonies in the culture medium was chosen for identification, investigation of its tyrosinase activity, and characterization of its metabolic product.

Molecular identification of a novel strain

A selected bacterial isolate with code HM24 was identified molecularly, via 16srRNA gene sequence analysis in the GenBank database in the NCBI system. According to the findings and constructed phylogenetic tree presented in Figure 2, HM24 was 99.97% related to the genus microbacterium, which belonged to the actinobacteria phylum.

Producing tyrosinase in different substrates

Considering the natural presence of L-tyrosine in sources such as soybean meal, peanut meal, and yeast extract (D'Incecco *et al.*, 2024; Guo *et al.*, 2023; Morris and Thompson, 1962; Zheng and Van Huystee, 1991), these sources were utilized alongside standard L-tyrosine. The results demonstrated that after seven days, a change of color and turbidity in culture media containing various tyrosine sources revealed successful bacterial growth and tyrosinase enzyme activity (Fig. 3).

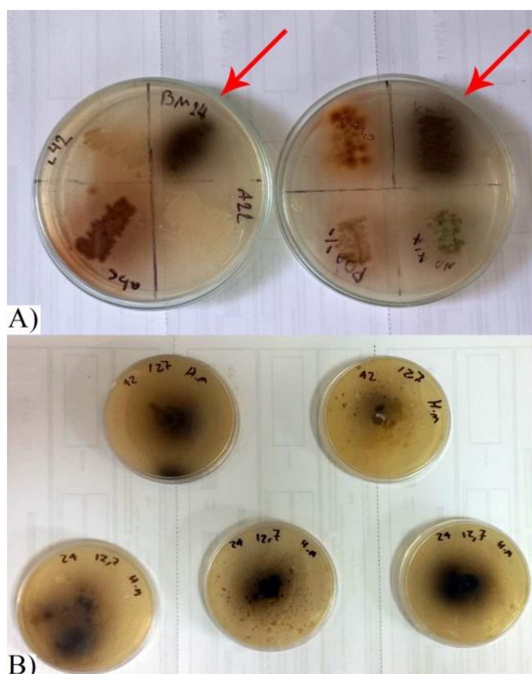

Fig. 1. Actinobacterial isolates on tyrosine Agar (ISP7) through the activity of tyrosinase enzyme: A) Out of 20 cultured strains, two actinobacterial colonies present the dark-brown color (indicated by the red arrow). B) Strain 24 presents a darker color in Tyrosine Agar than strain 24 in 3 repetitions.

Evaluation of tyrosinase enzyme activity

The tyrosinase enzyme activity in the Tyrosine medium, GYP, Peanut medium, and soybean medium was very low on days 1 and 2. Intriguingly, the Tyrosine medium yielded the highest enzyme activity during the first two days compared to other mediums (47.46 U/ml and 92.01 U/ml, respectively). From day 4 onward, the activity of tyrosinase increases in peanut, GYP, and soybean medium. Specifically, this increase is nearly 9-fold (35.48U/ml) in the soybean medium, while the highest activity is observed in the peanut medium (120.14 U/ml) and tyrosine broth (97.42 U/ml). On day 5, enzyme activity significantly increased across all culture media. The highest increase was associated with the soybean medium, which shows nearly a 6-fold increase (223.52 U/ml). Following that, the peanut medium exhibited the second-highest activity (220.44 U/ml). On day 6, a remarkable increase in the activity of this enzyme in all culture media was observed. Above all, the peanut medium exhibited the highest activity (315.72 U/ml), followed in descending order by the soybean meal medium (250.74 U/ml), tyrosine broth (158.02 U/ml), and GYP medium (125.16 U/ml). The activity of the tyrosinase enzyme significantly decreased in all culture media on day 7. In tyrosine broth, the activity decrease was less pronounced than in other media. In particular, the reduction in activity was 66.65 U/ml. The respective reductions in the soybean meal, GYP, and peanut extract media were 187.66, 105.18, and 263.08 U/ml, respectively (Table 1 and Fig. 4).

