INTRODUCTION
1.0 Introduction

1.1 Aromatic amino acid metabolism in bacteria

Bacteria are the most diverse group of microorganisms and they thrive in diverse habitats. Bacteria uses wide range of organic compounds as carbon source for their growth and can metabolise both aliphatic and aromatic hydrocarbons. Next to carbohydrates, aromatic hydrocarbons are the most widely distributed group of organic molecules (Teufel et al., 2010). Lignin polymer, monolignols, phenolic compounds and aromatic amino acids are a few abundantly found natural aromatic compounds in nature (Carmona et al., 2009). Aromatic amino acids are one such group of natural aromatic compounds (Carmona et al., 2009; Teufel et al., 2010). These are of plant and microbial origin, found in nature mainly due to plant and animal decay, and also by release of plant root exudates. Copious amounts of meta-tyrosine found in the root exudates of fine fescue cultivar Festuca rubra L. ssp. commutata (Tengfang et al., 2011). Concentrations of aromatic amino acids in nature varies greatly and their absolute concentrations in natural conditions is still unknown (Phillips et al., 2004). Derivatives of aromatic amino acids such as indolic (Ryu and Patten, 2008) and phenolic (Badri et al., 2013) compounds also present in root exudates.

Aromatic amino acids and their derivatives which are present in root exudates are utilised by bacteria for their growth (Ryu and Patten, 2008; Tengfang et al., 2011). Aromatic amino acids are excellent sources of carbon and nitrogen and some bacteria utilise aromatic amino acids for its growth as sole source of carbon and nitrogen or nitrogen sources (Tengfang et al., 2011). Bacteria are able to degrade/transform aromatic amino acids (L-phenylalanine, L-tyrosine and L-tryptophan) under oxic/anoxic conditions into an array of metabolites (Carmona et al., 2009; Ismail and Gescher, 2012). Anoxic aromatic metabolism via central benzoyl-CoA pathway was studied in denitrifying, phototrophic and fermentative bacteria (Carmona et al., 2009). Aromatic amino acids (L-tryptophan, L-phenylalanine, L-tyrosine) catabolism was also reported in Lactobacillus casei and Lactobacillus helveticus via transamination and dehydrogenation reactions resulting in the production of aromatic metabolites (Gummalla and Broadbent, 2001). Lactobacillus casei and Lactobacillus helveticus catabolised L-tryptophan to indole-3-pyruvic acid and indole-3-lactic acid (a chemically liable compound), L-phenylalanine to phenylpyruvic acid, phenyllactic acid and L-tyrosine to 4-hydroxyphenylpyruvic acid and 4-hydroxyphenyllactic acid (Gummalla and Broadbent, 2001). Bacteria are able to transform L-phenylalanine/L-tyrosine to phenolic compounds and L-tryptophan to indolic compounds under
oxic and anoxic conditions (Smith and Macfarlane, 1996; Diaz et al., 2001; Mechichi et al., 2002). Like other aromatic amino acid, L-phenylalanine is also used as carbon or nitrogen source by bacteria under oxic and anoxic conditions.

1.2 Anoxic L-phenylalanine catabolism in bacteria

1.2.1 L-phenylalanine catabolism as carbon source

Bacteria utilise L-phenylalanine as a sole source of carbon for its growth and catabolise L-phenylalanine to TCA cycle intermediates under anoxic conditions. L-Phenylalanine catabolism via phenylacetic acid was reported in *Escherichia coli* and *Pseudomonas putida* (Teufel et al., 2010). Complete anaerobic degradation of L-phenylalanine to TCA cycle intermediates via cinnamic acid, phenylpyruvic acid, phenylacetic acid, phenylacetyl-CoA and benzoyl-CoA was reported in *Desulfobacula toluolica* Tol2 (Wohlbrand et al., 2013). L-Phenylalanine was used as sole source of carbon by *Thauera* and *Azoarcus* strains under anoxic conditions and complete degradation of L-phenylalanine to TCA cycle intermediates via benzoyl-CoA was reported in denitrifying bacterium, *Thauera aromatica* (Schneider et al., 1997; Laempe et al., 2001; Fuchs, 2008; Carmona et al., 2009) (Fig. 1). In bacteria such as *Escherichia coli* (Diaz et al., 2001) and *Rhodococcus* sp. strain RHA1 (Navarro-Llorens et al., 2005) L-phenylalanine degraded via phenylacetic acid (PA) catabolic pathway and which further catabolised to acetoacetic acid and fumaric acid (Abe-Yoshizumi et al., 2004).

