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A Review on the Genetics of Aliphatic and Aromatic Hydrocarbon Degradation

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Abstract Because of the high diversity of hydrocarbons, degradation of each class of these compounds is activated by a specific enzyme. However, most of other downstream enzymes necessary for complete degradation of hydrocarbons maybe common between different hydrocarbons. The genes encoding proteins for degradation of hydrocarbons, including the proteins required for the uptake of these molecules, the specific enzyme used for the initial activation of the molecules and other necessary degrading enzymes are usually arranged as an operon. Although the corresponding genes in many phylogenetic groups of microbial species show different levels of diversity in terms of the gene sequence, the organisation of the genes in the genome or on plasmids and the activation mode (inductive or constitutive), some organisms show identical hydrocarbon-degrading genes, probably as a result of horizontal gene transfer between microorganisms.

Keywords Genetics · Hydrocarbons · Alkanes · Aromatics

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Introduction

Due to the high diversity of the molecular structures of hydrocarbons, each class of compound has to be degraded by a specific enzyme [1]. Through the catabolic process, hydrocarbon molecules are first taken up by specific microbes, and then converted to simple organic molecules [70, 78]. Based on the microbial species and the community in which the degrader species lives, these simple organic molecules may then be used by the same organism or may be released into the environment and further catabolised by other microbial members of the community [27]. In terms of molecular investigations, such synergistic relationships suggest the probable presence of corresponding degradation genes in more than one member of the microbial community [7]. This review describes the genes involved in the degradation of different types of aliphatic and aromatic hydrocarbons in both aerobic and anaerobic conditions.

All hydrocarbon oxygenases, including both monooxygenases and aromatic-ring-hydroxylating dioxygenases, are classified into a large family of iron-sulphur-flavoproteins containing enzymes capable of transferring one or two electrons to their substrates [113, 115]. Despite the existence of some monooxygenases that are structurally homomultimer (α_6) and physiologically related to each other [38], the aromatic-ring-hydroxylating dioxygenases usually have a heterohexamer ($\alpha_3\beta_3$) structure. Based on the sequence diversity of their α subunits, these aromatic hydroxylating dioxygenases are sub-classified into four groups: toluene/benzene oxygenase, toluate/benzoate oxygenase, naphthalene oxygenase and biphenyl oxygenase [47, 93]. Furthermore, based on their native substrates, the aromatic ring hydroxylating dioxygenases are classified into four groups: the toluene/biphenyl family (specialised for initial oxidative attack to benzene, toluene, chlorobenzenes, isopropylbenzene and biphenyl), the naphthalene family (for activation of naphthalene, phenanthrene, nitrotoluene and nitrobenzene), the benzoate family (for catabolism of toluate, benzoate, anthranilate, isopropylbenzoate, trichlorophenoxyacetate, 2-chlorobenzoate) and finally the phthalate family (for the initial catabolism of aromatic acids such as phenoxybenzoate, *p*-toluene sulfonate, phthalate, vanillate, 3-chlorobenzoate) [41, 42]. In addition to these classified enzymes, several oxygenase enzymes have been identified which are specific for different substrates, such as salicylate, *o*-halobenzoate, 3-phenylpropionate, dibenzodioxin, aniline and dehydroabeitate [28, 76].

The full range of genes required for hydrocarbon degradation and their genetic organisation is not fully understood in many organisms. Although most of the genetic elements involved in the catabolism of aliphatic hydrocarbons are ordered in inducible operon structures with several coregulated genes present in the same transcription unit, in some cases, these operons are expressed constitutively [15, 120]. Both inducible and constitutive genes can also be located on chromosomes either in the form of an operon unit or as separate genes [19, 88]. Based on the catabolic genes present and their homology in endonuclease restriction patterns, DNA rearrangements and electrophoretic mobility, plasmids containing hydrocarbon-degrading genes are divided into three groups: the plasmids containing alkane degradation genes (like the OCT plasmid) [88], the plasmids containing naphthalene and salicylate degradation genes (such as the NAH plasmids) [75] and finally, the plasmids containing toluene- and xylene-oxidising genes (like the TOL plasmids) [75].

The Alkane Degradation Genes

As an outline, biodegradation of hydrocarbons starts with oxidation of the substrate molecules by an electron-carrier-dependent monooxygenase system, producing the corresponding

alcohol [60]. After conversion of the hydrocarbons to their corresponding alcohol, the alcohols are further oxidised and broken down to smaller molecules that finally are utilised via the central catabolic reactions of the cells (reaction 1) [89].

$$\text{Reaction 1: R-CH2OH} \xrightarrow[\text{Dehydrogenase}]{\text{Alcohol}} \text{R-CH2O} \xrightarrow[\text{Dehydrogenase}]{\text{Aldehyde}} \text{R-COOH}$$

Based on the chain length of the aliphatic hydrocarbons utilised, *n*-alkane utilising organisms are classified into three groups: methanotrophs, gaseous alkane-utilising (C2 to C4) microorganisms and finally liquid alkane-catabolising (C5 to C20) microorganisms [89, 112]. Furthermore, based on the molecular structure and the supporting electron transport system, monooxygenases are classified into rubredoxin-dependent enzymes and (bacterial and fungal) cytochrome P450-containing monooxygenases [29, 90]. The rubredoxin-dependent enzymes are composed of a rubredoxin reductase, a rubredoxin and an alkane hydroxylase [45]. In most bacteria, the rubredoxin-dependent monooxygenases are encoded by the gene *alkB* while some bacteria, such as *Acinetobacter* sp., express the enzyme encoded by the *alkM* gene [114].

Pseudomonas putida GP01, for instance, uses a monooxygenase to convert *n*-alkanes (C6–C10) into their fatty acids (Fig. 1) [88]. The *alk* gene organisation in this strain is located on OCT plasmid and encodes the enzymes required for degradation of C5–C13 *n*-alkanes [88]. In *P. putida*, it is postulated that the *alkL gene* is involved in import of *n*-alkanes into the bacterial cells [37]. Furthermore, it has been shown that changes in the configuration of an outer-membrane protein encoded by *blc* in *Alcanivorax borkumensis* Sk2 can lead to the transport of short-length chain hydrocarbons into the cells [55]. Furthermore, it is believed that the long-length chain fatty acid transporter proteins (*FadL*) in many bacteria participates in the transportation of long-length chain hydrocarbons into the cell [6]. The alkanes are initially oxidised by a trimer alkane hydroxylase (a complex of alkane monooxygenase, rubredoxin and rubredoxin reductase encoded by *alkB*, *alkG* and *alkK*, respectively), which are integrated into the inner cell membrane of the bacterium via the product of *alkB* [88]. The resulting alcohol is further oxidised by the products of *alkJ* and *alkH* (respectively for an alcohol

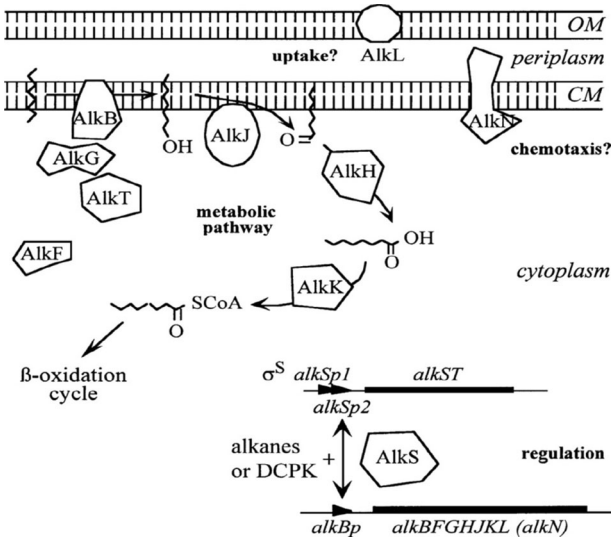


Fig. 1 The position and role of alkane-degrading proteins in *Pseudomonas putida*. *alkB* alkane hydroxylase, *alkF* rubredoxin 1, *alkG* rubredoxin 2, *alkH* aldehyde dehydrogenase, *alkJ* alcohol dehydrogenase, *alkL* outer-membrane protein, *alkN* chemotactic transducer of *Pseudomonas aeruginosa*, *alkT* rubredoxin reductase, *alkS* positive regulator [116]

dehydrogenase and aldehyde dehydrogenase) into an aldehyde and acid, respectively, which is activated by addition of a CoA to the acid (through the action of the product of *alkK*). These genes are organised as *alkBFGHJKL* on the OCT plasmid and are controlled by the action of the products of another operon (*alkST*) located 40 kb away from the first operon [40]. The same operon structure (*alkBFGHJKL*) exists in *P. putida* P1, but *alkST* has been moved to a position upstream of the operon and also the *alkL* and *alkN* genes are not separated by an insertion sequence (IS) [116]. In *Acinetobacter* sp. strain ADP1, the three subunits of alkane hydroxylase (specialised for C6–C12 alkanes), alkane monooxygenase, rubredoxin and rubredoxin reductase, are encoded by *alkM*, *rubA* and *rubB*, respectively [109]. With the exception of *Rhodococcus eryithropolis*, none of the rubredoxin reductase genes are situated near a hydroxylase gene probably because of the involvement of the rubredoxin reductases in other metabolic pathways as well [102]. This well-organised aliphatic hydrocarbon-degrading gene cluster is not always observed in all aliphatic hydrocarbon-degrading bacteria. *Acinetobacter* HOI-N, for instance, is a hydrocarbon-degrading bacterium that contains a set of aliphatic hydrocarbon-degrading enzymes located at three separate loci on the chromosome: the gene encoding the alkane hydroxylase is located at a considerable distance from the genes specified for the alcohol dehydrogenase as well as aldehyde dehydrogenase [5, 85].

