Pharmacological and Toxicological Appraisal of Promising Enzyme-tyrosinase Isolated from Soil Bacteria-an Update

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AA designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author KRG performed whole experimental work and author HAK managed the interpretation and analyses of bacterial work. Author RM managed the literature searches and author SA managed the molecular part of work. Author JAQ gave the approval of study design and final draft of this manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: The membrane associated tyrosinase is an enzymatically active monomeric glycoprotein which is purified to only a low degree. It has gained importance in the present era due to its antioxidant and immunomodulatory properties as well as applications in industry. Moreover its role in the synthesis of melanin in skin for protection from UV radiations also paved the way towards the better understanding of this enzyme.

Study Design: Biochemical and molecular characterization of tyrosinase producing soil bacterium had been followed by the assessment of its antioxidant, cytotoxic and anti-cancer activities.

Place and Duration of Study: Whole work had been completed at the Microbiology and Molecular biology labs of IMBB during 2018-2019.

Methodology: Tyrosinase producing species were identified by biochemical characterization followed by their genomic DNA sequencing and BLAST analysis while crude tyrosinase was
characterized by Bradford's methods, tyrosinase activity and total protein activity, followed by their molecular characterization on SDS-PAGE. The antioxidant and free radical scavenging properties of tyrosinase were evaluated via DPPH, ABTS and FRAP assays and cell proliferation inhibition and the cytotoxicity was calculated via antitumor and MTT assays.

**Results:** *P. putida, B. cereus, B. mycoides, M. luteus, K. pneumonia* were found to be tyrosinase producing species while their SDS-PAGE analysis showed that the molecular mass of crude tyrosinase was about 39 kDa. Protein contents, total tyrosinase and specific tyrosinase activity was found to be highest in tyrosinase from *B. mycoides* [0.008±0.06 mg/ml, 1500±0.06 U/ml and 346820.8±0.03 U/mg of tyrosinase respectively]. The results of biological activities of crude tyrosinase vary from bacteria to bacteria. Tyrosinase from *P. putida* showed higher antioxidant [66.4±0.01% in DPPH assay, 32.04±0.06% in ABTS assay and 320.6±0.06 in FRAP assay], antitumor [67.8±0.01%] and cytotoxic activity [39±1.0% cell viability], followed by *B. Cereus* tyrosinase [64.7±0.06% antioxidant power in DPPH assay, 53.41±0.03% in ABTS assay and 159.6±0.06% in FRAP assay, 46.46±0.01% antitumor and 43±0.75% cell viability].

**Conclusion:** The study revealed that tyrosinase isolated from different bacterial strains depicted optimal percentage of anti-oxidative, anti-proliferative and cellular viability and can be used in the near future for medical and industrial purposes.

