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Review

Potential pharmacological applications of enzymes associated with bacterial metabolism of aromatic compounds

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Many purple anoxygenic bacteria contribute significantly to the catabolic and anabolic processes in the oxic/anoxic zones of several ecosystems. However, these bacteria are incapable of degrading the benzenoid ring during the biotransformation of aromatic hydrocarbons. The key enzymes in the aromatic amin acids metabolism of purple bacteria include 3,4-dihydroxyphenylalanine minotransferase (EC 2.6.1.49), 3,4-dihydroxyphenylalanine reductive deaminase (EC 4.3.1.22), 3,4-dihydroxyphenylalanine oxidative deaminase (EC 1.13.12.15), L-tryptophan aminotransferase (EC 2.6.1.27), 3,4-dihydroxyphenylalanine aminotransferase (EC 2.6.1.49), phenylalanine ammonia lyase (EC 4.3.1.24), tyrosine ammonia lyase (EC 4.3.1.23), phenylalanine/tyrosine ammonia lyase (EC 4.3.1.25), phenylacetate-CoA ligase (EC 6.2.1.30); histidine ammonia lyase (EC 4.3.1.3), tryptophanase (EC 4.1.99.1), tryptophan 2,3-dioxygenase (EC 1.13.11.11) and kynurenineformidase (EC 3.5.1.49). These enzymes have biological significance since these are known to have highly antioxidant, anti-cancer, anti HIV, antifungal/microbial, cyclooxygenase inhibitory phytohormonal activities and also display an impressive array of pharmacological applications viz. pigmenta, toxins, enzyme inhibitors, pesticides herbicides, antiparasitics, mycotoxins, antitumor agents, cytotoxic activities and growth promoter of animal and plants. Here, we reviewed anoxygenic bacterial novel enzymes and their biotechnological applications.

Key words: Alkylester, biotransformation, bioprospect, indigo, indolmycin, purple bacteria, violacein.

INTRODUCTION

Anoxygenic phototrophic bacteria preferably grow by a photoheterotrophic metabolism with organic substances as electron donors during their photosynthetic activity. However, phototrophic purple non-sulfur bacteria (PNSB)

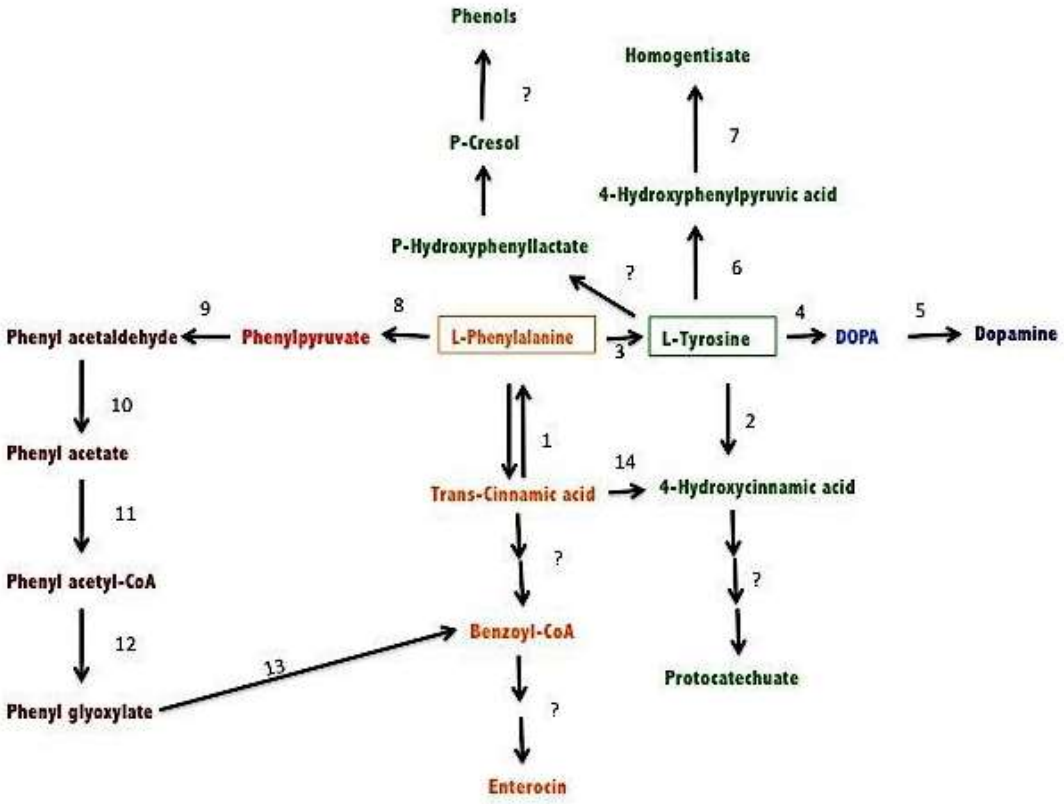

Figure 1. General microbial catabolic pathway of phenylalanine and tyrosine. [(1)L-phenylalanine ammonia lyase (PAL; EC 4.3.1.24);2) L-tyrosine ammonia lyase (TAL; EC 4.3.1.23); 3) phenylalanine hydroxylase; 4) tyrosine dehydrogenase; 5) 3,4-dihydroxyphenylalanine decarboxylase (DDC; EC 4.1.1.27); 6) tyrosine transaminase (TAT; EC2.6.1.5); 7) 4-hydroxyphenylpyruvate dioxygenase; 8) phenylalanine aminotransferase (PATs; EC 2.6.1.5); 9) phenylpyruvate decarboxylase (PDC); 10) phenyl acetaldehyde dehydrogenase; 11) phenyl acetate Co-A ligase (EC 6.2.1.30); 12) phenyl acetate Co-A α-oxidizing enzyme; 13) phenylglyoxylateoxidoreductase; 14) cytochrome P450; DOPA=3,4-dihydroxyphenylalanine; ? = Indicates not known.]

belong to a physiological group of photosynthetic prokaryotes, and are distributed in four different phyla that are able to grow under anaerobic conditions and carry out photosynthesis without oxygen liberation. These bacteria are widely distributed in anoxic habitats of various ecosystems (Bertoldi et al., 1999). They lack photosystem-II and carry out anoxygenic photosynthesis. They have metabolic versatility and depend on electron donors viz. reduced sulfur compounds, hydrogen and organic compounds, which get more reduced than water. The purple non- sulfur bacteria are capable of metabolizing a wide range of aliphatic organic compounds and are versatile in inducing metabolic routes in response to nutritional changes in the environment. The catabolism of L-phenylalanine/ L-tyrosine takes place through several aerobic or anaerobic routes (Rother et al., 2002). Catabolism of L-phenylalanine/L-tyrosine by these bacteria is carried out by a peripheral pathway resulting in the formation of homogensitate as a central intermediate (Figure 1).

Homogensitatedioxygenase is involved in the opening of the aromatic ring of homogensitate producing maleylacetoacetate and fumarylacetoacetate. Finally these substrates are hydrolyzed by a specific hydrolase forming fumarate and acetoacetate. In photosynthetic bacteria, catabolism of L- tyrosine is also crucial since homogentisate is a precursor in the biosynthesis of photosynthetic pigments.