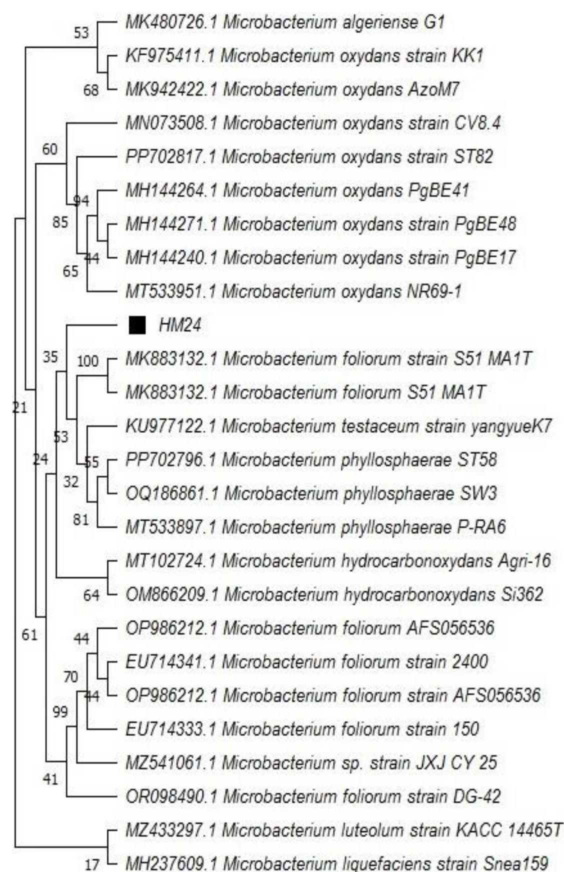

Fig. 2. Neighbor-joining phylogenetic tree based on 16 S rRNA gene sequences. It shows the relationship between isolate HM24 and species of the genus microbacterium in the phylum actinobacteria.

SPF of pigment

The SPF of this pigment produced by the HM24 in the tyrosine broth medium was 134.3608, indicating its high potential for use in sunscreen products. The UV absorption of the produced pigment in the tyrosine broth decreased as the wavelength increased (Table 2).

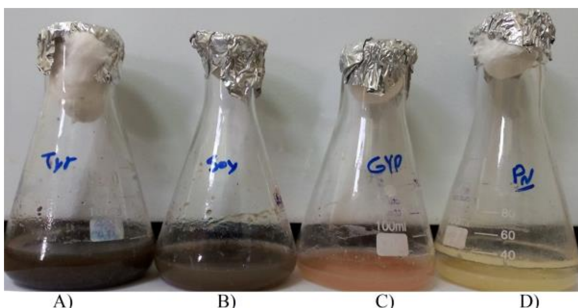

Fig. 3. Bacterial growth and tyrosinase enzyme activity of isolate HM24 cultured in different culture media: Change in color and turbidity resulted from HM24 growth in A) Tyrosine broth, B) Soybean broth, C) GYP broth, and D) peanut broth.

Antioxidant activity of pigment

The dark pigment produced by the HM24 in the tyrosine broth medium displayed maximum antioxidant activity at a concentration of 5 mg/l. Compared to vitamin C, it neutralized free radicals and biological oxidants at 69%. At 10 mg/l, the antioxidant activity decreased to 39%; at 20 mg/l, it increased to 47% (Fig.4).

Discussion

The microbial tyrosinases exist in different microorganisms such as Actinobacteria (*Streptomyces castaneoglobisporus*), Bacillus (*Bacillus megaterium*), and fungi (*Agaricus bisporus*) (Faccio *et al*., 2012), have various industrial applications, including the breakdown of phenolic compounds, melanin production, and removal of phenol from industrial wastewater. Phenol and phenolic compounds are common pollutants in effluents from various industries, such as steel, metals, carbon conversion, oil refining, resins, plastics, agricultural products, pharmaceuticals, and dyes (Chakraborty *et al*., 2015).

Tyrosinase enzymes are crucial for removing harmful phenolic compounds. Given the genetic similarities between actinobacterial and human tyrosinase, these bacteria represent a promising source for developing novel tyrosinase inhibitors. This study aimed to isolate and identify actinobacterial strains with tyrosinase-producing capabilities as well as to assess their enzyme activity in the presence of suitable substrates. Most of the tyrosinase active isolates of actinobacteria are isolated from soil samples; HM24 is isolated from Mahan, Kerman, Iran soil using ISP7 (Tyrosine Agar) medium, the same as the method used by Eskandari and Etemadifar (2021) for by *Dietzia schimae NM3*. Shahrokh *et al.* (2022) isolated *Streptomyces sp.* from the Maranjab Desert and the Hormoz Island using Tyrosine Agar as well. El-nagger and colleagues isolated *Streptomyces glaucescens NEAE-H* from soil samples of Egypt and Saudi Arabia using Peptone Yeast extract Iron agar and Tyrosine agar Abou-Dobara assessed tyrosinase production of *Streptomyces malachitorectus*, *Streptomyces iakyrus*, and *Streptomyces echinatus* isolated from the Hampoeil Cave using Tyrosine Agar (Abou-Dobara *et al*., 2019; El-Naggar and El-Ewasy, 2017; Eskandari and Etemadifar, 2021; Shahrokh *et al*., 2022).