**Keywords:** Tyrosinas; soil bacteria; antioxidant; cytotoxic; anti-cancer.

1. **INTRODUCTION**

The membrane associated tyrosinase is an enzymatically active monomeric protein, which actively catalyzes monophenols to the corresponding catechol’s for the biosynthesis of melanin via hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine or DOPA and oxidation of DOPA to dopaquinone [1]. Ultimately, this dopaquinone is converted into melanin pigments. This mechanism of action of tyrosinase is applied in enormous biotechnological and environmental aspects. Therefore, tyrosinases have gained much attraction of scientists towards its molecular characterization and bioengineering studies [2]. The synthesis of tyrosinase takes place in rough endoplasmic reticulum and is purified to merely a low degree due to the difficulty in obtaining it in pure form [3]. Tyrosinases are purified through various methods in addition to the column chromatography using hydroxyapatite, DEAE-cellulose and size exclusion gel chromatography [4]. The production of microbial tyrosinase is extracellular method which is then secreted into the growth medium but some micro-organisms produce tyrosinase intracellularly [5]. The extracellular tyrosinase originated from bacteria is a short length sequence protein as compared to plant and fungal tyrosinase protein with an encoded 30kDa of protein TYR gene [6]. The clear zone of dark brown pigmentation appearing in the culture media confirms the extracellular production of tyrosinase by bacterium [7,8]. The best purified and characterized tyrosinases are derived from *Streptomyces glaucescens*, *Neurosporarass* and *Agaricus bisporus*. However, the preclinical studies regarding tyrosinase inhibition are mostly conducted using mushroom tyrosinase due to its active commercial availability. The antitumor effect of tyrosinase was also studied *in vitro* [9]. Tyrosinase is able to suppress DNA polymerase activity and its 2,5-dihydroxy derivative inhibits uracil, whereas the 3, 4-dihydroxy derivative carries out the inhibition of thymine and leucine incorporation into nucleic acid and proteins of melanoma cells [10]. Phenol-L-glutaminyl-4-hydroxybenzene [GHB], is oxidized by tyrosinase to a quinone and a second oxidation product, which both suppress the energy production by mitochondrial and synthesis of proteins and nucleic acids [11]. The quinone produced is able to block the tumor growth in mice with cultured murine B-16 melanoma cells and L1210 leukemia cells [12]. But the tumor progression is promoted in the aforementioned cultured cells due to the absence of tyrosinase production. These contradictory observations have come in front concerning the possible mutagenic role of tyrosinase. These preclinical trials describe the cytotoxic effect of tyrosinase. The mutagenic effect of tyrosinase is also elucidated when the tyrosinase was observed to enhance the mutagenic activity of quinoid and phenolic compounds. Whereas, its mutagenic response was suppressed by catalase, dimethyl sulfoxide, superoxide dismutase and glutathione, which indicates the role of quinoid and phenolic compounds in the production of reactive oxygen species [13]. Keeping in view the importance of microbial tyrosinase in clinical and pharmaceutical domains, it is necessary to...
elucidate the biochemical and molecular pathway of production and mode of action of tyrosinase [14]. The current research work elucidates several important insights into the biochemical and molecular characteristics as well as the cascades of mechanism of action of the microbial tyrosinases which leads to the cost-effective synthesis of tyrosinase revealing its applications in pharmaceutical, food bioprocessing and environmental areas.

2. MATERIALS AND METHODS

2.1 Sample Collection

Research work was performed in the Microbiology Laboratory at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore during a period of November, 2018 to December, 2019. 1.0 g Soil samples were collected from grounds of The University of Lahore and University of the Punjab, Lahore and Public Park, from 6-inch depth for bacterial isolation.

2.2 Identification of Bacteria

2.2.1 Nitrate reduction test

This test confirmed the presence of Enterobacteriaceae family which hydrolyses nitrate into nitrogenous components. Few drops of sulfanilic acid were added in test tube having bacterial colonies in media [prepared and autoclaved for 20 min at 121°C] and were placed in incubator at 37°C for 24 hours. Positive bacterial culture showed red color [15].

2.2.2 Citrate utilization test

Bacteria having citrate permease are capable of using sodium citrate in the absence of fermenting sugars and utilize citrate as a source of energy in the form carbon. The citrate enzyme produces acetate and oxaloacetic acid which were converted to carbon dioxide and pyruvic acid. These bacteria had been detected by Citrate utilization test was performed by streaking bacterial isolates, incubated for 24 hours at 37°C and examined the color [16].

2.2.3 Triple Sugar Iron Agar [TSIA] test

Triple sugar iron [TSI] agar test is used to differentiate among the gram negative rods which are carbohydrate fermenting and produce hydrogen sulphide. TSI agar media was prepared, autoclaved, kept in slanting position to prepare slants on which bacterial colony had been streaked and incubated for 24 hours and then noted the medium color [17].

2.2.4 Rapid Urease Test [RUT]

Urease enzyme is produced by proteus species [gram negative rod] for which broth medium was prepared and autoclaved at 121°C for 20 minutes. When temperature of broth medium lowered to room temperature then urea was added into it. Bacterial isolates were inoculated with loop and incubated for 24 to 48 hours in incubator at 37°C. Urease positive bacteria changed the color of media in to deep pink [18].

