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# Biodegradation of polycyclic aromatic hydrocarbons

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**Abstract**

The pathway for the microbial degradation of simple polycyclic aromatic compounds *viz.* naphthalene, anthracene, phenanthrene and substituted naphthalenes are described. Emphasis has been given to the diversity of metabolic reactions for the degradation of naphthalene in bacterial, fungal, algal and mammalian systems. A brief account of the genetics of naphthalene catabolic plasmids with special reference to the plasmid *NAH7* has been given. This is followed by a brief discussion of our present knowledge of the metabolism of phenanthrene, anthracene, methylnaphthalenes and naphthoic acids.

**Keywords:** Polycyclic aromatic hydrocarbons, microorganisms, biodegradation, oxygenases.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds containing carbon and hydrogen with fused benzene rings in linear, angular or cluster arrangements. These compounds have a large (negative) resonance energy. This results in thermodynamic stability which manifests itself in their chemical properties. PAHs may also contain alkyl and nitro substituent groups. The complexity in thesee molecules can be generated by substitution of an aromatic carbon with nitrogen, oxygen or sulfur, giving rise to heterocyclic molecules.

PAHs are universal products of the combustion of organic matter like fossil fuels, saturated and unsaturated hydrocarbons, peptides and carbohydrates. PAHs and their alkyl homologs are widely distributed in soil and aquatic environments[1,2]. Occurrence of PAHs in the environment is due to both natural and anthropogenic processes[3]. Some major sources of PAHs are forest and prairie grass fires, volcanic ash, heat and power generation, refuse burning, motor vehicle emission, industrial processes, petroleum leakage and spills, fallout from urban air pollution, coal liquification and gasification and cigarette smoke[4].

Several theories on PAH synthesis by pyrolysis have been proposed and extensive reviews on this subject are available[3,5,6]. The type of PAHs formed depends on the pyrolysis temperature. At high temperatures (2000°C), unsubstituted PAHs are the principal products. At intermediate temperatures (400–800°C), synthesis of alkyl substituted and unsubstituted compounds takes place. In contrast, petroleum, which is synthesised at low temperatures (80–150°C), contains PAHs with two or three alkyl substituents as the major components[3].

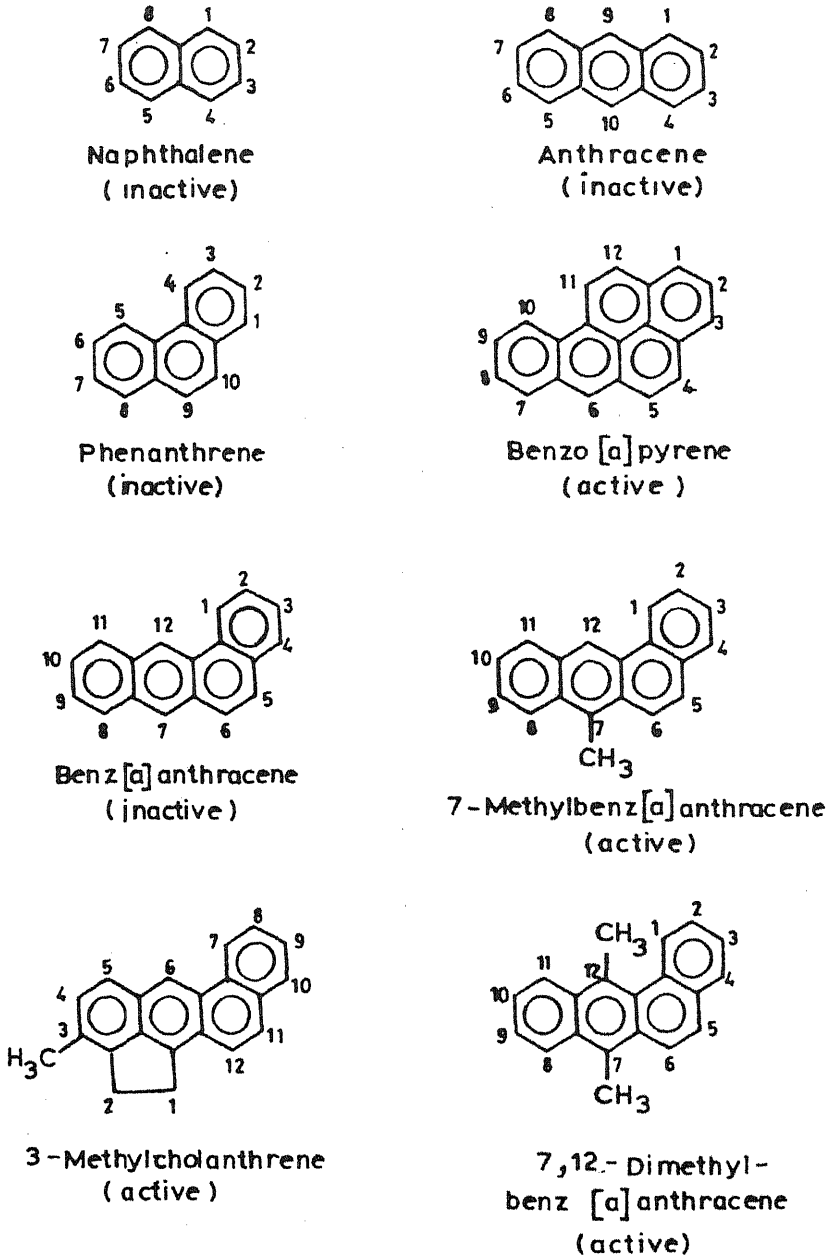


FIG. 1. The structure of polycyclic aromatic hydrocarbons and their biological activity.

The mutagenic and carcinogenic natures of several PAHs make studies of the various aspects of these compounds extremely important. Historically, physician John Hill recognised the relationship between PAHs and carcinogenesis and documented the high rate of nasal cancer as a consequence of excessive use of tobacco snuff[7]. Similarly, Pott also

noted such a relationship in his report on the high rate of scrotal skin cancer in chimney sweepers[8]. In 1932, Kennaway and Heiger showed that dibenz (a,h) anthracene caused cancer in mouse skin[9]. Yamagiwa and Ichikawa induced tumors on the ears of rabbits by repeated application of coal tar[10]. Some of the PAHs found in environmental samples and their biological activity as a carcinogen are summarised in Fig. 1.

Simple PAHs like, naphthalene and subsitituted naphthalenes, are among the most toxic components present in the water soluble fraction of crude and fuel oils[11–13]. During the last few decades, the enormous increase in human activity has resulted in an increase in the concentration of PAHs in the urban environment[14].

The role of microorganisms in maintaining steady state concentrations of environmental chemicals is well established. These activities ensure a smooth operation of the Earth's carbon cycle. It has been generally accepted that all biosynthetic compounds are degraded by microorganisms. However, manmade compounds which are structurally different from naturally occuring compounds and of recent origin, may not be metabolised by microorganisms, since sufficient time has not elapsed for the evolution of the requisite enzyme systems[15,16]. Although most of the aromatic hydrocarbons in the environment do not have a biosynthetic origin, they do have a natural pyrolytic origin and must have been in contact with living organisms throughout evolution. Thus, it is understandable that microorganisms can degrade a few PAHs.

As many excellent reviews on the microbial degradation of PAHs are available[4,17,21] in this article, we will be discussing the microbial degradation of simple PAHs *viz.* naphthalene; anthracene, phenanthrene and substituted naphthalenes, which form part of complex and carcinogenic PAHs like benzo[a]pyrene, benzo[a]anthracene, 3-methyl-cholanthrene, 7,12-dimethylbenz[a] anthracene, etc.

## 2. Metabolism of naphthalene

### 2.1. *Bacterial oxidation of naphthalene*

The ability of bacteria to utilise naphthalene as the sole source of carbon and energy was reported as early as 1927[22,23]. Later Strawinski and Stone isolated salicylic acid from culture filtrates of *P. aeruginosa* grown on naphthalene[24]. Since that time, a number of investigators have identified salicylate as an intermediate in naphthalene degradation[25–28]. However, the metabolic sequence and the enzymatic reactions leading to the degration of naphthalene were first described by Davies and Evans[29].