The primary step in the catabolism of L-phenylalanine/ L-tyrosine is either transamination or ammonia lyase process. In *Rhodobactercapsulatus* and *Pseudomonas putida*, L- phenylalanine and L-tyrosine are converted into

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phenylpyruvate and 4-hydroxypyruvate by a non- specific aromatic aminotransferase, respectively. In most cases, L-phenylalanine is converted into L-tyrosine in the presence of phenylalanine hydroxylase (Copeland et al., 2005). Besides, trans-cinnamic acid and p-hydroxycinnamic acid are the products of L-phenylalanine catabolism when the reaction is catalyzed by L-phenylalanine/L-tyrosine ammonia lyase (PAL/TAL; EC 4.3.1.25) (Kevin et al., 2006). In *R. capsulatus* and *Rhodobactersphaeroides*, p-hydroxycinnamic acid biosynthesis from L-tyrosine has been reported to be catalyzed by TAL (Duran et al., 1983). In *Erwiniaherbicola*, 3,4-dihydroxyphenylalanine (DOPA) biosynthesis has been reported from L-tyrosine. On the other hand, some methanogens convert L-tyrosine into p-cresol and phenol. The downstream processing of L-phenylpyruvate occurs through phenyl acetaldehyde leading to the formation of phenyl acetate, phenyl glyoxylate culminating in the biosynthesis of benzyl CoA (Figure 1) (Lee et al., 2004; Herrera and Ramos, 2007). Ammonia-lyases belong to the family of enzymes that catalyze deamination of amino acids. The well-studied enzymes are L- phenylalanine/ L-tyrosine ammonia lyase (PAL/TAL) and L-histidine ammonia lyase (HAL). Deamination of tyrosine to p-hydroxycinnamic acid (pHCA) is catalyzed by the PAL/TAL enzyme. Phenylalanine ammonia lyase (PAL; EC 4.3.1.24) has been found in both higher plants and various microorganisms. It is a bifunctional enzyme. It uses tyrosine also as a substrate, and therefore is also called TAL. Among various microbial PAL/TAL enzymes (Samaha et al., 1997), the enzyme from the fungus, *Rhodotorulaglutinis* (RgTAL) has been reported to have the highest TAL activity. The members of ammonia lyase family contain a conversed “Ala, Ser, Gly” amino acids motif (Table 1) that undergoes autocatalytic cyclization to generate a 3,5-dihydro-5-methylidene-4H-imidazol-4-one (MIO) group and acts as the catalytic electrophile for elimination of ammonia and a non-acidic β-proton from the amino acid substrate (Samaha et al., 1997). This mechanism has been supported by the X-ray crystal structure of the *Pseudomonas*-HAL and *Rhodotorulaglutinis*-PAL (Juana et al., 1997). It is worthy to note that most of the PAL and TAL enzymes are strongly inhibited by their products.

In plants, this enzyme catalyzes the first reaction of the phenylpropanoids pathway and converts phenylalanine to trans-cinnamic acids (CA). Further hydroxylation of trans-cinnamic acid produces para-hydroxycinnamic acid (pHCA), which plays a pivotal role in the production of a diverse array of plant secondary met abolites. The PAL from some bacteria and plants deaminates phenylalanine to trans-cinnamic acid (CA), which is ultimately converted into secondary metabolites such as lignins, flavonoids and coumarins in plant and several antibiotic compounds in bacteria. Human’s recombinant phenylalanine ammonia lyase has been explored for the treatment of phenylketonuria (PKU) by metabolizing excess dietary phenylalanine (Li et al., 2003). This enzyme is highly selective for L-tyrosine and synthesizes para-hydroxycinnamic acid (4-coumaric acid, pHCA) as a protein co-factor or antibiotic precursor in microorganisms. The TAL enzyme from the photosynthetic bacterium *R. sphaeroides* has been identified, cloned and functionally expressed in *Escherichia coli.* The 4-coumaric acid serves as antibiotic precursor in microorganisms and as a cofactor for the synthesis of a small 14 kDa water soluble protein designated as photoactive yellow protein (PYP) in *Ectothiorhospirahalophilia*, *Rhodospirillumsalexigens* and *Chromatiumsalexigenes*. Many research groups have reported that, TAL metabolic engineering of flavonoid and resveratrol biosynthesis pathways require 4-coumaric acid as a precursor (Regina et al., 2004). Since TAL forms 4-coumaric acid directly from L-tyrosine and uses it in heterologous expression systems, it circumvents the need to express both PAL and 4-coumaric acid hydroxylase, a membrane bound cytochrome P450 enzyme for conversion of L-phenylalanine to 4-coumaric acid. Structural and functional studies of TAL identified a histidine residue in the active site, which is essential for controlling substrate preference for L-tyrosine over L-phenylalanine (Bartling et al., 1994). Phenylalanine ammonia lyase and tyrosine ammonia lyase have been reported in a few microorganisms with possible involvement in the biosynthesis of secondary metabolites similar to their plant counterparts (Bartling et al., 1994). These compounds are of interest due to their potential use as starting material for chemical and enzymatic conversion to a wide array of commercially valuable biomecules including, flavors, fragrances, pharmaceuticals, biocosmetics and other secondary metabolites (Hoshino et al., 1990). Two potential microbial routes for the biosynthesis of pHCA from aromatic amino acids have been envisioned (Figure 1). In such reactions, the enzyme has been designated as tyrosine ammonia lyase and reaction product is 4-coumaric acid. There are reports on the identification, characterization, cloning and functional expression of a TAL from anoxygenic phototrophic bacteria in *E. coli*. The PAL/TAL has been identified in *R. capsulatus* (integrated Genomics accession number RRC01844) *R. sphaeroides* (integrated genomics accession numbers YP-355075) and in *Saccharothrixespanaensis* (Gene bank accession numbers ABC88669) (Table 1). The sequence is homologous to plant PAL from *Petroselinumcrispum* (CAA57056, 30% identical and 48% similar) (Hoshino et al., 1990). However, it has more homology to bacterial histidine ammonia lyase (HAL; EC 4.3.1.3) from *Pseudomonas putida* (A35251, 36% identical and 54% similar).The amino acid ammonia lyases were thought to use dehydroalanine as an electrophile in the reaction mechanism but the three dimensional structure of PAL and HAL indicated that these enzymes have a MIO group

Table 1. Few key enzymes involved in aromatic amino acids catabolism.

Enzyme(EC.No.)	Organism	Co-factor	Substrate	Co-substrate	Products	GeneBankNo.
PAL(EC4.3.1.24)	*Rhodotorulagluti- nis/R.rubra/ Rhodobactercapsulatus*	MIO	Phenylalanine	NA	*Trans*cinnamic acid	ABC88669
PAL/TAL (EC4.3.1.25	*Rhodotorul rubra* /Streptomycesmaritimus	MIO	Phenylalanine/ Tyrosine	NA	CA/pHCA	AF254925/AAF81735
TAL (EC.4.3.1.23)	*Rhodobacter sphae- roides/Rhodobactercapsulatus*	NA	Tyrosine	NA	PhCA	YP3550755
HAL(EC4.3.1.3)	*Rhodopseudomonas putida*	NA	Histidine	NA	Urocanicacid	RRC01844
WAT (EC2.6.1.27)	*Brevibacteriaum linens/Clostridium sporogenes/Enterrobactercloacae*	PLP	Tryptophan	α-KGA	Indolepyruvic acid	AK102509
Phenylalanine/Tyrosineami- notransferase(EC.2.6.1.5)	*Klebsiellapneumoniae*	PLP	Tyrosine	α-KGA	4-Dihydroxyphe nylpyruvicacid	NC.000913.1
DOPA Transaminase (EC2.6.1.49)	*Enterobacter cloacae/ Alcaligens faecalis/ Erviniagerbicola*	PLP	L-DOPA	α-KGA	3,4-Dihydroxyp henylpyruvic acid	D55724
HistidineTransamiase(EC2.6.1.38)	*Pseudomonas acidovo- rans*	PLP	Histidine	α-KGA	Imidazol-5 yl	AE0013810
TDC (EC4.1.1.28)	*Streptococcusfaecalis*	PLP	Tryptophan	NA	Tryptamine	M25151
DDC (EC 4.1.1.28)	*Streptococcusfaecalis*	PLP	L-DOPA	NA	Dopamine	UO8597