1.2.2 L-Phenylalanine catabolism as nitrogen source

Amino acids also serves as nitrogen source and bacteria utilizes aromatic amino acids as sole source of nitrogen. Utilisation of aromatic amino acids as sole source of nitrogen leads to the production of aromatic acids and alcohols (Wei et al., 2013). The catabolic pathways of amino acids involving chemical reactions to produce respective alcohols or carboxylic acids containing one carbon less than the starting amino acid is referred as Ehrlich’s pathway (Hazelwood et al., 2008; Ravasio et al., 2014). In general branched-chain amino acids (valine, leucine and isoleucine) (Wei et al., 2013), aromatic amino acids (L-phenylalanine, L-tyrosine and L-tryptophan) and sulphur-containing amino acid (methionine) are catabolised via Ehrlich’s pathway which led to the production of respective fusel acids and alcohols (Somers et al., 2005). The enzyme reactions involved in the Ehrlich’s pathway are transamination, decarboxylation and reduction/oxidation reactions (Hazelwood et al., 2008). Transformation of aromatic amino acid, such as L-phenylalanine via Ehrlich’s pathway was studied in bacteria...
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(Behrends et al., 2009; Afzal et al., 2013; Banerjee et al., 2014). Bacteria catabolise L-phenylalanine via phenylpyruvic acid and phenylacetaldehyde to phenylacetic acid and the enzymes involved in the transformation are aromatic aminotransferase, first enzyme that catalyses the conversion of L-phenylalanine to phenylpyruvic acid, pyruvate decarboxylase which convert phenylpyruvic acid to phenylacetaldehyde and aldehyde dehydrogenase converts phenylacetaldehyde to phenylacetic acid (Wei et al., 2013) (Fig. 1).

Fig. 1. Anoxic catabolism of L-phenylalanine via central intermediate benzoyl-CoA by denitrifying and phototrophic bacteria (Schneider et al., 1997).

Reaction numbers 1, L-phenylalanine:2-oxoglutarate aminotransferase; 2, L-phenylalanine NAD+ oxidoreductase; 3, L-phenylalanine ammonia-lyase; 4, β-oxidation pathway; 5, phenylpyruvate decarboxylase; 6, phenylpyruvate:acceptor oxidoreductase; 7, phenylacetaldehyde dehydrogenase; 8, phenylacetaldehyde dehydrogenase (CoA-acylating); 9, phenylacetate-CoA ligase; 10, phenylacetyl-CoA α-oxidizing enzyme; 11, phenylglyoxylate : acceptor oxidoreductase; TCA cycle, tricarboxylic acid cycle. Bold arrows indicate anoxic L-phenylalanine degradation in Thauera aromatica DSM698. Shaded region indicate the Ehrlich’s pathway.
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A branch point of Ehrlich’s pathway leads to the conversion of phenylpyruvic acid to phenyllactic acid, catalysed by lactate dehydrogenase (EC 1.1.1.27). Phenyllactic acid production was reported in lactic acid bacteria (Li et al., 2007; Mu et al., 2009) and propionibacteria (Thierry and Maillard, 2002). L-Phenylalanine catabolism to phenylacetic acid via Ehrlich’s pathway and phenyllactic acid production from phenylpyruvic acid was reported in lactic acid bacteria under anoxic condition (Svanstrom et al., 2013; Cortes-Zavaleta et al., 2014). L-Phenylalanine conversion to homogentisic acid via L-tyrosine, 4-hydroxyphenylpyruvic acid under anoxic conditions was reported in Rhodobacter capsulatus B10S (Saez et al., 1999). L-Phenylalanine conversion to 3,4-dihydroxybenzoic acid (protocatechuic acid) via L-tyrosine, 3,4-dihydroxy L-phenylalanine (DOPA) under anoxic conditions is reported in Rhodobacter sphaeroides (Ranjith et al., 2007b) (Fig. 2). Some of the intermediates like phenylacetic acid, mandelic acid and phenylglyoxylic acid were also observed in the L-phenylalanine transformation by lactic acid bacteria (Nierop Groot and de Bont, 1999). Apart from using L-phenylalanine under anoxic conditions, bacteria also catabolise L-phenylalanine for their growth as carbon/nitrogen source under oxic conditions (Arias-Barrau et al., 2004).

