World Journal of Microbiology and Biotechnology (2022) 38:2
https://doi.org/10.1007/s11274-021-03186-0

REVIEW

Application of bacterial tyrosinases in organic synthesis

Mayowa Agunbiade[1] · **Marilize Le Roes-Hill**[1]

Received: 11 October 2021 / Accepted: 6 November 2021

Abstract
Bacterial tyrosinases, as in the case of other bacterial oxidative enzymes, have been found to possess biochemical characteristics that typically make them more suited to applications requiring special operational conditions such as alkaline pH, high or low temperature, the presence of organic solvents, and the presence of inhibitors. Even though a great deal is known about fungal tyrosinases, bacterial tyrosinases still vastly remain underexplored for their potential application in organic synthesis. A literature survey in particular highlights the gaps in our knowledge pertaining to their biochemical properties. Bacterial tyrosinases have not only shown promise in the synthesis of medically important compounds such as L-3,4-dihydroxyphenylalanine (L-DOPA) and melanin but have also seen application in cross-linking reactions of proteins and the polymerization of environmental pollutants. Their ability to catalyse *o*-hydroxylation reactions have shown some degree of promise in the biocatalytic conversion of resveratrol to piceatannol, tyrosol to hydroxytyrosol, and many more. In this review, we will explore the world of bacterial tyrosinases, their current applications, and future perspectives for the application of these enzymes in organic synthesis.

Keywords Bacteria · Cross-linking · L-DOPA · Melanin · Organic synthesis · Tyrosinase

Introduction

Tyrosinases (EC 1.14.18.1) are type-3 copper-containing monooxygenases known to catalyze two types of reactions: the *o*-hydroxylation of phenols to catechols (monophenolase activity) and subsequent oxidation of catechols to *o*-quinones (diphenolase activity) in the presence of molecular oxygen (Fig. 1) (Claus and Decker 2006; Faccio et al. 2012). Furthermore, the non-enzymatic polymerization of the active quinones results in melanin formation as seen in skin, hair colouration, as well as browning in fruit and vegetables. Moreover, tyrosinases are responsible for wound healing in plants and the melanin produced by bacteria, protection of DNA from UV damage and other environmental stresses (Sánchez-Ferrer et al. 1995; Claus and Decker 2006; Fairhead and Thöny-Meyer 2011; Faccio et al. 2012). Due to their protective abilities, it is not surprising that tyrosinases have been isolated from various sources such as animals, plants, fungi, and bacteria (Gasparetti et al. 2010; Roy et al. 2014; Le Roes-Hill et al. 2015; Harir et al. 2018). The tyrosinase produced by *Agaricus bisporus* (the common white button mushroom) has been widely explored in biotechnology mainly because of its commercial availability (Fairhead and Thöny-Meyer 2011). However, its low solvent stability and low temperature fluctuation tolerance have demanded the need for alternative tyrosinases that can be used as a substitute in biocatalysis reactions (Fairhead and Thöny-Meyer 2011; Subrizi et al. 2014).

Bacterial tyrosinases

Studies focused on bacterial tyrosinases, where sequence data and/or biochemical data and applications are provided, have been reported for only a limited number of bacterial genera: *Aeromonas*, *Bacillus*, *Burkholderia*, *Marinomonas*, *Nitrosopumilus*, *Pseudomonas*, *Ralstonia*, *Rhizobium*, *Shewanella*, *Stenotrophomonas*, *Streptomyces*, *Verrucomicrobium* (Table 1). It is interesting to note that the producer-strains were all isolated from diverse environments, including soil, a termite mound, rock, seawater, a marine

✉ Marilize Le Roes-Hill
LeroesM@cput.ac.za

Mayowa Agunbiade
AgunbiadeM@cput.ac.za

[1] Applied Microbial and Health Biotechnology Institute, Cape Peninsula University of Technology, PO Box 1906, 7535 Bellville, South Africa

Published online: 24 November 2021

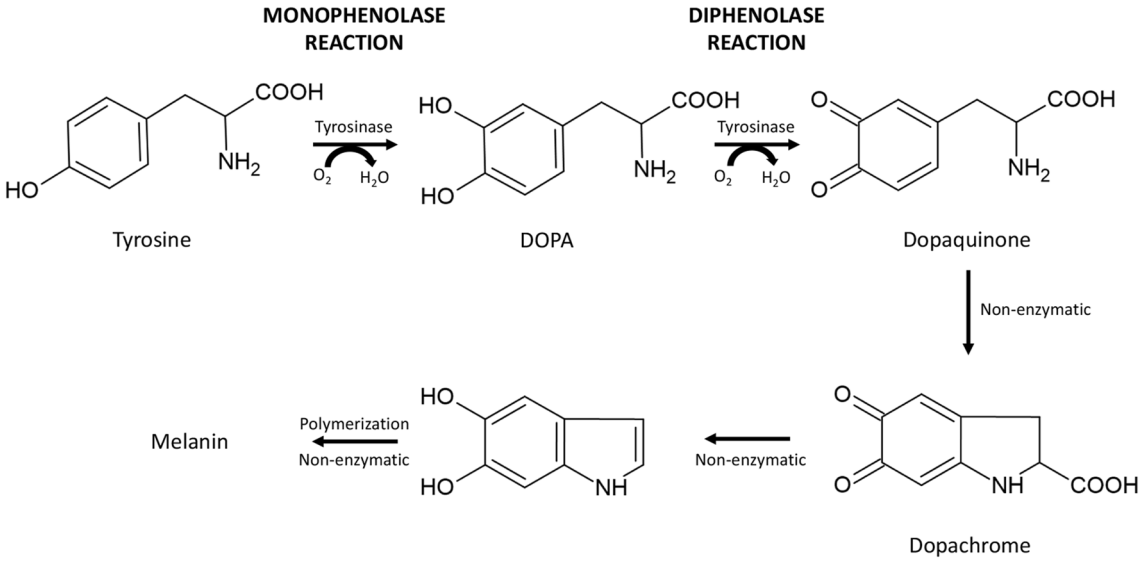

Fig. 1 The monophenolase and diphenolase reaction catalysed by tyrosinase. Non-enzymatic oxidation of dopaquinone results in the formation of dopachrome and ultimately, polymerization results in the formation of melanin

metagenome, root nodules, a legume, oyster (*Crassostrea virginica*), and freshwater eutrophic habitats. The first bacterial tyrosinases were isolated from *Streptomyces glaucescens* (Lerch and Ettlinger 1972) and *Streptomyces nigrifaciens* (Nambudiri et al. 1972), but the validation of the enzyme produced by the latter strain as a true tyrosinase is yet to be confirmed (Fairhead and Thöny-Meyer 2010, 2011). Many more publications report on the production of tyrosinases from bacterial strains, but without corresponding sequence information. Conversely, some studies report the presence of a tyrosinase (e.g., based on mRNA studies or genomic studies), but without any corresponding biochemical data. The existence of such incomplete data sets makes it difficult to evaluate the distribution of this important oxidoreductase among bacteria.

Fairhead and Thöny-Meyer (2011) broadly grouped bacterial tyrosinases into five groups based on their sequence-structure properties (Fig. 2). Type I include the streptomycete tyrosinases that require the presence of a caddie protein. The streptomycete tyrosinase bicistronic operon contains two genes known as *melC1* and *melC2*. The structural gene, *melC2*, encodes for an inactive apotyrosinase (30–35 kDa in size) which requires the assistance of MelC1 (helper protein/chaperone; 13–19 kDa in size) for its secretion and activation (Claus and Decker 2006). The MelC1 caddie protein aids with the incorporation of copper atoms into the active site of MelC2, thereby forming a complex with MelC2. Since MelC1 contains a twin arginine translocation (TAT) signal peptide sequence, it further facilitates the export of the enzyme in its active form through the TAT pathway (Yang and Chen 2009) Chen et al. (1992) proposed two potential models for the activation of the apotyrosinase. In Model 1, the presence of histidines in MelC1 may be responsible for the binding of copper. Binding of MelC1 with MelC2 then allows for the incorporation of the copper while at the same time MelC1 acts as a helper protein/molecular chaperone to allow for correct folding of MelC2. Alternatively, Model 2 proposes that MelC1 is only involved in the correct folding of the apotyrosinase, which then allows for copper incorporation to take place. Although Chen et al. (1992) proposed that MelC1 is later released from the complex and is not required for tyrosinase activity, it has since been shown through structural data that MelC1 plays a key role in the co-ordination of copper in the active, extracellular tyrosinase (for example see Fig. 3A). Yang and Chen (2009) also showed the existence of a *melD1/melD2* bicistronic gene cluster, which encodes for an intracellular tyrosinase responsible for protecting the producer strain against plant phenolics. Similarly, another *melC1/melC2* homologous pair, *griE/griF*, has been detected in *Streptomyces griseus*, where it plays a role in the synthesis of the antibiotic grixazone (Suzuki et al. 2006).

Type II tyrosinases are similar to the streptomycete tyrosinases, but do not require a caddie protein for their secretion (e.g. the *Bacillus megaterium* tyrosinase). Type III tyrosinases contain a C-terminal extension that needs to be removed in order to activate the enzyme. These tyrosinases therefore share similar features to fungal tyrosinases, with the tyrosinase from *Verrucomicrobium spinosum* being one such example. Type IV tyrosinases are much smaller in size and are only active in a homodimer form (e.g. *Bacillus thuringensis*), while Type V tyrosinases contain multifunctional polyphenol oxidases, which seem to exhibit both tyrosinase and laccase-like activities (e.g. *Marinomonas*

Table 1 Bacterial tyrosinases for which sequence data have been reported and/or biochemical and application data

Bacterium	Sequence accession number(s)	Biochemical properties reported	Applications reported	Reference
Aeromonas media strain WS	ACD40043.1	Molecular weight (58 kDa) Optimum pH (L-DOPA = 11.0; L-tyrosine = 9.0) Optimum temperature (70 °C for native; 50 °C for recombinant) Kinetic data (L-DOPA) Effect of inhibitors (EDTA, Glutathione, Arbutin, NaCl, Phenylthiourea, Tropolone)	None	Wan et al. 2009
Bacillus aryabhattai strains TCCC 111983	MT271685	Molecular weight (35 kDa) Optimum pH (5.0) Optimum temperature (60 °C) Kinetic data (L-tyrosine, L-DOPA) Effect of metal ions (Mn^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Co^{2+}, K^{+}, Cu^{2+}) Effect of inhibitors (EDTA, SDS, Triton X-100, Mercaptoethanol, DTT, PMSF, NaCl, EGTA	Dye decolorization of three azo dyes (chrysoidine G, adizol black B, and acid orange 74), one triphenylmethane dye (crystal violet), and one anthraquinonic dye (reactive blue 19)	Wang et al. 2021
Bacillus megaterium strains ATCC 10778, BCRC 10608 (=NBRC 15308=ATCC 14581)	EEM62437.1 B2ZB02 pdb: 3NM8 KGJ77254	Molecular weight (35-36 kDa) Optimum pH (7.0-7.5) Optimum temperature (50 °C) Kinetic data (L-tyrosine, L-DOPA, D-DOPA) Effect of inhibitors (EDTA, mercaptoethanol, glutathione, diethyldithio-carbamate) Effect of organic solvents (methanol, ethanol, acetone, 2-propanol, DMSO)	**Strain ATCC 10778**: Regio-selective o-hydroxylation of 7,4'-dihydroxyisoflavone (daidzein) to produce 7,3',4'-trihydroxyisoflavone (3'-ODI). Conversion of genistein and resveratrol to orobol and piceatannol, respectively, with ca. 95 % conversion yield. Production of a unique melanin biopolymer. **Strain name not defined**: application in the cross-linking of soy glycenin, stabilising it in an oil-in-water emulsion, improving its potential application in food. In conjunction with glucose dehydrogenase, the tyrosinase was applied in the production of hydroxytyrosol from tyrosol. **Strain BCRC 10608**: Biotransformation of the soy isoflavone glycosides daidzin and genistin	Shuster and Fishman 2009; Shuster Ben-Yosef et al. 2010; Goldfeder et al. 2013; Isaschar-Ovdat et al. 2015, 2016; Chiang et al. 2016, 2017; Hosseini-Abari et al. 2016; Lee et al. 2016, 2018; Isaschar-Ovdat and Fishman 2017; Son et al. 2018; Hosseini-Abari and Tayebi 2019; Deri-Zenaty et al. 2020; Hosseini-Abari 2020; Park et al. 2020
Burkholderia thailandensis strain E264 (ATCC 700388)	WP_011400974.1	Optimal pH (5.0) Kinetic data (L-tyrosine, L-DOPA)	Conversion of daidzin to o-hydroxydaidzin	Son et al. 2018
Marinomonas mediterranea strain MMB-1 (ATCC 700492^{T}, CECT 4803^{T})	AAV4996.1	None	None	Lopez-Serrano et al. 2002; Sanchez-Amat et al. 2010

Table 1 (continued)