Table 1. Mean of tyrosinase enzyme activity (U/ml) of isolate HM24 in different culture media during seven days.

Culture medium	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Tyrosine broth	47.46	92.01	94.73	97.42	111	158.02	91.55
Soybean	4.4	4.8	14.58	35.48	223.52	250.74	63.08
GYP	10.4	40.06	59.46	79.64	151.96	125.16	19.98
Peanut	19.55	37.06	84.92	120.14	220.44	315.72	52.64

L-tyrosine serves as a crucial biological substrate for producing phenol-based compounds, including L-DOPA and melanin. The tyrosinase enzyme facilitates this process, and L-tyrosine is naturally present in sources like peanuts, yeast, soybeans, eggs, and dairy products (D'Incecco *et al*., 2024; Guo *et al*., 2023; Morris and Thompson, 1962; Zheng and Van Huystee, 1991). The activity of the tyrosinase enzyme varies depending on the source of L-tyrosine used and can be influenced by factors such as bacterial strain type and isolation location. In this research, the maximum enzyme activity of HM24 in the tyrosine broth was 158.02 u/ml, while the best activity was associated with the peanut extract medium (315 U/ml), which is still acceptable compared to other studies. Also, it can be considered that the low activity of tyrosinase enzyme can be related to the lag phase of bacterial growth, and higher activity in

Tyrosine broth can be due to the bacteria's easy access to the amino acid L-tyrosine and the decrease of its activity in day 7 can indicate the depletion of its substrate, L-tyrosine. In a study conducted by Shahrokh *et al*. (2022), the maximum enzyme activity of *Streptomyces sp.* was reported to be 35 U/ml in a culture medium containing yeast.

Table 2. UV absorptions of the dark metabolite in different wavelengths from 280-400 nm.

Wavelength*	Abs	Wavelength*	Abs
399.9714	0.057995	334.9983	0.227554
395.0317	0.062137	330.0115	0.267833
389.9336	0.07716	325.0214	0.316138
384.986	0.077301	320.0282	0.386034
380.0347	0.084772	315.0317	0.483219
374.9247	0.089348	310.0322	0.634186
369.9658	0.097973	305.0296	0.875991
365.0032	0.107692	300.024	1.24611
360.0371	0.115895	295.0155	1.764441
355.0674	0.126271	290.004	2.633781
349.9387	0.139608	284.9897	3.215547
344.962	0.163557	SUM	13.43608
339.9818	0.190301	-	-

*=Wavelength= (nm); SPF: 10×1×13.43608=134.3608

However, in another study by El-nagger and colleagues, the maximum tyrosinase activity of *Streptomyces glaucescens NEAE-H* was 5454.27 U/ml, while the minimum activity was 996.05 U/ml in an optimized tyrosine broth medium. Also, in the study by Eskandari and Etemadifar (2021), the tyrosinase enzyme activity produced in the tyrosine broth medium by *Dietzia schimae NM3* was reported to be 2010 u/ml. In 2019 a study reported the tyrosinase activity of *Streptomyces malachitorectus*, *Streptomyces iakyrus*, and *Streptomyces echinatus* on tyrosine medium; 1.2 U/ml, 4.8 U/mL and 8.4 U/mL, respectively (Abou-Dobara *et al*., 2019; El-Naggar and El-Ewasy, 2017; Eskandari and Etemadifar, 2021; Shahrokh *et al*., 2022).