**Fig. 2. Biotransformation of L-phenylalanine under anoxic conditions in Rhodobacter sphaeroides OU5 (Ranjith et al., 2007b)**

DOPA, 3,4-Dihydroxy L-phenylalanine; DOPP, 3,4-dihydroxyphenylpyruvic acid; DOPL, 3,4-dihydroxyphenyllactic acid; DOPAc, 3,4-dihydroxyphenylacetic acid; PC, protocatechuic acid; DPPA, 3,4-dihydroxyphenylpropionic acid; DOPAATS, 3,4-dihydroxyphenylalanine aminotransferase; DOPARDA, 3,4-dihydroxyphenylalanine reductive deaminase.
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1.3 Oxic L-phenylalanine metabolism in bacteria
1.3.1 Degradation of L-phenylalanine as carbon source

Bacteria utilise L-phenylalanine as sole source of carbon, carbon and nitrogen under oxic conditions. Oxidative degradation of aromatic amino acids by bacteria led to TCA cycle intermediates (Arias-Barrau et al., 2004; Camarero et al., 2008). Under oxic conditions L-phenylalanine is completely catabolised via two different pathways i.e homogentisic acid or phenylacetic acid pathway in bacteria (Arias-Barrau et al., 2004; Khan et al., 2013). In homogentisic acid dependent L-phenylalanine catabolism, L-phenylalanine was hydroxylated to L-tyrosine further converted to 4-hydroxyphenylpyruvic acid followed by hydroxylation to homogentisic acid which on ring cleavage with homogentisate dioxygenase leads to fumarate and acetoacetate (Arias-Barrau et al., 2004). Complete L-phenylalanine degradation into fumarate and acetoacetate via homogentisic acid was reported in different groups of bacteria. L-phenylalanine and L-tyrosine were completely catabolised via homogentisic acid in Streptomyces setonii 75Vi2 (Pometto and Crawford, 1985) and Psuedomonas putida U (Arias-Barrau et al., 2004).

Under oxic conditions, degradation of L-phenylalanine to TCA cycle intermediates through central phenylacetic acid pathway along with production of tropodithietic acid was reported in Pheobacter galleciensis (Berger et al., 2012) (Fig. 3). Some bacteria are capable of operating multiple catabolic pathways for the transformation of L-phenylalanine and L-tyrosine under oxic/anoxic conditions (Jimenez et al., 2002). A facultative methylotrophic bacterium, Nocardia sp. 239 capable of utilising L-phenylalanine and L-tyrosine as source of carbon, nitrogen and energy was reported (De Boer et al., 1988). Catabolism of both L-phenylalanine and L-tyrosine through homogentisic acid was reported in bacteria which further converts to TCA cycle intermediates (De Boer et al., 1988). Oxidative degradation of phenylacetic acid into phenylacetyl-CoA was reported in Azoarcus evansii in which L-phenylalanine was metabolised into phenylacetic acid (Mohamed et al., 2002).
Fig. 3. Oxic degradation of L-phenylalanine via phenylacetyl-CoA in Pheobacter gallaeciensis (Berger et al., 2012).

Phe, L-phenylalanine; PP, phenylpyruvic acid; PhAc-CoA, phenylacetyl-CoA; PhAc, phenylacetic acid; TDA, tropodithietic acid; TCA cycle, tricarboxylic acid cycle; TyrB, aromatic amino acid transaminase; PaaK, phenylacetate-CoA ligase; PaaG, 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase; PaaJ, 3-oxoadipyl-CoA/3-oxo-5,6-dehydrosuberyl-CoA thiolase; PaaF, 2,3-dehydroadipyl-CoA hydratase; PaaH, 3-hydroxyadipyl-CoA dehydrogenase; PaaZ, oxepin-CoA hydrolase/3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase.
1.3.2 L-phenylalanine as sole source of nitrogen