The alkane hydroxylating enzymes involved in initial activation of long-chain length aliphatic hydrocarbons (>C18) are evolutionary distinct from the previously mentioned enzymes involved in hydroxylation of short-length chain hydrocarbons. For instance, *AlmA*, encoded by *alma*, is an alkane monooxygenase belonging to the flavin-binding family in *Acinetobacter* sp. DSM17874, *Alcanivorax* and many other bacteria which involves in initial activation of the hydrocarbons with bigger than C32 [119]. Furthermore, *LadA* is a thermophilic alkane hydroxylase, belonging to flavin-dependent oxygenase, obtained from *Geobacillus thermodenitrificans* NG80-2 with the ability to hydroxylate C15–C36 alkanes [59].

The genes involved in degradation of alkane hydrocarbons are downregulated by two regulatory systems, cytochrome ubiquinol oxidase (*Cyo*) and the global regulatory protein Crc [18] to ensure expression of these genes just in certain physiological conditions. The *Cyo* gene product is known as a global regulatory factor able to regulate carbon metabolism and respiration. This factor suppresses the expression of alkane-degrading genes in the presence of easily metabolised carbon sources [18]. The *Crc* gene product is a RNA-binding protein with the ability to stop the mRNA translation via binding to the 5′end of the mRNAs responsible for production of both the regulatory factor *alkS* and alkane-degrading proteins [40].

Cycloaliphatic Compounds

The cycloaliphatic hydrocarbons, like cyclopentane, methylcyclopentane and cyclohexane are degraded by a large range of bacteria [16]. *Acinetobacter* strain SE19, for instance, uses six chromosomal catabolic genes, arranged as *chnBER* ORF and *chnADC* ORF for the degradation of cycloaliphatic hydrocarbons [16]. Through this reaction, cyclohexanone monooxygenase (encoded by *chnB*) and NAD(P)H-dependent aldehyde dehydrogenases (encoded by *chnE*) convert cyclohexane into cyclohexanone, which is further oxidised by the products of the *chnADC* ORFs. The *chnADC*, which is located in opposite direction of the *chnBER* ORF, encodes cyclohexanol dehydrogenase, 6-hydroxyhexanoic acid dehydrogenase

and caprolactone hydrolase, respectively, by *chnB*, *chnE* and *chnR*, to produce an end product of oxohexanoic acid. In the same way (Fig. 2), *Rhodococcus ruber* SC1 uses a cyclododecane monooxygenase and a NAD(P)H-dependent aldehyde dehydrogenases to oxidise cyclododecane first to cyclododecanol and then to cyclododecanone [51]. A cyclododecanone monooxygenase, encoded by *cddA* oxidises it into a lactone oxacyclotridecan-2-one (lauryl lactone), which is first hydroxylated by lauryl lactone esterase (encoded by *cddB*) to 12-hydroxydodecanoic acid and then is oxidised twice by two dehydrogenases (12-hydroxylauric acid dehydrogenase and 12-oxolauric acid dehydrogenase, encoded respectively by *cddC* and *cddD*, to make a 12-oxolauric acid and finally a DDDA (dodecanedioic acid) [51]. Although different bacteria utilise the same genes for the degradation of cyclododecane, their gene organisation may be different. In *R. ruber* SC1, the gene cluster is arranged as *cddABCDXY* with two-space ORFs between *cddABCD* and *cddXY*, while the gene order in *chn* cluster of *Acinetobacter* sp. strain SE19 is random [51].

The Plasmids Containing Naphthalene and Salicylate Degradation Genes

Several aromatic-degrading bacteria are able to convert mono/multiple cyclic aromatic hydrocarbons into salicylate, which undergoes a meta-cleavage to present the products to tricarboxylic acid cycle (TCA) [43]. As a prototype dioxygenase enzyme, a (Rieske-type two-iron two-sulphur centre containing) naphthalene dioxygenase (NOD; encoded by *nahAaAbAcAd*) inserts two oxygen atoms into the aromatic ring of a broad range of aromatic hydrocarbons, such as naphthalene, phenanthrene and anthracene, converting them to corresponding dihydrodiol*s*, such as *cis*-naphthalene dihydrodiol and *cis*-phenanthrene dihydrodiol, respectively [9, 44]. Next, a *cis*-dihydrodiol dehydrogenase (encoded by *nahB*) dehydrogenates the dihydrodiols to make 1,2-dihydroxynaphthalene, which is subjected to meta-cleavage by 1,2-dihydroxynaphthalene dioxygenase (*nahC*) to form 2-hydroxychromene-2-carboxylic acid (Fig. 3). After an enzymatic *cis* to *trans* isomerisation (by an isomerase encoded by *nahD*), the side-chain at the *trans*-unsaturated bond of the *trans-o*-hydroxybenzylidenepyruvate product is cleaved by a hydratase-aldolase (encoded by *nahE*) to produce a salicylaldehyde. The product is finally dehydrogenated by NAD-dependent salicylaldehyde dehydrogenase to salicylate (encoded by *nahF*). Depending on the bacterial strain, the covalent bond of the aromatic ring of salicylate is cleaved between two adjacent carbon atoms with hydroxyl groups (meta-cleavage) or between a carbon with a hydroxyl group and its adjacent carbon with a carboxyl group (ortho-cleavage) [97]. In most cases, like *P. putida* PpG7 (containing NAH7 plasmid) and *P. putida* R1 (containing SAL1 plasmid), bacteria use a meta-cleavage reaction on salicylate (Fig. 4) [80] in which bacteria salicylate hydroxylase (*nahG*) convert salicylate

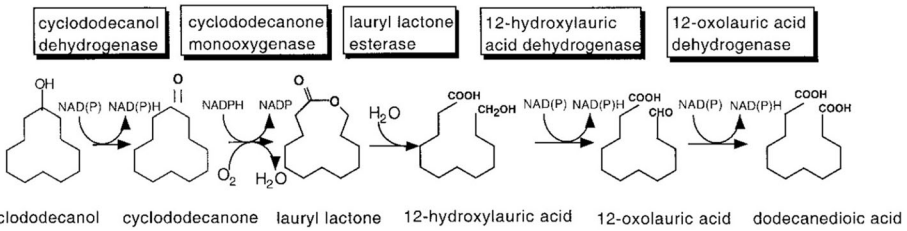


Fig. 2 The metabolic pathway for degradation of cyclododecanol by *Rhodococcus ruber* SC1 [51]

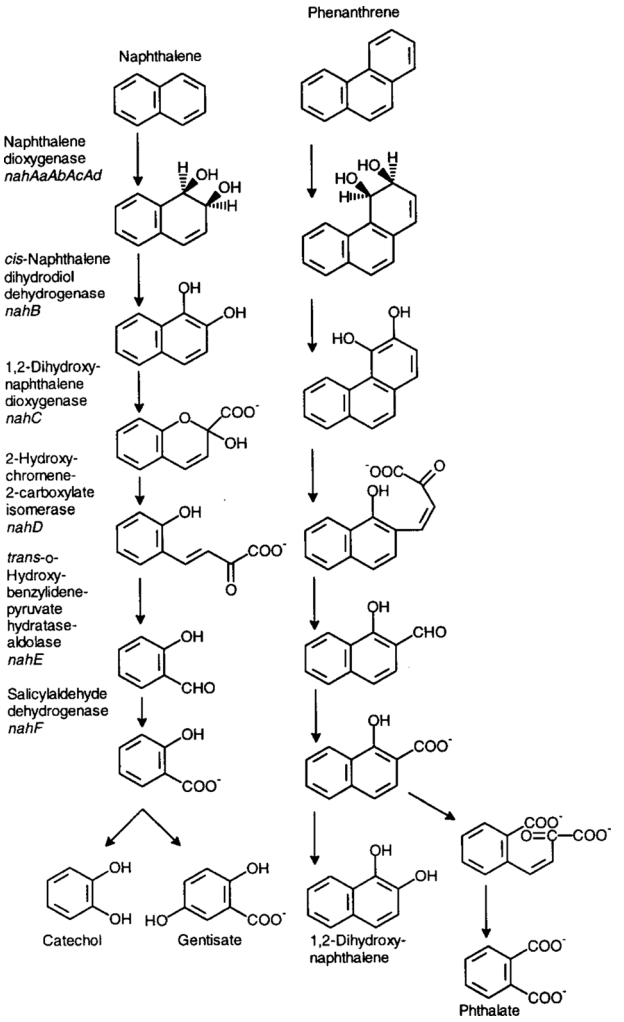


Fig. 3 The metabolic pathway for degradation of naphthalene and phenanthrene [31]

into catechol. The product is oxidised by catechol oxygenase (*nahH*) to 2-hydroxymuconic semialdehyde. From here this intermediate can pass two different ways: in one way, the molecule is directly hydrolysed by a hydroxymuconic semialdehyde hydrolase (*nahN*) into 2-Oxo-4-pentenoic acid, while through a second pathway, the product is acted on by 2-hydroxymuconic semialdehyde dehydrogenase (*nahI*) and *4-oxalocrotonate isomerase* (*nahJ*) to produce 2-hydroxymuconic acid and 4-oxalocrotonic acid before conversion by 4-oxalocrotonate decarboxylase (*nahK*) into 2-Oxo-4-pentenoic acid. This intermediate is the substrate for 2-Oxo-4-pentenoate hydratase (*nahL*) and is converted to 4-hydroxy-2-oxovaleric acid which is broken by 2-Oxo-4-hydroxypentanoate aldolase (*nahM*) into pyruvic acid and acetaldehyde. The acetaldehyde is converted by Acetaldehyde dehydrogenase (*nahO*) into acetyl-CoA. In the ortho-cleavage pathway, on the other hand, bacteria use three subsequent enzymes, 2-oxo-4-hydtoxypentanoate aldolase (*nahM*), catechol 1,2-oxygenase (*carA*) *and cis*-muconate lactonising enzyme (*catB*) to convert catechol into succinate and acetyl-CoA (Fig. 4) [117].