The degradation of naphthalene in bacteria is inititated by double hydroxylation of one of the aromatic rings to form a dihydrodiol intermediate. Walker and Wiltshire reported that an unidentified gram negative organism oxidises naphthalene to D-*trans*-1,2-dihyroxy-1,2-dihydronaphthalene[25]. Subsequently, *trans*-naphthalene dihydrodiol was also detected in three *Pseudomonas* strains and a *Nocardia* strain metabolizing naphthalene[30]. Gibson and his coworkers isolated a mutant strain of *Pseudomonas putida*, which converts naphthalene to dihydrodiol naphthalene. A detailed analysis of dihydrodiol by NMR spectroscopy provided conclusive proof that it is a *cis*-naphalene dihydrodiol. Re-

duction of this metabolite with hydrogen in the presence of palladium gave (-)-2(S)-hydroxy-1,2,3,4-tetrahydronaphthalene, which established the structure of bacterial naphthalene[31]. In addition, experiments with $^{18}O_2$ showed that both atoms of oxygen in *cis*-naphthalene dihydrodiol come from a single molecule of oxygen[32]. Similar results have been reported for *Pseudomonas* sp. NCIB 9816[33]. The enzyme involved in the conversion of naphthalene to *cis*-naphthalene dihydrodiol is a naphthalene dioxygenase which has three protein components[34].

The first component of naphthalene dixoygenase accepts electrons from NADH and is termed as NADH-ferredoxin$_{NAP}$ reductase. This reductase has been purified to homogeneity and has an Mr of 36,300. It is an iron-sulfur flavoprotein[35]. The purified protein contains one mole of FAD per mole of enzyme protein, 1.8 g atoms of iron and 2.0 g atoms of acid-labile sulfur and shows absorption maxima at 278, 340, 420 and 460 nm with a broad shoulder at 540 nm. It also catalyses the reduction of cytochrome c, dichlorophenol indophenol, nitroblue tetrazolium and ferricyanide. It functions as an NAD(P)H oxidoreductase in the naphthalene dixoygenase system.

The second component of naphthalene dixoygenase has been purified to homogeneity[36]. The protein contains 2.0 g atoms each of iron and acid-labile sulfur and its Mr is calculated to be 13,600. It is a ferredoxin type of protein that functions as an intermediate electron transfer component in naphthalene dioxygenase activity.

The third and terminal component of naphthalene dioxygenase is an oxygenase component. It has been purified from *P. putida* NCIB 9816[37]. The purified enzyme oxidises naphthalene only in the presence of NADH, oxygen and the other two components. The Mr of the negative enzyme is estimated to be 158,000. On SDS-PAGE it gave two bands with Mr of 55,000 and 20,000, suggesting a $\alpha_2\,\beta_2$ quaternary structure for this component. It is an iron-sulfur protein with 6.0 g atoms of iron and 4.0 g atoms of acid labile sulfur. The oxidized enzyme shows absorption maxima at 566, 462, and 344nm. The enzyme has also been found to bind to naphthalene. Thus, this oxygenase component takes part in the terminal step of naphthalene oxidation. The overall scheme of the reaction of naphthalene dioxygenase is shown in Fig. 2.

The second step in the bacterial oxidation of naphthalene is the conversation of *cis*-1,2-dihydroxy-1,2-dihydronaphthalene to 1,2-dihydroxynaphthalene. This reaction is catalysed by an NAD dependent naphthalene *cis* (+) dihydrodiol dehydrogenase. This enzyme is a homotetramer with a subunit Mr of 25,500. It can act on other *cis*-dihydrodiols, but is highly stereospecific to (+) isomer of *cis*-1,2-dihydroxy-1,2-

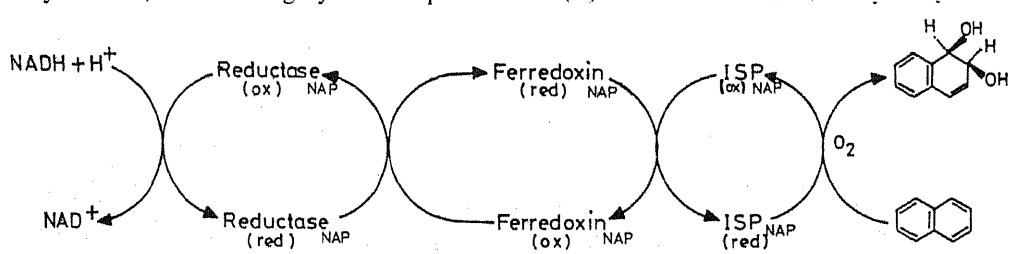


FIG. 2. Proposed electron transport scheme for naphthalene dioxygenase.

dihydronaphthalene and cannot act on *trans*-1,2 dihydroxy-1,2-dihydronaphthalene[38]. Davies and Evans proposed that 1,2-dihydroxy naphthalene is enzymatically cleaved by a dioxygenase to yield *cis*-2′-hydroxy-benzal pyruvic acid, which is then converted *via* a series of reactions to salicylate and pyruvate[29]. However, Bansley showed that *Pseudomonas* sp. NCIB 9816 and other pseudomonads oxidise, which is enzymatically converted by an isomerase to *cis*-2′-dihydroxybenzal pyruvate[39]. Eventually, Patel and Barnsley purified 1,2-dihydroxy naphthalene dioxygenase from *P. putida*. It has a native Mr of over 275,000 and a subunit Mr of 19,000. The enzyme requires $Fe^{++}$ for activity[40]. However, the enzyme purified from *P. putida* CSV86, in our laboratory, has a native Mr of 316,000 and a subunit Mr determined by SDS-PAGE of 32,000 and requires $Fe^{++}$ for activity[41]

The generated *cis-ortho*-hydroxybenzalpyruvic acid is further metabolised to salicylate. In 1964, Davies and Evans showed that cell free extracts prepared from naphthalene grown cells contained an aldolase which converted both *cis* and *trans*-hydroxybenzalpyruvate into salicylaldehyde and a three carbon fragment identified as pyruvate. The same cell free extract contained an NAD-specific dehydrogenase that oxidises salicylaldehyde to salicylate.

In most cases, the salicylate derived from naphthalene is oxidized to catechol, which is the substrate for ring fission by the *meta* or the *ortho* payway giving rise to 2-hydroxy muconic semialdehyde or *cis*, *cis*-muconic acid, respectively. However, the formation of gentisic acid during naphthalene metabolism by *P. fluorescens* and *P. alcaligens* has been reported[42] and the metabolism of salicylate *via* gentisate has also been reported[43,44]. The pathway for the catabolism of naphthalene is pseudomonads in shown in Fig. 3.

### 2.2. *Metabolism of naphthalene in fungi*

A wide variety of taxonomically and phylogenetically different fungi are known to metabolise naphthalene[45–47]. Studies on the fungal metabolism of naphthalene have shown that the reactions involved are different from those reported for the bacterial oxidation of naphthalene.

Unlike bacteria, fungi incorporate only one atom of molecular oxygen into one of the naphthalene rings to give rise to naphthalene 1,2-oxide. This reaction is catalysed by a cytochrome P-450 dependent monooxygenase. the arene oxide generated is highly unstable, and it could be converted to 1-naphtol (major) and 2-napthol (minor) by rearrangement *via* the NIH shift mechanism. Another route for further oxidation of the arene oxide is by enzymatic hydration catalysed by epoxide hydrolase to form(+)*trans*-(1S,2S)-dihydroxy-1,2-dihydronaphthalene. The formation of *trans*-dihydrodiolnaphthalene, 9:4 ratio of 1-naphthol and 2-naphthol and occurrence of NIH shift strongly suggest the formation of naphthalene 1,2-oxide as the initial product in the oxidation of naphthalene in fungi[48–50].