Under oxic conditions, many hydroxylases and oxygenases such as monooxygenases and dioxygenases catalyse the bioconversion reactions (Wang et al., 2011; Leiros et al., 2013) and play significant role in aromatic catabolism. Biotransformation of L-phenylalanine into different aromatic metabolites in the presence of oxygen were reported in bacteria (Berger et al., 2012). Bioconversion of L-phenylalanine into stilbene (4-isopropyl-3,5-dihydroxystilbene) under oxic conditions was reported in *Photorhabdus luminescens* (Joyce et al., 2008). L-phenylalanine transformed into trans-cinnamic acid and then to cinnamoyl-CoA which further conjugate with leucine derivative by an enzyme cyclase to form stilbene (Joyce et al., 2008). In the presence of oxygen some of the monooxygenases and dioxygenases transform L-phenylalanine into aromatic metabolites like L-tyrosine and 4-hydroxyphenylpyruvic acid, homogentisic acid in bacteria (Carreira et al., 2001; Arias-Barrau et al., 2004).

Oxic metabolism of L-phenylalanine via L-tyrosine, homogentisic acid leads to production of pigments such as melanin in bacteria. For example, *Psuedomonas sp* (Arias-Barrau et al., 2004; Hunter and Newman, 2010) (Fig. 4) and *Schwenella colweliana* (Turick et al., 2008) transformed L-tyrosine into pyomelanin via 4-hydroxyphenylpyruvic acid and homogentisic acid in presence of oxygen. Oxic L-phenylalanine and L-tyrosine biotransformation by a thermophilic *Geobacillus stearothermophilus* bacterium produced aromatic compounds like phenylpyruvic acid, phenylacetic acid, phenylacetaldehyde, phenylethylamine, trans-cinnamic acid, cinnamaldehyde and protocatechuic acid, benzaldehyde, homovanillic acid, homogentisic acid (Afzal et al., 2013). Under oxic conditions also L-phenylalanine catabolised via Ehrlich’s pathway in bacteria (Hazelwood et al., 2008) when L-phenylalanine is used as sole source of nitrogen.
Fig. 4. Oxic L-phenylalanine degradation via homogentisic acid dependent pathway reported in *Psuedomonas putida* (Arias-Barrau et al., 2004).

PhhA, L-phenylalanine hydroxylase; TyrB, aromatic amino acid transaminase; Hpd, hydroxyphenylpyruvic acid dioxygenase; HmgA, homogentisic acid 1,2-dioxygenase; HmgC, maleylacetoacetic acid isomerase; HmgB, fumarylacetoacetase.