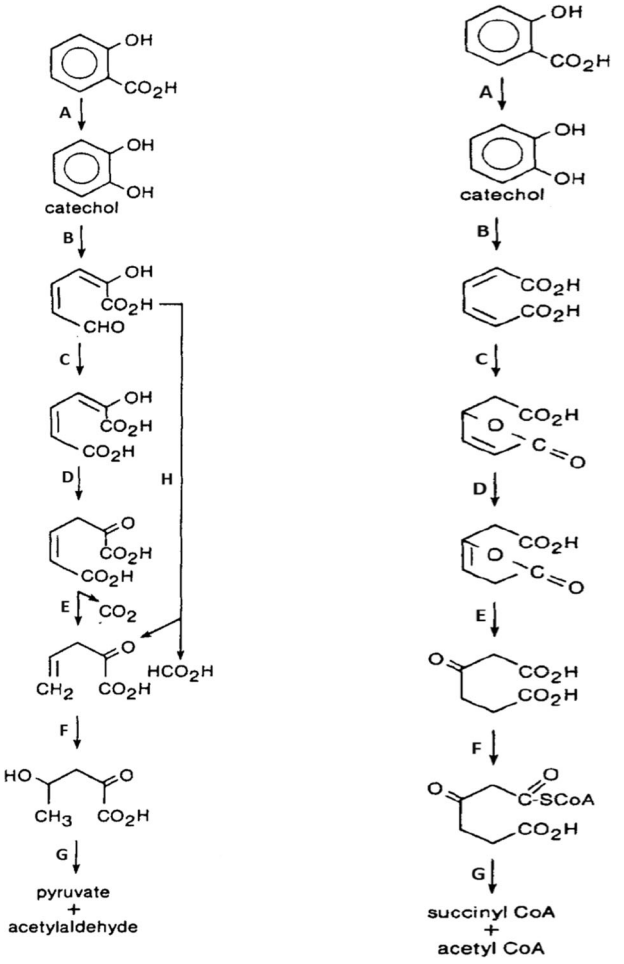


Fig. 4 Degradation of catechol via meta-cleavage reaction (*left*) and ortho-cleavage reaction (*right*) [122]

Through the gentisate pathway, bacteria employ an alternative pathway in which gentisate (2,5-dihydroxybenzoate) is subjected to a ring cleavage by gentisate1,2-dioxygenas (*BagI*) to produce a maleylpyrovate [3, 64]. The product can directly hydrolyse into pyruvate and malate or may go into another process in which the product is first isomerised by an isomerase (*bagKL*) into fumarylpyruvate before hydrolysing (*bagK*) to pyruvate and fumarate [65]. Several other bacteria, such as *Salmonella typhimurium* and *Pseudomonas alkaligenese* are also able to use this system to degrade other substrates, such as 3-hydroxybenzoate and xylenol, respectively, through conversion to gentisate as an intermediate [21, 30].

Although most of the genes responsible for degradation of naphthalene identified in different bacteria show 99–100 % homology with their counterparts in other strains, the location (plasmid or chromosome) and organisation of these gene clusters may be different in each strain (Fig. 5) [62]. However, there is a lower similarity between those genes identified in mycobacterial species and those in other bacteria probably due to the origin of the genes being from different sources or, less likely due to a greater rate of genetic changes in the mycobacterial genes [50]. NAH plasmids are a group of highly homologous plasmids, which

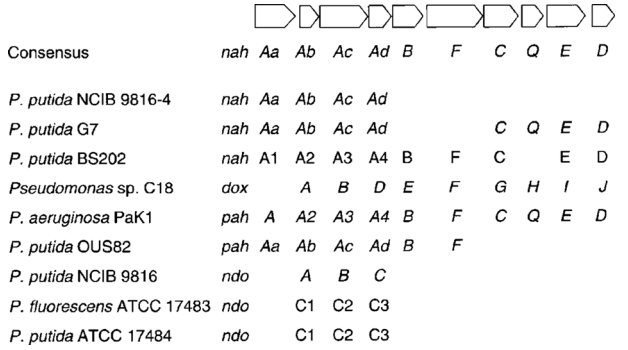


Fig. 5 The gene organisation in different strains of *Pseudomonas* sp. [31]

carry naphthalene catabolic genes. These plasmids can be distinguished by their restriction endonuclease digestion patterns [10]. The gene sequences of all identified NAH plasmids, such as NAH7 in *P. putida* PpG7, pNL1 in *Novosphingobium aromaticivorans* F199, pND6-1 in *Pseudomonas* sp. strain ND6 and pWW60-1 in *P. putida* NCIB9816 are highly conserved, with 90–100 % homology in the gene sequences [61, 75, 80]. NAH7 in *P. putida* PpG1carries two separate operons of which the NAH operon is specialised for conversion of PAHs, including naphthalene, anthracene and phenanthrene, to salicylate (*nahAaAbAcAdBFCED*) and the Sal operon is used for the catabolism of salicylate to catechol and further to TCA cycle intermediates (*nahGTHINLOMKJ*) [75]. In addition, *nahX* (with an unknown function) and *nahY* (a chemotaxic transducer protein) are located downstream of the *nahJ* [75, 81]. The product of the *nahY* gene acts as a methyl-accepting chemotaxis protein for cell attraction towards naphthalene via flagella-dependent movement [81]. A *nahR* gene located between these two operons positively regulates the expression of both of the operons [33, 75].

The Plasmids Containing Toluene- and Xylene-Oxidising Genes

Through several pathways, bacteria insert one or more hydroxyl groups into aromatic rings to form a catechol (Fig. 6), which is later cleaved for further catabolism. In one of these pathways, the toluene-degrading genes in *P. putida*, located on the TOL plasmid, degrade this molecule into benzoic acid, *cis*-benzoate dihydrodiol and finally to catechol that in turn is cleaved for further oxidation processes (Fig. 6; pathway A). Conversion of toluene into benzoate is performed by *xylA*, benzylalcohol dehydrogenase (*xylB*) and benzaldehyde dehydrogenase (*xylC*), while the next process, oxidation of toluate to catechol, is carried out by the products of the *xylD*, *xylE*, *xylF* and *xylG* genes. The first group of enzymes for the production of benzoate (encoded by *xylCAB*) is located on a plasmid, while the genes responsible for conversion of toluate into catechol can be found on both plasmids and chromosomes (Fig. 7) [48, 57]. Catechol and its derivatives are cleaved via one of two meta-cleavage activities. In one pathway, 2-hydroxymuconic semialdehyde is directly converted via hydroxymuconic semialdehyde hydrolase (HMSH; encoded by *xylF*) into 2-oxopent-4-enoate or its derivatives [36]. In the second pathway, 2-hydroxymuconic semialdehyde is first oxidised by 2-hydroxymuconic semialdehyde dehydrogenase (HMSD; encoded by *xylG*) to its corresponding dioates before isomerisation (encoded by *xylI*) to 2-oxopent-4-enoate (Fig. 8) [36]. This last product is finally hydrolysed (by the *xyl*K-encoded enzyme) to 4-hydroxy-2-oxovalerat before cleavage by 4-hydroxy-2-oxovalerate aldolase (encoded by *xylJ*) into pyruvate and

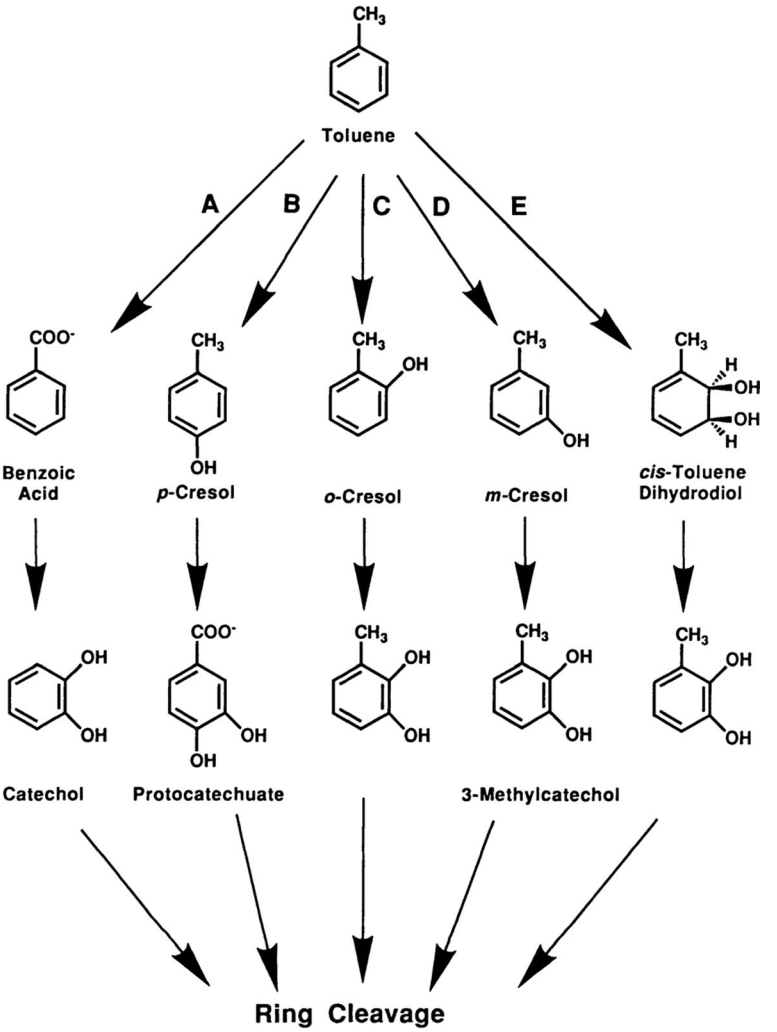
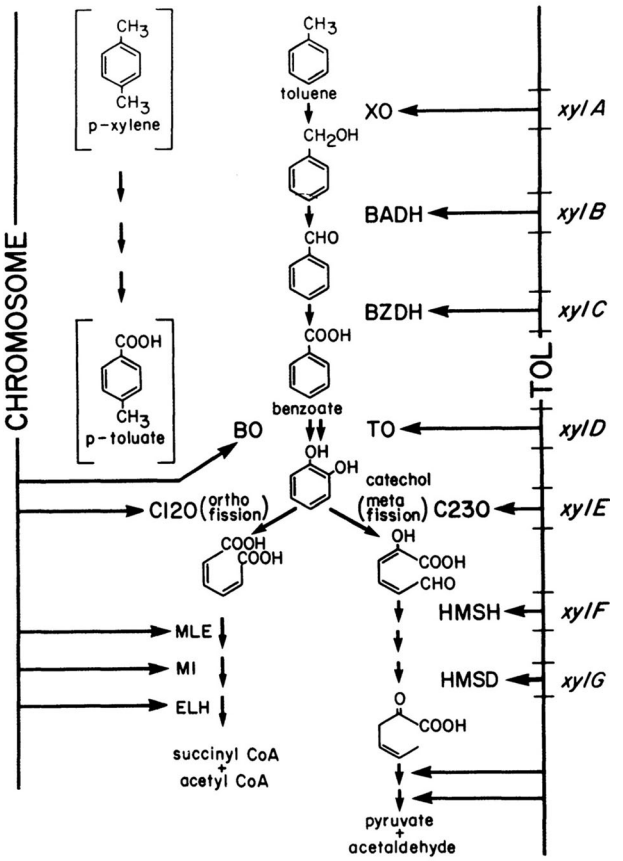