2.3 Biochemical Conformation of Tyrosinase Producing Bacteria

Tyrosinase screening medium/ T-medium was prepared for the confirmation of tyrosinase producing bacteria from the previous isolated pure bacterial species by streaking on T-medium. Bacterial species with brown-black colony indicated the presence of tyrosinase in prospective bacterial species after incubating the plates for 72 hour at 37°C and 175rpm [19].

2.4 Molecular Identification of Tyrosinase Producing Bacteria

All isolated bacterial strains were identified through sequencing of genome.

2.4.1 DNA extraction

Genomic DNA had been isolated by using TE buffer and RNAase by the modified protocol of El-Ashram et al., [2016] while it was further confirmed on 1.7 % TAE agarose gel by using TBE buffer.

2.4.2 PCR for genome sequencing

PCR had been performed for molecular identification of bacterial strains. Master mix [1 ul of forward and reverse 16Sr RNA primer [ R=5’-TACGGYTACCTTGTGACTT-3’, F=5’- AGRGTTGATYMTGGCAG-3’] and 1U Taq polymerase],1ul bacterial DNA and injection water was mixed in Eppendorf tube and run the Thermal cycler for PCR at 94°C for 5 mint and 30 second at 94°C for denaturation, 56°C for 30 seconds [annealing] and 72°C for 2 mint and 72°C for 10 mint [for elongation]followed by 30 cycles. PCR product has been confirmed by running 1.5% electrophoresis gel followed by visualization under UV light [20].
2.5 Production and Partial Purification of Tyrosinase Enzyme

After the confirmation of tyrosinase producing strains, those bacterial species were inoculated on broth T-medium [1.5 g yeast extract, 1.5 g tryptone, 5 g NaCl, 1% L-tyrosine and 100 mM CuSO4 at pH 7.0] and incubated for 72 hours at 37°C in shaking incubator. Color of broth medium changed to dark brown due to production of tyrosinase which was partially purified by centrifugation of broth medium at 5000 rpm for 10 minutes by discarding the pellet and saving the supernatant at 4°C [18].

2.6 Pharmacological Activities of Partially Purified Enzyme

2.6.1 Antioxidant activity

To determine the antioxidant activity of tyrosinase, DPPH, ABTS and FeCl3 reducing power assays had been used.

2.6.1.1 DPPH assay

To assess the scavenging activity of tyrosinase against DPPH, 1 ml sample was mixed with 1 ml of methanolic solution of 0.1 Mm DPPH solution by keeping Ascorbic acid as standard, left for 30 minutes at 25°C in dark and measured absorbance at 517nm. Lower absorbance showed higher capability of scavenging free radicals [21].

\[
\% \text{ DPPH scavenging effects} = \frac{A_0 - A}{A_0} \times 100
\]

Where, \( A_0 \) is the absorbance of negative control [0.1 mM DPPH solution]; \( A \) is the absorbance in presence of Enzyme. The results were reported as IC50 values and ascorbic acid equivalents [AAE, mg/g] of crude tyrosinase.

2.6.1.2 ABTS assay

ABTS radicals showed scavenging activity by decolorize it. The solution was prepared by mixing of 7 mM ABTS and 2.4mM potassium persulfate, left at room temperature for 13.50 hours, diluted in ethanol and checked the absorbance of 0.70 ± 0.02 at 734nm for ABTS by keeping Ascorbic acid as a standard. 1.0ml ABTS solution was added into 1.0ml sample by keeping whole experiment in dark and measured absorbance at 734nm after 30 minutes [22].

\[
\% \text{ scavenging effect} = \frac{AB - AA}{AB} \times 100
\]

Where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard.

2.6.1.3 FRAP assay

2.5 ml of 0.2M sodium phosphate buffer, 1ml sample and 1% potassium ferric-cyanide solution had been added, vortexed well and incubated at 50°C for 20 minutes. Then 2.5ml of 10% trichloroacetic acid was added into mixture and centrifuged for 10 minutes at 3000 rpm. 2.5ml distilled water and 0.5 ml of 0.1 ferric chloride was added to supernatant and measured the absorbance at 700 nm by keeping Ascorbic acid as standard [23].