### 1.3.3 Production of pigments and antibiotics from L-phenylalanine by bacteria

Bacteria produce an array of aromatic compounds as a result of L-phenylalanine metabolism under oxic conditions, some of them are pigments such as melanins (Leiros et al., 2013; Banerjee et al., 2014; Coelho-Souza et al., 2014), anthroquinones (Li et al., 2013; Malak et al., 2013), stilbenes (Li et al., 1995; Joyce et al., 2008) and antibiotics such as tropodithietic acid (Berger et al., 2012), enterocin (Xiang and Moore, 2005), actinorhodin (Taguchi et al., 2012). A red pigment identified as anthraquinone derivative; 1,6-dihydroxy-4-methoxy-9,10-anthraquinone and an antibiotic identified as hydroxystilbene derivative; 3,5-dihydroxy-4-isopropylstilbene were reported in entomopathogenic bacterium, *Xenorhabdus luminescens* from L-phenylalanine (Richardson et al., 1988). *Streptomyces maritimus*, a marine bacterium, produces an antibiotic called enterocin (a polyketide) under anoxic condition where L-phenylalanine is converted to trans-cinnamic acid and β-oxidation of trans-cinnamic acid to benzoyl-CoA which on further converts to enterocin (Xiang and Moore, 2002). Production of antibiotic tropodithietic acid from L-phenylalanine through central phenylacetic acid pathway was reported in *Pheobacter gallaeciensis* (Berger et al., 2012) (Fig. 6).
Phenylpropanoid pathway is widely distributed among plant kingdom. Pigments such as anthocyanins and flavonoids were biosynthesised from L-phenylalanine through phenylpropanoid pathway (Fig. 7) (Ghasemzadeh et al., 2012). Trans-cinnamic acid, coumaric acid, chalcone are the key intermediates of phenylpropanoid pathway and the key enzymes of phenylpropanoid pathway are phenylalanine ammonia-lyase (PAL) (EC 4.3.1.24) and chalcone synthase (CHS) (EC 2.3.1.74). Phenylalanine ammonia-lyase convert L-phenylalanine to trans-cinnamic acid and chalcone synthase involves in the ring cyclisation and formation of a compound called chalcone, a precursor for the different flavonoids and anthocyanins (Maskeya et al., 2003). Phenylalanine ammonia-lyase (PAL) (Xiang and Moore, 2005) and chalcone synthase (CHS) are the rate limiting steps in the phenylpropanoid pathway (Fowler and Koffas, 2009). A novel bacterial L-phenylalanine ammonia-lyase (EncP) was identified in Streptomyces maritimus (Xiang and Moore, 2002) (Fig. 5). The PAL gene which is homologous to PAL gene sequence of plants was observed in the genome of Rhodobacter capsulatus B10S. The genome project of Rhodobacter capsulatus B10S (www.integratedgenomics.com) has an open reading frame (ORF) of PAL gene (1626 bp, contig 58-60, ORF 1844) located upstream of Photoactive Yellow Protein (PYP) gene (Kyndt et al., 2004).

Anthocyanins and flavonoids biosynthesis is widely distributed among plants. However, some bacteria and fungi transform flavonoids/anthocyanins by glycosidases. Some of the isoflavonoids were isolated from bacteria and fungi whereas their biosynthetic origin was not proven (Maskeya et al., 2003). A few actinomycetes were reported to transform isoflavonoids which most probably not reflect the de novo biosynthesis rather the presence of glycosidases can transform the flavonoid derivatives (Maskeya et al., 2003; Yuan et al., 2007). However, many bacteria have PAL and CHS genes of phenylpropanoid pathway in their genome whereas the functionality of those genes is not yet proved. Some photosynthetic bacteria such as Rhodobacter sphaeroides ATCC 17029 (gi|126102442), Pheospirillum molischianum DSM120, Pheospirillum fulvum MGU-K5, Rhodomicrobium vannielli also consists PAL and CHS genes in their genomes whereas functionality of these genes is unknown.
Fig. 5. Biotransformation of L-phenylalanine via trans-cinnamic acid and biosynthesis of benzoyl-CoA derived antibiotic, enterosin in *Streptomyces maritimus* (Xiang and Moore, 2002)

Double arrow reactions in the pathway indicate the multiple steps. EncP, phenylalanine ammonia-lyase gene associated to enterosin biosynthetic gene cluster.
Fig. 6. Biosynthetic pathway of an antibiotic compound, Tropodithetic acid

Multiple arrows indicate the presence of multiple reactions in the pathway

TDA, tropodithetic acid, TCA cycle, tricarboxylic acid cycle.
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Fig. 7. Phenylpropanoid pathway observed in plants (Ghasemzadeh et al., 2012)

PAL/TAL, L-Phenylalanine/L-Tyrosine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-hydroxy-cinnamate lyase; CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; IFS, Isoflavone synthase; IOMT, Isoflavone O-methyltransferase; I2'H, iso flavone 2'-hydroxylase; IFR, isoflavone reductase; VR, vestitone reductase; DFR, dihydroflavonol 4-reductase; DMID, 7,2'-di hydroxy,4'-methoxyiso flavonol dehydratase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3' hydroxylase; F3'5'H, flavonoid 3'5' hydroxylase; FS1/FS2, flavone synthase; LDOX, leucoanthocyanidin dioxygenase OMT, O-methyltransferase; PAL, phenylalanine ammonia-lyase; RT, rhamnosyl transferase; UFGT, UDP flavonoid glucosyl transferase; STS, stilbene synthase; FLS, flavanol synthase.
1.4 Melanin biosynthesis in bacteria

Melanins are enigmatic pigments, important natural biopolymers with multiple functions, yet their structures are not well understood (Manivasagan et al., 2013). L-Tyrosine is the precursor for the melanin biosynthesis. Melanin is also produced from L-phenylalanine via L-tyrosine by some bacteria like *Rhizobium* species (Mercado-Blanco et al., 1993). Melanins have radioprotective and antioxidant properties (Dadachova et al., 2008; Khajo et al., 2011). Oxic L-phenylalanine/L-tyrosine catabolism lead to the production of melanin pigment by a thermophilic *Geobacillus stearothermophilus* (Afzal et al., 2013).