Bacterium	Sequence accession number(s)	Biochemical properties reported	Applications reported	Reference
Candidatus *Nitrosopumilus koreensis*	AFS80363.1	Molecular weight (47 kDa) Optimum pH (6.0) Optimum temperature (20 °C) Kinetic data (L-tyrosine, L-DOPA)	None	Kim et al. 2016
Pseudomonas aeruginosa strain PAO1	NP_251082.1	Kinetic data (L-tyrosine, D-tyrosine, L-DOPA, ferribactin)	Capable of acting on large peptidic substrates.	Nadal-Jimenez et al. 2014; Wibowo et al. 2020
Ralstonia solanacearum strain GMI1000	NP518458	Molecular weight (55 kDa) Kinetic data (D-tyrosine, L-tyrosine, 4-fluorophenol, 4-chlorophenol, 4-bromophenol, 4-iodophenol)	Biotransformation of 4-fluorophenol to 4-fluorocatechol	Hernández-Romero et al. 2005; Molloy et al. 2013; Davis et al. 2018
Rhizobium etli strain CFN42	AAM54973.1	Optimum pH (6.5-7.5) Optimum temperature (50 °C) Kinetic data (L-DOPA)	Production of melanin	Cabrera-Valladares et al. 2006; Laguna-Muñoz et al. 2006; Chávez-Béjar et al. 2013
Rhizobium meliloti strain GR4	EMBL: P33180	None	None	Mercado-Blanco et al. 1993
Shewanella colwelliana	AAA26510	Molecular weight (41 kDa)	None	Fuqua et al. 1991
Pseudomonas maltophila (renamed as *Stenotrophomonas maltophila*)	AAC16658	Molecular weight (18 kDa)	None	Wang et al. 1999, 2000
Streptomyces avermitilis strain MA4680 (ATCC 31267)	KP198295.1 (AIY72671.1) and WP_010982575.1	Optimum pH (8.0) Kinetic data (L-tyrosine, L-DOPA, resveratrol, piceatannol)	Production of a tissue adhesive hydrogel. Conversion of resveratrol to piceatannol as well as conversion of daidzein to 3'-ODI.	Lee et al. 2012, 2015, 2016; Kim et al. 2018; Son et al. 2018
Streptomyces castaneoglobisporus strain HUT6202	Q83WS2 and 2ZMX	Molecular weight (MelC2: 38 kDa) Kinetic data (L-DOPA)	None	Ikeda et al. 1996; Kohashi et al. 2004
Streptomyces cyaneofuscatus	ATX68182 and ATX68183	Molecular weight (MelC2: 30 kDa) Optimum pH (7.0) Optimum temperature (55 °C) Kinetic data (L-DOPA) Effect of metal ions (Mn^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Co^{2+}, Cu^{2+}) Effect of inhibitors (EDTA, SDS, Ascorbic acid, L-cysteine, sodium metabisulfite, NaCl) Effect of organic solvents (methanol, ethanol, acetone, acetonitrile, DMSO)	Production of melanin	A Khatib et al. 2018; Harir et al. 2018
Streptomyces griseus strain IFO13350	BAD99128.1 (GriE); BAD99129.1 (GriF)	Optimum pH (6.5-8.5) Optimum temperature (55 °C) Kinetic data (*o*-aminophenol, 3,4-AHBAL, 2-amino-4-methylphenol, 3,4-dihydroxybenzaldehyde, catechol, L-DOPA)	Grixazone antibiotic production	Suzuki et al. 2006

Table 1 (continued)

Bacterium	Sequence accession number(s)	Biochemical properties reported	Applications reported	Reference
Streptomyces kathirae strain SC-1	MelC1 (AIL88697.1) and MelC2 (AIL88698.1)	Molecular weight (MelC2: 30 kDa) Optimum pH (6.2) Optimum temperature (45 °C) Kinetic data (L-DOPA, L-tyrosine) Effect of metal ions (Mn^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Co^{2+}, K^{+}, Al^{3+}, Cu^{2+}) Effect of inhibitors (EDTA, SDS, Tween-80, Triton X-100, $NaNO_2$, Ascorbic acid, mercaptoethanol, DTT, thiourea)	Melanin production	Guo et al. 2015
Streptomyces pharetrae strainCZA14	KR030068 (MelC2), KR030066 (MelC1)	Molecular weight (MelC2: 32.6 kDa) Optimum pH (5.5-9.5 depending on substrate) Optimum temperature (30-40 °C) Kinetic data (*p*-cresol, L-DOPA) Effect of metal ions (Mn^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Co^{2+}, Cu^{2+}) Effect of inhibitors (EDTA, SDS, Ascorbic acid, Arbutin, L-cysteine, sodium metabisulfite, NaCl) Effect of organic solvents (methanol, ethanol, acetone, acetonitrile, 2-propanol, DMSO)	Application in cross-linking reactions	Le Roes-Hill et al. 2015
Streptomyces polyantibioticus strain SPR	KR030067 (MelC2), KR030065 (MelC1)	Molecular weight (MelC2: 30.5 kDa) Optimum pH (5.5-9.0 depending on substrate) Optimum temperature (22-35 °C) Kinetic data (*p*-cresol, L-DOPA) Effect of metal ions (Mn^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Co^{2+}, Cu^{2+}) Effect of inhibitors (EDTA, SDS, Ascorbic acid, Arbutin, L-cysteine, sodium metabisulfite, NaCl) Effect of organic solvents (methanol, ethanol, acetone, acetonitrile, 2-propanol, DMSO)	Application in cross-linking reactions	Le Roes-Hill et al. 2015

Table 1 (continued)

Bacterium	Sequence accession number(s)	Biochemical properties reported	Applications reported	Reference
Streptomyces sp. strain REN-21	Sequence provided in manuscript	Molecular weight (44 kDa) Optimum pH (7.0) Optimum temperature (35 °C) Kinetic data (L-DOPA, DL-DOPA, D-DOPA, L-tyrosine, DL-tyrosine, D-tyrosine) Effect of organic solvents (methanol, ethanol, acetone, 2-propanol, DMSO, 1-propanol)	None	Ito and Oda 2000; Ito and Inouye 2005; Rodakiewicz-Nowak and Ito 2005
Verrucomicrobium spinosum strain No. 4136	WP_081452337.1	Molecular weight (36.6 kDa) Optimum pH (6.0-7.0) Optimum temperature (45 °C) Kinetic data (L-tyrosine, L-DOPA)	Cross-linking of various proteins, including the production of lipase cross-linked enzyme aggregates. Enzymatic crosslinking of C-phycocyanin to amino-modified polystyrene beads thus conferring a blue fluorescence. Production of a protein with adhesive properties. L-DOPA production from L-tyrosine.	Fairhead and Thöny-Meyer 2010; Jus et al. 2012; Ren et al. 2013; Faccio et al. 2014; Axambayeva et al. 2018; Tan et al. 2019; Winroth 2019

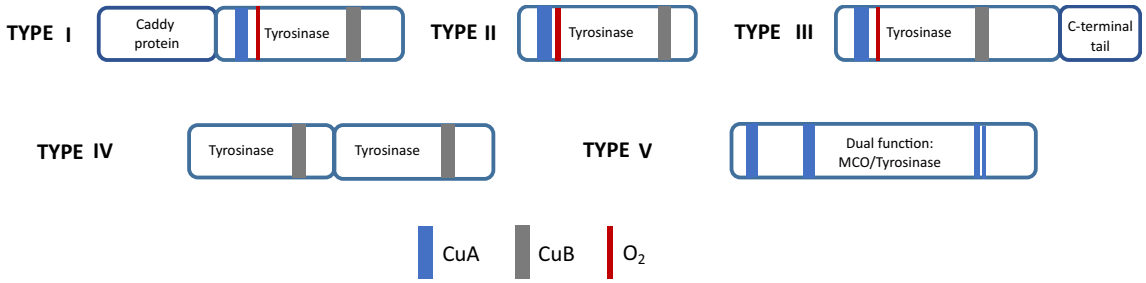

Fig. 2 Arbitrary assigned classification/grouping of bacterial tyrosinases as described by Fairhead and Thöny-Meyer (2011). CuA and CuB bands represent the presence of the copper binding motifs found in tyrosinases, while the O_2 band serves as an indicator for the oxygen binding site. *MCO* multicopper oxidase

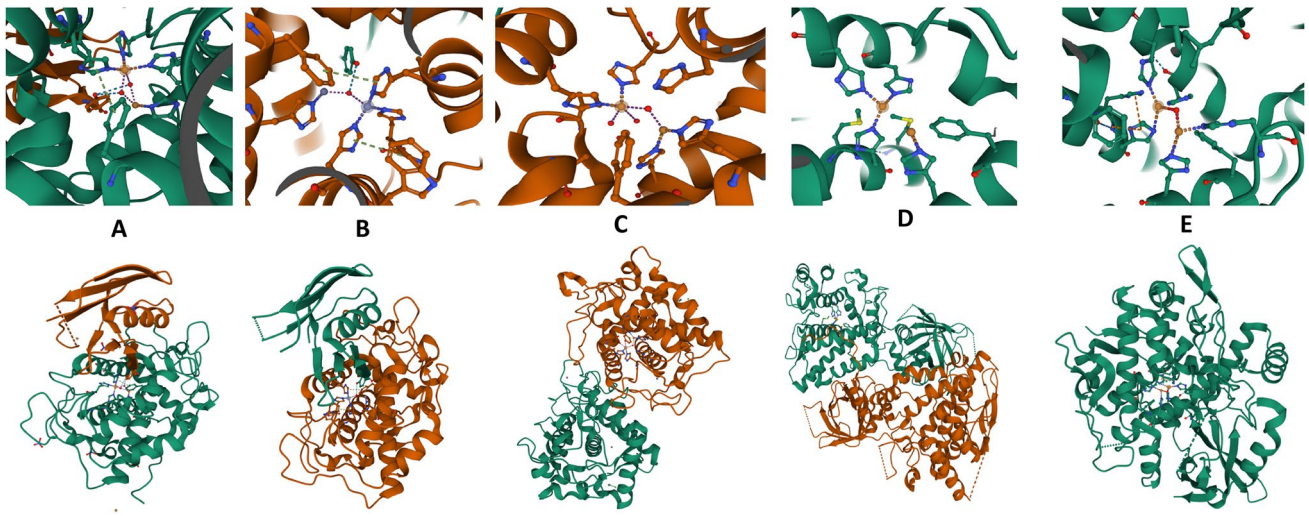

Fig. 3 Structural features of bacterial tyrosinases showing the co-ordination of the copper atoms as well as the structure of the active enzymes. A: *Streptomyces castaneoglobisporus* (PDB: 2ZMX) (Matoba et al. 2006); B: *Streptomyces avermitilis* (PDB: 6J2U; unpublished) (copper substituted with zinc); C: *Bacillus megaterium* (PDB: 3NM8) (Sendovski et al. 2011); D: *Pseudomonas aeruginosa* (PDB: 6RRQ) (Wibowo et al. 2020); and E: *Burkholderia thailandensis* (PDB: 5ZRE) (Son et al. 2018). Images were created using Mol* (Sehnal et al. 2018) and obtained from the RCSB PDB. Protein chains are represented in ribbon format. For images A and B, the green ribbons represent the apotyrosinase that requires activation by the caddie protein (represented in orange-brown); for images C and D the colour differentiation shows the crystallisation of the proteins in homo-dimeric form; while for image E, the single colour indicates that the protein crystallised in monomeric form

mediterranea). The classification of Types IV and V as tyrosinases are therefore in question since they do not share the 'typical' structural features of tyrosinases such as the presence of the binuclear type-3 copper center, but merely exhibit the 'typical' biochemical features of tyrosinases (e.g. the ability to utilize L-tyrosine as a substrate, a feature that other related oxidoreductases such as laccases, do not share) (Fairhead and Thöny-Meyer 2011).

The binuclear type-3 copper center of tyrosinases consists of two copper atoms (CuA and CuB) which are coordinated by conserved histidine residues and make up the active site – a feature shared by all tyrosinases (except Types IV and V as shown in Figs. 2, 3) (Huber and Lerch 1988). The CuA binding motif shared by bacterial tyrosinases has a signature sequence of H-Xn-H-X8-H, while the CuB signature sequence motif is H-X3-H-Xn-H, where Xn represents a set of undefined amino acids (Faccio et al. 2019). To date, only a few bacterial tyrosinase structures have been elucidated, and include those of *Streptomyces castaneoglobisporus* (Matoba et al. 2006), *Streptomyces avermitilis* (unpublished; PDB: 6J2U), *B. megaterium* (Sendovski et al. 2011), *Pseudomonas aeruginosa* (Wibowo et al. 2020), and *Burkholderia thailandensis* (Son et al. 2018). The co-ordination of the two copper atoms can be seen in all the structures elucidated to date, and the importance of the caddie protein in the formation, co-ordination, and stabilization of these copper atoms in streptomycete tyrosinases, can also be seen (Figs. 3A, B).