Fig. 6 Five possible pathways for degradation of toluene [124]

propionaldehyde [36]. The gene cluster on pTOL in *P. putida* is ordered as *xyl XYZLTEGFJQKIH* where *xylZ* and *xylL* encodes for 1,2-dioxygenase and 1,2-dihydroxycyclohexa-3,5-diene-carboxylate dehydrogenase, respectively and other genes downstream to *xylL* involve in the lower catabolic pathway [48].

Different strains of bacteria harbour quite similar operons in terms of DNA sequence and gene organisation. *Sphingomonas yanoikuyae* B1 harbours a *xylXYEFGJQKIHT* operon where in addition to internal gene rearrangements, the *xylL and xylZ* genes have moved to a separate place on the genome [49]. Furthermore, the operon *bphR1-bphA1A2*(*orf3*)*bphA3A4BCX0X1X2X3D* in *Burkholderia* sp. strain LB400 and *Pseudomonas pseudoalcaligenes* KF707, responsible for degradation of biphenyls to pyruvate and acetaldehyde, is highly homologous to the operons for degradation of toluene (Fig. 9) [25]. The corresponding operon in *Pseudomonas* KKS102 is broken into a



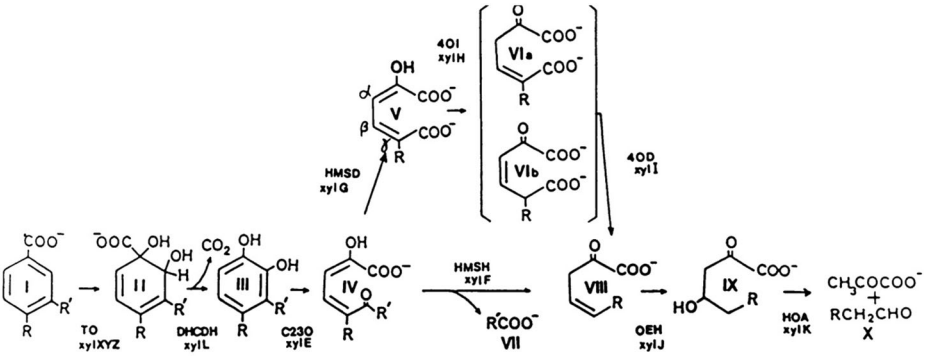


Fig. 7 Gene organisation of toluene and xylene degradation on chromosome and TOL plasmid of *P. putida*. *XO* xylene oxidase, *BADH* benzyl alcohol dehydrogenase, *BZDH* benzaldehyde dehydrogenase [98]

Fig. 8 The metabolic pathway for the degradation of toluate and benzoate via the meta-cleavage process. The 2-hydroxymuconic semialdehyde intermediate can oxidise via two different reactions. It may be oxidised directly by hydroxymuconic semialdehyde hydrolase (*HMSH*; into 2-oxopent-4-enoate or may be first converted by HMSD (2-hydroxymuconic semialdehyde dehydrogenase into a dioates before isomerisation to 2-oxopent-4-enoate. *TO* toluate 1,2-dioxygenase, *DHCDH* 1,2-dihydroxycyclohexa-3,5-diene-carboxylate dehydrogenase, *C230* catechol 2,3-dioxygenase, *40D* 4-oxalocrotonate decarboxylase, *OEH* 2-oxopent-4-enoate hydratase, *HOA* 4-hydroxy-2-oxovalerate aldolase. xylE to xylZ are names of the genes specified for the degrading enzymes [36]

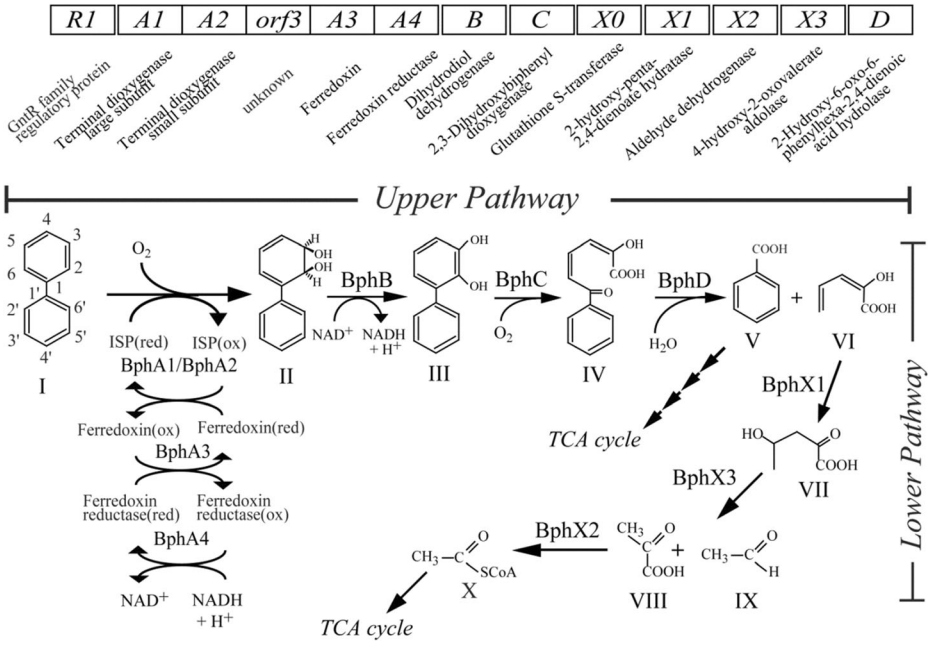


Fig. 9 The degradation pathway of biphenyl into acetyl-CoA and the order of the corresponding genes (*bph* operon) in *Pseudomonas pseudoalcaligenes* KF707. The biphenyl dioxygenase (consisting of two subunits of the terminal dioxygenase and ferrodoxin encoded respectively by bphAlA2A3A4) inserts two oxygen atoms into biphenyls (I) to yield dihydrodiols (II). The product is further oxidised by dihydrodiol dehydrogenase (encoded by bphB) to 2,3-dihydroxybiphenyl (III), which undergoes a cleavage at the meta-position by the product of the *bphC* gene (2,3-dihydroxybiphenyl dioxygenase) to yield 2-hydroxy-6-oxo-6-phenylhexa- 2,4-dienoate (IV). This molecule is broken by 2-hydroxy-6-oxo-6phenylhexa-2,4-dienoic acid hydrolase (encoded by bphD) into benzoic acid (V) and 2-hydroxypenta-2,4-dienoate (VI). A hydratase, encoded by (bphX1) converts the 2-hydroxypenta-2,4-dienoate to 4-hydroxy-2-oxovalerate (VII), which is cleaved by 4-hydroxy-2-oxovalerate aldolase (encoded by bphX3) into pyruvate (VIII) and acetaldehyde (XI). The acetaldehyde is then activated by acetaldehyde dehydrogenase (encoded by bphX2) and glutathione-*S*-transferase (encoded by bphX0) through the addition of CoA (X) before entering the TCA cycle. *ISP* iron-sulphur protein [25]

bphEGF(ORF4)*A1A2A3BCD*(ORF1)*A4* operon, which allows the catabolic reaction of biphenyls to proceed to 2-hydroxypenta-2,4-dienoate and benzoic acid, and the gene cluster *bphEGF* located 4 kbp upstream of the first operon, which encode for hydratase, aldolase and dehydrogenase and convert these intermediate products into pyruvate and acetaldehyde [77]. Finally, the gene cluster for the degradation of biphenyl in *Rhodococcus* sp. RHA1 is distributed between several linear plasmids, referred to as RHA1, RHA2 and RHA3. Most of the genes for initiating the catabolism of biphenyl (*bphA1A2A3A4CB*) are located on RHA1, while the *bhpDEF* cluster is placed on pRHL2 [108].