1.4.1 Occurrence

Melanin is a dark brown to black colored ochronotic pigment produced by all groups of biological kingdom. Melanins are widely distributed among plants, animals and microorganisms (Gessler et al., 2014). Melanin biosynthesis is L-tyrosine dependent in almost all the systems. Melanin exist in different forms like amorphous, crystalline. Melanin is predominantly found in the hair and skin of humans. Eumelanin and pheomelanin biosynthesis is abundant in vertebrates. Allomelanin is found in plants, fungi and bacteria (Singh et al., 2013).

1.4.2 Types of melanin and biosynthetic pathway of melanin

Different types of melanins were described in plants, animals, fungi and bacteria: eumelanin, pheomelanin, allomelanin, pyomelanin, neuromelanin. Eumelanins and pheomelanins were of animal origin. Eumelanin and pheomelanins are present in the human abundantly in hair and skin (Piletic et al., 2010). Eumelanins are black or brown colored pigments derived from L-tyrosine. Polymers of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) are called eumelanins (Glass et al., 2012). Pheomelanins are synthesised from the same precursor, L-tyrosine while cysteine is involved during the pheomelanin synthesis (Napolitano et al., 2008). Pheomelanins are polymers of benzothiazine units and has both nitrogen and sulfur in their structures (Napolitano et al., 2008). Allomelanins are most heterogenous group of polymers, synthesised from nitrogen free precursors (Tarangini and Mishra, 2013) and they are formed by oxidation or polymerisation of dihydroxynaphthalene [Di-(DHN)] or tetrahydroxynaphthalene [tetra-(DHN)]. Pyomelanins are dark brown colored pigment synthesised extracellularly by oxidative polymerisation of
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homogentisic acid, an intermediate of L-tyrosine catabolism (Turick et al., 2008; Almeida-Paes et al., 2012; Zheng et al., 2013; Coelho-Souza et al., 2014).

Different types of melanins were biosynthesised through a common precursor L-tyrosine (Fig. 8). L-tyrosine catabolism leads to the production of eumelanin, pheomelanin, pyomelanin, neuromelanin. L-tyrosine catabolism leads to the production of pyomelanin through 4-hydroxyphenylpyruvic acid and homogentisic acid. Homogentisic acid on autooxidation and copolymerisation produces pyomelanin (Leiros et al., 2013; Coelho-Souza et al., 2014). The key enzymes involved in pyomelanin biosynthesis are tyrosine aminotransferase which converts L-tyrosine to 4-hydroxyphenylpyruvic acid and 4-hydroxyphenylpyruvate dioxygenase converts 4-hydroxyphenylpyruvic acid to homogentisic acid (Chai et al., 2012). Biosynthesis of eumelanin is 3,4-dihydroxyphenylalanine (DOPA) dependent. L-tyrosine transforms into DOPA and dopaquinone and the reactions were catalysed by tyrosinase (Apte et al., 2013). Dopaquinone further cyclise into 5,6-dihydroxyindole (DHI) and 5,6 dihydroxyindole-2-carboxylic acid (DHICA). Eumelans are polymers of DHI and DHICA (Glass et al., 2012). Dopaquinone with cysteine together form benzothiazine derivatives which lead to pheomelans (Thureau et al., 2012). Dopaquinone is common for both eumelanin and pheomelanin biosynthesis. Neuromelanin was synthesised from L-tyrosine via tyramine, Dopamine and Dopaminochrome. Transformation of L-phenylalanine to L-tyrosine also leads to production of different types of melanin in bacteria (Singh et al., 2013) (Fig. 8).