Although these reactions are similar to those reported fror mammalian systems, their stereo specificity differ[51–54]. The *C. elegans* enzyme produces the (+)-(1S,2S) isomer of *trans*-dihydrodiol naphthalene while the mammalian system (enzyme purified from rat,

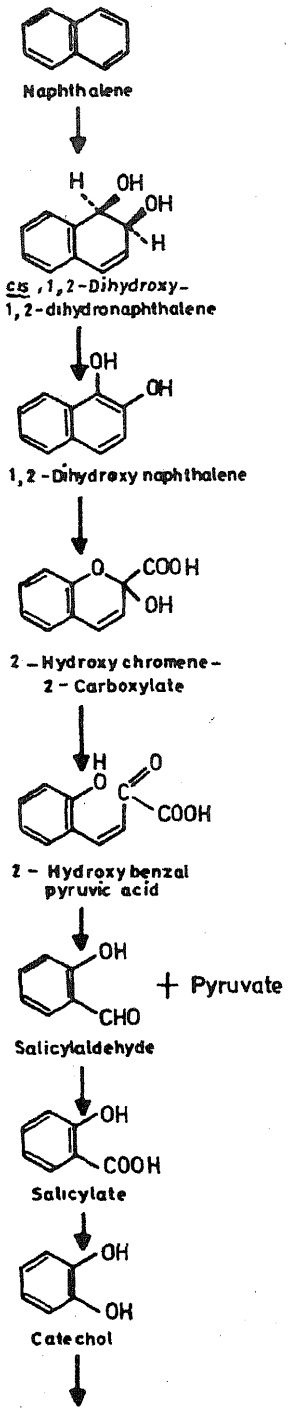


FIG. 3. Pathway for the degradation of naphthalene by bacteria.

rabbit, mice and pig microsomes) produces the (-) (1R,2R) isomer 52. In addition to these compounds, *C. elegans* produces 4-hydroxy-1-tetralone, 1-naphthol, 2-naphthol, *trans*-hydrodiol naphthalene, 1,2-naphthoquinone and 1,4-naphtho-quinone. It is possible that the *trans*-dihydrodiol naphthalene may be converted to 1,2-dihydro-xynaphthalene and 1-naphthol to 4-hydroxy-1-tetralone *via* the formation of 1,4-dihy-droxynaphthalene. The naphthoquinones accumulate due to aerial oxidationof the corresponding dihyroxy naphthalenes, Interestingly, bacteria and cyanobacteria also produce 4-hydroxy-1-tetralone when they are incubated with 1-naphthol[49,55-58].

Fungi also possess the ability to form glucuronide and sulfate conjugates of phenolic aromatic hydrocarbons. Cerniglia and his co-workers isolated 1-naphthyl glucuronic acid and 1-napthyl sulfate as the major water soluble metabolites from the spent medium[59]. In addition, the activity of UDP-glucuronosyl transferase has been demonstrated in the cell free extracts of *C. elegans*[60]. This is probably to overcome the toxic effects of 1-naphthol by glucuronide and sulfate conjugation making it more water soluble. This is then eliminated from the system through the detoxification process, in a manner similar to that found in mammalian systems. Thus, fungi could serve as a model system to study the mechanism of detoxification and chemical carcinogenesis in mammals. The pathway(s) for the fungal oxidation of naphthalene is shown in Fig.4.

### 2.3. *Metabolism of naphthalene in algae*

The prokaryotic and eukaryotic algae possess the ability to oxidize naphthalene. Three strains of cyanobacteria, *Agmenellum quadruplicatum* PR-6, *Coccochloris elabens*-17A and *Oscillatoria* sp. JCM, when grown photoautotrophically in the presence of radioactive naphthalene oxidized it to form 1-naphthol, *cis*-dihydrodiol naphthalene and 4-hydroxy-1-tetralone[58,61]. $^{18}O_2$ incorporation studies in *Oscillatoria* sp. JCM showed 57% of this oxygen to be present on 1-naphthol. These results suggest that the algae could oxidize napthalene to form naphthalene 1,2-oxide as an intial product, which could isomerise to form 1-naphthol and subsequently to 4-hydroxy-1-tetralone. This pathway for generation of 1-napthol is similar to that described previously for fungi and mammals. An NIH shift mechanism is also implicated for the formation of 1-naphthol by cyanobacteria[62]. The formation of cis-dihydrodiol naphthalene could be similar to a dioxygenase reaction described earlier for bacteria. The further fate of this compound could not be demonstrated. However, Ellis has demonstrated that fresh water algae can oxidize phenol and catechol to carbon dioxide[63]. Thus, it appears that like bacteria, algae may have the potential to degrade naphthalene to carbon dioxide and water. Apart from Cyanobacteria, five green algae, one red algae, one brown alga and two diatoms were found to oxidize naphthalene[64].

## 3. Genetics of naphthalene catabolism in pseudomonads

Pseudomonads are a diverse group of organisms which have the ability to utilise various carbon compounds as the sole source of carbon and energy. Many of these organisms have been shown to carry plasmid bearing genes coding for the enzymes involved in the metabolism of compounds such as salicylate, camphor, naphthalene, toluence, xylene and chlorinated aromatic hydrocarbons[65]. These plasmids are termed as degradative

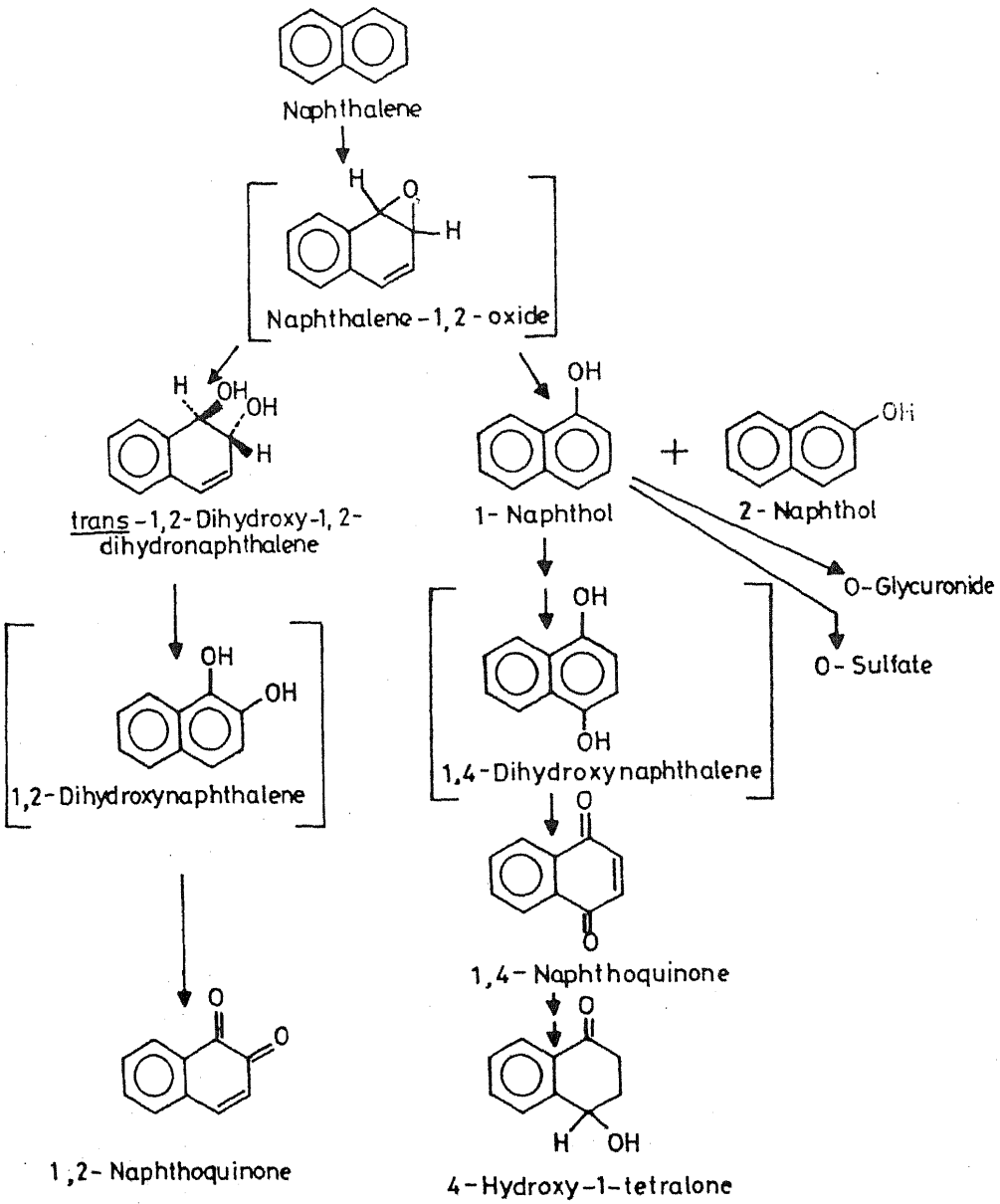


FIG. 4. Oxidation of naphthalene by fungi.