Through a completely different pathway in *P. putida* Fl (Figs. 6d and 10), a multimeric enzyme referred to as toluene dioxygenase (encoded by tod *C1C2BA*) converts toluene and many other aromatics into (+)-*cis*-1(S),2(R)-dihydroxy-3-methylcyclohexa-3,5-diene [17, 100]. This reaction is driven by the protons and electrons originating from NADH that are passed through an electron transport system composed of a reductase (encoded by *todA*), a ferrodoxin (encoded by *todB*) and an iron-sulphur protein (ISP; encoded by *todC1* and *todC2*). A NAD^+-dependent-*cis*-toluene dihydrodiol dehydrogenase (encoded by *todD*) oxidises the dihydrodiol to form 3-methylcatechol, which is cleaved twice by 3-methylcatechol-2,3-dioxygenase

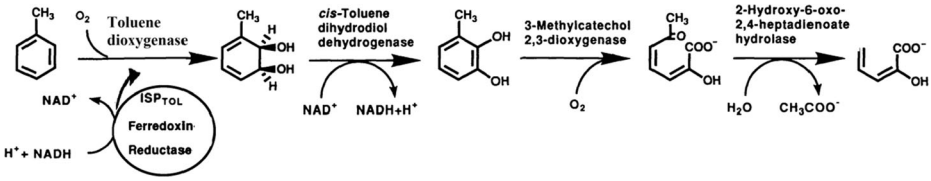


Fig. 10 Conversion of toluene into 2-hydroxypenta-2,4-dienoate by *P. putida* F1. The electron originating from NADH, and H^+ is passed through an electron chain system, respectively, consisting of reductase, ferrodoxin and an iron-sulphur protein (*ISP*) to catabolise toluene into toluene dihydrodiol dehydrogenase [100]

(encoded by *todE*) to 2-hydroxy-6-oxo-2,4-heptadienoate and then by 2-hydroxy-6-oxo-2,4-hepladlenoale hydrolase (encoded by *todF*) to 2-hydroxypenta-2,4-dienoate and acetate. All of the toluene-degrading genes are ordered as *todFClC2BADE* gene cluster.

Nitroaromatic Compounds

Nitroaromatic compounds (NACs) are synthetic molecules broadly utilised in different industries as plastics, pharmaceuticals, precursors for dyes, explosives and pesticides [35]. Although there are many different types of nitroaromatics, 2,4,6-trinitrotoluene (TNT), dinitrotoluenes and nitrotoluenes are the most abundant environmental pollutants [8, 24]. Based on the gene capacity and type of the original nitroaromatic compound, microorganisms use oxidative and/or reductive degrading pathways to convert these NACs completely to CO_2 and H_2O or partially to an organic compound [4]. While aerobic bacteria use both the catabolic systems, anaerobic bacteria are able to use only the reductive degrading mechanism to catabolise NACs [4].

The oxidative reactions are triggered through the reaction of a mono/di-oxigenase enzyme, releasing a nitrite and dihydroxy aromatic compounds. The substrate specificity and the intermediate and final products are unique based on the substrate, the type of oxygenases used in the reaction and the organisms involved in the degradation. The monooxygenase systems, for instance, are able to react with different substrates, including 2-nitrophenol (*P. putida* B2) [123], 4-nitrophenol (*Moraxella* sp.) [103] and 4-methyl-5-nitrocatechol (*Pseudomonas* sp. strain DNT) [34], 2-nirrotoluene (from *Acidovorax* sp. JS42) [56], nitrobenzene *Comamonas* sp. strain JS765 [73], 3-nitrobenzoate [52], 1,3-dinitrobenzene (*Burkholderia cepacia* R34) [46], 2-chloronitrobenzene (*Pseudomonas stutzeri* strain ZWLR2-1) [63] and 2,4-dinicrotoluene [104]. The monooxygenases belonging to the two-component flavin-diffusible monooxygenase (TC-FDM) family in *Moraxella* sp., *Pseudomonas* sp. strain ENV2030, *Rhodococcus* sp. strain PN1, *Rhodococcus opacus* SAO101 and many other bacteria, oxidise 4-nitrophenol in expense of two NADPH and a molecular oxygen to hydroquinone and releases a nitrite molecule [79]. The members of this family can be divided into two homology groups: the phenol 2-monooxygenase and phenol 4-monooxygenase groups. While the members of the first group (such as phenol monooxygenase (*PheA*), nitrophenol monooxygenase (*NphA1*) and 4-hydroxyphenylacetate monooxygenase (*HpaB*), hydroxylate the ortho group of phenols, members of the second group, including 2,4,6-trichlorophenol monooxygenases (*TcpA*), 2,4,5-trichlorophenol monooxygenase (*TftD*), PNP monooxygenase (*NpcA*) and 4-chlorophenol monooxygenase (*CphC*-I), hydroxylate their para position. Following release of nitrite in both bacteria, the products are directed into normal cell metabolism that leads to production of maleylacetic acid and further to β-ketoadipate. The *npd* gene cluster in *Arthrobacter* sp. JS443, responsible for catabolism of *p*-nitrophenol, consists of three genes, *npdB* (hydroxyquinol 1,2-

dioxygenase), *npdA1* (*p*-nitrophenol monooxygenase) and *npdA2* (*p*-nitrophenol hydroxylase) [79]. This cluster is 85 % similar to the *cph I* gene cluster found in *A. chlorophenolicus* A6 (Fig. 11) [79]. However, while *cph* gene cluster are regulated by products of two genes (cphR and cphS), these regulatory genes are combined into a single gene, called as *npdR* [74, 79].

The multicomponent NAC dioxygenase system, such as NBDO, NDO, DNTDO, 3-NTDO, 2NTDO and TNT dioxygenase, consists of a terminal iron-sulphur oxygenase, an iron-sulphur ferredoxin and a flavoprotein reductase to substitute a hydroxyl group to the ring by a nitrite [101]. NAC dioxygenases are usually non-specific enzymes able to react with several NACs, such as nitrotoluenes, dinitrobenzenes and nitrophenols and non-nitrogen aromatic hydrocarbons as well. These dioxygenases use the electrons transferred by two other components to the system to add two oxygen atoms into the ring to produce catechol intermediates [101]. The dioxygenase genes show highly similarity with the sequence and gene structure of the naphthalene, which belongs to Rieske non-heme iron dioxygenases [22]. The genes responsible for expression of these subunits are organised in a sequence of a reductase (*mntAa*), followed by one or two other ORF, the ferredoxin subunits, the large subunit of oxygenase (*MntAc*)and its small subunit (*MntAd*). These genes are under control of a regulator (*mntR*) located at upstream of the functional dioxygenase gene. Although the two ORFs between genes reductase and ferredoxin in many strains are inactive, in several cases, such as DNTDO identified in *Burkholderia* sp. strain DNT and NDO identified in *Ralstonia* sp., are responsible for expression of two subunits of salicylate-5-hydroxylase, which accept the electron released

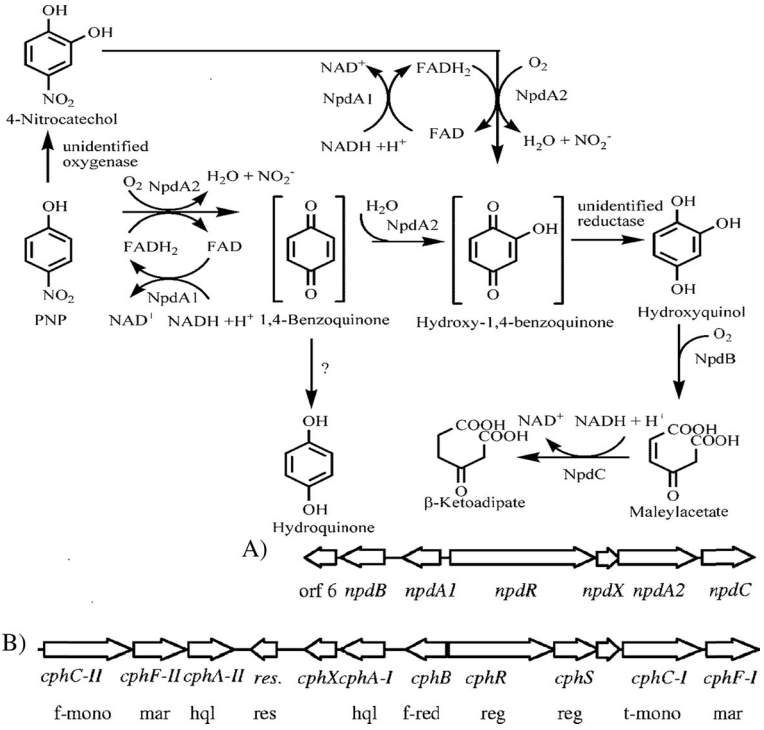


Fig. 11 The metabolic pathway of *p*-nitrophenol (*PNP*) and the *npd* gene cluster in *Arthrobacter* sp. JS443 (**a**) and *Arthrobacter chlorophenolicus* A6 (**b**); *f-mono* flavoprotein monooxygenase, *mar* maleylacetate reductase, *hql* hydroxyquinol 1,2-dioxygenase, *res* resolvase pseudogene, *f-red* flavin reductase, *reg* transcriptional regulator, *t-mono* TC-FDM [79]

from reductase and ferredoxin. As a prototype NAC, 2,4-dinitrotoluene (2,4-DNT) (Figs. 12 and 13) is initially oxidised by a tetramer dioxygenase (encoded by *dntAaAbAcAd*) to release NO_2^-, converting it to 4-methyl-5-nitrocatechol (MNC). While in *Acidovorax* sp. strain JS42 this operon is regulated by a transcriptional activator (*NtdR*) located immediately upstream of the operon [82], there is a gap between the *dntAaAbAcAd* and its upstream regulator (*dntR*) in *B. cepacia* R34 [106]. This intermediate is affected by the MNC monooxygenase (encoded by *dntB*) to produce 2-hydroxy-5-methylquinone (HMQ), which is further oxidised by HMQ reductase (encoded by *dntC*) to 2,4,5-trihydroxytoluene (THT). THT should undergo extradiol ring fission by THT oxygenase (encoded by *dntD*) to produce 2,4-dihydroxy-5-methyl-6-oxo-2,4-hexadiennoic acid (DMOH). This intermediate is converted by bifunctional DMOH isomerase (encoded by *dntG*) first to 4-hydroxy-2-keto-5-methyl-6-oxo-3-hexenoic acid and further to pyruvic acid and methlmalonic acid semialdehyde. The last product is bound by a CoA-dependent methylmalonate semialdehyde dehydrogenase (encoded by *dntE*) to CoA-SH in conjunction with NAD^+ to produce propionyl-CoA.