Based on structural studies, it is predicted that during substrate oxidation, the enzyme active site can exist in one of three oxidative states: *oxy*, *met*, and *deoxy*. In the *oxy* state, the two copper ions are bridged by dioxygen, resulting in a peroxide linkage, and allows for the deprotonation of the monophenolic substrate, ultimately resulting in the *ortho*-hydroxylation of the substrate. Reaction with the substrate converts the enzyme into the *met* form, which in turn is converted to the *deoxy* form when oxidation of the diphenolic compound occurs (the two-electron reduction reaction). Once the *deoxy* form binds dioxygen, it is restored to the *oxy* state (Agarwal et al. 2019; Faccio et al. 2019; Hamann et al. 2017). The enzyme is required to be in the *oxy* state to allow for the monophenol monooxygenase type reaction, whereas the *met* and *oxy* forms are required for diphenolase activity (*met* tyrosinase is unable to hydroxylate monophenols) (Fig. 4) (Agarwal et al. 2019; Hamann et al. 2017).

Production of bacterial tyrosinases

For bacterial tyrosinases to be a potential replacement for the commercially available mushroom tyrosinase, one of the obstacles that needs to be overcome, is their large-scale production. The first description of the production of mushroom (*A. bisporus*) tyrosinase showed a reproducible yield of 21.6 mg/ml purified tyrosinase (from 10.9 kg of mushroom; Kertesz and Zito 1965). However, it is a convoluted process requiring multiple steps of acetone and ammonium sulphate precipitation and fractionation, purification with calcium acetate, and Sephadex G-100 chromatography (Kertesz and Zito 1965). It has been well established that various factors including the DNA of microorganisms, medium composition, pH, temperature, inducers, copper (essential metal cofactor), and inhibitors contribute immensely to tyrosinase production in bacteria (Faccio et al. 2012). For most bacterial tyrosinases, it is yet to be determined whether their tyrosinases are constitutively expressed or whether they are inducible (Popa and Bahrim 2011) – aspects which are important when making use of the native strain for expression of the enzyme. Some bacteria such as streptomycetes and selected bacilli have the potential to produce tyrosinases extracellularly, which are synthesized intracellularly before their transportation and secretion into the growth medium (Baumann et al. 1976). Tyrosinase production by *S. glaucescens* was induced by L-phenylalanine, L-methionine, and L-leucine, while the addition of L-tyrosine had no effect (Baumann et al. 1976). Tyrosinase production by *Streptomyces antibioticus* was induced by the presence of L-methionine, copper sulphate and D-glucose (Katz and Betancourt 1988). Furthermore, the tyrosinase produced by *Streptomyces cyaneofuscatus* was cultured in modified phenoxazinone production medium (MPPM), supplemented with chloroform and copper sulphate as inducers (Harir et al. 2018). Similarly, *Streptomyces polyantibioticus* strain SPR^T and *Streptomyces pharetrae* strain CZA14^T were cultured in the same complex medium (MPPM) and the addition of copper sulphate resulted in the induction of tyrosinase production in both strains, while L-methionine led to a weak effect on tyrosinase production (Le Roes-Hill et al. 2015). In contrast, tyrosinase produced by *Streptomyces kathirae* attained its optimum production when grown in high concentration of yeast extract (37 g/L), salts (NaCl and $CaCl_2$), amylodextrin

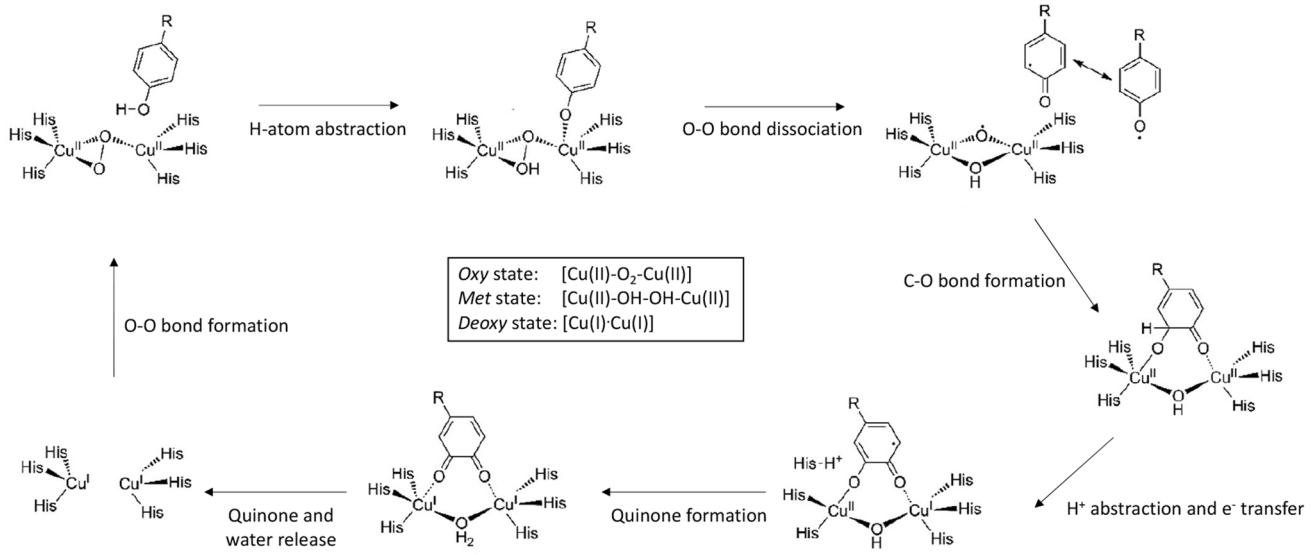

Fig. 4 Proposed catalytic reaction for tyrosinases which results in the formation of the three oxidative states of the enzyme active site, *oxy*, *met*, and *deoxy*, which results in the *ortho*-hydroxylation of monophenols, and the subsequent two-electron reduction required for the oxidation of diphenolic compounds (adapted from Hamann et al. 2017)

(carbon source) and copper sulphate as an inducer (Guo et al. 2014). Even though it is clear that potential precursor amino acids and the metal co-factor, copper, act as inducers, the triggers and cues for the overproduction of tyrosinases from native strains remains an aspect that re-quires further investigation.

Many attempts have been made to produce tyrosinases by overexpressing bacterial tyrosinases in bacterial hosts. Recombinant strains offer the possibility of an enhanced protein production level, better growth, and improved productivity when compared to non-recombinant production systems. The bicistronic nature of the tyrosinases produced by streptomycetes often provide some challenges. However, there have been a few success stories. *Streptomyces castaneoglobisporus* tyrosinase was expressed in a complex with its caddie protein, ORF378, in *E. coli* (maximum yield of 11 mg/L) (Kohashi et al. 2004) as well as in *Streptomyces lividans* (Ikeda et al. 1996). *Streptomyces lividans* was also used as an expression host for tyrosinases from *S. antibioticus* (Katz et al. 1983; Lee et al. 1988), *S. glaucescens* (Hintermann et al. 1985), and *S. kathirae* (Guo et al. 2015). The *E. coli* BL21 expression host was used to produce tyrosinase from *Streptomyces* sp. strain REN-21, resulting in the production of 54 mg/L of the enzyme after a 16 h incubation (Ito and Inouye 2005). For the expression of the tyrosinase produced by *S. antibioticus*, *P. fluorescens* was used as an expression host since it exhibited better benefits over other bacterial hosts tested (Son et al. 2012). Some of the features or characteristics it possesses over other hosts includes the possibility of culturing in high-cell-density fermentation (Chew et al. 2005), the presence of a TAT system for the secretion of the tyrosinase, which could eliminate the rigorous procedures of purifying streptomycete tyrosinase from other intracellular proteins from lysed cells, and its ability to utilize the well-documented ATP-binding cassette (ABC) transporter system. Interestingly, extracellular tyrosinase secretion levels were far higher in its non-natural translationally conjugated fusion protein form than in the natural complex of two separate polypeptides (Ryu et al. 2019). This study therefore showed the great potential of using *P. fluorescens* as a stable expression host, obtaining a yield of 26 mg/L for a bacterial tyrosinase by exploiting the expression strain's secretory machinery. The bicistronic nature of the streptomycete tyrosinase encoding operon, has also complicated expression. However, great success has been achieved through the use of the pETDuet expression vector (Lee et al. 2015, 2016; Son et al. 2018), which was also applied in the co-expression of the *B. megaterium* tyrosinase and a P450 monooxygenase for the controlled production of melanin (Park et al. 2020). Another expression system of note, is the surface-displayed tyrosinase of *B. megaterium* which was tethered to the cell surface of *Bacillus subtilis* spores via the CotE anchor protein, resulting in enhanced enzyme activity, stability, and reusability for application in the conversion of genistein to orobol (Hosseini-Abari and Tayebi 2019). Even though these reports are promising, the protein yield is not always reported, making it difficult to gauge progress in terms of protein yields (21.8 mg/ml) obtained during the purification of *A. bisporus* tyrosinase.

Biochemical properties of bacterial tyrosinases

The successful application of bacterial tyrosinases in biocatalytic reactions requires an understanding of the biochemical properties of the enzyme of interest. Important aspects to consider include functionality under different pH and temperature conditions, the ability to oxidize certain substrates, and the effect of metal ions, inhibitors (including reducing agents such as L-ascorbic acid) and organic solvents on enzyme function. As such, it is interesting to note that very few studies provide a full overview of the biochemical properties of bacterial tyrosinases. To date, there are only a few studies reported in the literature where sequence data (partial or complete) have been provided (Fuqua et al. 1991; Mercado-Blanco et al. 1993; Ikeda et al. 1996; Wang et al. 1999, 2000; Ito and Oda 2000; Lopez-Serrano et al. 2002; Kohashi et al. 2004; Ito and Inouye 2005; Hernández-Romero et al. 2005; Rodakiewicz-Nowak and Ito 2005; Cabrera-Valladares et al. 2006; Laguna-Muñoz et al. 2006; Suzuki et al. 2006; Shuster and Fishman 2009; Wan et al. 2009; Fairhead and Thöny-Meyer 2010; Sanchez-Amat et al. 2010; Shuster Ben-Yosef et al. 2010; Jus et al. 2012; Lee et al. 2012, 2015, 2016, 2018; Chávez-Béjar et al. 2013; Goldfeder et al. 2013; Molloy et al. 2013; Ren et al. 2013; Faccio et al. 2014; Nadal-Jimenez et al. 2014; Isaschar-Ovdat et al. 2015, 2016; Guo et al. 2015; Chiang et al. 2016; Hosseini-Abari et al. 2016; Kim et al. 2016, 2018; Chiang et al. 2017; Isaschar-Ovdat and Fishman 2017; Al Khatib et al. 2018; Axambayeva et al. 2018; Davis et al. 2018; Harir et al. 2018; Son et al. 2018; Hosseini-Abari and Tayebi 2019; Tan et al. 2019; Winroth 2019; Deri-Zenaty et al. 2020; Hosseini-Abari 2020; Park et al. 2020; Wibowo et al. 2020; Wang et al. 2021). Of these studies, eight provide information on the effect of metal ions, nine on the effect of inhibitors, and five on the effect of organic solvents. The latter is quite surprising considering the widespread use of organic solvents not only in biocatalytic reactions, but also by various industries, requiring enzymes that would be able to function in the presence of organic solvents (Doukyu and Ogino 2010). In contrast to their eukaryote counterparts, bacterial tyrosinases have been shown to be stable in the presence of organic solvents and are often also activated in the presence of water-soluble organic solvents: the tyrosinase produced by *B. megaterium* showed 170% relative activity in the presence of 30% (v/v) dimethyl sulfoxide (as compared to the reaction in an optimal buffer solution)

(Shuster and Fishman 2009); the tyrosinase produced by *Streptomyces* sp. REN-21 retained 44% of its activity in the presence of 50% (v/v) ethanol, while a tyrosinase isolated from a mushroom only exhibited 6% activity under the same experimental conditions (Ito and Oda 2000); and the tyrosinases produced by three streptomycete strains exhibited > 80% residual activity in the presence of 30% (v/v) methanol, with enhanced activity observed for the tyrosinase produced by *S. polyantibioticus* when incubated for 20 h at 4 °C in the presence of 2-propanol (10%, v/v), ethanol (10–20%, v/v), and methanol (10–20 %, v/v) (Le Roes-Hill et al. 2015; Harir et al. 2018).