plasmids. Some of the degradative plasmids characterised to fine detail are TOL, CAM, OCT and NIC[66–70] for the degradation of toluence, camphor, octane and nicotine, respectively. The genes responsible for the degradation of naphthalene are also found to be associated with plasmids. Naphthalene catabolic plasmids isolated from various pseudomonads are listed in Table 1. All these plasmids are large in size, but are amenable to

**Table I**
**Naphthalene catabolic plasmids in pseudomonads***

| *Plasmid* | *Size (kb)* | *Incompatibility group* | *Pathway*[a] | *Host* |
|---|---|---|---|---|
| NAH7 | 83 | P9 | *meta* | *P. putidaG1* (ATCC 17453) |
| NAH2 | 116 | nd[b] | *meta* | *P. putida* NCIB 9816–3 |
| NAH3 | 116 | nd | *meta* | *P. putida* NCIB 9816–2 |
| pWW60 | 87 | P9 | *meta* | *P. putida* PGBI (NCIB 9816–3) |
| pWW60-1 | 87 | P9 | *meta* | *P. putida* PaW701 |
| pDTG1 | 81 | nd | *meta* | *P. putida* (NCIB 9816–4) |
| pND140 | nd | P9 | *meta* | nd |
| pND160 | nd | P9 | *meta* | nd |
| pDTG15 | | nd | *meta* | *P. putida* C84–1 |
| pBS3 | nd | P7 | *meta* | *P. fluorescence* BS243 |
| pBS2 | nd | P7 | nd | *P. putida* BS238 |
| pBS4 | 173 | P7 | gentisate[c] | *P. fluorescence* BS291 |
| NPL-1 | nd | P9 | upper pathway[d] | *P. putida* 12A |

*Source: Reference 76 a: catechol ring opening
b: nd, not determined c: gentisate as an intermediate
d: degradation of naphthalene to salicylate

physical isolation and biochemical and genetic analysis. They belong to the incompatability groups P7 or P9. The first and the best-studied naphthalene catabolic plasmid is NAH7.

### 3.1. *The NAH7 plasmid*

Dunn and Gunsalus, in 1973 showed for the first time the presence of a naphthalene catabolic plasmid NAH in *P. putida* strain G7 (ATCC 17485) which was of naphthalene ($Nah^+$) and salicylate ($Sal^+$) utilizing phenotype[71]. This plasmid was subsequently analysed in detail in the derivatives of *P. putida* strain G1 (PpG1) (ATCC 17453) and was renamed NAH7. The plasmid is large, 83 Kb in size, conjugative in nature and can be cured by treatment with mitomycin C[72]. Subsequently, the presence of NAH7 was confirmed by the isolation of plasmid DNA from a PpG1 derivative, which when introduced into an isogenic strain lacking the plasmid, reestablished the $Nah^+$ $sal^+$ phenotype[73].

The genes on NAH7 coding the enzymes for the first 11 steps of the naphthalene degradative pathway have been mapped (Fig.5). Their organisation and direction of transcription have been determined by generating a series of polar mutations on NAH7 using transposon Tn5[74]. The pattern of enzyme induction in these polar mutants revealed the presence of two *nah* operons. The first operon includes the genes *nahABCDEF*, coding

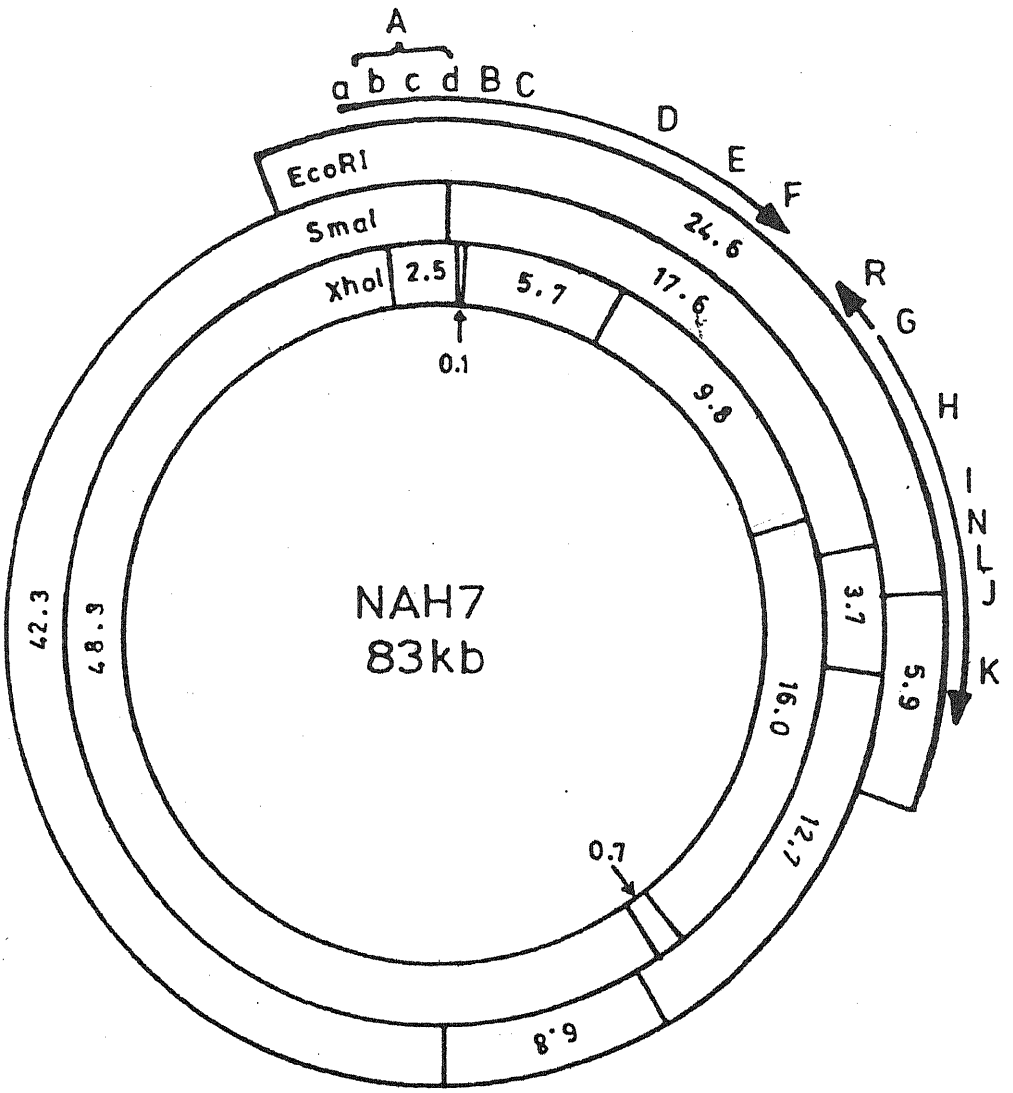


FIG. 5. Restriction map of NAH7 plasmid. The naphthalene catabolic genes are marked by capital letters and their directions of transcription are marked by arrows. The numbers denote kilobases.

for the conversion of naphthalene to salicylate (upper pathway), and the second operon includes the genes *nahGHIJK*, coding for the oxidation of salicylate *via* the catechol *meta* cleavage pathway to acetylaldehyde and pyruvate (lower pathway). The polarity of each NAH7 mutation revealed that the transcription of the first operon is from *nahA* to *nahF* and the second operon is transcribed from *nahG* to *nahK*. The same gene order has been demonstrated by electron microscope heteroduplex mapping of Tn5 insertions in each of the NAH7::Tn5 molecules[75].