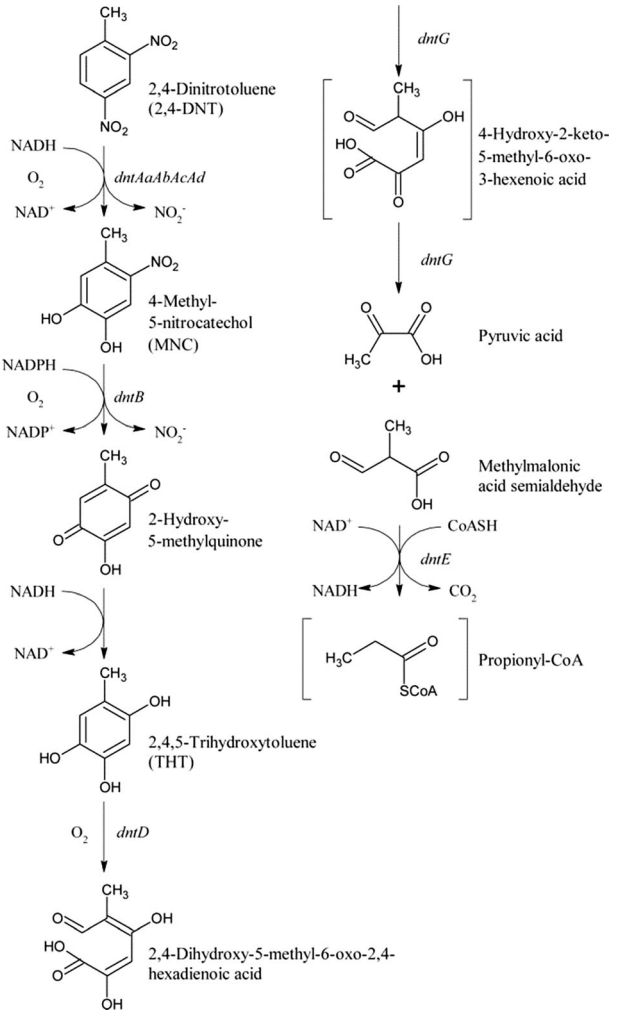


Fig. 12 Oxidative catabolism of 2,4-dinitrotoluene in *Burkholderia cepacia* R34. *dntAaAbAcAd* 2,4-DNT dioxygenases, *dntB* methylnitrocatechol monooxygenase, *dntD* the extradiol ring fission enzyme, *dntG* bifunctional isomerase/hydrolase, *dntE* CoA-dependent methylmalonate semialdehyde dehydrogenase [106]

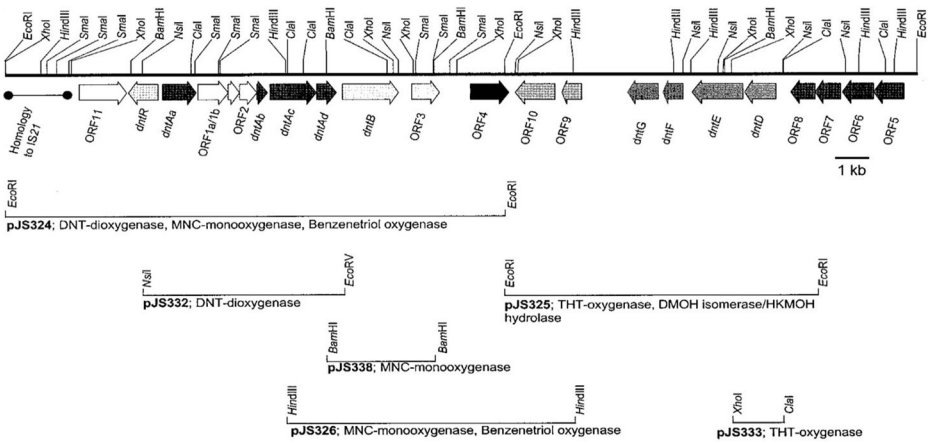


Fig. 13 Physical map of region encoding 2,4-DNT pathway genes in *Burkholderia cepacia* R34 [46]

Dibenzothiophene

Since a carbon group of dibenzothiophene (DBT) and its derivatives is substituted with sulphur, their degradation requires an initial set of reactions to remove this sulphur [67]. The genes involved in desulfurization of dibenzothiophene (*DszA*, *DszB* and *DszC*) are located on an operon. These genes remove the sulphur through a four step reaction referred to as the 4S desulfurization pathway [84, 99] (Fig. 14). The two first reactions are catalysed by the action of dibenzothiophene monooxdase (*DszC*) in which DBT is converted initially to dibenzothiophene-sulfoxide and later to dibenzothiophene sulfone ($DBTO_2$) [2, 68]. The product of *DszA* ($DBTO_2$ monooxygenase) converts this intermediate to 2-(2-hydroxyphenyl)-benzene sulfonate (HBPS) [2, 68], which then is more metabolised by HBPS-desulfinase (*DszB*) releases sulphur from the compound and leaves 2-hydroxybiphenyl (HBP). The first three steps are O_2-dependent oxidative reactions and require an electron and hydrogen transportation system, composed of $FMNH_2$ and NAD(P)H as a reductant [2, 68]. Oxidation of NAD(P)H to NAD(P) is catalysed by action of a flavin reductase, which is encoded by *DszD* [2, 68]. This later genes is located at a separate locus from *dszABC* [2, 68]. Following desulfurisation of these compounds, the product is a simple aromatic compound which is metabolised by the aromatic degradation system as mentioned before. Although there is not enough evidence for the regulation of these genes, it has been shown that expression of the operon is under the control of a repressor and is limited in the presence of different readily bioavailable sulphur sources, such as SO_4^{2-}, casamino acids, methanesulfonic, taurine, cysteine and methionine [71].

Anaerobic Degradation of Hydrocarbons

Several phylogenetically and physiologically distinct microorganisms degrade hydrocarbons through anaerobic metabolic pathways utilising the reduction of unusual electron acceptors, such as sulphate, thiosulfate, nitrate, nitrite, nitrous oxide, metal ions and carbonate, or using anoxygenic phototrophic reactions involving the donation of electrons and hydrogen for substrate catabolism activities [32]. These anaerobic bacteria degrade hydrocarbons via five

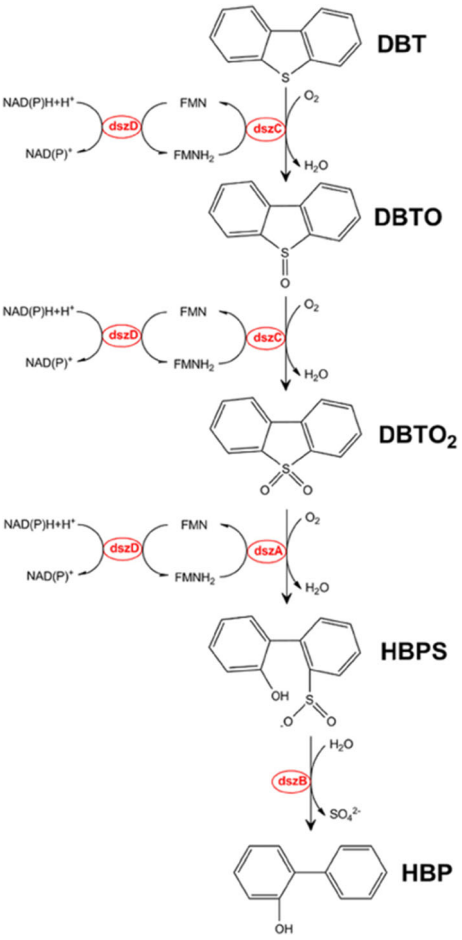


Fig. 14 The dibenzothiophene desulfurizing pathway in *Gordonia* sp.; *dszA* dibenzothiophene sulfone ($DBTO_2$) monooxygenase, *dszB* 2-(2-hydroxyphenyl)-benzene sulfonate desulfinase, *dszC* dibenzothiophene monooxdase, *dszD* NAD(P)H/FMN oxidoreductase, *DBT* dibenzothiophene, *DBTO* dibenzothiophene-sulfoxide, *$DBTO_2$* dibenzothiophene sulfone, *HBPS* 2-(2-hydroxyphenyl)-benzene sulfonate, *HBP* 2-hydroxybiphenyl (taken from http://2012.igem.org/File:12SJTU_desulpathway1.png)

different pathways: (a) addition of a fumarate to methylene or methyl groups of hydrocarbons [118], (b) oxygen-independent hydroxylation of 2nd or 3rd terminal C-atoms (to make secondary or tertiary alcohols) [107], (c) carboxylation of unsubstituted carbon atoms of aromatics [11], (d) hydration of the double and triple bond of alkenes and alkynes [66] and (e) reverse methanogenesis [111].