Bacterial tyrosinases are active across a wide pH range: pH 5–11 (L-DOPA) and pH 5–9 (L-tyrosine) and temperature range: 22–70 °C (see Table 1). Typical substrates used in these studies include L-DOPA, and L-tyrosine, with D-tyrosine and o-aminophenol also being reported. Different substrates oxidized by bacterial tyrosinases (in addition to L-DOPA and L-tyrosine), include a range of phenolic compounds: 3-hydroxyanthranilic acid, 4-methylcatechol, 4-*tert*-butylcatechol, N-acetyl-L-tyrosine, caffeic acid, (+)-catechin, (±)-catechin, catechol, chlorogenic acid, *o*-coumaric acid, *p*-coumaric acid, *p*-cresol, epicatechin, *p*-hydroxybenzoic acid, orcin, phloroglucin, pyrogallol, resorcinol, resveratrol, tyramine, and L-tyrosine ethyl ester (Table 1). It is due to their ability to oxidize phenolic molecules such as these (as well as phenolic compounds associated with proteins) that bacterial tyrosinases have proven potential for biotechnological applications. For instance, the application of tyrosinases in the production of L-DOPA, the bioremediation of phenols and dyes, melanin production, cross-linking reactions, synthesis of *o*-hydroxylated polyphenols, and other applications (Lantto et al. 2007; Selinheimo et al. 2007; Nagatsua and Sawadab 2009; Martorell et al. 2012). In the next section, we explore the current applications of bacterial tyrosinases and reiterate the future perspectives for the application of these enzymes in organic synthesis.

Application of bacterial tyrosinases in organic synthesis

L-3,4-dihydroxyphenylalanine production

L-DOPA is an amino acid analogue and a precursor to dopamine and is typically employed in the treatment of Parkinson's disease, a neurodegenerative disorder that is linked with a compromised level of the neurotransmitter, dopamine, in the brain (Xu and Pu 2016; Agarwal et al. 2019). According to the survey conducted by the Global Burden of disease center in 2013, Parkinson's disease occurs in about 53 million people and has resulted in a high death rate globally. Furthermore, it has been reported that elderly people suffer from this ailment with a manifestation of symptoms associated with rigidity and tremor (Surwase et al. 2012a, b). Based on the challenges associated with this ailment, the demand and global market for L-DOPA have increased tremendously to about 250 tons yearly (Min et al. 2015). To meet these demands, Monsanto commercialized a chemical process to produce L-DOPA. This chemical process occurs under harsh conditions resulting in a low conversion rate and poor enantioselectivity (Algieri et al. 2012). Alternatively, L-tyrosine can serve as a precursor for L-DOPA, catalyzed by tyrosinase (Fig. 5) (Algieri et al. 2012). However, this approach is still hampered by low productivity (22.54 mg/L/hr), a low conversion (10.2 ± 0.03%), and low stability of the catalyst, tyrosinase. To improve the level of production, a tyrosinase with a high monophenolase to diphenolase ratio would be required, and in order to improve stability, the tyrosinase would have to be immobilized. To realise this, the *V. spinosum* tyrosinase was co-expressed with a polyhydroxyalkanoate (PHA) synthase gene, resulting in the production of an in situ immobilized tyrosinase on PHA nano-granules with the ability to synthesise L-DOPA (Tan et al. 2019). Productivity of L-DOPA obtained in this study

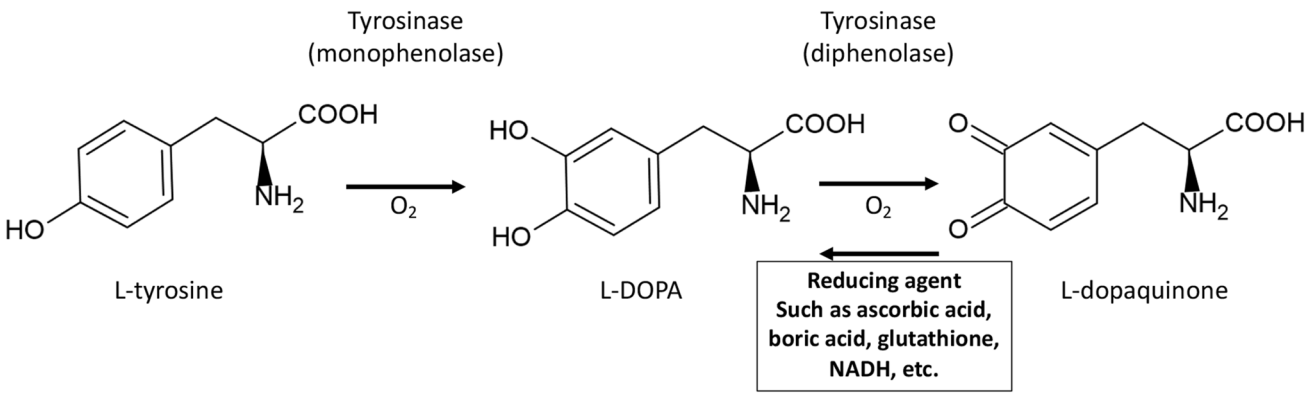

Fig. 5 L-tyrosine conversion to L-DOPA as catalysed by tyrosinase. To prevent L-dopaquinone formation and subsequent polymerization, a reducing agent is often employed (Algieri et al. 2012)

was 148.70 mg/L/hr, with a conversion rate of the substrate, L-tyrosine, of 90.62 %. In addition, the enzyme proved to be stable after 8 cycles of repeated use, thereby presenting an economically viable alternative to commercial processes for L-DOPA production. The tyrosinases produced by *Ralstonia solanacearum* (Davis et al. 2018) and *Candidatus Nitrosopumilus koreensis* (Kim et al. 2016) with their high ratio of monophenolase to diphenolase activity, could potentially also find application in the production of L-DOPA and are worth exploring in future studies.

Bioremediation of phenols and dyes

The discharge of untreated wastewater containing phenols and dyes into the ecosystem poses a major health threat. Hence, there is an urgent need to mitigate the negative impact of the toxic compounds and their derivatives in the environment. Phenolic compounds are usually found in wastewaters of the pulp and paper, textile, dye, petroleum refineries, resin, and plastics industries (Brasquet et al. 1999). Bacterial tyrosinases have been shown to have the potential to remove micropollutants from the environment: *Bacillus thuringiensis* tyrosinase removed 4-chlorophenol (60% removal), phenol (80% removal), and 2,4-dichlorophenol (100% removal) in buffer solutions; *S. antibioticus* tyrosinase removed 3-chlorophenol and 4-chlorophenol in buffer solutions; while a sodium alginate encapsulated *Streptomyces espinosus* tyrosinase removed 60% of phenol in an aqueous solution within a 4 h incubation period (Ba and Kumar 2017). The application of tyrosinases in bioremediation is based on their ability to oxidise compounds that eventually result in polymerization (the same as what happens during melanin formation). The polymers can either be filtered or allowed to precipitate and can then be removed from the aqueous environment.

Optimal remediation of toxic phenolic pollutants in wastewaters can either be achieved with tyrosinase-producing strains or with the enzyme in an immobilized state (Jadhav et al. 2010; Saratale et al. 2011). The application of immobilized tyrosinases in water treatment has proven to exhibit excellent efficiency in terms of stability, reusability, and extended viability (López-Molina et al. 2003; Kameda et al. 2006). In a study conducted by Abdollahi et al. (2018), tyrosinase immobilized on magnetic iron oxide nanoparticles efficiently removed 70% of a 2.5 g/L phenol solution in synthetic wastewater at 35 °C and neutral pH. Furthermore, 99% and 58% of phenol removal were maintained after the 3rd and 7th re-use cycle, respectively. Thus, affirming the use of tyrosinase as a nano-biocatalyst that can be applied in the removal of phenolic toxic pollutants. Furthermore, the co-immobilization of tyrosinase and magnetic nanoparticles on graphene oxide, resulted in tyrosinase aggregates that efficiently re-mediated phenol and bisphenol A in water. The result revealed optimum removal efficiency of 98 % of phenol within 39 h and 74.5 % of bisphenol A within 2 h. In addition, over 56% of the initial activity of the enzyme aggregates was maintained after reusing in phenol remediation for five times, thereby validating the potential of the enzyme in phenol removal in wastewater (Liu et al. 2016). The application of laccases and peroxidases in dye decolourization are well-established (Lonappan et al. 2017; Sun et al. 2017). Even though catecholase activity is inhibited by selected azo dyes, tyrosinase has been applied in dye decolourization (Dubey et al. 2007). Interestingly, the decolourization and detoxification of the azo dyes RY 107, reactive black 5, reactive red 198, and direct blue 71 by *Brevibacterium* sp. strain VN-15 resulted in almost 100 % decolourization (Franciscon et al. 2012). The fact that so few dye decolourization analyses have been performed using tyrosinases emphasizes the fact that the removal of micropollutants and dyes by bacterial tyrosinases is one area that is vastly underexplored.

Melanin production

Melanin is a macromolecule formed by the oxidative polymerization of phenolic or indolic compounds. It is a protective agent that is produced by a diverse group of organisms and is typically black or brown in colour (Franciscon et al. 2012). Biological roles that have been identified, include protection against UV, free radical adsorption (oxidative stress mediation), chelation of toxic metals, scavenging of phenolic compounds, as a buffer against environmental stresses, plays a role in pathogenesis and possibly play a role in host root cell penetration by *Frankia* species and nitrogen fixation, nodulation efficiency and symbiosis-associated stress resistance by *Rhizobium* species (Piñero et al. 2007; Yuan et al. 2007; Fairhead and Thöny-Meyer 2011; El-Naggar and El-Ewasy 2017). Melanin is widely used in medicine, pharmacology, and cosmetics, and due to its redox chemistry, has been explored for electrical conductivity in electrical materials (Park et al. 2020).

Commercially available melanin is made from cuttlefish (*Sepia officinalis*) extracts (available at 100 mg or 1 g scale), which is not sustainable, or is chemically synthesized through the oxidation of tyrosine with hydrogen peroxide (e.g. melanin available from Sigma-Aldrich available at 100 mg, 250 mg, or 1 g scale). Production of melanin by bacteria (see Fig. 1) is an economically viable alternative and environmentally friendly. Guo et al. (2015) described the overexpression of the tyrosinase from *S. kathirae* strain SC-1 for enhanced melanin production. The authors successfully increased melanin production at 28.8 g/L, the highest ever reported for bacterial melanin production (Pavan et al.

2020). Melanin biosynthesis is initiated from L-tyrosine via a series of enzymatic and non-enzymatic reactions. To 'control' the chemical composition of melanin, Park et al. (2020) introduced 3-hydroxyindole and 3-oxoindole generated by cytochrome P450 monooxygenase (CYP102G4; produced by *Streptomyces cattleya*). A tyrosinase produced by *Bacillus megaterium* and CYP102G4 were co-expressed from a L-tryptophan-converting *E. coli* strain to produce melanin. Despite the use of different analytical tools, it was not possible to elucidate the precise structure of the melanin synthesized but the authors could propose the chemical composition. Other bacterial strains reported to produce melanin at levels greater than 1 g/L, include: *Pseudomonas* sp. strain WH001 55 (7.6 g/L), *Pseudomonas stutzeri* strain HMGM-7 (6.7 g/L and 7.2 g/L), *Bacillus safensis* (6.9 g/L), *Brevundimonas* sp. strain SGJ (6.8 g/L), a recombinantly expressed tyrosinase from *Rhizobium etli* (6 g/L, 3.22 g/L), *Streptomyces lusitanus* strain DMZ-3 (5.29 g/L), *Streptomyces* sp. strain ZL-24 (4.24 g/L), and *Nocardiopsis alba* strain MSA10 (3.4 g/L) (Pavan et al. 2020). El-Naggar and El-Ewasy (2017) successfully produced extracellular melanin (0.35 g/L) from *S. glaucescens* strain NEAE-H which exhibited anticancer and antioxidant activities. The brown and black melanin produced by the tyrosinase from *Bacillus licheniformis* strain MAL was also reported to exhibit anticancer activity (Shalaby et al. 2019). Unfortunately, most of the studies reported in the review by Pavan et al. (2020) did not confirm (for example via sequence- and function-based studies) whether a tyrosinase or another related enzyme was responsible for the melanin production.