PAL = phenylalanine ammonia lyase; TAL = tyrosine ammonia lyase; HAL = histidine ammonia lyase; WAT=tryptophan aminotrans- ferase; DOPAATS= 3,4-dihydroxyphenylalanine aminotransferase; TDC= tryptophan decarboxylase; DDC= 3,4-dihydroxyphenylalanine decarboxylase;PLP= Pyridoxal-5-phosphateDOPA= 3,4- dihydroxyphenylalanine; pHCA= p-hydroxycinnamic acid; CA/pHCA= Cinnamic a c i d /p-hydroxycinnamicacid;α- KGA=2-oxoglutarate;MIO=3,5-dihydro-5-methylidene-4H-imidazol-4-one; NA= not applicable).

for substrate activation. The compounds such as phenyl pyruvate, indole pyruvate and keto acids with more than six carbon atoms in a straight chain served as substrate for the decarboxylase, and a subsequent step is the formation of phenyl acetaldehyde from phenyl acetate, a reaction catalysed by a dehydrogenase where NAD± is a cofactor. Phenyl acetate is a known intermediate in the microbial metabolism of various aromatic substrates including phenylalanine (Wolfram and Oliver, 2002). In *Thauera aromatic* and *Rhodobacter sp*. strain RHA1, phenyl acetate is oxidized under anoxic conditions to a common intermediate benzyl CoA via phenyl acetyl CoA (Wolfram and Oliver, 2002). Phenyl acetyl CoA is formed from phenyl acetate by specific phenyl acetate CoA ligase in both the aerobic and anaerobic pathways (Chen et al., 2005). Phenylglyoxylate is oxidized to benzyl CoA by the phenylglyoxylate: acceptor oxidoreductase complex enzyme, which ultimately transfers electrons to NAD± Deaminases are a group of enzymes that catalyze the elimination of ammonia from organic substitutes and play an important role in the nitrogen cycle. Microbial L-amino acid deaminases identified so far include: L-tyrosine, L-phenylalanine, and L-arginine. In a route, 4-coumaric acid is formed from L-phenylalanine in a two-step process where PAL removes the (pro3S) hydrogen and NH3 from phenylalanine to yield trans-cinnamic acid (CA). In the next step, a cytochrome P450 enzymesystem hydroxylates CA to produce 4-coumaric acid. The most natural

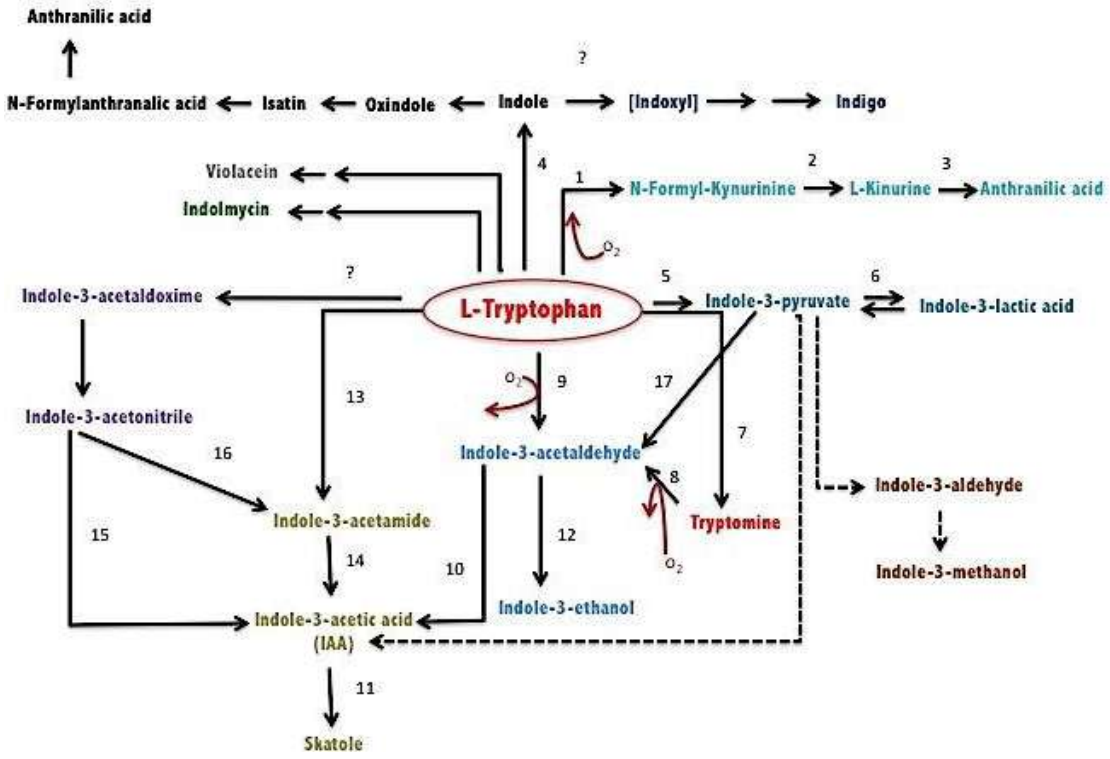

Figure 2. Metabolism of L-tryptophan in bacteria.[Tryptophan 2,3 dioxygenase (EC 1.13.11.11); 2) kynurenineformidase (EC 3.5.1.49); 3) kynureninase (EC 3.7.1.3); 4) tryptophanase (EC4.1.99.1); 5) Tryptophan aminotransferase (EC 2.6.1.27); 6) indole lactate dehydrogenase (EC 1.1.1.110);7) Tryptophan decarboxylase (EC 4.1.1.28); 8) tryptamine oxidase (EC 1.4.3.4); 9) Tryptophan side chain oxidase (EC 4.1.1.43); 10) indole acetaldehyde dehydrogenase (EC 1.2.1.3); 11) indole acetic acid oxidase; 12) indole acetaldehyde dehydrogenase; 13) Tryptophan 2-monooxygenase (EC 1.13.12.3); 14) indoleacetamide hydrolase (EC 3.5.1.0); 15) Nitrilase; 16) Nitrile hydratase;17) indole-3-pyruvic acid decarboxylase. The dotted lines (---) indicate a spontaneous reaction].