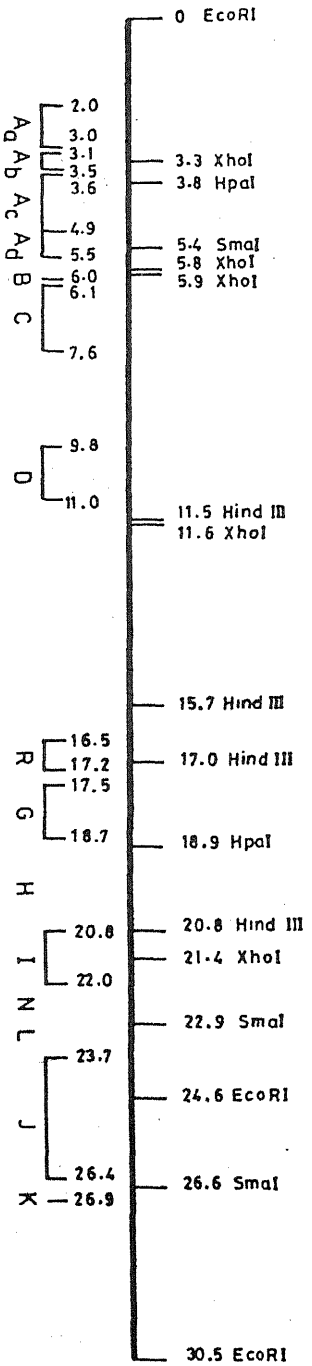


FIG. 6. Restriction and genetic maps of the region of NAH7 plasmid containing the naphthalene catabolic genes. The numbers denote kilobases. Naphthalene catabolic genes are marked by capital letters.

By restriction mapping of NAH7::Tn5 plasmids (Fig.6), all the *nah* genes have been located within the 30 kb region of the NAH7 plasmid[74,76]. The flavoprotein gene *nah* $A_a$ is upstream from the ferredoxin gene, $nahA_b$, which is followed by the two genes $nahA_c$ and $nahA_d$, encoding two subunits of the terminal dioxygenase[77]. The *nahC*, which codes for 1,2-dihydroxy naphthalene dioxygenase, has been cloned and sequenced recently[78]. The amino acid sequence, deduced from the nucleotide sequence of *nahC*, does not show any significant homology with the amino acid sequence deduced from *nahH* (which codes for catechol1 2,3-dioxygenase) or other extradiol or intradiol dioxygenases[78,79].

Ensley and his co-workers cloned a 10.5 kb EcoRI-HinIII DNA fragment of NAH7 into *E. coli*-HB101. The resultant recombinant plasmid, termed as pE317, could transform naphthalene into salicylate[80]. Thus, it shows that the 10.5 kb fragment contains the genes coding for the upper pathway of naphthalene catabolism. In this recombinant *E. coli* HB101, these genes are expressed contitutively.

### 3.2. *Regulation of naphthalene catabolic genes on NAH7 plasmid*

The *nah* operons on NAH7 are inducible. They are expressed only in the presence of an inducer and the product of a regulatory gene. Both the *nah* operons, which contain all the genes essential for naphthalene oxidation can be induced by salicylate and 2-aminobenzoic acid[74,81,82]. They are also induced when the cells are grown in the presence of naphthalene. However, naphthalene is not an inducer of NAH7 encoded naphthalene oxidation pathway. Effective induction in the presence of naphthalene requires the expression of the *nah ABCDEF* operon from *nahA* to at least the *nahD* gene. However, induction does not depend on the expression of the lower *nah* operon. Also, Tn5 insertion in *nahA*, *nahB*, *nahC* and *nahD* resulted in the complete blockage of naphthalene induced induction, and the same insertion in *nahG* and *nahI* regions did not show any effect[76]. These results show that, the substrate naphthalene and its initial oxidation products like, *cis*-naphthalene dihydrodiol, 1,2-dihydronaphthalene, and 2-hydroxychromene-2-carboxylic acid do not induce the *nah* operons on NAH7. It was eventually concluded that due to the low level constitutive expression of the first *nah* operon, naphthalene gets converted to the inducer, namely salicylate. Thus, salicylate (or possibly salicylaldehyde acts as an inducer of both *nah* operons. The same conclusion was reached for the naphthalene catabolic pathway specified by *Pseudomonas* sp. ATCC 17483[83].

The role of a regulatory gene in the induction of *nah* operons was predicted by the analysis of a third class of Tn5 insertion mutations which yielded a $nah^-$ $sal^-$ phenotype[74]. These mutations are mapped within a small region of NAH7 upstream of the *nahG* gene. The mutation and deletion of this region resulted in the complete loss of the ability of the bacterium to degrade naphthalene. This results in a $nah^-$ $sal^-$ phenotype, which can be fully corrected in the presence of a wild type allele in a *trans* position[84,85]. These mutations are pleiotropic, recessive and negative. This indicates that the product of the regulatory gene *nahR*, is required to activate both *nah* operons. Further studies showed that in a *nahR*::Tn5 mutant, the salicylate induced mRNA synthesis of *nahA* to *nahG* genes is

completely blocked[81]. This suggests a role for the *nahR* gene product at the level of transcription.

The potential binding site of the *nahR* gene product has been investigated by detailed analysis of promoter regions of both *nah* operons. The map of Tn5 insertion mutations in *nahA*, *nahR* and *nahG* regions revealed that the promoters of both the operons are in close proximity to each other[74, 84]. For more accurate mapping of these promoters, the DNA regions containing promoters have been cloned and mapped by identifying the regions protected from $S_1$ nuclease digestion. The nucleotide sequence of these promoter regions has been determined[86]. The results of such studies revealed the presence of two start sites separated by a 10 bp stretch for each of the two *nah* operons.

The approximate transcription initiation site and direction of transcription of the *nahR* have been determined by $S_1$ nuclease mapping. The direction of transcription of the *nahR* gene is opposite to that of the *nahG* gene[86]. As in the *nah* promoters, two apparatent start sites separated by a small A + T rich region were detected in the *nahR* promoter region. Various restriction fragments from the *nahR* region have been cloned and tested for their transacting activities in order to determine the size of the *nahR* gene product[87]. The presence of a 1.8kb HindIII-PstI region including approximately 350 bases upstream from the first base of the putative *nahR* coding region and 1.4kb downstream from the same site, activated the expression of the *nahA* and *nahG* genes. Thus, it appears that the size of the *nahR* gene may not be more than 1.4kb, which can code for a polypeptide of 51kD. The product specified by the 1.8kb region in an *E. coli* maxicell system, a polypeptide of 36kD was identified. As this polypeptide was not detected in the strains containing *nahR* deletions, it was concluded to be the *nahR* gene product.

The binding of the *nahR* gene product to the promoter region was shown by gel retardation assay. Extracts of *E. coli* or *P. putida* containing the functional *nahR* gene showed a specific binding to the DNA fragment containing both the *nah* promoters. Partially purified nahR protein protects both promoter sequences between -82 and -47. Analysis of mutants in this region showed that the nahR protein is involved in transcription activation[88].

The mRNA of the *nahR* is synthesised both in the absence or presence of salicylate, indicating that *nahR* is probably synthesised constitutively. The nahR protein may exist in two forms, an inactive form (nahRi) and an active form (nahRa), which are in equilibrium. In the absence of an inducer, nahRi is the predominant form, and presence of an inducer shifts the equilibrium towards the formation of nahRa[86,88]. Such, self regulationof *nahR* is similar to other regulatory proteins like L-arabinose and maltose regulons from *E. coli* and several other positively regulated systems[89].

## 4. Plasmids from strains of *P. putida* NC1B 9816

*Pseudomonas* sp. NC1B 9816 was first isolated by Davis and Evans and later identified as *P. putida*[29]. Differences in the regulation of the naphthalene catabolic pathway by this strain used in different laboratories have been reported[82,90,91]. Based on studies on the regulation and induction of the enzymes of upper and lower pathways of naphthalene deg-

radation, the *P. putida* NCIB 9816 was subclassified into three strains NCIB9816-2, NCIB 9816-3 and NCIB 9816-4[91–93]. In all these strains the naphthalene catabolic genes were found to be on plasmids.