Cross-linking of materials

The cross-linking of proteins plays an important role in biomedical engineering fields, food processing industry, leather, and the textile fabrication industry (Heck et al. 2013). The application of toxic chemicals such as formaldehyde and glutaraldehyde as chemical cross-linkers in industries has posed a threat because of their toxicity and challenges associated with their removal from finished products (Petite et al. 1990; Barbani et al. 1995; Jus et al. 2012). Thus, there is a need to explore cross-linking enzymes that are environmentally friendly. Tyrosinases, transglutaminases, peptidases, laccases, peroxidases, and amino oxidases have served as potential cross-linking agents in improving the properties of materials (Heck et al. 2013). Addition of small phenolic compounds could enhance enzymatic cross-linking reactions that could be hindered by limited numbers of functional groups (e.g. lysyl, tyrosyl, cysteinyl and histidinyl residues) on the target protein (Fig. 6) (Selinheimo et al. 2007, 2008; Fairhead and Thöny-Meyer 2010; Heck et al. 2013). Le Roes-Hill et al. (2015) demonstrated that poor or limited cross-linking was observed when bovine serum albumin, horse heart cytochrome C, and myoglobin were used as substrates, which could be because of limited or lack of tyrosyl side chains. Furthermore, tyrosinases produced by *S. polyantibioticus* and *S. pharetrae* effectively cross-linked casein and gelatin without the aid of phenolic compounds, thus suggesting their potential application in the production of biological frames for tissue engineering (Jus et al. 2012; Le Roes-Hill et al. 2015). Jus et al. (2012) also demonstrated the cross-linking of gelatin by the tyrosinase produced by *V. spinosum*, while Axambayeva et al. (2018) showed the application of the *V. spinosum* tyrosinase in the production of a protein with adhesive properties similar to that of mussel foot proteins. The tyrosinase from *V. spinosum* has also successfully been employed in the formation of cross-linked enzyme aggregates (CLEAs) of the *Candida antarctica* lipase (in the presence of phenol), and the cross-linking of the photosynthetic blue-fluorescent C-phycocyanin to polystyrene beads with potential application in the biomedical and diagnostic fields (Faccio et al. 2014). Not only does this protein have light harvesting properties, but it is also known

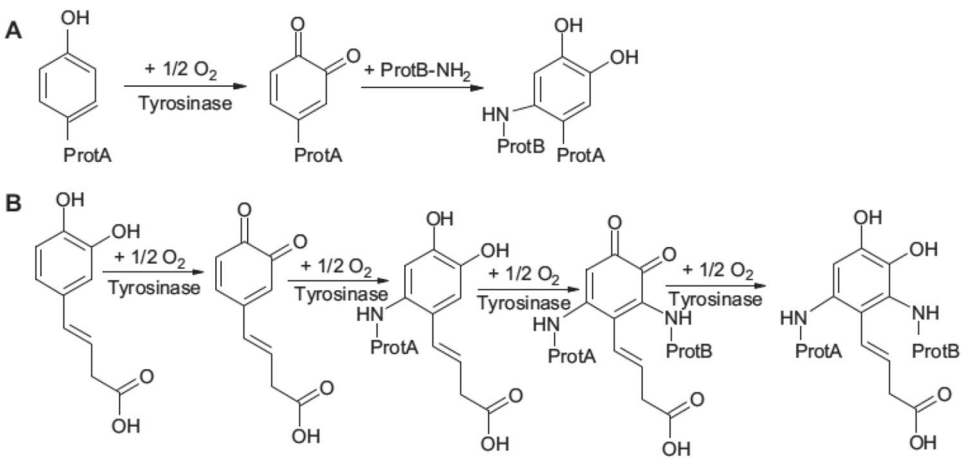

Fig. 6 The different types of cross-linking reactions either in the absence (**A**) or presence (**B**) of a phenolic compound

to have antioxidant, anti-diabetic, and anti-tumour properties, making it an ideal candidate for surface functionalization of materials.

Kim et al. (2018) explored the application of a *S. avermitilis* tyrosinase in the production of a hydrogel based on tyramine-conjugated hyaluronic acid and gelatin. Hydrogels are attractive biomaterials for application in regenerative biomedical applications. Hydrogels are biocompatible, have a high water-content and favourable mechanical properties. For a strong adhesion to biological tissue, a maximum amount of covalent or non-covalent interactions are required. Since biological tissue is typically negatively charged, a hydrogel that is mostly positively charged is required. The combination of tyramine-conjugated hyaluronic acid and gelatin results in a predominantly positively charged hydrogel. In vivo application of the hydrogel resulted in a negligible immune reaction and therefore its potential for application in tissue engineering and regenerative medicine (Kim et al. 2018).

Despite the significance of cross-linking enzymes in medicine for tissue restoration and biomimetic tissue adhesives, it is worthy to note that oxidoreductases have equally played a pivotal role in cross-linking applications in the food industry (Broderick et al. 2005; Raucci et al. 2019). One such example is the application of the *B. megaterium* tyrosinase. Due to their nutritional value and ability to improve texture, soy proteins such as glycinin and beta-conglycinin are widely used in food products. However, glycinin has a low emulsifying ability compared to other soy proteins. The *B. megaterium* tyrosinase was successfully employed in the cross-linking of glycinin, thereby stabilizing it in oil-in water emulsions and its subsequent application in food products (Isaschar-Ovdat et al. 2015).

Synthesis of *o*-hydroxylated polyphenols

Isoflavones are a group of polyphenolic compounds known to have health protective properties, exhibiting antioxidative, anticancer and anti-inflammatory effects (Hosseini-Abari and Tayebi 2019). It is therefore not surprising that they have found application in the food industry as food ingredients and as pharmaceutical supplements. One such example, is genistein (4′,5,7-trihydroxyisoflavone). It exhibits anticancer and anti-angiogenesis properties and attenuates cancer cell proliferation. Hydroxylation of isoflavones has been shown to increase their antioxidant properties, e.g. the bioconversion of genistein to orobol by a surface displayed *B. megaterium* tyrosinase [expressed on the surface of *B. subtilis* spores and anchored by CotE; (Hosseini-Abari and Tayebi 2019)] and subsequent methylation of orobol by the *Streptomyces peuceticus o*-methyltransferase to methoxyisoflavones, exhibited anti-melanoma activity (Chiang et al. 2017). The *o*-hydroxylation of polyphenols by tyrosinases is often difficult to control. Many studies have shown the need to add boric acid or ascorbic acid to prevent the auto-oxidation of the products [e.g. the 3′hydroxylation of daidzin and genistin by recombinant expressed *B. megaterium* tyrosinase; (Chiang et al. 2016)] and the need to regenerate the tyrosinase. The main problem is that most bacterial tyrosinases are active from pH6.0–9.0 and the use of boric acid and ascorbic acid will reduce the bioactivity of the tyrosinase. Son et al. (2018)

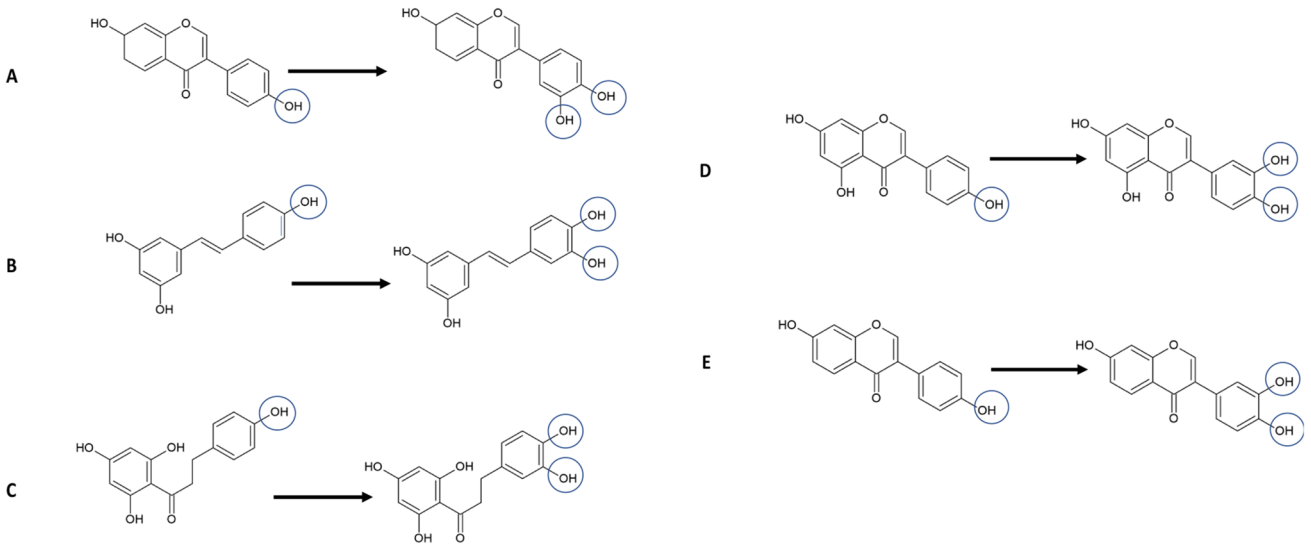

Fig. 7 The *o*-hydroxylation of polyphenols by bacterial tyrosinases. **A** Daidzin to 3′-hydroxydaidzin; **B** resveratrol to piceatannol; **C** phloretin to 3-hydroxyphloretin; **D** genistein to orobol; and **E** daidzin to 7,3′,4′-trihydroxyisoflavone (3′-ODI)

described a bacterial tyrosinase produced by *B. thailandensis* that functions at low pH and therefore could be applied in the conversion of daidzin to 3′-hydroxydaidzin, resveratrol to piceatannol, and phloretin to 3-hydroxyphloretin (Fig. 7). Daidzin, as with genistein, has been shown to have protective effects against breast and prostate cancer, diabetes, and cardiovascular diseases. Conversion of daidzin to 7,3′,4′-trihydroxyisoflavone (3′-ODI) was shown by the surface displayed *B. megaterium* tyrosinase (Fig. 7) (Hosseini-Abari 2020).

Resveratrol is a phytoalexin that naturally occurs in grapes, peanuts, and berries (Lee et al. 2012). As with other phytochemicals, *trans*-resveratrol has been shown to have excellent antioxidant potential and can be applied as an anticancer, anti-inflammatory and blood sugar lowering supplement and drug. Piceatannol is a resveratrol derivative (C3′ hydroxylated) which is also found in plants and is known to act as a tyrosine kinase inhibitor and suppresses cancer cell growth and proliferation (Lee et al. 2012). Various attempts have been made to apply enzymes (mainly P450 monooxygenases) in the conversion of resveratrol to piceatannol, but often results in a poor yield. Tyrosinase, with the ability to oxidise *o*-hydroxylation, is therefore an excellent candidate. However, the presence of a reducing agent such as NADH, glutathione or L-ascorbic acid is required to prevent the formation of a quinolic compound and subsequent polymerization. Lee et al. (2012) explored an alternative approach in the regioselective hydroxylation of *trans*-resveratrol by the tyrosinase from *S. avermitilis* strain MA4680. Instead of using a reducing agent, which would be costly at industrial scale, they explored the use of phenolic compounds as inhibitory compounds. The highest relative production was achieved in the presence of 1 mM catechol (100%), followed by 1 mM hydroquinone (97.1%), 1 mM NADH (64.2%) and 1 mM L-ascorbic acid (63.7%).

Other applications and future perspectives

Even though tyrosinases have a broad substrate specificity and can accept 3- and 4-substitued phenols as substrates, 2-substituted phenols and phenolic derivatives are typically competitive inhibitors (Zolghadri et al. 2019) as was shown for the tyrosinase produced by *S. antibioticus* and other bacterial and fungal tyrosinases (Marino et al. 2011; Davis et al. 2018; Zolghadri et al. 2019). The synthesis of substituted catechols by chemical means employs aggressive reagents, harsh reaction conditions and results in a poor yield. The use of tyrosinases for the synthesis of these substituted catechols is difficult to control and requires a tyrosinase with a high ratio of monophenolase to diphenolase activity. The tyrosinase produced by *R. solanacearum* exhibits such a high ratio and was successfully employed in the production of 4-halocatechols (Davis et al. 2018). Halocatechols exhibit biological activity and can be used in the functionalization of catechol building blocks for the synthesis of pharmaceuticals.

Hydroxytyrosol is a phenolic compound mainly found in olives. The compound is of great interest due to its great antioxidant potential and role in the protection of blood lipids from oxidative damage, its anti-carcinogenic, anti-inflammatory, and antimicrobial properties. Extraction of hydroxytyrosol from olives or olive oil wastewater typically results in low concentrations, with maximum yields of 1.5 g/L (Deri-Zanaty et al. 2020). Tyrosinases have the potential to hydroxylate the monophenol, tyrosol to hydroxytyrosol, but rapid oxidation to 3,4-quinone-phenylethanol occurs if the reaction is not controlled. Various researchers attempted the use of ascorbic acid to control the reaction, but often results in the inhibition of the enzyme. Deri-Zenaty et al. (2020) explored the use of a glucose dehydrogenase for the regeneration of NADH that can act as a reducing agent (Fig. 8), similar to the control of the conversion of resveratrol to piceatannol by the *Streptomyces lincolnensis* tyrosinase as described by Lee et al. (2015). The coupled reaction of the glucose dehydrogenase and the tyrosinase produced by *B. megaterium*, resulted in the conversion of tyrosol to hydroxytyrosol and 3,4-quinone-phenylethanol and the reduction of the latter to hydroxytyrosol, with a final yield of 7.68 g/L (Deri-Zenaty et al. 2020).

With the advent of next generation sequencing, vast numbers of bacterial genomes and environmental metagenomes have been submitted to public databases. A search of these databases reveals the abundance of bacterial tyrosinase genes. Therefore, there is a vast sequence space that still needs to be explored, especially the 'rare' type of tyrosinases that exhibit a high monophenolase to diphenolase activity ratio with potential for application in tightly controlled autooxidation reactions such as the *o*-hydroxylation of phenolic and polyphenolic compounds. An overview of the literature also made it abundantly clear that bacterial tyrosinases have great potential for application in biocatalytic reactions that can be used to replace chemical processes. However, to understand how bacterial tyrosinases can be applied in specific reactions, especially in systems containing organic solvents, metals, and other potential inhibitors, we need more biochemical and structural data. Very few studies report on these biochemical characteristics and without a link between sequence space, structure, and biochemistry, it reduces the chances of a more widespread application of bacterial tyrosinases.