Many anaerobic bacteria, including denitrifying microorganisms, sulphate-reducing bacteria, methanogenic consortia and metal-reducing (Mn(IV), Fe(III)) bacteria, are able to activate hydrocarbons via terminal or sub-terminal addition of a carbonic group, such as fumarate, to a carbon atom of the hydrocarbon [96]. Conversion of toluene into (*R*)-benzylsuccinate is a common example in which a trimer benzylsuccinate synthase (BSS) enzyme (encoded by *bbsABC*) adds a fumarate to the substrate (Fig. 15) [118]. Then, after addition of a CoA to the product via the action of succinyl-CoA/benzylsuccinate CoA-transferase (encoded by *bbsEF*), the benzylsuccinyl-CoA undergoes an oxidation step, a hydration and another oxidation respectively by benzylsuccinyl-CoA dehydrogenase (encoded by *bssG*), phenylitaconyl-CoA hydratase (encoded by *BbsH*) and (5)3-hydroxyacyl-CoA dehydrogenase (encoded by *BbsCD*) before cleavage (by benzoylsuccinyl-CoA thiolase (encoded by *BbsB*) into a benzyl-CoA and

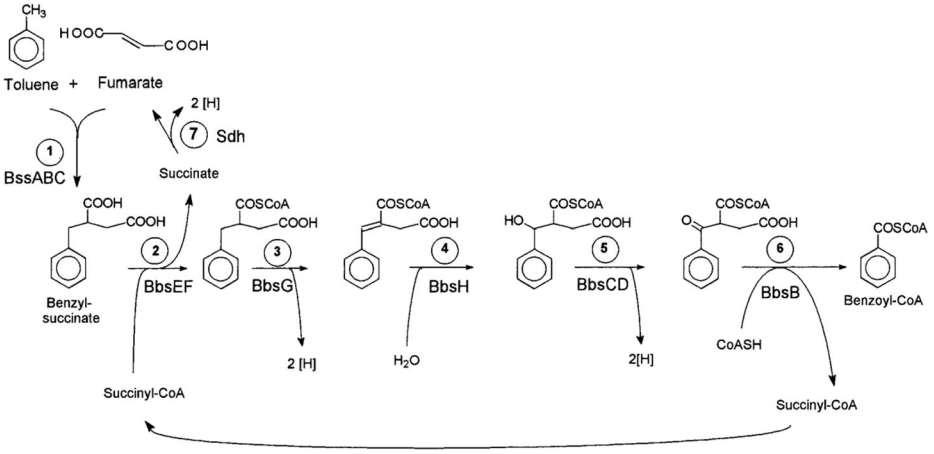


Fig. 15 The catabolic pathway for anaerobic toluene degradation. *1*, Benzylsuccinate synthase, encoded by BssABC; *2*, succinyl-CoA/benzylsuccinate CoA-transferase, encoded by BbsEF; *3*, benzylsuccinyl-CoA dehydrogenase, encoded by BbsG; *4*, phenylitaconyl-CoA hydratase, encoded by BbsH; *5*, 3-hydroxyacyl-CoA dehydrogenase, encoded by BbsCD; *6*, benzoylsuccinyl-CoA thiolase, encoded by BbsB; *7*, succinate dehydrogenase, encoded by *Sdh* [58]

a succinate. All of these genes are clustered as a *bbsABCDEFGHI* operon (*bbs* is abbreviation of beta-*oxidation* of benzylsuccinate) and is controlled by a regulatory factor (TdiSR) that activates this operon in the absence of O_2 and the presence of toluene [39]. The naphthyl-2-methyl-succinate synthase in SRBs, encoded by *nmsABC*, specific to transfer a fumarate to naphthalene is similar to the corresponding enzyme involved in the biodegradation of toluene [94]. Furthermore, the smallest subunit of alkylsuccinate synthases, involved in addition of a fumarate to alkanes through anaerobic degradation, is highly similar to the corresponding subunit of benzylsuccinate synthetase, encoded by *bssC* [20].

n-alkane and cycloalkanes are metabolised with similar strategy by sulphate-reducing microorganisms in which a fumarate addition step activates these substrates to yield alkylsuccinates and cycloalkylsuccinate derivatives, respectively [72, 86]. The metabolism of cyclohexane by a nitrate-reducing microorganism to cyclohexylsuccinate [72] and of ethylcyclopentane by a sulphate-reducing organism to cyclopentylsuccinate [86] have been reported before. In the case of the anaerobic degradation of hexadecane by this mechanism (Fig. 16), alkylsuccinate synthetase (encoded by *assABC*) binds a fumarate to the substrate to produce 1-methylpentadecyclisuccinate. This intermediate is converted by methylmalonyl-CoA mutase (encoded y mcm) to 2-(2-methylhexadecyl)malonate, which is decarboxylated by the activity of a carboxyl transferase to produce 4-methyloctadecanoate. This last product is directed into the beta-oxidation pathway for further catabolism [14]. In the genome of *Desulfatibacillum alkenivorans* AK-01, these genes are located at two different loci (*assA1* and *assA2*), and there is no similarity between them [14] (Fig. 17).

Several denitrifying bacteria use an oxygen-independent hydroxylation process for the degradation of some aromatics in which a trimer (α, β and γ) molybdenum-containing ethylbenzene dehydrogenase (EBDH; encoded by *ebdABC*) hydroxylates the terminal carbon of this molecule to produce (*S*)-1-phenylethanol [83, 107]. The (*S*)-1-phenylethanol is converted into acetophenone and then to benzoylacetate and finally to benzoylacetyl-CoA as a result of reactions involving NAD-dependent (*S*)-1-phenylethanol dehydrogenase,

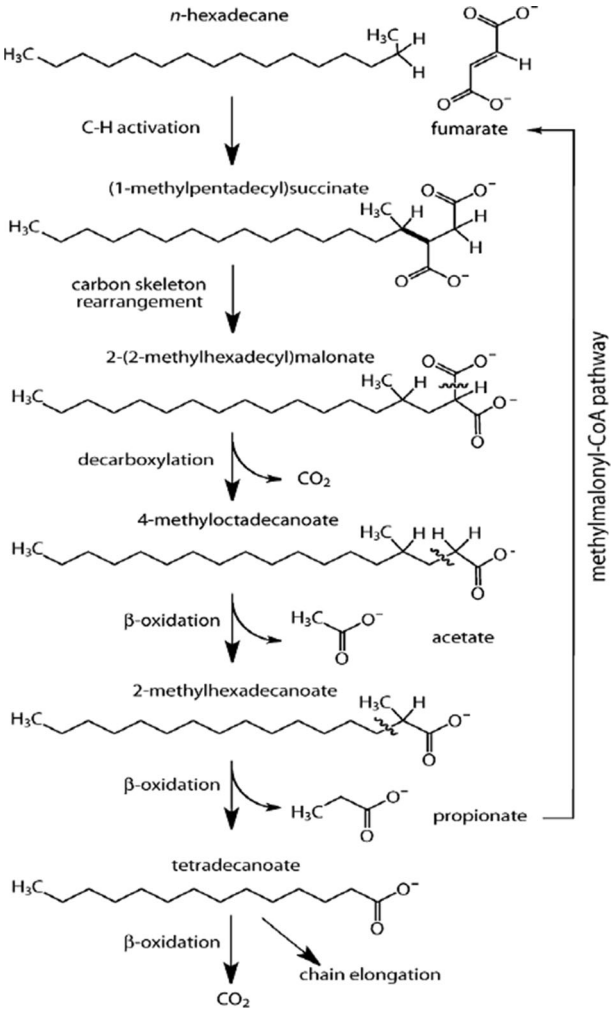


Fig. 16 Biodegradation of *n*-hexadecane in *Desulfatibacillum alkenivorans* AK-01 [14]

acetophenone carboxylase (APC) and benzoylacetate-CoA ligase (BAL), respectively [83, 107]. The gene cluster for these three subunits of ethylbenzene dehydrogenase, along with the genes encoding (*S*)-1-phenylethanol dehydrogenase (*ped*) and a chaperone-like protein (encoded by *ebdD*) (necessary for transferring molybdenum into ethylbenzene dehydrogenase) are located on one operon, whereas the genes encoding APC (subunits A, B, C, D and E) and BAL are present on another operon [83, 107].

Addition of a carboxyl group to substrates is an alternative reaction utilised for the anaerobic catabolism of hydrocarbons by several sulphate and nitrate-reducing bacteria [95]. For instance, after conversion of propylene to acetone by *Xanthobacter autotrophicus* strain Py2, the acetone is carboxylated by acetone carboxylase at the expense of a CO_2 and an ATP to produce acetoacetate [11]. This multimeric ($\alpha 2 \beta 2 \gamma 2$) enzyme is encoded by three genes: *acxA*, *acxB* and *acxC* which encode the β, α and γ subunits, respectively, which are clustered

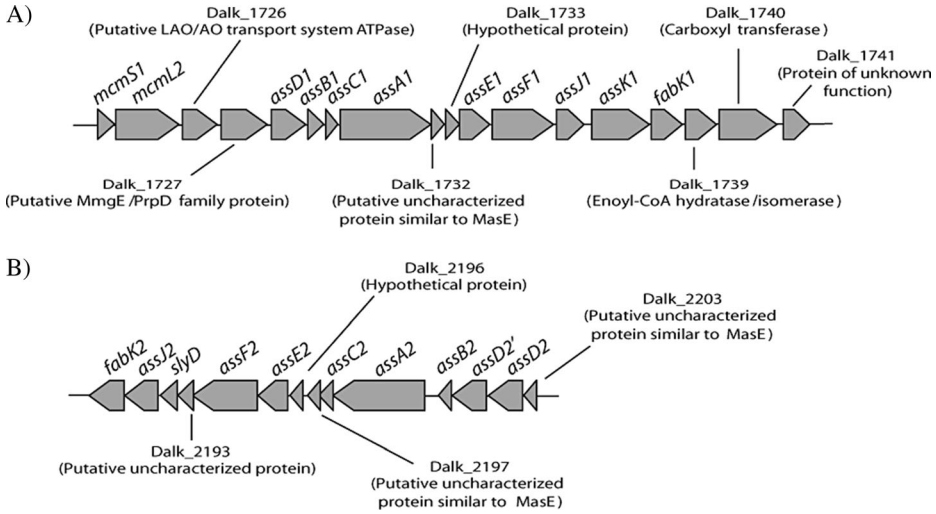


Fig. 17 Gene organisation of the enzymes involved in hexadecane degradation in anaerobic conditions (in the *D. alkenivorans* AK-01 genome). **a** Alkylsuccinate synthase locus 1, **b** locus 2; *mcmS1 and mcmS2* small and large subunits of methylmalonyl-CoA mutase, *assD1* alkylsuccinate synthase (I) glycyl radical activating enzyme, *assB1* alkylsuccinate synthase beta subunit, *assC1* alkylsuccinate synthase gamma subunit, *assD1* alkylsuccinate synthase alpha subunit, *assE1* a chaperone protein, *assF1* an uncharacterised protein, *assJ1* enoyl-CoA hydratase/isomerase, *assK1* AMP-dependent synthetase and ligase, *fabK1* enoyl-(acyl-carrier protein) reductase II, *fabK2* putative enoyl-(acyl-carrier protein) reductase II, *assJ2* enoyl-CoA hydratase/isomerase, *slyD* putative peptidyl-prolyl *cis-trans* isomerase (fkbp-type), *assF2* putative uncharacterised protein, *assE2* chaperone protein, *assC2* gamma subunit of alkylsuccinate synthase (II), *A2* alpha subunit of alkylsuccinate synthase (II), *B2* beta subunit of alkylsuccinate synthase (II), *assD2' and assD2* alkylsuccinate synthase (II) glycyl radical-activating enzyme [14]

in an operon as *acxABC* and its expression is regulated by a gene (*AcxR*) upstream of the *acxABC* cluster [11].