PAL/TAL enzymes from either plants or microbial sources prefer to use L -phenylalanine rather than L-tyrosine as their substrate. In addition to their ability to convert L-phenylalanine to trans-cinnamic acid, also accept L-tyrosine, tamate, serine and cytosine as substrate. Based on the mechanism, these are further classified as: oxidative, reductive and hydrolytic deamination (Nieminen et al., 2002). The 3,4- dihydroxyphenylalanine decarboxylase purified from a mutant strain of *E. coli* is a homodimeric enzyme belonging to the family of pyridoxal-5-phosphate enzymes. During catalysis, there is also generation of aromatic amine. DOPA decarboxylase catalyzes not only the decarboxylation of L-aromatic amino acids but also side reactions including half-transamination of aromatic amino acid and oxidative deaminase of aromatic amines.

Decarboxylation of L-aromatic amino acids is the main reaction where the enzyme has been identified to catalyze the side reactions that are the oxidative deamination of aromatic amines and the half transamination of aromatic amino acids accompanied by a pictet-spengler reaction. The reaction specificity of DOPA decarboxylase does not change in the presence or absence of oxygen. The enzyme catalyzes reaction between 3, 4-dihydroxyphenylalanine and 2-oxoglutarate to form 3, 4-dihydroxyphenyl pyruvic acid and L-glutamate. This enzyme has been reported from animals and bacteria that is *Alcaligenesfaecalis* IAM 1015 and *Enterobacter cloacae*. The phenylpyruvate decarboxylase (EC. 4.1.1.43) and phenyl acetaldehyde dehydrogenase enzymes catalyze the reaction of phenyl pyruvate to phenyl acetate through phenyl acetaldehyde and also catalyze the non-oxidative decarboxylation of phenyl pyruvate where diphosphothiamin (DPT) and $Mg^{2\pm}$ act as cofactors. While a variety of chemical transformations related to the aerobic degradation of L-tryptophan and most of the genes and corresponding enzymes involved therein have been predominantly characterized in eukaryotes, relatively little is known about these pathways in bacteria (Figure 2). In *E. coli* and many other bacteria, the non-oxidative degradation of L-tryptophan to indole, pyruvate and ammonia occurs by pyridoxal-5-phospahte dependent tryptophanase (Tryptophan indole-lyase EC 4.1.99.1). The oxidative degradation of

exogenous L- tryptophan via the anthranilate pathway has been implicated as a sole source of carbon and nitrogen. The gene encoding kynureninase (KYN, EC 3.7.1.3) has been cloned from *Pseudomonas fluorescens* and corresponding enzyme homologous to eukaryotic KYN has been characterized. The tryptophan 2, 3-dioxygenase (EC 1.13.11.42) activity has been described in some bacteria and the corresponding genes have been identified. Phenylalanine hydroxylase enzyme converts phenylalanine to tyrosine. It is an iron-containing protein that requires a co-factor, (6R)-L-erythro-5, 6, 7, 8-tetrahydrobiopterin (pterine) (Schneider et al., 1997). Tyrosine/phenylalanine aminotransferase (EC 2.6.1.5) from *Bacillus caldolyticus*catalyzes the conversion of tyrosine into 4-hydroxyphenylpyruvic acid and also phenylalanine to phenylpyruvate. The transformation of 4-hydroxyphenylpyruvate to homogentisate is catalyzed by 4-hydroxyphenylpyruvate dioxygenase. DOPA is synthesized f r o m L-tyrosine of *Erwiniaherbicola* in a one-step oxidation reaction catalyzed by tyrosine phenol-lyase (EC 4.1.99.2). In *E. coli*, histidine ammonia lyase enzyme is apparently ubiquitous (Kodach et al., 2006). It is a red colored enzyme, which converts histidine into urocanic acid and ammonia. The enzyme catalyzes the non-oxidative elimination of the α-amino group of histidine and is closely related to the important plant enzyme, phenylalanine ammonia lyase and is further metabolized to glutamate (Srinivas et al., 2002). It is widely distributed in higher plants and bacteria viz. *Pseudomonas fluorescens*, *Pseudomonas aerusinosa*, *Pseudomonas putida*, *Pseudomonas testosteron* and *Aerobacteraerogenes*, filamentous fungi and yeast. The whole genome sequence of photosynthetic bacterium *Rhodobactersphaeroides* enzyme indicated the presence of probable histidine ammonia lyase.

ENZYMES MODE OF ACTION

Tryptophan indole-lyase is a pyridoxal-5-phosphate dependent enzyme found in many bacteria. It catalyzes the reversible hydrolytic decomposition and α, β-elimination of L-tryptophan leading to formation of indole, pyruvate and ammonia. Tryptophanase is a homotetrametic protein in which each monomer binds to one molecule of PLP, and forms an aldimine bound with a lysine residue (Srinivas et al., 2002). It has been shown that the tnaA gene encoding tryptophanse is necessary for biofilm formation in *E. coli*. It has been cloned from a virulent strain of *Haemophilesinfluenzae*, and the production of indole has been correlated with the ability of the strain to cause some infectious diseases viz. septicemia, epiglottitis and meningitis. Therefore, tryptophanase is a convenient target for elaboration of efficient inhibition, and can be used for treatment of meningitis. Tryptophan aminotransferase (EC 2.6.1.27) enzyme belongs to the family of transferase, and it catalyzes the conversion of L- tryptophan into indole-3-pyruvic acid in the presence of 2-oxoglutarate (Figure3) and pyridoxal-5- phasphate in *Enterobacter cloacae* (Table 1). It has also been reported in plants and animals. In the anthranilic acid pathway, tryptophan is first converted to formylkynurenine by the enzyme, tryptophan 2,3-dioxygenase and the latter is involved in the formation of kynurenine and anthranilic acid by kynurenineformidase (Figure 2). Indole-3-pyruvic acid decarboxylase catalyzes the conversion of indole-3-pyruvic acid to indole-3-acetaldehyde and it has been isolated from *Azospirillumbrasilense* and *Paenibacillspolymyxa* E681 (Figure 2).

Indole-3-pyruvic acid can also be converted into indole acetaldehyde non-enzymatically (Panke et al., 2004). L-tryptophan is also converted into indole-3-acetaldehyde and IAA via the intermediate tryptapmine (Figure 2). The initial metabolism of tryptophan to tryptamine is catalyzed by tryptophan decarboxylase followed by conversion of tryptamine to indole-3-acetaldehyde by tryptamine oxidase. Indole lactate dehydrogenase (EC 1.1.1.110) enzyme has been isolated from *Clostridium saprogenic*. It catalyzes the conversion of indole-3-pyruvic acid in to indole-3-lactic acid, a reversible reaction and NAD± is used as a cofactor. Indole-3-acetonitrile is converted into IAA in the presence of nitrilase (Figure3). This enzyme has been identified in higher plants belonging to families Cruciferae, GramineaeMusaceae and also in microbes (*Klebsiellaozonae*, *Alcaligenesfaecalis* and *Rhodococcusrhodochrous*). Nitriles could be hydrolyzed directly to their corresponding acids in the presence of specific nitrilases or via a two-steps process involving an initial conversion to an amide by nitrile hydratase followed by hydrolysis of the amide to an acid by amidase or by acetamide hydrolase (Figure 2) (Koga, 1995). Indole-3-acetic acid (IAA) biosynthesis pathways in bacteria have been studied. The identification of intermediates led to five different pathways using L-tryptophan as a precursor for IAA biosynthesis (Figure 2).