![](image)

**Fig. 8.** An overview of melanin biosynthetic pathways present in human, plants, fungi and bacteria (Singh et al., 2013). DOPA, 3,4-dihydroxyphenylalanine.
1.5 Aromatic amino acid catabolism in purple bacteria

Anoxygenic phototrophic bacteria utilise aromatic amino acids as sole source of carbon/nitrogen. *Rhodopsuedomonas palustris* catabolise/degrade aromatic amino acids and heterocyclic aromatic compounds (Larimer et al., 2004; Oda et al., 2008). *Rhodobacter sphaeroides* utilises L-phenylalanine as sole source of nitrogen and produces phenolic compounds such as caffeic acid, protocatachuic acid, gallic acid and alkyl esters of gallic acid (Kumavath et al., 2010a). *Rhodobacter sphaeroides* OU5 utilised L-phenylalanine as sole source of nitrogen under anoxic conditions and produced aryl metabolites such as L-tyrosine, 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylpyruvic acid (DOPP), 3,4-dihydroxyphenyllactic acid (DOPLA), 3,4-dihydroxyphenylacetic acid (DOPAc), 3,4-dihydroxybenzoic acid (protocatachuic acid; PC). The catabolic pathway of L-phenylalanine was demonstrated with enzyme activities such as DOPA transaminase (DOPAATS) and DOPA reductive deaminase (DOPARDA). DOPA transaminase convert DO\textsubscript{P}A into DO\textsubscript{P}A phenylpyruvic acid (DOPAPP) and DOPA reductive deaminase convert DOPA to 3,4-dihydroxyphenylpropionic acid. A novel DOPA reductive deaminase enzyme was further purified and characterised (Ranjith et al., 2007b). Anoxic catabolism of L-phenylalanine to homogentisic acid *via* L-tyrosine and 4-hydroxyphenylpyruvic acid is reported in *Rhodobacter capsulatus* B10S. L-phenylalanine hydroxylated to L-tyrosine and transminated to 4-hydroxyphenylpyruvic acid and finally oxidised to homogentisic acid by *Rhodobacter capsulatus* B10S (Saez et al., 1999). A novel indole terpenoid ether called rhodethrin was biosynthesised by *Rhodobacter sphaeroides* OU5 when grown in the presence of L-tryptophan as sole source of nitrogen (Ranjith et al., 2007a).

Aromatic amino acids (L-tryptophan, L-phenylalanine and L-tyrosine) metabolism as sole source of nitrogen was also reported in *Rubrivivax benzoatilyticus* JA2 under oxic and anoxic conditions. L-Tryptophan catabolism *via* indole-3-pyruvic acid, indole-3-acetaldehyde, indole-3-acetic acid is reported in *R. benzoatilyticus* JA2 when grown in the presence of L-tryptophan as sole source of nitrogen (Kumavath et al., 2010b). Further multiple catabolic pathways of L-tryptophan were reported in this organism (Mujahid et al., 2011). *Rubrivivax benzoatilyticus* JA2 utilised L-phenylalanine as sole source of nitrogen but not as carbon source. *Rubrivivax benzoatilyticus* JA2 able to produce L-tryptophan in the presence of aniline (Mujahid et al., 2014). *Rubrivivax benzoatilyticus* JA2 transformed L-phenylalanine into rubrivivaxin under anoxic conditions (Kumavath et al., 2011). Genome sequence of *Rubrivivax*
benzoatilyticus JA2 revealed the diverse metabolic pathways of aromatic metabolism and also possible multiple catabolic pathways of L-phenylalanine (Mohammed et al., 2011).

1.6 Metabolic responses in bacteria under varied growth conditions

The changes/responses of bacteria to different physiological conditions at the metabolic level were not extensively studied. Metabolic responses varies with the surrounding environmental conditions (Frimmersdorf et al., 2010) such as pH, temperature (Azizan et al., 2012; Ye et al., 2012), salinity (Kol et al., 2010), and some physiological changes like oxic/anoxic growth modes (Frick and Wittmann, 2005; Chen et al., 2011; Schellenberger et al., 2012). Global view of metabolome revealed the metabolic variations in Psuedomonas aeruginosa when grown under different carbon sources and growth modes (Frimmersdorf et al., 2010). Metabolomics is a wholistic approach to know the metabolic responses of the system towards surrounding conditions. Metabolic changes were monitored over the course of time in batch cultures of well studied organisms, Escherichia coli and Pseudomonas aeruginosa using time-resolved metabolic footprinting (TReF) (Behrends et al., 2009). Metabolomics approach offered a promising avenue to fingerprint the intestinal microbiota and its interaction with the host (Marcobal et al., 2013). Metabolomics approach provided snapshot of bacterial metabolic adaptations to changing conditions (Chen et al., 2011).