### 4.1. *Plasmids from strain NCIB 9816-3*

In this strain two plasmids have been detected, one large (termed as Nah2, 116kb) and another small cryptic plasmid[92]. However, in the same strain, Cane and Williams reported the size of the large plasmid to be 87 kb and designated it as pWW 60-1 and a small cryptic plasmid[93]. All the genes of the naphthalene degradation pathway are on the large plasmid. This plasmid contains all the genes responsible for the conversion of naphthalene to catechol. Further, ring cleavage of catechol occurs by the *ortho* cleavage pathway and the necessary genes are on the chromosome. However, selection of growth on 2-methylnaphthalene, whose effective degradation requires a nonspecific catechol metabolic pathway, has led to the isolation of mutants with a fully functional catechol *meta* cleavage pathway. Each of the mutants carried a plasmid identical to pWW 60-1 except for the presence of a small deletion of 1.2 to 1.5kb between *nahG* and *nahH* gene. This shows that the pWW 60-1 harbours the genes for the lower *meta* pathway of naphthalene degradation, but is silent. The deletionof 1.2-1.5kb fragment between *nahG* and *nahH* seems to activate this lower pathway wherein catechol is cleaved by *meta* fission.

Studies by transposon mutagenesis on pWW 60-1 revealed the presence of two operons as in the case of NAH7. Most interestingly, the two *nah* operons on pWW 60-1, contrary to their counterparts of NAH7 , are transcribed in opposite directions[94].

### 4.2. *Plasmids from the strain NCIB 9816-4*

This strain harbours a single, 81kb, conjugative plasmid designated as pDTG1 which is involved in the degradation of naphthalene[91]. The 15kb EcoRI fragment of pDTG1 has been cloned in *E. coli* using the plasmid pKT230 as a vector[80]. The resultant recombinant plasmid was called as pDTG113. The *E. coli* cells containing pDTG113, when grown in the presence of naphthalene accumulate salicylate, suggesting that the 15kb EcoRI fragments harbor the genes of the upper pathway of naphthalene degradation[91]. Within this 15kb EcoRI fragment, an 8kb EcoRI-XhoI region harbours *nahABC*, the first three genes of the upper pathway[94].

Recently, Denome and his colleagues cloned and sequenced a 9.8kb DNA fragment which encodes dibenzothiophene-degrading enzymes from soil isolate *Pseudomonas* strain C18[95]. The cloned DNA fragment was found to encode enzymes capable of metabolising dibenzothiophene, naphthalene and phenanthrene. This DNA sequence was found to contain 9 OFRs and was designated as doxABCDEFGHIJ. At the nucleotide level doxABD is identical to the *ndoABC* gene that encodes naphthalene dioxygenase. The doxG protein is 97% identical to NahC (1,2-dihyroxynaphthalene dioxygenase) of *P. putida*. The DoxE has 37% identity with *cis*-toluence dihyrodiol dehydrogenase. DoxF is similar to the aldehyde dehydrogenases of many organisms. The DOX proteins convert naphthalene to salicylate and phenanthrene to 1-hydroxy-2-naphthoic acid. Comparison

**Table II**
**Various transposons involved in the aromatic hydrocarbon degradation.**

| *Transposon* | *Size (kb)* | *Degradation property* | *Reference* |
|---|---|---|---|
| Tn 4651 | 56 | Xylene/Toluene | 98 |
| Tn 4653 | 70 | Xylene/Toluene | 98 |
| Tn 4655 | 55 | Naphthalene | 99 |
| Tn 5280 | 17 | Chlorobenzene | 100 |
| Tn 5271 | 17 | Chlorobenzene | 101 |
| Tn 4371 | 59 | Biphenyl/4- | 102 |

of the DOX sequence with the restriction map of cloned naphthalene catabolic pathway genes (NAH) revealed many conserved restriction sites. Also the gene arrangement is identical to that proposed for NAH[95].

## 5. Transposons in hydrocarbon degradation

Transposable elements including both transposons and insertion sequences (IS) are common in various *Pseudomonas* species. These elements are responsible for antibiotic and heavy metal resistance. Earlier studies indicated that the genes encoding the degradation of hydrocarbons were transposable[96,97]. Recently, these elements were shown to be involved in the degradation of the aromatic hydrocarbons. Various transposons involved in the degradation of aromatic hydrocarbons are listed in Table II.

In 1990, Tasuda and Iino reported that the genes present on NAH7 plasmid, responsible for the naphthalene degradation are present on the defective transposons[98]. The naphthalene degrading genes on NAH7 plasmid were found to be transposable only in the presence of transposases encoded by Tn1721 subgroup of the class II transposons. This new naphthalene transposon is designated as Tn4655 (Fig. 7). This transposon is transposed by a two step process involving formation of cointegrate followed by its subsequent resolution. In contrast to the defects in the transacting factor for the first step, a functional system for the second step was encoded within a 2.4kb region[99].

## 6. Metabolism of phenanthrene and anthracene

A considerable amount of information exists on the microbial degradation of phenanthrene and anthracene. Anthracene and phenanthrene and their metabolites are not actually toxic, carcinogenic, or mutagenic. However, presence of these structures in carcinogenic compounds like benzo (a)pyrene, benz(a)anthracene, and 3-methylcholanthrene, allowed the use of these compounds as model substrates.

Pure culture of bacteria and microbial population isolated from fresh water and marine environments have the ability to degrade these compounds as the sole source of carbon and energy[103–110].

The initial reaction involved in the degradation of phenanthrene is oxidation at the 3,4-position to form the dihydrodiol. Colla and his coworkers isolated 3,4-dihydroxy-3,4-dihydrophenanthrene from the culture filtrates of *Flavobacterium*[104]. Subsequent

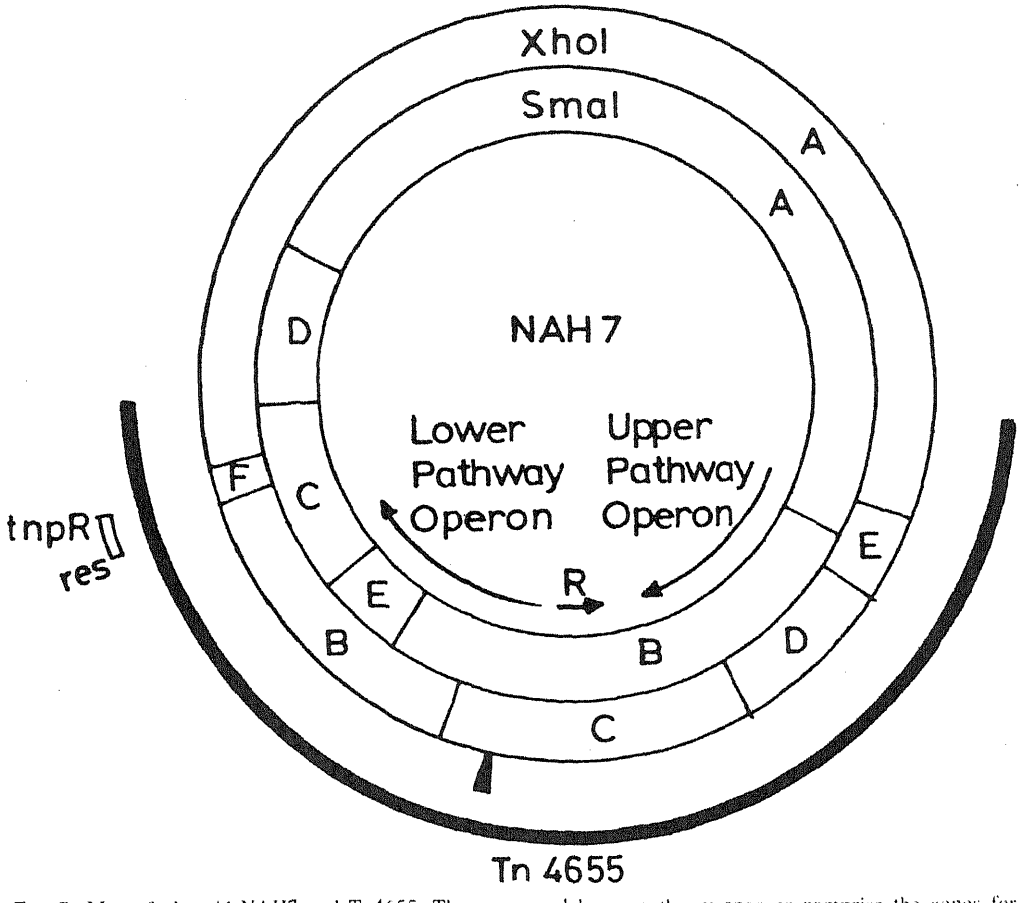


FIG. 7. Map of plasmid NAH7 and Tn4655. The upper and lower pathway operons comprise the genes for conversion of naphthalene to salicylate and salicylate to the central metabolites *via* catechol.