2.6.2 Antitumor activity

For the growth of Agrobacterium tumefaciens, Yeast extract media was prepared and incubated at 25°C for 48 hours. Potato discs were sterilized with 20% Clorox. The size of discs was 0.5cm thick. These discs were rinsed with sterilized distilled water. A. tumafaciens was suspended in phosphate buffer saline to 1×10⁹ CFU. Paclitaxel was used as a positive control while DMSO as negative control. Discs were kept in 24-well culture plates and added 50ul of the sample, 15% Agar water and bacteria and incubated at 25°C for 2 weeks, after which Lugol’s Reagent had been added. Potato discs were turned in to blue color due to the inhibition of tumors after staining but they remained colorless when tumor were still present [12]. Tumor inhibition was calculated by following this formula

\[
\% \text{ tumor inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

2.6.3 Cytotoxic assay

In microculture tetrazolium [MTT] assay, HepG2 [Hepatocellular carcinoma cell line or liver cancer cell line] cell lines in cryo cylinder and then cry well was thawed to soften from frozen. For the removal of DMSO, cell line was centrifuged. After centrifugation supernatant was discarded and pellet was suspended in DMEM medium supplemented with 10% FBS, 1g/L streptomycin and 100mg/L penicillin. Cells were placed in 96 well plate by keeping density of cells at 1×10⁶ per well. Plate was incubated at 37°C for 24 hours in 5% CO₂ incubator, after which cells were attached to surface. 100 ul of partially purified enzyme had been loaded in each well, incubated for 24 hours, added with 25 ul of 5mg/ml MTT, again incubated for 4 hours, added100 ul of SDS
as a stop solution and measured the absorbance after 4 hours at 570 nm [24].

3. RESULTS

3.1 Confirmation of Tyrosinase Producing Bacterial Isolates

Tyrosinase producing bacteria showed colonies of black brown color on T-medium [Fig. 1] while results of biochemical characterization showed that *P. putida*, *B. cereus*, *B. mycoides*, *M. luteus* and *K. pneumonia* had been isolated from the soil samples [Table 1].

3.2 Molecular Identification of Tyrosinase Producing Isolates

PCR products of isolates were sequenced from Advance Bioscience International. 16S rRNA gene sequence was analyzed by sequence alignment through nucleotide BLAST, 99% or 98% identity of sequence with 16S rRNA gene sequenced bacterial species and showed that isolate 1 was *P. putida*, isolate 2 was *B. cereus*, isolate 3 was *B. mycoides*, isolate 4 was *M. luteus* and isolate 5 was *K. pneumonia*. Identification at the genus level was defined as > 97% identity of the 16S rRNA gene sequence with the sequence of its closest bacteria relative in the Gene Bank database [Figs. 2-8].

3.3 Production and Partially Purification of Tyrosinases

Tyrosinase is a fundamental enzyme principally used in melanin production and in different pharmaceutical industries. Till this moment there are scarce publications on bacterial tyrosinases. Before incubation period, color of the medium was light yellow [Fig. 9A] while after incubation period broth color was changed to dark brown due to tyrosinase production which was actually due to the production of crude/ partially purified tyrosinase after centrifugation [Fig. 9B]. Five bacteria had been isolated from the soil. These isolates could grow aerobically and reported a promising monophenolase and di-phenolase activities in the presence of tyrosine and Cu$^{2+}$ only. This result suggested that the microorganism could gain energy through the tyrosine oxidation. The bacterial isolates were *P. putida*, *K. pneumonia*, *M. luteus*, *B. mycoides* and *B. cereus* with 96.22%, 98.04%, 96.33%, 97.47% and 96.22% similarity respectively [Figs. 4-8].