The discovery of Indole-3-acetic acid as a plant growth regulator coincided with the first indication of the molecular mechanisms involved in tumorigenesis induced by *Agrobacterium*. It was later found that not only plants but also microorganisms including bacteria and fungi are able to synthesize indole-3-acetic acid (Phi et al., 2008). The production of IAA by *Enterobacter* and *Pseudomonas* strains harboring the gene for indole-3-pyruvic acid decarboxylase were higher than those produced by the same strains without the gene (Elsa et al., 2004). A number of studies have shown that IAA can be a signalling molecule in microorganisms for both IAA producing and IAA non-producing species. Catabolism of L- tryptophan to indole-3-acetic acid occurs via five distinct routes. Indoleacetamide pathway involves tryptophan 2-monooxygenase (EC 1.13.12.3) and indoleacetamide hydrolase (EC 3.1.1.51). Tryptophan side-chain oxidase pathway and tryptophan side chain oxidase (EC.4.1.1.43) activity have been found in

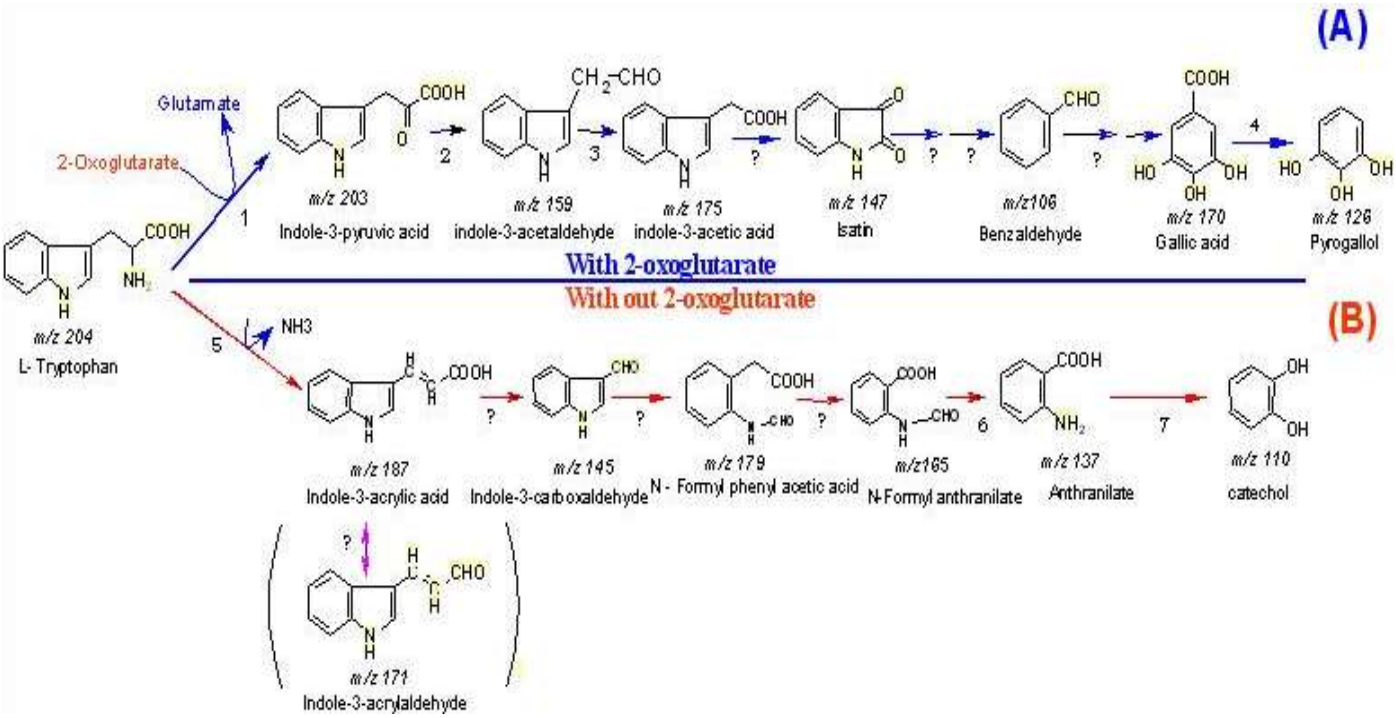

Figure3. Proposed pathways of L-tryptophan catabolism by *Rhodobactersphaeroides* OU5. (A = in the presence of 2-oxoglutarate; B= in the absence of 2-oxoglutarate). [The proposed pathway is based on the metabolites identified in this study and the evidence for some of the enzymes is from the literature. 1 = Tryptophan aminotransferase (EC2.6.1.27); 2=Indole-3-pyruvicacid decarboxylase (EC 4.1.1.74), 3 = Aldehyde dehydrogenase (EC 1.2.3.1)/Aldehyde oxidase (EC 1.2.3.1); 4 = Gallate decarboxylase (EC=4.1.1.59); 5= Tryptophan ammonialyase; 6=N-Formylanthranilate. deformylase (EC 3.5.1.68) and 7 = Anthranilate dehydrogenase (EC 1.1.1.95). ? = Enzymes not identified].

Pseudomonas fluorescens CHA0. In this pathway tryptophan is directly converted to indole-3acetaldehyde bypassing indole-3-pyruvic acid, which can be oxidized to IAA. In *Alcaligenesfaecalis*, indole-3-acetonitrile pathway is known and conversion of indole-3- acetonitrile to IAA by a specific nitrilase has been reported (David et al., 2001). In indole-3-pyruvate pathway, the initial step is the conversion of tryptophan to indole-3-pyruvic acid by an aminotransferase. In a rate limiting step, indole-3-pyruvic acid is decarboxylated to indole-3-acetaldehyde by indole pyruvate decarboxylase enzyme. In the last step, indole-3-acetaldehyde is oxidized in IAA in *Azospirillumbrasilense*, *Pseudomonas putida* and *Paenibacillspolymyxa*E681.5. Tryptamine pathway has been identified in *Azospirillum* and *Bacillus cereus* where conversion of exogenous tryptamine to IAA takes place (Figure 2). On the other hand, tryptophan-independent pathway of indole-3-acetic acid biosynthesis has been demonstrated in *Azoperillumbrasilense* by feeding experiments with labelled precursors (Duran et al., 1994). The indole-3-acetic acid production has been reported in a few of anoxygenic phototrophic bacteria viz. *Rhodopseudomonaspalustris*, *Rhodobactersphaeroides*, *Rubrivivaxgelatinosus* and *Rubrivivaxtennius*) in L-tryptophan grown culture where tryptophan production occurs not only from L-tryptophan but also from indole and glycine.

Indole-3-acetic acid has been shown to be involved in light/ horseradish peroxidase (HRP) activation and it has been suggested as a new photodynamic cancer therapy by forming free radicals such as indolyl, statolyl and perxoyl radicals which can cause lipid peroxidation (Hoshino et al., 1990). The combination of IAA and HRP showed cytotoxicity to mammalian cells including G361 human melanoma cells and human pancreatic cancer BXPC-3 cells. In *Agrobacterium tumefaciens* and Rhizobium sp., nitrile hydratase and amidase activities have been identified indicating the conversion of indole-3-acetonitrile to indole-3-acetic acid via indole-3-acetamide.