One aspect that is also not widely explored, is the potential application of bacterial tyrosinases in novel antibiotic production. Michalik et al. (1975) reported the involvement of a phenol oxidase in the biosynthesis of the antibiotic, lincomycin that is produced by *S. lincolnensis*.

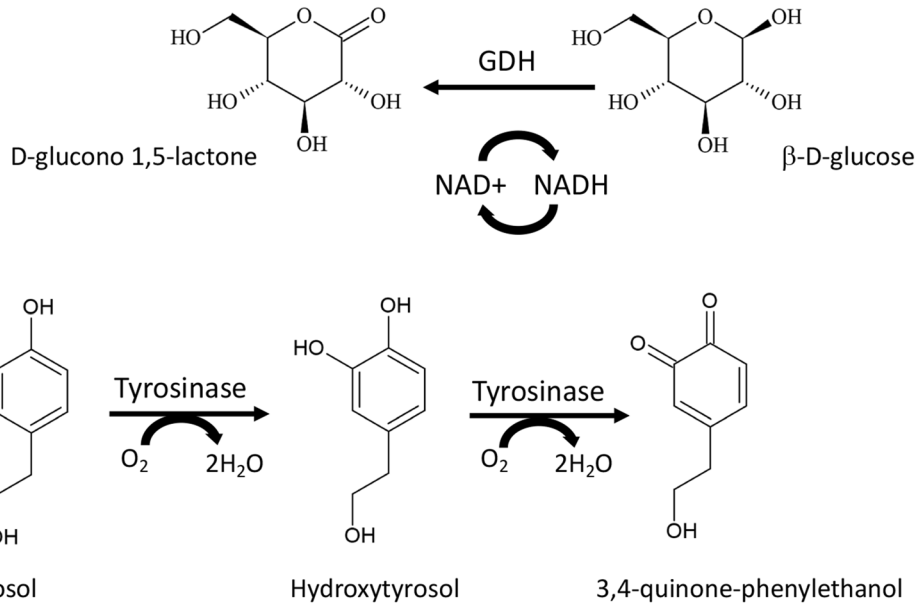

Fig. 8 Conversion of tyrosol to hydroxytyrosol in the presence of NADH as reducing agent (*GDH* glucose dehydrogenase, *tyrosinase Bacillus megaterium* tyrosinase)

Similarly, the GriE/GriF tyrosinase produced by *S. griseus* is involved in the production of the antibiotic grixazone (Suzuki et al. 2006). Understanding the role of these enzymes in the production of these antibiotics can serve as a guide in the synthesis of novel antibiotics. Alternatively, learning more about the biochemical and structural features of bacterial tyrosinases will allow for the development of artificial metalloenzymes that could mimic either the monophenolase and/or diphenolase activity of bacterial tyrosinases such as the ones shown to play a role in antibiotic production. Fujieda et al. (2012) demonstrated how these metalloenzymes can be modified into a catechol oxidase with potential application in melanin production and other types of reactions. It is clear from the information presented here that bacterial tyrosinases offer many advantages over their eukaryotic counterparts: ease of recombinant expression allows for increased production of the enzyme; many can function under harsh conditions without the need for activation (with the exception of the *V. spinosum* tyrosinase which requires activation); and their kinetic efficiency, especially towards monophenols (Kim et al. 2016; Davis et al. 2018), are several times higher than tyrosinases derived from other organisms (Cabanes et al. 1987; Espín et al. 2002; Zaidi et al. 2014; Agarwal et al. 2019). There is therefore a vast scope of areas that still need to be explored for this interesting group of underexplored bacterial enzymes.

Author contributions Conceptualization: Mayowa Agunbiade, Marilize Le Roes-Hill; Writing – original draft preparation: Mayowa Agunbiade, Marilize Le Roes-Hill; Writing—review and editing: Mayowa Agunbiade, Marilize Le Roes-Hill. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by the National Research Foundation (NRF) of South Africa, grant number 105873, and the Cape Peninsula University of Technology funded the postdoctoral fellowship for Dr Agunbiade. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

Declarations

Conflict of interest The authors, Mayowa Agunbiade and Marilize Le Roes-Hill, declare no conflict of interest.

References

Abdollahi K, Yazdani F, Panahi R, Mokhtarani B (2018) Biotransformation of phenol in synthetic wastewater using the functionalized magnetic nano-biocatalyst particles carrying tyrosinase. https://doi.org/10.1007/s13205-018-1445-2. 3Biotech 8

Agarwal P, Singh M, Singh J, Singh RP (2019) Microbial tyrosinases: A novel enzyme, structural features, and applications. In: Shukla P (ed) Applied Microbiology and Bioengineering. Academic Press, pp 3-19. https://doi.org/10.1016/B978-0-12-815407-6.00001-0

Al Khatib M, Harir M, Costa J, Baratto MC, Schiavo I, Trabalzini L, Pollini S, Rossolini GM, Basosi R, Pogni R (2018) Spectroscopic characterization of natural melanin from a *Streptomyces cyaneofuscatus* strain and comparison with melanin enzymatically synthesized by tyrosinase and laccase. Molecules 23:1916. https://doi.org/10.3390/molecules23081916

Algieri C, Donato L, Bonacci P, Giorno L (2012) Tyrosinase immobilised on polyamide tubular membrane for the L-DOPA production: Total recycle and continuous reactor study. Biochem Eng J 66:14–19. https://doi.org/10.1016/j.bej.2012.03.013

Axambayeva AS, Zhaparova LR, Shagyrova ZS, Ramankulov EM, Shustov AV (2018) Unusual stability of a recombinant *Verrucomicrobium spinosum* tyrosinase to denaturing agents and its use for a production of a protein with adhesive properties.

Appl Biochem Biotechnol 185:736–754. https://doi.org/10.1007/s12010-017-2686-y
Ba S, Kumar VV (2017) Recent developments in the use of tyrosinase and laccase in environmental applications. Crit Rev Biotechnol 37:819–832. https://doi.org/10.1080/07388551.2016.1261081
Barbani N, Giusti P, Lazzeri L, Polacco G, Pizzirani G (1995) Bioartificial materials based on collagen: 1. Collagen cross-linking with gaseous glutaraldehyde. J Biomat Sci-Polym E 7:461–469. https://doi.org/10.1163/156856295X00535
Baumann R, Ettlinger L, Hütter R, Kocher HP (1976) Control of melanin formation in *Streptomyces glaucescens*. In: Arai T (ed) Actinomycetales: The boundary microorganisms. Toppan Co Ltd, Tokyo-Singapore, pp 55–63
Brasquet C, Subrenat E, Le Cloirec P (1999) Removal of phenolic compounds from aqueous solution by activated carbon cloths. Water Sci Technol 39:201–205. https://doi.org/10.1016/S0273-1223(99)00277-2
Broderick EP, O'Halloran DM, Rochev YA, Griffin M, Collighan RJ, Pandit AS (2005) Enzymatic stabilization of gelatin-based scaffolds. J Biomed Mater Res B 72B:37–42. https://doi.org/10.1002/jbm.b.30119
Cabanes J, García-Cánovas F, Lozano JA, García-Carmona F (1987) A kinetic study of the melanization pathway between L-tyrosine and dopachrome. Biochim Biophys Acta 923:187–195. https://doi.org/10.1016/0304-4165(87)90003-1
Cabrera-Valladares N, Martínez A, Piñero S, Lagunas-Muñoz VH, Tinoco R, de Anda R, Vázquez-Duhalt R, Bolívar F, Gosset G (2006) Expression of the *melA* gene from *Rhizobium etli* CFN42 in *Escherichia coli* and characterization of the encoded tyrosinase. Enzyme Microb Technol 38:772–779. https://doi.org/10.1016/j.enzmictec.2005.08.004
Chávez-Béjar MI, Balderas-Hernandez VE, Gutiérrez-Alejandre A, Martinez A, Bolívar F, Gosset G (2013) Metabolic engineering of *Escherichia coli* to optimize melanin synthesis from glucose. Microb Cell Fact 12:108. https://doi.org/10.1186/1475-2859-12-108
Chen L-Y, Leu W-M, Wang K-T, Lee Y-HW (1992) Copper transfer and activation of the *Streptomyces* apotyrosinase are mediated through a complex formation between apotyrosinase and its *trans*-activator MelC1. J Biol Chem 267:20100-20107. PMID: 1400328
Chew LC, Ramseier TM, Retallack DM, Schneider JC, Squires CH, Talbot HW (2005) Pseudomonas fluorescens. In: Gellissen G (ed) Production of recombinant proteins: novel microbial and eukaryotic expression system. Weinheim, Wiley-VCH, pp 45–62
Chiang C-M, Wang D-S, Chang T-S (2016) Improving free radical scavenging activity of soy isoflavone glycosides daidzin and genistin by 3'-hydroxylation using recombinant *Escherichia coli*. Molecules 21:1723. https://doi.org/10.3390/molecules21121723
Chiang C-M, Chang Y-J, Wu J-Y, Chang T-S (2017) Production and anti-melanoma activity of methoxyisoflavones from the biotransformation of genistein by two recombinant *Escherichia coli* strains. Molecules 22:87. https://doi.org/10.3390/molecules22010087
Claus H, Decker H (2006) Bacterial tyrosinases. Syst Appl Microbiol 29:3–14. https://doi.org/10.1016/j.syapm.2005.07.012
Davis R, Molloy S, Quigley B, Nikodinovic-Runic J, Solano F, O'Connor KE (2018) Biocatalytic versatility of engineered and wild-type tyrosinase from *R. solanacearum* for the synthesis of 4-halocatechols. Appl Microbiol Biotechnol 102:5121–5131. https://doi.org/10.1007/s00253-018-8994-5
Deri-Zenaty B, Bachar S, Rebroš M, Fishman A (2020) A coupled enzymatic reaction of tyrosinase and glucose dehydrogenase for the production of hydroxytyrosol. Appl Microbiol Biotechnol 104:4945–4955. https://doi.org/10.1007/s00253-020-10594-z
Doukyu N, Ogino H (2010) Organic solvent-tolerant enzymes. Biochem Eng J 48:270–282. https://doi.org/10.1016/j.bej.2009.09.009
Dubey SK, Pandey A, Bajaj AK, Misra K (2007) Some commercial azo dyes as inhibitors of mushroom tyrosinase DOPA oxidase activity. J Pharmacol Toxicol 2:718–724. https://doi.org/10.3923/jpt.2007.718.724
El-Naggar NE, El-Ewasy SM (2017) Bioproduction, characterization, anticancer and antioxidant activities of extracellular melanin pigment produced by newly isolated microbial cell factories *Streptomyces glaucescens* NEAE-H. Sci Rep-UK 7:42129. https://doi.org/10.1038/srep42129
Espín JC, Varón R, Fenoll LG, Gilabert MA, García-Ruíz PA, Tudela J, García-Cánovas F (2002) Kinetic characterization of the substrate specificity and mechanism of mushroom tyrosinase. FEBS J 267:1270–1279. https://doi.org/10.1046/j.1432-1327.2000.01013.x
Faccio G, Kruus K, Saloheimo M, Thöny-Meyer L (2012) Bacterial tyrosinases and their applications. Process Biochem 47:1749–1760. https://doi.org/10.1016/j.procbio.2012.08.018
Faccio G, Kämpf MM, Piatti C, Thöny-Meyer L, Richter M (2014) Tyrosinase-catalyzed site-specific immobilization of engineered C-phycocyanin to surface. Sci Rep-UK 4:5370. https://doi.org/10.1038/srep05370
Faccio G, Richter M, Thöny-Meyer L (2019) Bioprospecting for microbial tyrosinases. In: Abdel-Aziz SM, Garg N, Aeron A, Jha C, Nayak SC, Bajpai VK (eds) Microbial Catalysts, vol 2. Nova Science Publishers, New York, pp 1–16
Fairhead M, Thöny-Meyer L (2010) Cross-linking and immobilisation of different proteins with recombinant *Verrucomicrobium spinosum* tyrosinase. J Biotechnol 150:546–551. https://doi.org/10.1016/j.jbiotec.2010.10.068
Fairhead M, Thöny-Meyer L (2011) Bacterial tyrosinases: Old enzymes with new relevance to biotechnology. New Biotechnol 29:183–191. https://doi.org/10.1016/j.nbt.2011.05.007
Franciscon E, Grossman M, Paschoal JA, Reyes FG, Durrant L (2012) Decolorization and biodegradation of reactive sulfonated azo dyes by a newly isolated *Brevibacterium* sp. strain VN-15. https://doi.org/10.1186/2193-1801-1-37. SpringerPlus 1:37
Fujieda N, Hasegawa A, Ishihama K-I, Itoh S (2012) Artificial dicopper oxidase: Rational reprogramming of bacterial metallo β-lactamase into a catechol oxidase. Chem Asian J 7:1203–1207. https://doi.org/10.1002/asia.201101014
Fuqua WC, Coyne VE, Stein DC, Lin C-M, Weiner RM (1991) Characterization of *melA*: a gene encoding melanin biosynthesis from the marine bacterium *Shewanella colwelliana*. Gene 109:131–136. https://doi.org/10.1016/0378-1119(91)90598-6
Gasparetti C, Faccio G, Arvas M, Buchert J, Saloheimo M, Kruus K (2010) Discovery of a new tyrosinase-like enzyme family lacking a C-terminally processed domain: Production and characterization of an *Aspergillus oryzae* catechol oxidase. Appl Microbiol Biotechnol 86:213–226. https://doi.org/10.1007/s00253-009-2258-3
Goldfeder M, Kanteev M, Adir N, Fishman A (2013) Influencing the monophenolase/diphenolase activity ratio in tyrosinase. Biochim Biophys Acta 1834:629–633. https://doi.org/10.1016/j.bbapap.2012.12.021
Guo J, Rao Z, Yang T, Man Z, Xu M, Zhang X (2014) High-level production of melanin by a novel isolate of *Streptomyces kathirae*. FEMS Microbiol Lett 357:85–91. https://doi.org/10.1111/1574-6968.12497
Guo J, Rao Z, Yang T, Man Z, Xu M, Zhang X, Yang S-T (2015) Cloning and identification of a novel tyrosinase and its overexpression in *Streptomyces kathirae* SC-1 for enhancing melanin

production. FEMS Microbiol Lett 362:fnv041. https://doi.org/10.1093/femsle/fnv041