3.4 Biochemical Characterization of Tyrosinase

Most important factors with a great influence on tyrosinase production is the nutritional factors including C, N factors and copper concentrations in the medium astyrosinase structure consists mainly of copper [an essential micronutrient found in trace amounts in nature], so it is an essential element to tyrosinase enzyme activity and had been added to tyrosinase production medium. The result proved that the tyrosinase activity had been increased by the increase of protein concentration who have C and N as their structural elements [0.008±0.06 mg/ml protein content by *B. mycoides*] and the same species gives rise to maximum tyrosinase activity [1500±0.06 U/ml]. Moreover those bacterial species which have less amount of tyrosinase may have less Nitrogen in soil as production of this enzyme is also triggered by nitrogen depletion, although some nitrogen sources do not affect the enzyme activity. The brown color for *P. putida*, *K. pneumonia*, *M. luteus*, *B. mycoides* and *B. cereus* isolates was yielded on L-tyrosine broth medium [Fig. 9A and B]. Moreover alanine is the dominating amino acid in the prokaryotic tyrosinase sequences while in Gram-positive bacteria there are also high amounts of charged residues [arginine, aspartate] and arginine residues favor not only the formation of salt bridges, but may simultaneously bind to aromatic stacked rings [25].

3.4.1 Protein content in bacterial tyrosinase

Protein contents of tyrosinase [mg of protein/ml of tyrosinase] from different isolates showed that different source of tyrosinase showed different values of protein content. Protein contents was found to be highest in tyrosinase from *B. mycoides* [0.008±0.06 mg/ml] and *B. cereus* [0.0077±0.01 mg/ml], followed by *P. putida* [0.0067±0.01 mg/ml] and *K. pneumonia* [0.0052±0.01 mg/ml], while it was least in tyrosinase of *M. luteus* [0.0035±0.06 mg/ml] [Fig. 10].

3.4.2 Total tyrosinase activity

Maximum of total tyrosinase activity had been shown by *B. mycoides* and *B. cereus* [1500±0.06 U/ml and 1200±0.01 U/ml respectively] followed by *P. putida* [1000±0.01 units/ml] and *K. pneumonia* [500±0.01 U/ml] while it was least in *M. luteus* [300±0.01 U/ml] [Fig. 11].
3.4.3 Specific tyrosinase activity

It was calculated by Units of enzyme/mg protein contents and had been found to be 346820.8±0.03 U/mg of tyrosinase by *B. mycoides*, and 286806.8±0.01 U/mg by *B. cereus* followed by *P. putida* [149476.8±0.01 U/mg] and *K. Pneumonia* [64432.9±0.01 U/mg] while least tyrosinase activity had been shown by *M. Luteus* [37174.7±0.011] [Fig. 12].

3.5 Evaluation of Biological Activities of Partially Purified Tyrosinase

3.5.1 Antioxidant activity of tyrosinase

3.5.1.1 DPPH radical scavenging by tyrosinase

Tyrosinase from different bacterial isolates had shown variant scavenging activity. *P. putida* had higher antioxidant power [66.4±0.01%] followed by *K. pneumonia* [65.2±0.01%] and *B. cereus* [64.7±0.06%], while *B. mycoides* and *M. luteus* had 50±0.06% antioxidant power [Fig. 13].

3.5.1.2 ABTS radical scavenging by tyrosinase

Tyrosinases showed variable antioxidant activity against ABTS oxidant. Tyrosinase from *M. luteus* had highest scavenging activity [74.14±0.01%] while *P. putida* tyrosinase had least power to scavenging ABTS [32.04±0.06%]. Tyrosinase from *B. mycoides* and *K. pneumoniae* had almost same activity [50.85±0.01% and 50.42±0.03% respectively] while *B. Cereus* Tyrosinase had shown 53.4±0.03% antioxidant activity [Fig. 13].

3.5.1.3 FRAP radical scavenging by tyrosinase

Tyrosinases form different bacteria had different power of scavenging free ion. *P. putida* tyrosinase had shown maximum activity [320.6±0.06%], followed by *M. luteus* [205±0.01%], *B. cereus* [159.6±0.06%] and *B. mycoides* [159.3±0.03%] while *K. pneumoniae* tyrosinase had least activity [125.3±0.03%] [Fig. 13].

3.5.2 Antitumor activity of tyrosinase

Percentage of tumor inhibition by tyrosinase varied from bacteria to bacteria. *K. pneumoniae* tyrosinases showed highest tumor inhibition [74.30±0.01%] followed by tyrosinases from *P. putida* and *B. mycoides* who had similar capacity of tumor inhibition [67.8±0.01%]. *M. luteus* tyrosinase inhibited tumors only up-to 57±0.01% while *B. cereus* tyrosinase had least power to inhibit potato tumors [46.46±0.01%] [Fig. 14].