Novel potential enzyme

Tryptophan ammonia lyase (Kumavathet al., 2015) which adds to research knowledge of L- tryptophan metabolism and to the list of ammonia lyases (L-phenylalanine ammonia lyase (PAL; EC 4.3.1.24), L- tyrosine ammonia lyase (TAL; EC 4.3.1.23), PAL/ TAL (EC 4.3.1.25) L-serine ammonia lyase (SAL; EC 4.3.1.17) and L-histidine

ammonia lyase (HAL; EC 4.3.1.3) reported so far, hence went for purification and characterization of tryptophan ammonia lyase enzyme. Tryptophan ammonia lyase was isolated, purified and characterized as a ~225 kDaheterotetramer. The novelty of WAL was confirmed through MALDI-TOF (MS/MS) finger printing analysis. Among the four subunits, the 55 kDa subunit had ~54% score with a hypothetical protein in *Rhodobactersphaeroides* 2.4.1 while the remaining three had less significant match in database (NCBS, MSDB and SwissPort),the partial N-terminal sequences of WAL matched with the probable histidine ammonia lyase of *Rhodobactersphaeroides* 2.4.1. The molecular evidence of this enzyme is in progress.

Enzymes and their products

The production of secondary metabolites has been shown from aromatic amino acids. The metabolism of aromatic amino acids in microorganisms has been widely studied. Lots of microorganisms have evolved secondary metabolic pathways with the capacity to produce compounds displaying an impressive array of pharmacological applications which include pigments, toxins, enzyme inhibitors, pesticides, herbicides, antiparasitcs, mycotoxins, antitumor agents, antibiotics cytotoxicity activities and growth promotors of animal and plants (Bourinbaiar and Hung, 1994).

Indoles and its derivatives

Indoles are known for antimicrobial activities. In addition, molecules like indole-3-acetic acid (IAA) and indole-3-propionic acid (IPA), are natural auxins. Esters of indole acetic acid, indolemyoinositol esters; sphestrin and rhodestrin were reported to have phytoharmonal activity. The isolated rhodethrin also showed the phenol esters are an important group of bio- genic molecules reported from plants, bee propolis (Kumavathet al., 2011), yeasts like *Candida* and from a marine bacterium; microbulbifer. Alkyl esters of phenols are of biotechnological significance since they have antioxidant (Ranjith et al., 2008), anticancer, anti HIV and antimicrobial activities. The cytotoxicity of rhodophestrol against U937 (human leukemic monocyte lymphoma cell line) was determined (apoptosis bodies formation) on this cancer cell line even at low concentrations (50 nM). It has COX-I and COX-II inhibitory activity. Rhodophestrol is a potential anticancer and thus worth exploiting from *Rubrivivaxbenzoatilyticus* (Fonnum and Larsen, 1965). The literature suggested the production of other phenols during the bioprocessing of an aromatic amino acid like L-phenylalanine by *Rubrivivaxbenzoatilyticus*. Though most of theseare representatives of free phenols, which many researchers have already identified as microbial products, the microbially produced phenol terpenoids and related conjugates are novel bio-molecules, which are worth exploiting. Indoles are extensively produced by the chemical industries for variety of applications including, pharmaceuticals, pesticides and dyes. They are widely used as analgesics, anti-inflammatory agents antihypertensive, anti HIV compounds and phytohormones (Ahn et al., 2004). Many indole esters are found as COX-2 selective enzyme inhibitors. They were also found to have anti lipid peroxidation activity and anti-superoxide formation. Indole esters are also found to have more phytohormonal activity than their corresponding acids in the auxin bioassay.

Rhodethrin

Rhodethrinhas A molecular mass of m/z 279 [M±H] ± and was named as rhodethrin (3-hydroxy-6-(1H-indole-33-yloxy)-4-methyl-hexanoic acid). This compound differs from sphestrin or rhodestrin in having an ether linkage rather than ester linkage and in the length of the terpenoid side chain. Esters of the myoinositol-indole acetate were only reported from plants. This metabolite was not a product of L-phenylalanine. The LC-MS analysis revealed a more wide range of indoles including those purified (m/z 279 m/z and 129m/z) and the other metabolites identified include indole-3-acrylaldehyde, idole-3-carboxaldehyde, indole-3-acetic acid and rhodethrin. It presumes the molecular mass of 171 (m/z) as indole-3-acrylaldehyde; this compound is not available in the literature, but a related compound indole-3-acrylic acid in known. Excretion of indole-3-acrylic acid in the urine and feces of L-tryptophan fed animals was attributed to the intervention of intestinal microorganisms. Thus, indole-3-acrylaldehyde may be an intermediate of L-tryptophan metabolism in *Rhodobactersphaeroides*. Production of indole-3-carboxaldehyde was reported earlier from an Acetenobacter sp. and by an unidentified fungus. Microbial production of indole-3-acetic acid is well known and was reported even from a few other purple bacteria (*Rhodopseudomonaspalustris, Rhodobactersphaeroides*, and *Rubrivivaxtenuis*) when grown on L- tryptophan or from indole ± glycine by *Rhodobactersphaeroides*. Low levels of some of the metabolites in the L-tryptophan induced culture supernatant were also observed in the absence of L-tryptophan, the notable one was indole-3-acetic acid. While in the absence of 2-oxoglutarate, none of the above metabolites were observed in LC-MS profiling One normally expects the production of indole by the enzymatic action of tryptophanase (EC 4.1.99.1) encoded by tnaA gene(Kar et al., 1999), which was not observed as confirmed through the LC-MS and enzyme assay.

Indole 3- acrylic acid

To the best of knowledge, the proposed production of indole 3-acrylic acid pathway is a novel biological

pathway. Indole 3-acrylic acid was not listed as a phyto-auxine(http://www.biologie.uni-hamburg.de/b online/e31/31.htm) though its microbial origin was suspected. Production of indole-3-acrylic acid and ammonia indicates that the enzyme as an ammonia lyase, since this family of enzymes cleaves at C-N bond of various substrates and results in the formation of ammonia and corresponding products.

Indigo

The blue dye indigo has been known since prehistoric times and is still one of the most economical important textile dyes. The first report of microbial indigo production was in 1928. It is biosynthesized in bacteria through the oxidation of indole by a naphthalene dioxygenase and subsequent oxidation and dimerization. The desire to achieve a competitive, alternative to the chemical production of indigo rejuvenated interest in microbial indigo production since many microorganisms expressing both monooxygenase and dioxygenase during growth on aromatic hydrocarbons have been shown to transform indole i n t o indigo. The work has been focused on the naphthalene dioxygenase from *Pseudomonas putida* PpG7 expressed in *Escherichia coli*. Some of the genes of indigo biosynthetic pathway have been cloned and used to construct “engineering bacteria”. More efficient fermentation systems for indigo production have been exploited with this kind of bacteria.

Violacein

Chromobacteriumviolaceum was first reported as an isolate from wet rice paste. One of the characteristics of this microorganism is the ability to produce a purple pigment known as violacein under aerobic conditions. The biological role of violacein in *Chromobacteriumviolaceum*, as well as its biosynthesis pathway and the role of tryptophan and other indole derivatives have been reported. Tryptophan appears to be the only precursor molecule in violacein biosynthesis. Its production is apparently essential for pigment production in *Chromobacteriumviolaceum*. The IUPAC name and molecular mass of violacein are (3-[1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2- oxo-3H-pyrrol-3-ylidene]-1,3-dihydro-2H-indol-2-one) and 343.34, respectively. Violacein has attracted interest owing to its important multiple biological activities and pharmacological potential such as antibiotic, bactericide, antitumoralgenotoxic properties (Forrest et al., 1993). The antioxidant efficiency has been reported against oxygen and nitrogen reactive species as a scavenger of hydroxyl, superoxide and nitric oxide radicals. In addition, it is capable of inducing apoptosis in cancer cell cultures and is effective against a panel of neoplastic cell lines including leukemia lineage cancer diseases.