Hamann JN, Herzigkeit B, Jurgeleit R, Tuczek F (2017) Small-molecule models of tyrosinase: From ligand hydroxylation to catalytic monooxygenation of external substrates. Coord Chem Rev 334:54–66. https://doi.org/10.1016/j.ccr.2016.07.009

Harir M, Bellahcene M, Baratto MC, Pollini S, Rossolini GM, Trabalzini L, Fatarella E, Pogni R (2018) Isolation and characterization of a novel tyrosinase produced by Sahara soil actinobacteria and immobilization on nylon nanofiber membranes. J Biotechnol 265:54–64. https://doi.org/10.1016/j.jbiotec.2017.11.004

Heck T, Faccio G, Richter M, Thöny-Meyer L (2013) Enzyme-catalyzed protein crosslinking. Appl Microbiol Biotechnol 97:461–475. https://doi.org/10.1007/s00253-012-4569-z

Hernández-Romero D, Solano F, Sanchez-Amat A (2005) Polyphenol oxidase activity expression in *Ralstonia solanacearum*. Appl Environ Microbiol 71:6808–6815.

Hintermann G, Zatchej M, Hutter R (1985) Cloning and expression of the genetically unstable tyrosinase structural gene from *Streptomyces glaucescens*. Mol Gen Genet 200:422–432. https://doi.org/10.1007/BF00425726

Hosseini-Abari A, Kim B-G, Lee S-H, Emtiazi G, Kim W, Kim J-H (2016) Surface display of bacterial tyrosinase on spores of *Bacillus subtilis* using CotE as an anchor protein. J Basic Microb 56:1331–1337. https://doi.org/10.1002/jobm.201600203

Hosseini-Abari A, Tayebi M (2019) Bioconversion of genistein to orobol by *Bacillus subtilis* spore displayed tyrosinase and monitoring the anticancer effects of orobol on MCF-7 breast cancer cells. Biotechnol Bioproc E 24:507–512. https://doi.org/10.1007/s12257-019-0067-9

Hosseini-Abari A (2020) Bioconversion of daidzein to 3′-ODI by *Bacillus subtilis* spore displayed tyrosinase. Biol J Microorg 8:59–63. https://doi.org/10.22108/bjm.2018.112719.1154

Huber M, Lerch K (1988) Identification of two histidines as copper ligands in *Streptomyces glaucescens* tyrosinase. Biochem 27:5610–5615. https://doi.org/10.1021/bi00415a032

Ikeda K, Masujima T, Suzuki K, Sugiyama M (1996) Cloning and sequence analysis of the highly expressed melanin synthesizing gene operon from *Streptomyces castaneoglobisporus*. Appl Microbiol Biotechnol 45:80–85. https://doi.org/10.1007/s002530050652

Isaschar-Ovdat S, Rosenberg M, Lesmes U, Fishman A (2015) Characterization of oil-in-water emulsions stabilized by tyrosinase-crosslinked soy glycinin. Food Hydrocolloid 43:493–500. https://doi.org/10.1016/j.foodhyd.2014.07.007

Isaschar-Ovdat S, Davidovich-Pinhas M, Fishman A (2016) Modulating the gel properties of soy glycinin by crosslinking with tyrosinase. Food Res Internat 87:42–49. https://doi.org/10.1016/j.foodres.2016.06.018

Isaschar-Ovdat S, Fishman A (2017) Mechanistic insights into tyrosinase-mediated crosslinking of soy glycinin derived peptides. Food Chem 232:587–594. https://doi.org/10.1016/j.foodchem.2017.04.016

Ito M, Oda K (2000) An organic solvent resistant tyrosinase from *Streptomyces* sp. REN-21: Purification and characterisation. Biosci Biotechnol Biochem 64:261–267. https://doi.org/10.1271/bbb.64.261

Ito M, Inouye K (2005) Catalytic properties of an organic solvent–resistant tyrosinase from *Streptomyces* sp. REN-21 and its high-level production in *E. coli*. J Biochem 138:355–362. https://doi.org/10.1093/jb/mvi150

Jadhav JP, Kalyani DC, Telke AA, Phugare SS, Govindwar SP (2010) Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. Bioresource Technol 101:165–173. https://doi.org/10.1016/j.biortech.2009.08.027

Jus S, Stachel I, Fairhead M, Meyer M, Thöny-Meyer L, Guebitz GM (2012) Enzymatic cross-linking of gelatine with laccase and tyrosinase. Biocatal Biotransfor 30:86–95. https://doi.org/10.3109/10242422.2012.646036

Kameda E, Langone MAP, Coelho MAZ (2006) Tyrosinase extract from *Agaricus bisporus* mushroom and its *in natura* tissue for specific phenol removal. Environ Technol 27:1209–1215. https://doi.org/10.1080/09593332708618736

Katz E, Thompson CJ, Hopwood DA (1983) Cloning and expression of the tyrosinase gene from *Streptomyces antibioticus* in *Streptomyces lividans*. J Gen Microbiol 129:2703–2714. https://doi.org/10.1099/00221287-129-9-2703

Katz E, Betancourt A (1988) Induction of tyrosinase by L-methionine in *Streptomyces antibioticus*. Can J Microbiol 34:1297–1303. https://doi.org/10.1139/m88-227

Kertesz D, Zito R (1965) Mushroom polyphenol oxidase I. Purification and general properties. Biochim Biophys Acta 96:447–462. https://doi.org/10.1016/0005-2787(65)90562-9

Kim H, Yeon YJ, Choi YR, Song W, Pack SP, Choi YS (2016) A cold-adapted tyrosinase with an abnormally high monophenolase/diphenolase activity ratio originating from the marine archaeon *Candidatus Nitrosopumilus koreensis*. Biotechnol Lett 38:1535–1542. https://doi.org/10.1007/s10529-016-2125-0

Kim S-H, Lee S-H, Lee J-E, Park SJ, Kim K, Kim IS, Lee Y-S, Hwang NS, Kim B-G (2018) Tissue adhesive, rapid forming, and sprayable ECM hydrogel via recombinant tyrosinase crosslinking. Biomaterials 178:401–412. https://doi.org/10.1016/j.biomaterials.2018.04.057

Kohashi PY, Kumagai T, Matoba Y, Yamamoto A, Maruyama M, Sugiyama M (2004) An efficient method for the overexpression and purification of active tyrosinase from *Streptomyces castaneoglobisporus*. Protein Express Purif 34:202–207. https://doi.org/10.1016/j.pep.2003.11.015

Laguna-Muñoz VH, Cabrera-Valladares N, Bolívar F, Gosset G, Martínez A (2006) Optimum melanin production using recombinant *Escherichia coli*. J Appl Microbiol 101:1002–1008. https://doi.org/10.1111/j.1365-2672.2006.03013.x

Lantto R, Puolanne E, Kruus K, Buchert J, Autio K (2007) Tyrosinase-aided protein cross-linking: Effects on gel formation of chicken breast myofibrils and texture and water-holding of chicken breast meat homogenate gels. J Agr Food Chem 55:1248–1255. https://doi.org/10.1021/jf0623485

Le Roes-Hill M, Palmer Z, Rohland J, Kirby BM, Burton SG (2015) Partial purification and characterisation of two actinomycete tyrosinases and their application in cross-linking reactions. J Mol Catal B-Enzym 122:353–364. https://doi.org/10.1016/j.molcatb.2015.10.012

Lee Y-HW, Chen B-F, Wu S-Y, Leu W-M, Liu J-J, Chen CW, Lo SJ (1988) A trans-acting gene is required for the phenotypic expression of a tyrosinase gene in *Streptomyces*. Gene 65:71–81. https://doi.org/10.1016/0378-1119(88)90418-0

Lee N, Kim EJ, Kim B-G (2012) Regioselective hydroxylation of trans-resveratrol via inhibition of tyrosinase from *Streptomyces avermitilis* MA4680. ACS Chem Biol 7:1687–1692. https://doi.org/10.1021/cb300222b

Lee N, Lee S-H, Baek K, Kim B-G (2015) Heterologous expression of tyrosinase (MelC2) from *Streptomyces avermitilis* MA4680 in *E. coli* and its application for ortho-hydroxylation of resveratrol to produce piceatannol. Appl Microbiol Biotechnol 99:7915–7924. https://doi.org/10.1007/s00253-015-6691-1

Lee S-H, Baek K, Lee J-E, Kim B-G (2016) Using tyrosinase as a monophenol monooxygenase: A combined strategy for effective inhibition of melanin formation. Biotechnol Bioeng 113:735–743. https://doi.org/10.1002/bit.25855

Lee P-G, Lee S-H, Hong EY, Lutz S, Kim B-G (2018) Circular permutation of a bacterial tyrosinase enables efficient polyphenol-specific oxidation and quantitative preparation of orobol. Biotechnol Bioeng 116:19–27. https://doi.org/10.1002/bit.26795

Lerch K, Ettlinger L (1972) Purification and characterization of a tyrosinase from *Streptomyces glaucescens*. Eur J Biochem 31:427–437. https://doi.org/10.1111/j.1432-1033.1972.tb02549.x

Liu N, Liang G, Dong X, Qi X, Kim J, Piao Y (2016) Stabilized magnetic enzyme aggregates on graphene oxide for high performance phenol and bisphenol A removal. Chem Eng J 306:1026–1034. https://doi.org/10.1016/j.cej.2016.08.012

Lonappan L, Rouissi T, Laadila MA, Brar SK, Hernandez Galan L, Verma M, Surampalli RY (2017) Agro-industrial-produced laccase for degradation of diclofenac and identification of transformation products. ACS Sustain Chem Eng 5:5772–5781. https://doi.org/10.1021/acssuschemeng.7b00390

López-Molina D, Hiner ANP, Tudela J, García-Cánovas F, Rodríguez-López JN (2003) Enzymatic removal of phenols from aqueous solution by artichoke (*Cynara scolymus* L.) extracts. Enzyme Microb Technol 33:738–742. https://doi.org/10.1016/S0141-0229(03)00208-4

Lopez-Serrano D, Sanchez-Amat A, Solano F (2002) Cloning and molecular characterization of a SDS-activated tyrosinase from *Marinomonas mediterranea*. Pigm Cell Res 15:104–111. https://doi.org/10.1034/j.1600-0749.2002.1o068.x

Marino SM, Fogal S, Bisaglia M, Moro S, Scartabelli G, De Gioia L, Spada A, Monzani E, Casella L, Mammi S, Bubacco L (2011) Investigation of *Streptomyces antibioticus* tyrosinase reactivity toward chlorophenols. Arch Biochem Biophys 5050:67–74. https://doi.org/10.1016/j.abb.2010.09.019

Martorell MM, Pajot HF, Rovati JI, Figueroa LIC (2012) Optimization of culture medium composition for manganese peroxidase and tyrosinase production during Reactive Black 5 decolourization by the yeast *Trichosporon akiyoshidainum*. Yeast 29:137–144. https://doi.org/10.1002/yea.2896

Matoba Y, Kumagai T, Yamamoto A, Yoshitsu H, Sugiyama M (2006) Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis. J Biol Chem 281:8981–8990. https://doi.org/10.1074/jbc.M509785200