$$CH_3{-}COCH_3 + CO_2 + ATP \rightarrow CH_3COCH_2COO + H^+ + AMP + 2Pi$$

Some microorganisms are able to anaerobically degrade alkenes and alkynes through the addition of H_2O to the unsaturated bond, producing the corresponding alcohols [66]. Using a monomeric thermostable acetylene hydratase (encoded by *AH* gene), for instance, *Pelobacter acetylenicus* adds a H_2O molecule to acetylene converting it to an acetaldehyde [66, 110]. In the degradation of β-myrcene (7-methyl-3-methylen-1,6-octadien) by *Castellaniella defragrans*, linalool dehydratase/isomerase (LDI; encoded by *ldi* gene) acts as a dual-function enzyme that desaturates linalool to myrcene before isomerisation to geraniol [12]. The molecule is then oxidised by two dehydrogenases, referred to geraniol dehydrogenase (GeDH) and geranial dehydrogenase (GaDH) (encoded by *geoA* and *geoB*, respectively) into geranial and geranic acid [12].

Several methanotrophic microorganisms use methyl-coenzyme M reductase (Mcr) for the initial activation of methane, which then binds to coenzyme B (CoBSH) via methyl-coenzyme M (CoMSH) to produce a complex of CoM-S-S-CoB-heterodisulfide and methane [111]. This enzyme consists of two of each of the α, β and γ subunits, one nickel atom and a tetrapyrrole cofactor (normally F_{430} factor or 17(2)-methylthio-F430) [91, 111]. The encoding genes for these subunits, *mcrBGA*, and two other additional genes with unidentified roles (*mcrC* and *mcrD*) are located on one operon [91, 111]. The *mcrA* gene is used as a marker to track methanogens and anaerobic methanotrophic microorganisms [91, 111].

Anaerobic Degradation of NACs

Several bacteria, such as sulphate-reducing bacteria and Clostridia, are able to degrade nitroaromatic compounds in anaerobic conditions. The anaerobic metabolism of these compounds is performed through the reduction of the nitro group. However, it has to be mentioned that this mechanism is not exclusive to anaerobic bacteria and many other bacteria, such as *Enterobacter* sp., *Nocardiodes* sp. and *Rhodococcus* sp., are able to metabolise NACs using similar reductive pathways. The reductive pathways can be proceed by two different processes depending on the microbial gene capacity. *Enterobacter cloacae* PB2 expresses a monomeric flavoenzyme, referred to as PETN reductase (PETNr), which enables this microorganism to reduce triply nitrated aromatic compounds, such as picric acid and TNT. Furthermore, presence of *PnrA* gene, encoding for an NADPH-dependent nitroreductase, enable several microorganisms to transform a variety of NACs, such as 3,5-dinitroaniline, 3- and 4-nitrobenzoate, 3-nitrotoluene, 2,4-DNT, TNT and 3,5-dinitrobenzamide.

Hydride transferases are a second reducing system in which the aromatic ring of some NACs, such as TNT and picric acid loss their nitrite. The initial reduction of TNT by PETNr, for instance, leads to production of hydride- and dihydride-Meisenheimer TNT complexes (H-TNT and 2H-TNT), which is further reduced through an unknown mechanism to release nitrite. *Nocardiodes simplex* FJ2-1A and *Rhodococcus erythropolis* are two bacteria with the ability to degrade picric acid using this mechanism. In these examples, a hydride is initially added to the aromatic ring by the activity of a F_{420}-dependent hydride transferase (*NpdI*) and F_{420} reductase (*NpdG*) to produce a hydride Meisenheimer complex. While in *N. simplex* the second hydride is added by the same enzyme, this step on *R. erythropolis* is performed by the product of *NpdC*/*NpdG*. The dihydride-Meisenheimer complex is later undergone a tautomerisation performed by the product of *NpdH* to release nitrite and various products.

Genetics of Microbial Adaptation to High Hydrocarbon Concentrations

A limitation of mineral nutrients is a common problem in oil contaminated marine environments due to an imbalanced C/P/N ratio [70]. Microorganisms can adapt themselves to these limitations using either their own gene products or through the creation of a cooperative relationship with other microbes to decrease this stress [69]. Due to such adaptation processes, the community of hydrocarbon-degrading microorganisms may show a sudden increase in cell mass after an initial temporary drop in the total number of microorganisms [70]. In addition to the genes responsible for degradation of hydrocarbons, many bacteria adapt their physiology to the shortage of mineral nutrition through increased expression of their existing ion transporters [92, 121]. *A. borkumensis* strain SK2, for instance, adapts itself to various mineral deficiencies through the induction of the genes responsible for different transport proteins such as *narKGHJI* cluster and *nrtCB-nasDTS* cluster (for reduction and uptake of nitrogen), *amt* (for uptake of ammonium), *phoBR* and *phoU-pstBACS* gene cluster (for uptake of phosphate), *znuAB* (for uptake of zinc), *modABC* (for uptake of molybdite), *mgtE* (for uptake of magnesium) and CorA-like MIT(for uptake of cobalt) [92].

In addition, one of the critical factors for biodegradation of hydrocarbons is the ability of the degrading microorganisms to be resistant to high concentrations of hydrocarbons, especially when the cells are suddenly exposed to large amounts of the compounds [26, 52]. A common resistance strategy to toxic solvents is to intensify the cell membrane density using isomerisation of *cis*-unsaturated fatty acids to their *trans* forms [69]. Furthermore, penetration

of the solvents into cells induces expression of several chaperons to refold the proteins denatured by the reagents [52]. Some bacteria tolerate high concentrations of solvents through expression of efflux pumps on their cell membrane, enabling them to export the toxic solvents out of the cells [26]. *P. putida* S12, for instance, is a highly resistant strain to organic compounds due to the expression of a solvent resistance pump (encoded by *srpABC*), which discharges several types of solvents, hydrocarbons included [105].

Furthermore, many bacteria use chemotactic strategies to improve their resistance to different toxic compounds, hydrocarbons included [23, 53]. The integral cell membrane associated proteins encoded by *alkN* [23] and *nahY* in *P. putida* [53], for instance, interact respectively with alkanes and naphthalene. These proteins act as a methyl-accepting chemotaxis factor that triggers a cell signalling pathway, regulating the flagella motor and resulting in cell attraction towards naphthalene [23, 53]. Interestingly, both chemotactic activities and solvent resistance abilities are gene-dose dependent, and the amounts of the corresponding genes and the level of gene activity in cells determine the level of cell susceptibility to a solvent [54]. Based on a study, performed by *Lacal* [54] on *P. putida* DOT-T1E, the presence of two alleles of chemoreceptor *mcpT* gene genes is enough to enable the cell to response strongly to different aromatic hydrocarbons. They showed that the level of *mcpT* gene expression in this strain was directly under control of the substrates, and there was an inverse relationship between the amounts of toluene and the level of gene methylation. The increases in the level of *McpT* methylation induce the activity of flagella motor using the autophosphorylation of *CheA* [54].

The behaviour of bacteria in the case of exposure to a carbon source, which is potentially toxic for the cells, depends to the reaction of signal transduction proteins. Although the signalling pathway leads to triggering a degradation shunt in the cells, some bacteria prefer to migrate away from these compounds. This decision making in *P. putida* DOT-T1E in the case of exposure to toluene depends on the activity of *TodS*/*TodT* two-component system and *TtgV* in the cells. Since affinity of the sensor kinase *TodS* to toluene is double in comparison with *TodV*, this signalling protein is activated in lower concentrations of the substrate, inducing the TOD pathway [13]. However, binding of toluene to *todV* in higher concentrations of toluene upregulates expression of *TtgGHI* efflux pump to enhance the cell's resistance to the toxicity of this compound [87].

Conclusion

Although there are some slight differentiations between many hydrocarbon-degrading genes in different phylogenetic groups of microbial species, the homology of DNA sequences and organisation of these genes as well as intensive overlapping of the activity of their products indicates that horizontal gene transfers have occurred between these groups. Slight differences in the corresponding hydrocarbon-degrading genes can change the ability of microbes to degrade hydrocarbons, including altering the time of expression (due to presence of inducible or constitutive promoters), the level of expression (due to the activity of the promoter) and the enzymatic activity of the product (due to sequence of amino acids and the protein configuration).

This information assists environmental microbiologists and biotechnologists to choose suitable/stronger hydrocarbon-degrading genes and the hydrocarbon resistance genes in order to create efficient genetic-engineered microorganisms (GEM). Furthermore, such information can be used for the selection of appropriate (non-genetically engineered) microbial consortia with higher hydrocarbon-degrading ability for use as an inoculum in the bioremediation of contaminated sites.

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