Following the observation of tyrosinase activity by strain HM24, the color change and darkening observed in all four cultures media could be indicative of pigment production, likely melanin or melanoid compounds and/or their precursors, such as L-DOPA. Tyrosine broth and Soybean medium presented a dark-brown color that can be a sign of producing melanin or other pigment, while given the high activity of tyrosinase in liquid cultures of peanut and GYP, the lack of darkening in the growth medium could result from the production of precursor molecules for pigments such as o-quinone or L-DOPA (Raval *et al*., 2012). Another significant biotechnological application of tyrosinase is its role in melanin and melanoid production. These compounds serve as important natural pigments and bioactive substances, offering a range of properties such as antioxidant activity, UV protection, anti-aging effects, anti-cancer properties, tissue repair, and antimicrobial activity. Moreover, they are found to be extensively used in cosmetics and pharmaceuticals. The production of these organic compounds directly relies on the activity of tyrosinase enzymes in microorganisms, acting as crucial chemical intermediates (Biyashev *et al*., 2023; Kiki, 2023; PUTRI *et al*., 2024; Sheefaa and Sivaperumal, 2022). SPF of the tyrosinase-based pigments has been extensively studied. In this study, the calculated SPF is 134.3608, which is significantly higher than the values reported in other research; Eskandari and Etemadifar (2021) reported an SPF value of 20.22 for the brown pigment produced by the bacterium *Dietzia schimae NM3*. Additionally, Shamila and colleagues reported a maximum SPF value of 19.07 (Eskandari and Etemadifar, 2021; Sharmila *et al*., 2020).

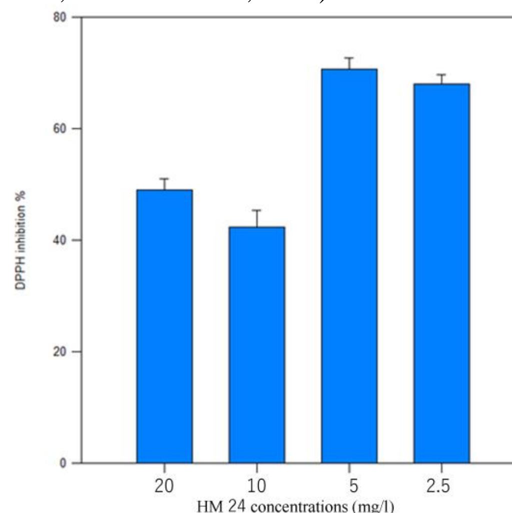

Fig. 4. Antioxidant activity of pigments: Antioxidant activity assay by DPPH in the different concentrations of HM24.

Free radicals are highly reactive molecules or unpaired electrons that can form during normal cellular processes or due to various factors such as environmental toxins, radiation, and stress and

can damage cellular components, leading to cell aging, tissue dysfunction, and the development of diseases like Alzheimer's, cancer, cataracts, and heart conditions. Antioxidants, on the other hand, act as protective agents; they neutralize free radicals by donating electrons, preventing them from causing harm. Common antioxidants include vitamins, minerals (like selenium), and phytochemicals in fruits, vegetables, and other plant-based foods. Promoting a balance between free radicals and antioxidants can support overall health and reduce the risk of chronic illnesses. Pigments exhibit robust antioxidant properties and offer various health benefits, including anti-aging effects, nervous system repair, anti-cancer properties, and anti-inflammatory effects (Lu *et al*., 2021; Rahaman *et al*., 2023). Ultimately, the maximum reported antioxidant activity by El-nagger, Eskandari, and Shamila was 89.6%, 188.9%, and 69.96%, respectively. However, in this study, the maximum antioxidant activity of the pigment produced by the tyrosinase enzyme is 69%, which is still acceptable compared to the previously mentioned values (Eskandari and Etemadifar, 2021; El-Naggar and El-Ewasy, 2017; Sharmila *et al*., 2020).

Considering the unique characteristics of strain HM24, which utilizes natural and readily available substrates such as soybean meal and peanut instead of pure L-tyrosine for tyrosinase enzyme activity, and the potential UV protection and Antioxidant ability of its pigment, this strain can be employed for industrial purposes. Additionally, it is recommended that the chemical structure of this pigment be identified for further investigation of its biological and other chemical properties. To obtain final confirmations, *in vivo* tests and cellular toxicity assessments should also be conducted for validation purposes.

Conflict of Interests

The authors declare no conflict of interest.