3.5.3 Percentage of cell viability by tyrosinase in cytotoxicity assay

Crude tyrosinases from *M. luteus* had shown maximum cell viability [95±1.5%] followed by *K. pneumonia* tyrosinase [60±1.0%] but tyrosinases from *B. mycoides*, *B. cereus* and *P. putida* had least cyto-protective activity as cell were less viable in them [45±0.5%, 43±0.75% and 39±1.0%]. The cell viability potential of tyrosinase in cytotoxicity depended on source of tyrosinase. These results show that tyrosinase from different bacterial species have different values of cell viability in cytotoxicity [Fig. 14].

[a] Isolate 2 produced brown color on media which shows positive result for tyrosinase

[b] Isolate 4 produced brown color on media which shows positive result for tyrosinase

[c] Isolate 5 produced brown color on media which shows positive result for Tyrosinase
Isolate 6 produced brown color on media which shows positive result for tyrosinase.

Isolate 7 produced brown color on media which shows positive result for tyrosinase.

Fig. 1. [a-e] Activity of bacterial isolates on medium containing L-tyrosine for confirmation of tyrosinase producing isolates.

Fig. 2. Gel picture of DNA of bacterial strains which were positive for Tyrosinase production. M= ladder [100bp DNA ladder] and lanes 1 – 5 show the DNA of P. putida, B. cereus, B. mycoides, M. luteus and K. pneumonia respectively.

Fig. 3. Gel picture of PCR product, lane M is a DNA ladder [1 kb] and lane 1-5 are amplified bands of 16S rRNA gene [1434 bp] of P. putida, B. cereus, B. mycoides, M. luteus and K. pneumonia respectively.
Fig. 4. Nucleotide BLAST of this sequence showed 96.22% identity with *P. putida*.

Fig. 5. Nucleotide BLAST of this sequence showed 96.22% identity with *B. cereus*.
Fig. 6. Nucleotide BLAST of this sequence showed 97.47% identity with *B. mycoides*

| Forward sequence |
|------------------|
| Fig. 6. Nucleotide BLAST of this sequence showed 97.47% identity with *B. mycoides* |

| Reverse sequence |
|------------------|
| Fig. 7. Nucleotide BLAST of this sequence showed 96.33% identity with *M. luteus* |
Fig. 8. Nucleotide BLAST of this sequence showed 98.04% identity with *K. pneumoniae*.

Fig. 9A. L-Tyrosine broth Media before incubation period
Fig. 9B. L-Tyrosine broth media by production of tyrosinase from bacteria after incubation and centrifugation

Fig. 10. Protein content in bacterial tyrosinases

Fig. 11. Total tyrosinase activity from bacterial tyrosinase
Fig. 12. Specific tyrosinase activity from bacterial tyrosinases

Fig. 13. Antioxidant analysis of bacterial tyrosinases compared with ascorbic acid [control]

Fig. 14. Tumor inhibitions and cytotoxic potential [%] of bacterial tyrosinases
Table 1. Biochemical characteristics of bacterial isolates obtained from soil

| Citrate use | Urease Activity | NO₃ Reduction | Triple Sugar Iron Test | Bacterial isolates |
|-------------|-----------------|----------------|-----------------------|-------------------|
| -           | -               | -              | K/K                   | P. putida         |
| -           | -               | +              | K/A                   | B. cereus         |
| -           | +               | +              | K/A                   | B. mycoides       |
| -           | +               | +              | K/K                   | M. luteus         |
| +           | +               | -              | R/AG                  | K. pneumonia      |

+ = positive, - = negative, H₂S= Hydrogen sulfide, K= Alkaline, A= Acid, AG= Acid gas