1.7 Definition of the problem

Bacteria are well known for their metabolic diversity among groups of microorganisms and they thrive under diverse habitats. Bacteria are capable of degrading and transforming different aromatic compounds which are second abundant class of hydrocarbons (Carmona et al., 2009). Aromatic amino acids are abundantly found group of natural aromatic compounds. Anoxyogenic phototrophic bacteria are capable of transforming aromatic amino acids like L-phenylalanine, L-tyrosine and L-tryptophan under different physiological conditions like oxic and anoxic conditions. *Rubrivivax benzoatilyticus* JA2, a betaproteobacterium transforms aromatic amino acids under oxic and anoxic conditions. *Rubrivivax benzoatilyticus* JA2 transforms L-phenylalanine to phenolic compounds (Kumavath et al., 2011) and L-tryptophan to indolic (Kumavath et al., 2010b; Mujahid et al., 2011) compounds under anoxic conditions. *R. benzoatilyticus* JA2 able to utilise aromatic amino acids as sole source of nitrogen but not as carbon source. Genomic insights also revealed *R. benzoatilyticus* JA2 lacks the gene coding for ring cleavage enzymes but genome sequence analysis of *R. benzoatilyticus* JA2 indicated the existence of multiple catabolic pathways of aromatic metabolism under oxic and anoxic...
conditions. Reports on aromatic amino acids catabolism in *Rubrivivax benzoatilyticus* JA2 revealed the capability of producing aryl metabolites by utilising aromatic amino acids as sole source of nitrogen. L-tryptophan catabolised via indole-3-pyruvic acid, indole-3-acetaldehyde and indole-3-acetic acid in *Rubrivivax benzoatilyticus* JA2 when given as sole source of nitrogen (Kumavath et al., 2010b). A novel compound, rubrivivaxin was isolated from *Rubrivivax benzoatilyticus* JA2 when L-phenylalanine was used as sole source of nitrogen (Kumavath et al., 2011).

Previous reports of L-phenylalanine catabolism in *R. benzoatilyticus* JA2 was targeted to few aryl metabolites produced under anoxic conditions whereas insights of oxic L-phenylalanine catabolism was not studied yet. Nevertheless the comprehensive insights of the L-phenylalanine metabolism under oxic and anoxic conditions was not studied in this organism and moreover, how changing oxic/anoxic conditions will influence L-phenylalanine metabolism is of particular interest. Wholistic study of L-phenylalanine metabolism under oxic and anoxic conditions may provide catabolic potential of *R. benzoatilyticus* JA2. Hence, *Rubrivivax benzoatilyticus* JA2 was used as a model organism, and a detailed study was taken up to elucidate the insights of L-phenylalanine metabolism in *Rubrivivax benzoatilyticus* JA2 and its catabolic potential under varying conditions. Metabolomics approach was employed to decipher the multiple L-phenylalanine catabolic pathways and metabolic responses of bacteria to oxic/anoxic conditions. L-phenylalanine catabolism under oxic and anoxic conditions in *R. benzoatilyticus* JA2 was studied by using LC-MS based stable isotopic studies. An attempt was made to know the metabolic changes under oxic and anoxic conditions when L-phenylalanine was used as sole source of nitrogen. Untargeted GC-MS based metabolomics approach was employed to know the overall metabolism under oxic and anoxic conditions.

1.8 Objectives

- Deciphering the insights of L-phenylalanine metabolism of *Rubrivivax benzoatilyticus* JA2
- Elucidating the metabolic responses of *Rubrivivax benzoatilyticus* JA2 to L-phenylalanine under oxic/anoxic conditions