References

Abou-Dobara, M., El-Sayed, A., El-Fallal, A., & Sauf, M. (2019). Survey for Tyrosinase production by Streptomyces species. *Journal of Egyptian Academic Society for Environmental Development. D, Environmental Studies*, 20(1), 79-90. https://dx.doi.org/10.21608/jades.2019.67696

Ait Assou, S., Anissi, J., Sendide, K., & El Hassouni, M. (2023). Diversity and antimicrobial activities of actinobacteria isolated from mining soils in Midelt Region, Morocco. *The Scientific World Journal*, 2023(1), 6106673. https://doi.org/10.1155/2023/6106673

Behera, S., & Das, S. (2023). Potential and prospects of actinobacteria in the bioremediation of environmental pollutants: Cellular mechanisms and genetic regulations. *Microbiological Research*, 273, 127399. https://doi.org/10.1016/j.micres.2023.127399

Biyashev, D., Siwicka, Z. E., Onay, U. V., Demczuk, M., Xu, D., Ernst, M. K., ... & Lu, K. Q. (2023). Topical application of synthetic melanin promotes tissue repair. *NPJ Regenerative Medicine*, 8(1), 61. https://doi.org/10.1038/s41536-023-00331-1

Chakraborty, I., Redkar, P., Munjal, M., Kumar, S. S., & Rao, K. B. (2015). Isolation and characterization of pigment producing marine actinobacteria from mangrove soil and applications of bio-pigments. *Der Pharmacia Lettre*, 7(4), 93-100. https://www.scholarsresearchlibrary.com/journals/der-pharmacia-lettre/

D'Incecco, P., Dallavalle, S., Musso, L., Rosi, V., Sindaco, M., & Pellegrino, L. (2024). Formation of di-Tyrosine in pasteurized milk during shelf storage. *Food Chemistry*, 435, 137566. https://doi.org/10.1016/j.foodchem.2023.137566

El-Naggar, N. E. A., & El-Ewasy, S. M. (2017). Bioproduction, characterization, anticancer and antioxidant activities of extracellular melanin pigment produced by newly isolated microbial cell factories *Streptomyces glaucescens* NEAE-H. *Scientific Reports*, 7(1), 1-19. https://doi.org/10.1038/srep42129

Eskandari, S., & Etemadifar, Z. (2021). Biocompatibility and radioprotection by newly characterized melanin pigment and its production from *Dietzia schimae* NM3 in optimized whey medium by response surface methodology. *Annals of Microbiology*, 71, 1-13. https://doi.org/10.1186/s13213-021-01628-6

Faccio, G., Kruus, K., Saloheimo, M., & Thöny-Meyer, L. (2012). Bacterial tyrosinases and their applications. *Process Biochemistry*, 47(12), 1749-1760. https://doi.org/10.1016/j.procbio.2012.08.018

García-Molina, P., García-Molina, F., Teruel-Puche, J. A., Rodríguez-López, J. N., García-Cánovas, F., & Muñoz-Muñoz, J. L. (2022). Considerations about the kinetic mechanism of tyrosinase in its action on monophenols: A review. *Molecular Catalysis*, 518, 112072. https://doi.org/10.1016/j.mcat.2021.112072

Guo, X., Wu, X., Ma, H., Liu, H., & Luo, Y. (2023). Yeast: A platform for the production of L-tyrosine derivatives. *Yeast*, 40(5-6), 214-230. https://doi.org/10.1002/yea.3850

Hazarika, S. N., & Thakur, D. (2020). Actinobacteria. *In Beneficial Microbes in Agro-Ecology* (pp. 443-476). Academic Press.

Irshad, G., Naz, F., Ghuffar, S., Khalid, A. R., Arif, S., Maqsood, A., ... & Din, R. U. (2023). Molecular studies of postharvest fungal peach fruit rots; *Fusarium sporotrichioides, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Cladosporium pseudocladosporioides*. *Pakistan Journal of Phytopathology*, 35(2), 349-356. https://doi.org/10.33866/phytopathol.035.02.0910

Kiki, M. J. (2023). Biopigments of microbial origin and their application in the cosmetic industry. *Cosmetics*, 10(2), 47. https://doi.org/10.3390/cosmetics10020047

Lee, Y. K., Kim, H. W., Liu, C. L., & Lee, H. K. (2003). A simple method for DNA extraction from marine bacteria tht produce extracellular materials. *Journal of Microbiological Methods*, 52(2), 245-250. https://doi.org/10.1016/S0167-7012(02)00180-X

Liao, L., Su, S., Zhao, B., Fan, C., Zhang, J., Li, H., & Chen, B. (2019). Biosynthetic potential of a novel Antarctic actinobacterium Marisediminicola antarctica ZS314T revealed by genomic data mining and pigment characterization. *Marine Drugs*, 17(7), 388. https://doi.org/10.3390/md17070388