studies by Evans and coworkers led to the suggestion that phenanthrene is oxidised to *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene, which undergoes enzymatic dehydrogenation to form 3,4-dihydroxy phenanthrene[105]. However, later Gibson and his colleagues isolated a mutant of *P. putida* and *Beijerinckia* species that oxidised naphthalene and biphenyl to *cis*-naphthalene dihydrodiol[32], and *cis*-biphenyl dihyrodiol[111], respectively. When these strains were grown on succinate in the presence of phenanthrene, two dihydrodiol intermediates were detected[107]. The major product (less than 90%) is readily dehydrated to 3-phenanthrol, and analysis of its NMR spectrum clearly established its structure as (+)-*cis*-3,4-dihyroxy-3,4-dihydrophenanthrene. The minor product was identified as *cis*-1,2-dihyroxy-1,2- dihydrophenanthrene. The true intermediate in the phenanthrene degradation is 3,4-dihydrodiol. Support for its involvement in the degradation pathway comes from the isolation of 1-hydroxy-2-naphthoic acid from the culture filtrates of *Pseudomonas* sp. Further, sequential induction experiments suggested 3,4-dihydroxyphenanthrene, 1-hydroxy-2-naphthoic acid, salicylate and catechol as intermediates in phenanthrene metabolism[103–105].

In an elegant series of experiments, Evans and his colleagues showed that the cell free extract prepared from phenanthrene grown *Pseudomonas* sp. contained a ferrous iron dependent oxygenase that oxidised 3,4-dihydroxyphenanthrene to an orange coloured ring fission product, *cis*-4-(1-hydroxynaphth-2-yl)-2-oxobut-3-enoic acid[105]. Further, when crude extracts were supplemented with NAD, both 3,4-dihydroxyphenan threne and 1-hydroxynaphthaldehyde were converted to 1-hydroxy-2-naphthoic acid. The generated naphthoate was converted to 2-hydroxymuconic semialdehyde by intact cells. These results suggested that 1-hydroxy-2-naphthoic acid undergoes oxidative decarboxylation to 1,2-dihydroxynaphthalene before being metabolised to salicylate and catechol. The proposed pathway for the degradation of phenanthrene is given in Fig. 8.

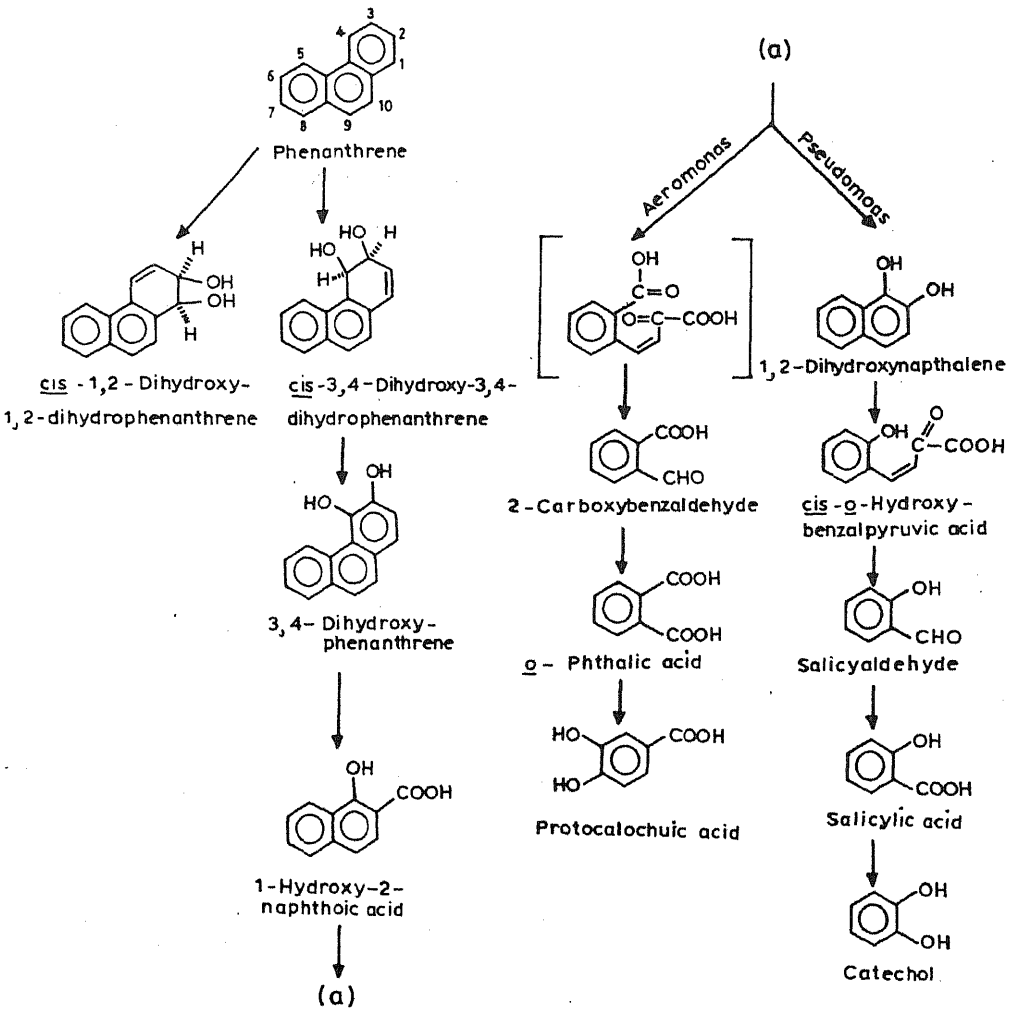


FIG. 8. Pathways for the degradation of phenanthrene by bacteria.

Kiyohara and his colleagues[112] proposed a different pathway for the degradation of phenanthrene (Fig. 8). Phenanthrene grown cells of *Aeromonas* sp., S45P1, oxidized phenanthrene to 1-hydroxy-2-naphthoic acid, 2-carboxybenzalde-hyde, o-phthalic acid, and protocatechuic acid. However, cells failed to oxidise salicylate and catechol. The enzyme responsible for the conversion of 1-hydroxy-2-naphthoic acid to phthalate has not been purified. Preliminary studies[113] suggest that an oxygenase, similar to gentisate[114], homogentisate[115] and 1,4-dihydroxynaphthoate[116] oxygenase, cleaves 1-hydroxy-2-naphthoate between C-1 and C-2. Subsequent reactions leading to 2-carboxybenzaldehyde would then involve a hydration and an aldol fission similar to those proposed in the conversion of 1,2-dihyroxynaphthalene to salicylate.

The initial step involved in the metabolism of anthracene is the double hydroxylation of one of the rings giving rise to *trans*-1,2-dihydroxy-1,2-dihydroanthracene[105, 117]. Subsequent studies by Gibson and his coworkers showed that *P. putida* strain[119] and a *Beijerinckia* sp. strain B-836, both oxidized anthracene to (+)-*cis*-1,2-dihydroxy-1,2-dihydroanthracene[107]. In each case the dihydrodiol was shown to be the optically pure 1(R), 2(S) enantiomer. Evans and coworkers further reported the metabolism of anthracene by soil pseudomonads[105]. They also found that cell yield with anthracene was extremely low. However, they were able to confirm the sequential induction experiments of Rogoff and Wender[103] which suggested that the 2-hydroxy-3-naphthoate, salicylate, and catechol were intermediates in the anthracene degradation pathway. In addition, anthracene grown cells were able to oxidise, 1,2-dihydroxy anthracene and 2-hydroxy-3-naphthoate rapidly. The cell free extract prepared from the cells converted 1,2-dihydroxyanthracene to *cis*-4-(2-hydroxynapth-3-y1)2-oxo-but-3-enoic acid and 2-hydroxy-3-naphthaldehyde. When the ring fission product or 2-hydroxy-3-naphthaldehyde was incubated with cell free extract and NAD, 2-hydroxy-3-naphthoic acid was the only detectable product. This acid was first isolated from the culture filtrate of *Pseudomonas* sp.[103]. The further metabolism of this compound has not been established. The anthracene grown cells failed to oxidise salicylate and catechol. The further metabolism of 2-hydroxy-3-naphthoate and its role in the anthracene metabolism remain to be elucidated. The pathway proposed for anthracene metabolism by bacteria is shown in Fig. 9.