Mercado-Blanco J, Garcia F, Fernandez-Lopez M, Olivares J (1993) Melanin production by *Rhizobium meliloti* GR4 is linked to non-symbiotic plasmid pRmeGR4b: Cloning, sequencing, and expression of the tyrosinase gene *mepA*. J Bacteriol 175:5403–5410. https://doi.org/10.1128/jb.175.17.5403-5410.1993

Michalik J, Emillianowicz-Czerska W, Switalski L, Raczyska-Bojanowska K (1975) Monophenol monooxygenase and lincomysin biosynthesis in *Streptomyces lincolnensis*. Antimicrob Agents Chemother 8:526–531. https://doi.org/10.1128/aac.8.5.526

Min K, Park K, Park DH, Yoo YJ (2015) Overview on the biotechnological production of L-DOPA. Appl Microbiol Biotechnol 99:575–584. https://doi.org/10.1007/s00253-014-6215-4

Molloy S, Nikodinovic-Runic J, Martin LB, Hartmann H, Solano F, Decker H, O'Connor KE (2013) Engineering of a bacterial tyrosinase for improved catalytic efficiency towards D-tyrosine using random and site directed mutagenesis approaches. Biotechnol Bioeng 110:1849–1857. https://doi.org/10.1002/bit.24859

Nadal-Jimenez P, Koch G, Reis CR, Muntendam R, Raj H, Jeronimus-Stratingh CM, Cool RH, Quax WJ (2014) PvdP is a tyrosinase that drives maturation of the pyoverdine chromophore in *Pseudomonas aeruginosa*. J Bacteriol 196:2681–2690. https://doi.org/10.1128/JB.01376-13

Nagatsua T, Sawadab M (2009) L-DOPA therapy for Parkinson's disease: Past, present, and future. https://doi.org/10.1016/S1353-8020(09)70004-5. Parkinsonism Relat D Suppl 1 2009:S3-8

Nambudiri AM, Bhat JV (1972) Conversion of p-coumarate into caffeate by *Streptomyces nigrifaciens*. Purification and properties of the hydroxylating enzyme. Biochem J 130:425–433. https://doi.org/10.1042/bj1300425

Park HA, Yang I, Choi M, Jang K-S, Jung JC, Choi K-Y (2020) Engineering of melanin biopolymer by co-expression of MelC tyrosinase with CYP102G4 monooxygenase: Structural composition understanding by 15 tesla FT-ICR MS analysis. Biochem Eng J 157:107530. https://doi.org/10.1016/j.bej.2020.107530

Pavan ME, López NI, Pettinari MJ (2020) Melanin biosynthesis in bacteria, regulation and production perspectives. Appl Microbiol Biotechnol 104:1357–1370. https://doi.org/10.1007/s00253-019-10245-y

Petite H, Rault I, Huc A, Menasche Ph, Herbage D (1990) Use of the acyl azide method for cross-linking collagen-rich tissues such as pericardium. J Biomed Mater Res 24:179–187. https://doi.org/10.1002/jbm.820240205

Piñero S, Rivera J, Romero D, Cevallos MA, Martínez A, Bolívar F, Gosset G (2007) Tyrosinase from *Rhizobium etli* is in-volved in nodulation efficiency and symbiosis-associated stress resistance. J Mol Microb Biotech 13:35–44. https://doi.org/10.1159/000103595

Popa CI, Bahrim G (2011) *Streptomyces* tyrosinase: Production and practical applications. Innov Romanian Food Biotechnol 8:1–7. http://www.bioaliment.ugal.ro/revista/8/paper%2081.pdf

Raucci MG, D'Amora U, Ronca A, Demitri C, Ambrosio L (2019) Bioactivation routes of gelatin-based scaffolds to enhance at nanoscale level bone tissue regeneration. Front Bioeng Biotechnol 7:27. https://doi.org/10.3389/fbioe.2019.00027

Ren Q, Henes B, Fairhead M, Thöny-Meyer L (2013) High level production of tyrosinase in recombinant *Escherichia coli*. BMC Biotechnol 13:18. https://doi.org/10.1186/1472-6750-13-18

Rodakiewicz-Nowak J, Ito M (2005) Effect of AOT on enzymatic activity of the organic solvent resistant tyrosinase from *Streptomyces* sp. REN-21 in aqueous solutions and water-in-oil microemulsions. J Colloid Interface Sci 284:674–679. https://doi.org/10.1016/j.jcis.2004.10.052

Roy S, Das I, Munjal M, Karthik L, Kumar G, Kumar S, Rao KVB (2014) Isolation and characterization of tyrosinase produced by marine actinobacteria and its application in the removal of phenol from aqueous environment. Front Biol 9:306–316. https://doi.org/10.1007/s11515-014-1324-0

Ryu J, Byun H, Park JP, Park J, Noh KH, Chung JH, Lee H, Ahn JH (2019) Tat-dependent heterologous secretion of recombinant tyrosinase by *Pseudomonas fluorescens* is aided by a translationally fused caddie protein. Appl Environ Microbiol 85:e01350–19. https://doi.org/10.1128/AEM.01350-19

Sanchez-Amat A, Solano F, Lucas-Elío P (2010) Finding new enzymes from bacterial physiology: A successful approach illustrated by the detection of novel oxidases in *Marinomonas mediterranea*. Mar Drugs 8:519–541. https://doi.org/10.3390/md8030519

Sánchez-Ferrer Á, Neptuno Rodríguez-López J, García-Cánovas F, García-Carmona F (1995) Tyrosinase: a comprehensive re-view of its mechanism. BBA-Protein Struct M 1247:1–11. https://doi.org/10.1016/0167-4838(94)00204-T

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2011) Bacterial decolorization and degradation of azo dyes: A review. J Taiwan Inst Chem E 42:138–157. https://doi.org/10.1016/j.jtice.2010.06.006

Sehnal D, Rose AS, Kovca J, Burley SK, Velankar S (2018) Mol*: Towards a common library and tools for web molecular graphics, MolVA/EuroVis Proceedings. https://doi.org/10.2312/molva.20181103

Selinheimo E, Autio K, Kruus K, Buchert J (2007) Elucidating the mechanism of laccase and tyrosinase in wheat bread making.

J Agr Food Chem 55:6357–6365. https://doi.org/10.1021/jf0703349

Selinheimo E, Lampila P, Mattinen ML, Buchert J (2008) Formation of protein-oligosaccharide conjugates by laccase and tyrosinase. J Agr Food Chem 56:3118–3128. https://doi.org/10.1021/jf0730791

Sendovski M, Kanteev M, Ben-Yosef VS, Adir N, Fishman A (2011) First structures of an active bacterial tyrosinase reveal copper plasticity. J Mol Biol 405:227–237. https://doi.org/10.1016/j.jmb.2010.10.048

Shalaby ASG, Ragab TIM, Helal MMI, Esawy MA (2019) Optimization of *Bacillus licheniformis* MAL tyrosinase: In vitro anticancer activity for brown and black eumelanin. Heliyon 5:e01657. https://doi.org/10.1016/j.heliyon.2019.e01657

Shuster V, Fishman A (2009) Isolation, cloning and characterization of a tyrosinase with improved activity in organic solvents from *Bacillus megaterium*. J Mol Microbiol Biotechnol 17:188–200. https://doi.org/10.1159/000233506

Shuster Ben-Yosef V, Sendovski M, Fishman A (2010) Directed evolution of tyrosinase for enhanced monophenolase/diphenolase activity ratio. Enzyme Microb Technol 47:372–376. https://doi.org/10.1016/j.enzmictec.2010.08.008

Son M, Moon Y, Oh MJ, Han SB, Park KH, Kim J-G, Ahn JH (2012) Lipase and protease double-deletion mutant of *Pseudomonas fluorescens* suitable for extracellular protein production. Appl Environ Microbiol 78:8454–8462. https://doi.org/10.1128/AEM.02476-12

Son HF, Lee S-H, Lee SH, Kim H, Hong H, Lee U-J, Lee P-G, Kim B-G, Kim K-J (2018) Structural basis for highly efficient production of catechol derivatives at acidic pH by tyrosinase from *Burkholderia thailandensis*. ACS Catal 8:10375–10382. https://doi.org/10.1021/acscatal.8b02635

Subrizi F, Crucianelli M, Grossi V, Passacantando M, Pesci L, Saladino R (2014) Carbon nanotubes as activating tyrosinase supports for the selective synthesis of catechols. ACS Catal 4:810–822. https://doi.org/10.1021/cs400856e

Sun H, Jin X, Long N, Zhang R (2017) Improved biodegradation of synthetic azo dye by horseradish peroxidase cross-linked on nano-composite support. Int J Biol Macromol 95:1049–1055. https://doi.org/10.1016/j.ijbiomac.2016.10.093

Surwase SN, Patil SA, Jadhav SB, Jadhav JP (2012a) Optimization of L-DOPA production by *Brevundimonas* sp. SGJ using response surface methodology. Microb Biotechnol 5:731–737. https://doi.org/10.1111/j.1751-7915.2012.00363.x

Surwase SN, Patil SA, Apine OA, Jadhav JP (2012b) Efficient microbial conversion of L-Tyrosine to L-DOPA by *Brevundimonas* sp. SGJ. Appl Biochem Biotech 167:1015–1028. https://doi.org/10.1007/s12010-012-9564-4

Suzuki H, Furusho Y, Higashi T, Ohnishi Y, Horinouchi S (2006) A novel o-aminophenol oxidase responsible for formation of the phenoxazinone chromophore of grixazone. J Biol Chem 281:824–833. https://doi.org/10.1074/jbc.M505806200

Tan D, Zhao J-P, Ran G-Q, Zhu X-L, Ding Y, Lu X-Y (2019) Highly efficient biocatalytic synthesis of L-DOPA using in situ immobilized *Verrucomicrobium spinosum* tyrosinase on polyhydroxyalkanoate nano-granules. Appl Microbiol Biotechnol 103:5663–5678. https://doi.org/10.1007/s00253-019-09851-7

Wan X, Chai B, Liao Y, Su Y, Ye T, Shen P, Chen X (2009) Molecular and biochemical characterization of a distinct tyrosinase involved in melanin production from *Aeromonas media*. Appl Microbiol Biotechnol 82:261–269. https://doi.org/10.1007/s00253-008-1742-5

Wang GL, Shen P, Yang L, Peng ZR (1999) Cloning and expression of tyrosinase gene from *Pseudomonas maltophila* in *E. coli*. Yi Chuan Xue Bao 26:274-279. https://doi.org/10.1111/j.1574-6968.2000.tb09035.x

Wang G, Aazaz A, Peng Z, Shen P (2000) Cloning and overexpression of a tyrosinase gene *mel* from *Pseudomonas maltophila*. FEMS Microbiol Lett 185:23–27. https://doi.org/10.1111/j.1574-6968.2000.tb09035.x

Wang F, Xu Z, Wang C, Guo Z, Yuan Z, Kang H, Li J, Lu F, Liu Y (2021) Biochemical characterization of a tyrosinase from *Bacillus aryabhattai* and its application. Int J Biol Macromol 176:37–46. https://doi.org/10.1016/j.ijbiomac.2021.02.042

Wibowo JP, Batista FA, van Oosterwijk N, Groves MR, Dekker FJ, Quax WJ (2020) A novel mechanism of inhibition by phenylthiourea on PvdP, a tyrosinase synthesizing pyoverdine of *Pseudomonas aeruginosa*. Int J Biol Macromol 146:212–221. https://doi.org/10.1016/j.ijbiomac.2019.12.252

Winroth L (2019) Recombinant expression of *Verrumicrobium spinosum* tyrosinase in *Escherichia coli*, its purification and characterisation, Bachelor Programme in Chemistry thesis, Uppsala University

Xu L, Pu J (2016) Alpha-synuclein in Parkinson's disease: from pathogenetic dysfunction to potential clinical application. Parkin-sons Dis-US. https://doi.org/10.1155/2016/1720621. 2016:1720621

Yang HY, Chen CW (2009) Extracellular and intracellular polyphenol oxidases cause opposite effects on sensitivity of *Streptomyces* to phenolics: A case of double-edged sword. PLoS ONE 4:7462. https://doi.org/10.1371/journal.pone.0007462

Yuan W, Burleigh SH, Dawson JO (2007) Melanin biosynthesis by *Frankia* strain Cel5. Physiol Plantarum 131:180–190. https://doi.org/10.1111/j.1399-3054.2007.00948.x

Zaidi KU, Ali AS, Ali SA, Naaz I (2014) Microbial tyrosinases: Promising enzymes for pharmaceutical, food bioprocessing, and environmental industry. Biochem Res Int 2014:1–16. https://doi.org/10.1155/2014/854687

Zolghadri S, Bahrami A, Khan MTH, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury AA (2019) A comprehensive review on tyrosinase inhibitors. J Enzym Inhib Med Ch 34:279–309. https://doi.org/10.1080/14756366.2018.1545767