4. DISCUSSION

Extracellular tyrosinases production in soil environment are most likely involved in detoxification of phenolic compounds of plants, humid matter formation and polymerization [26]. In some microorganism, tyrosinase produced intracellularly but it is also capable to secreteextracellularly, followed by its transportation into the growth medium [27]. When bacteria grow in a complex medium then enzymatic activities are in stationary phase. The L-tyrosine is a substrate and has positive effect on tyrosinase. Many researches prior that production of tyrosinase in a growth medium by microorganism are influenced by such factors like medium composition, genetics of microbes, presence of biosynthetic inhibitors, presence of enzyme inducer, number of tyrosinase producing cells, temperature, pH and duration of incubation [28]. Some bacteria have ability to produce enzyme and produce brown coloration in tyrosine agar medium and also in tyrosine broth medium which revealed the confirmation of tyrosinase producing isolates and these results showed similarity to an old study [29]. The brown coloration by tyrosinase was due to production of melanin by oxidative polymerization of phenolic compound. Results of tyrosinase characteristics and purity of the extracted enzyme was as similar as to the literature [30] in which size of tyrosinase is about 35 kDa from Bacillus megaterium. The purified tyrosinase is a monomer with a relative molecular mass of approximately 39,000kDa. Complicated hydroxylation mechanism at the active center is still not completely understood, because nothing is known about their tertiary structure. One main reason for this deficit is that its large amount from eukaryotic sources could not be isolated in sufficient quantities and purities for detailed structural studies. This is not the case for prokaryotic tyrosinases from different Streptomyces species, having been intensively characterized genetically and spectroscopically for decades. The Streptomyces tyrosinases are non-modified monomeric proteins with a low molecular mass of 30 kDa. They are secreted to the surrounding medium, where they are involved in extracellular melanin production. In the species Streptomyces, the tyrosinase gene is part of the melC operon. Next to the tyrosinase gene [melC2], this operon contains an additional ORF called melC1, which is essential for the correct expression of the enzyme [31]. Tyrosinases [EC 1.14.18.1] can oxidize various phenolic substrates in the presence of molecular oxygen [32]. The enzyme catalyzes monophenolase and di-phenolase activity [33] and these features make it a suitable candidate for the detoxification of waste water or soil contaminated with phenolic compounds, and/or aromatic amines [34,35]. Construction of biosensors to detect phenolic compounds [36], cross-linking reactions; biosynthesis of antioxidants e.g. hydroxyl-tyrosol, production of prodrugs e.g. L-DOPA or compounds suppressing melanoma [34].

The potato disc assay described a simple antitumor prescreening for the inhibition of tumor formation on potato disc and this inexpensive tumor assay system have many advantages as alternatives for animal trails in the research for new anticancer drugs This assay could be used to determine anticancer potential of tyrosinase. As tyrosinase inhibited tumor formation on potato discs. Many scientists have used these bioassays over the past 15 years for the purpose of quality control of bioactive natural compounds [37]. Tyrosinase had shown significant results of cell viability and tyrosinases from different bacterial species had showed different value of cell viability reported that results depending on the test agent used and cytotoxicity assay employed [38]. This may be due to their intensive biochemical investigations, but still the biological functions of bacterial tyrosinases are not fully understood. In strepto-mycetes, tyrosine is neither best substrate nor inducer of the
tyrosinases. The best-documented function of this enzyme is restricted to the formation of melanin, which protect the bacterial cells and spores against UV radiation [39]. For instance, the expression of the mel gene from P. maltophilia significantly increased the viability of B. thuringiensis. Melanin bind heavy metals that are otherwise toxic to the cells. They also confer protection against oxidants, heat, enzymatic hydrolysis, antimicrobial compounds and phagocytosis and thus can contribute to microbial pathogenesis [40,41].

5. CONCLUSION

This study shows that tyrosinase possess the remarkable antioxidative, antitumor and cytoprotective potential. Thus tyrosinases isolated from different strains of bacteria can be used as an effective therapy against free radicals as well as to control cell proliferation with reduced side effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Authors are highly acknowledged to Higher Education Commission [HEC], Pakistan for their financial support for the completion of this work as a part of SRGP [Project No. No:21-1431/SRGP/R & D/HEC/2017.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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