Lu, W., Shi, Y., Wang, R., Su, D., Tang, M., Liu, Y., & Li, Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *International Journal of Molecular Sciences*, 22(9), 4945. https://doi.org/10.3390/ijms22094945

Martín, J. F., Liras, P., & Sánchez, S. (2021). Modulation of gene expression in actinobacteria by translational modification of transcriptional factors and secondary metabolite biosynthetic enzymes. *Frontiers in Microbiology*, 12, 630694. https://doi.org/10.3389/fmicb.2021.630694

Mohammadi, M., Khaleghi, M., Shakeri, S., Hesni, M. A., Samandari-Bahraseman, M. R., & Dalvand, A. (2022). Isolation of Actinobacteria strains from environmental samples and assessment of their bioactivity. *Avicenna Journal of Clinical Microbiology and Infection*, 9(1), 8-15. https://doi.org/10.34172/ajcmi.2022.02

Morris, C. J., & Thompson, J. F. (1962). The isolation and characterization of γ-l-glutamyl-l-tyrosine and γ-l-glutamyl-l-phenylalanine from soybeans. *Biochemistry*, 1(4), 706-709. https://doi.org/10.1021/bi00910a026

Pavan, M. E., López, N. I., & Pettinari, M. J. (2020). Melanin biosynthesis in bacteria, regulation and production perspectives. *Applied Microbiology and Biotechnology*, 104(4), 1357-1370. https://doi.org/10.1007/s00253-019-10245-y

Putri, D. A., Astuti, R. I., & Wahyudi, A. T. (2024). The potency of yellow pigment extract from the marine bacterium *Pseudomonas oryzihabitans* SAB E-3 as an antioxidant agent. *Biodiversitas: Journal of Biological Diversity*, 25(6). http://dx.doi.org/10.13057/biodiv/d250627

Rahaman, M. M., Hossain, R., Herrera-Bravo, J., Islam, M. T., Atolani, O., Adeyemi, O. S., ... & Sharifi-Rad, J. (2023). Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. *Food Science & Nutrition*, 11(4), 1657-1670. https://doi.org/10.1002/fsn3.3217

Raval, K. M., Vaswani, P. S., & Majumder, D. R. (2012). Biotransformation of a single amino-acid L-tyrosine into a bioactive molecule L-DOPA. *The International Journal of Science and Research*, 2, 2250-3153. https://www.ijsrp.org/

Shahrokh, Z., Salimi, F., & Hamedi, J. (2022). Screening and molecular identification of tyrosinase-producing actinobacteria from soils of the Maranjab Desert, the Hampoeil

Cave, and the Hormoz Island. *Journal of Microbial Biology*, 11(41), 33-50. https://doi.org/10.22108/bjm.2021.124762.1320

Sheefaa, M. I., & Sivaperumal, P. (2022). Antioxidant activities from melanin pigment produced by marine actinobacterium of *Streptomyces* species. *Journal of Advanced Pharmaceutical Technology & Research*, 13(Suppl 1), S84-S87. https://doi.org/10.4103/japtr.japtr_338_22

Shivaveerakumar, S., & Hiremath, J. Isolation and biochemical characterization of potential isolates of Actinomycetes for the production of Tyrosinase from the campus of Davangere University. (2019). *International Journal of Pharmacy and Biological Sciences,* 9, 1550-1560. https://doi.org/10.21276/ijpbs.2019.9.1.203

Zarkogianni, M., & Nikolaidis, N. (2016). Determination of sun protection factor (SPF) and stability of oil-in-water emulsions containing Greek red saffron (Crocus Sativus L.) as a main antisolar agent. *International Journal of Advanced Research in Chemical Science*, 3(7), 1-7. https://dx.doi.org/10.20431/2349-0403.0307001

Zheng, X., & Van Huystee, R. B. (1991). Oxidation of tyrosine by peroxidase isozymes derived from peanut suspension culture medium and by isolated cell walls. *Plant Cell, Tissue and Organ Culture*, 25, 35-43. https://doi.org/10.1007/BF00033910

Zhu, Y., Shang, X., Yang, L., Zheng, S., Liu, K., & Li, X. (2020). Purification, identification and properties of a new blue pigment produced from Streptomyces sp. A1013Y. *Food Chemistry*, 308, 125600. https://doi.org/10.1016/j.foodchem.2019.125600