Indolmycin

Indolmycin is a secondary metabolite produced by *Streptomyces griseus* ATCC 1248 (formally *Streptomyces albus* BA 3972A), which was isolated from a sample of African soil. Indolmycin completely inhibits bacterial TrpRS (tryptophanyltRNAsynthetase) enzyme and it exhibits antimicrobial activity against gram positive and gram negative bacteria. Recently researchers have shown that indolmycin is active against *mycobacteria* and *Helicobacter pylori*. Indolmycin is also known as a major causative agent of chronic active gastritis.

Phenols and its derivatives

Alkyl esters of gallic acid

The chief source for obtaining gallic acid is through the hydrolysis of plant based products like tannins (Ahn et al., 2004). Microbial production of gallic acid has been reported using tannic acid as substrate. The esters iso-amyl- (iAG), n-amyl- (nAG), iso-butyl (iBG), n-butyl-(nBG) and isopropyl gallate (iPG) have been chemically synthesized from gallic acid. Gallic acid (3,4,5-trihydroxybenzoic acid) is an industrially important phenol and finds its applications in various fields. Alkyl esters of gallate form an important group of biogenic molecules which have been reported from plants, bee propolis and yeasts namely *Candida* (Forrest et al., 1993). These molecules are of biotechnological significance since these are known to have anti-oxidant, anticancer, anti HIV and antifungal/microbial activities (Forrest et al., 1993). Alkyl esters of gallic acid have antiviral, antibacterial, antifungal properties specifically against gram-positive bacteria (Kuniyoshi et al., 2003). The culture supernatants did not yield ammonia indicating that L-phenylalanine assimilation as nitrogen source is not through deamination process and hence possibility for transaminase activities is strong. Aromatic aminotransferase activity has been measured with L-phenylalanine, L-tyrosine and DOPA as substrates in the presence of 2-oxoglutarate and the substrate omission has been analyzed using HPLC. The enzyme activity has been reported to be 6, 10 and 21 units.mg $protein^{-1}.min^{-1}$. The increase in transaminase activity with the substrates L-phenylalanine < L-tyrosine < DOPA suggested that transamination occurred at the level of DOPA. DOPA consumption stagnated in the absence of supplemented keto acceptor, which restored only in the presence of 2-oxoglutarate (Figure 3). The transaminated product of DOPA has been extracted into ethyl acetate, concentrated and analyzed using LC-MS. A mass of 196 (m/z) indicated the product as 3,4-dihydroxyphenylpyruvic acid.

3, 4-dihydroxyphenylalanine reductive deaminase

Ammonia, the product of deaminase, has been observed only in the absence of added 2-oxoglutarate. The deamination product of DOPA was extracted (after acidifying; pH 4) into ethyl acetate, concentrated and analyzed using LC-MS. A mass of 182 (m/z) indicated the product as 3,4-dihydroxyphenylpropionic acid (DPPA) and the enzyme as reductive deaminase. The enzyme L-

phenylalanine ammonia lyase (PAL; EC. 4.3.1.24), which converts L-phenylalanine to trans-cinnamate is most commonly observed in plants and also in prokaryotes.

Biological applications

Anoxygenic phototrophic purple-non sulfur bacteria (PNSB) have the capability to degrade a wide range of low molecular weight aromatic hydrocarbons for growth (Poppe and Rétey, 2005) and thus help in the maintenance of biogeochemical cycles. The most extensively studied species among PNSB is *Rhodopseudomonaspalustris*, whose total genome analysis indicated the existence of at least five aromatic ring cleavage pathways, representing more aerobic (oxidative) mechanisms, than anaerobic. The other species of PNSB capable of utilizing benzoate for growth include: *Phaeospirillumfulvum*, *Rubrivivaxpurpurens*, *Rhodomicrobiumvannielli* and *Rhubrivivaxbenzoatiliticus*. On the other hand, the aromatic utilization for growth was not well reported among other purple bacteria, some incubation results indicated light dependent transformation of aromatic hydrocarbons by *Rubrivivaxgelatinosus*, *RhodobactercapsulatusRhodobacterblasticus* (Patten and Glick, 2002) and *Rhodobactersphaeroides* Correlation between L-phenylalanine consumption and simultaneous production of phenols during growth as sole nitrogen source by *Rhodobactersphaeroides* OU5 is similar to that observed with *Rhodobactercapsulatus* and differ from anaerobic chemotrophic bacterial metabolism, which produced benzoate.

Anoxygenic production of phenyl gallates

Gallate was the major product of L-phenylalanine metabolism in *Rubrivivaxbenzoatilyticus*. Gallate is a biotechnologically important compound (Ranjith et al., 2007), mainly produced by the hydrolysis of tannins and its microbial production by a few chemotrophic bacteria and fungi was observed earlier (Kumavathet al., 2007). Other phenols identified from the culture supernatant of *Rhodobactersphaeroides* include gallate, protocatechuate, catechol and caffeate, while homogensitate was reported from *Rhodobactercapsulatus*. Both gallate and caffeate had the same retention time under the experimental HPLC conditions used and their identity could be resolved and distinguished only through LC-MS analysis by their corresponding molecular masses.

Rubrivivaxineand rhodophestrol

Detailed characterization of these two of the purified metabolites based on FT-IR; 1H NMR, 13C NMR and mass analysis confirmed the structures as 3,4-dihydroxybenzoicacid -5-carboxy-4-hydroxy-3-methyl-pentylester and 2,3,4 tryhydroxybenzoylterpenoid ester. The hydrolysis of the ester bond and of rhodophestrol and 2,3,4-tryhydroxybenzoyl terpenoid ester by the enzyme esterase (EC 1.1.1.49) and identifying the corresponding phenol acid and alcohol through HPLC/LC-MS analysis helped in the confirmation of the novel metabolites, whose yield was about 100 µmole/(65µg)/ml-1. Rubrivivaxine and 0.2 µ mol 2,3,4-tryhydroxybenzoyl terpenoid ester.

Phenol esters were identified through hydrolysis of the ester bonds of the metabolites in different fractions using esterase enzyme and the corresponding acid and alcohol were analyzed using LC-MS (56). These are broadly categorized as gallate, caffeate, genisitate and protocatechuate esters. While the esterase hydrolyzed products represented the corresponding acids, the alcohols were hydroxyalkanoates, terpenols and phenols.

The marine bacterial isolate identified as belonging to the genus *Microbulbifer* could produce 4-hydroxybenoate alkyl esters (butyl, heptyl and nonyl 4- hydroxybenzoate esters; commonly called as “parabens”), which were antimicrobial, which differ from the isolated conjugated metabolites from phenylalanine of *Rubrivivaxbenzoatilyticus*. 3,4- Dihydroxyphenylalanine was identified as the major product of L-phenylalanine (L-tyrosine) metabolism of *Rhodobactersphaeroides* OU5 (Sunayana et al., 2005).

The literature evidences suggest that DOPA is the downstream product of L-phenylalanine or L-tyrosine in *Rhodobactersphaeroides* OU5, thus differ from the homogentisate pathway observed in other bac- teria. Based on the earlier evidence of production of DOPA by *Erwiniaherbicola* from L-tyrosine together with isolation of the enzyme DOPAATS, in this study and from other bacteria, we propose the bacterial DOPAATS pathway of L-phenylalanine catabolism (Figure 4). The further downstream products of this metabolism were identified through the LC-MS analysis (52).

The role of alternative enzymes involved in the deamination of DOPA is also shown in the pathway (Figure 4). The comparison of the three enzymes i n v o l v e d in ammonia metabolism of DOPA in *Rubrivivaxbenzoatilyticus* are shown in Table 2. Para-dimethylaminobenzaldehyde reagent (Ehrlich reagent) is the most commonly and widely used reagents in the identification of indoles.

This reagent gives pink to red colour when reacted with indole and its derivatives, while yellow to brown with anthranilate. The culture supernatant of L-tryptophan induced *Rubrivivaxbenzoatelitus* and *E. coli* were tested with Ehrlich reagent. The orange and pink coloured reactions were observed with *Rhodobactersphaeroides* and *E. coli*, and its suggesting variations in the products of L- tryptophan metabolism.

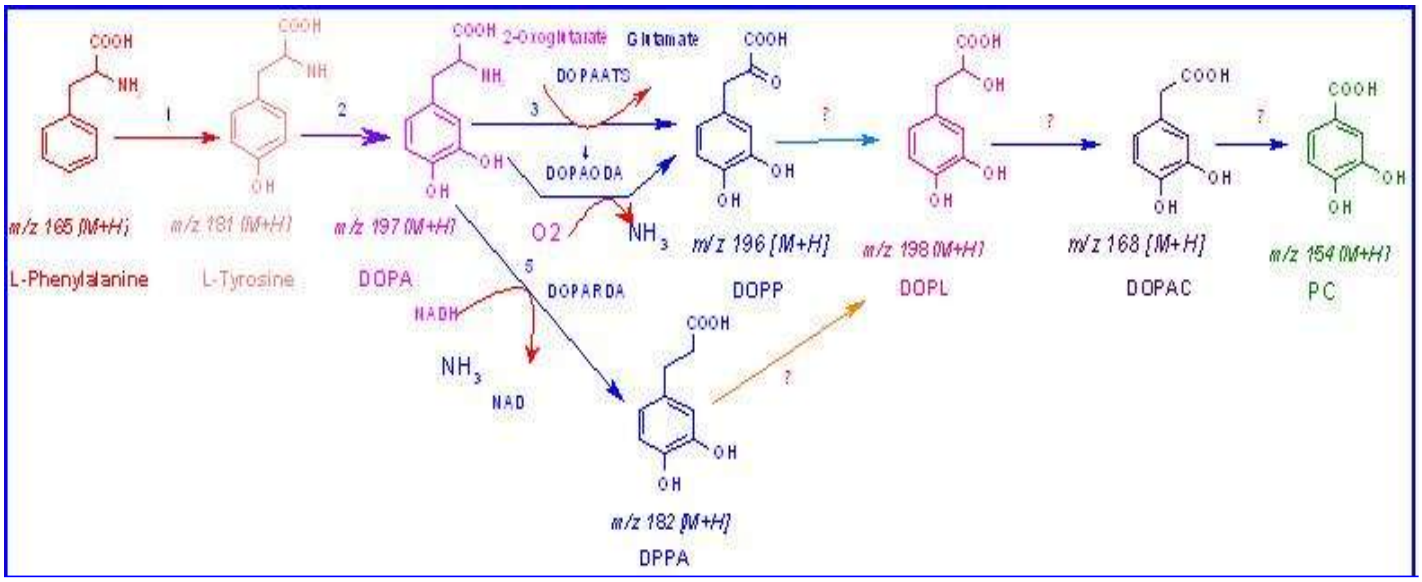

Figure 4.Proposed intracellular catabolism of L-phenylalanine by *Rubrivivaxbenzoatilyticus*. The pathway is based on experimental evidence observed in the present study. [1=L-phenylalaninehydrogenase;2=L-tyrosine dehydrogenase;3= 3,4-dihydroxyphenylalanine 2-oxoglutarate aminotransferase (DOPAATS;EC2.6.1.49);4=3,4-dihydroxyphenylalanine oxidative deaminase 5= 3,4- dihydroxyphenyalanine reductive Deaminase EC 4.3.1.22) ?= not knownone].

CONCLUSIONS

3,4-Dihydroxyphenylalanine as an intermediate of L-phenylalanine metabolism through intermediate of L-tyrosine in *Rhodobactersphaeroides*. Few enzymes reported in the ammonia metabolism of 3,4-dihydrophenylalanine 2-oxoglutarate aminotransferase; 3,4–dihydroxy- phenylalanine reductive deaminase and 3,4-Dihydroxy phenylalanine oxidative deaminase (Sunayana et al., 2005) were purified to homogeneity and characterized. An enzyme was identified in the downstream of L- tryptophan in *Rubrivivaxbenzoatilyticus*. Rhodethrin (Fonnum and Larsen, 1965) is one such novel molecule, which has Cox-2 inhibitory activity, cytotoxicity against cancer cell lines, phytoharmonal activity and antimicrobial activity. Rubrivivaxin is novel phenol terpenoid molecule, which has Cox-1 inhibitory activity, cytotoxicity against cancer cell lines (U937; Human leukemic monocyte lymphoma cell line) and antimicrobial activity.

This article suggested that production of a wide range of indoleterpenoids, and phenols during the bioprocessing of an aromatic amino acid like L-Tryptophan, L-phenylalanine and L-Tyrosine by *Rubrivivaxbenzoatilyticus*. Though most of these are representatives of free phenols, and indoles which most of the researchers had already identified as microbial enzyme products which are microbially produced phenol terpenoids and indoleterpenoids related conjugates are novel biomolecules, which are worth exploiting for microbial based drugs.

CONFLICT OF INTERESTS

The authors confirmed that this review article content have no conflict of interests.

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Table 2. Differentiating characters of the three enzymes of phenylalanine of *Rubrivivaxbenzoatilyticus*.involved in ammonia liberation /transformation of DOPA, an intermediate in the catabolism of L- phenylalanine of *Rubrivivaxbenzoatilyticus*.

Enzymes	Products	Co-substrates	Co-factors	K_m(µM)	K_{cat} (s^{-1})	M.Wt. (kDa)	Subunits(determinedby SDS-PAGE) (kDa)
L-DOPA-oxidative deaminase	DOPP	O_2	Nil	11.84±1.80	0.680±0.023	~190	Pentamer (54,43,34,25,22)
L-DOPA-reductive deaminase	DPPA	Nil	NADH	21.23±0.09	0.0636±3.0	~274	Hetero-tetra- mer (117,85,49,35)
L-DOPA-amino transfe- rase	DOPP	2-KGA	PLP	4.1	ND	ND	Homodimer
				0.35±0.045	0.29±3.0	~123	Heterodimer (60,63)

[Results are means ± SD of three different determinations done in duplicates. *Isolated from Pig brain. α-KGA = 2-oxoglutarate; PLP = Pyridoxal-5-phosphate; NADH = Nicotinamide adenine dinucleotide; Nil = No requirement; kDa = Kilo Dalton; DOPP =3,4-dihydroxyphenyl pyruvic acid; DPPA = 3.4-dihydroxyphenyl pro- pionic acid; DOPA= 3,4-dihydroxyphenylalanine. ND = Not deter- mined].

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