Although there have been numerous studies on the bacterial oxidation of these compounds, very little is known about the metabolism of these dompounds in the fungal systems. Cerniglia and his colleagues, in 1982 showed that, *Cunninghamella elegans* oxidises anthracene to *trans*-1,2-dihydroxy-1,2-dihydroanthracene and 1-anthryl-sulfate[118]. The formation of *trans* configuration and $^{18}O$ experiments suggested a mono-oxygenase catalysed reaction and indicated the prior formation of anthracene1-,2-oxide. These results are similar to those obtained in the studies on the mamalian metabolism of anthracene[106,119,120].

Phenanthrene is metabolised by rat hepatic microsomes and by highly purified cytochrome P-450 monooxygenase preparation to phenanthrene *trans*-1,2-, -3,4-, and -9, 10-dihydrodiols as well as phenolic products[120–123]. However, phenanthrene *trans*-9, 10-dihydrodiol is the major product. The fungus *C. elegans* oxidised phenanthrene at the

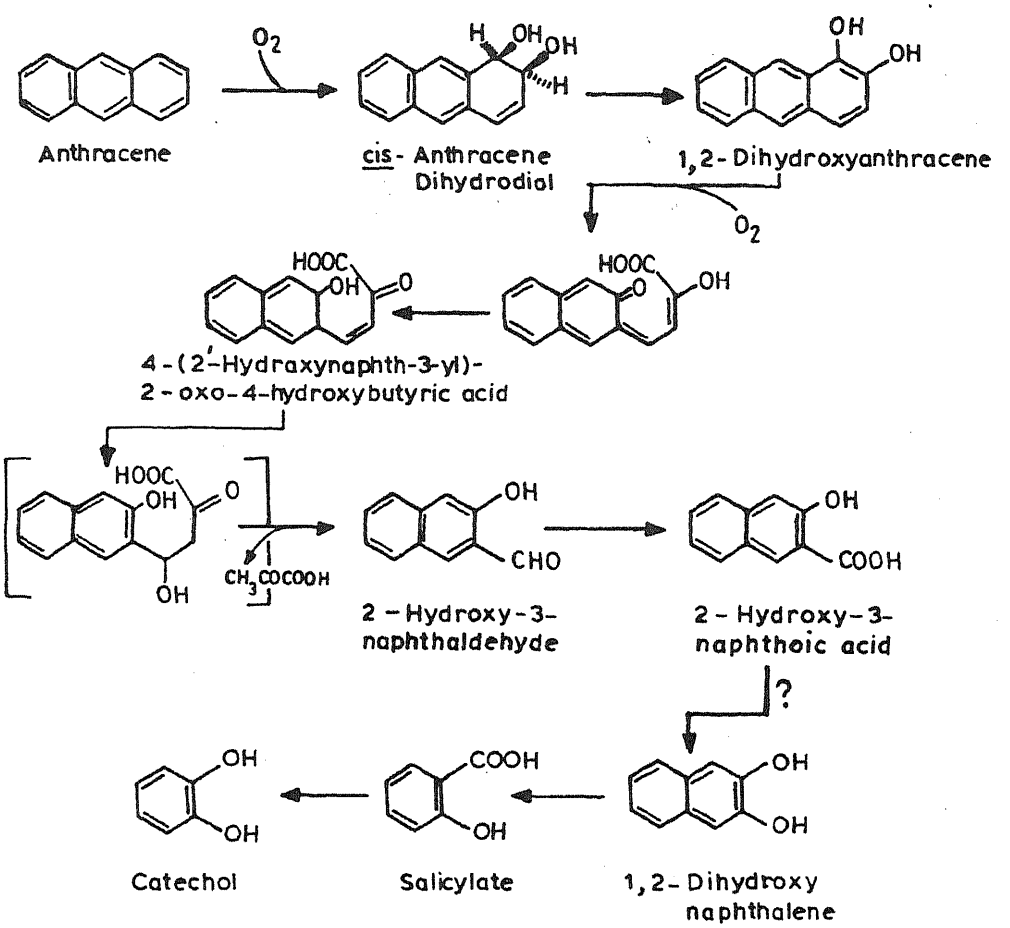


FIG. 9. Proposed pathway for the degradation of anthracene by bacteria.

1,2 and 3,4 positions to form phenanthrene *trans*-1, 2-and *trans*-3, 4-dihydrodiols[124]. The pathway for the metabolism of phenanthrene and anthracene in fungi and algae is shown in Fig. 10 and Fig. 11. respectively.

## 7. Metabolism of methylnaphthalenes

The presence of an alkyl substituent on the aromatic ring presents microorganisms with two alternatives in terms of oxidative degradation:

1. Hydroxylation of the aromatic ring leads to the formation of alkyl substituted diols which are substrates for ring fission.

2. Oxidation of the alkyl substituent results in the formation of an aromatic carboxylic acid.

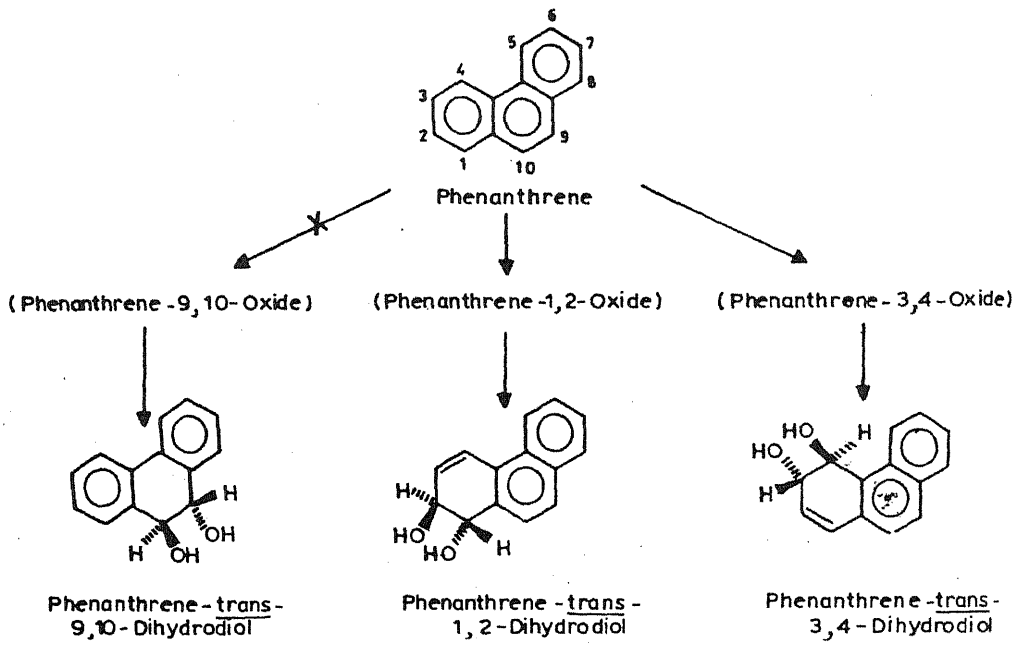


FIG. 10. Proposed reactions in the oxidation of phenanthrene by fungi.

The metabolism of alkyl substituted benzenes *viz.* xylene, toluene, ethylbenzene, and high alkylbenzenes has been studied in great detail[17,125–133]. However, there are only a few reports on the microbial metabolism of alkyl substituted naphthalenes.

In 1959, Rogoff and Wender reported the metabolism of methyl naphthalenes in pseudomonads[134]. These pseudomonad strains N-1a and P.-1, utilised 1-and 2-methylanaphthalene as the sole source of carbon and energy. Preliminary studies with washed cell suspensions suggested that, oxidation of methylnaphthalene takes place by two pathways. In one of the pathways, the unsubstituted aromatic ring is oxidised and subsequently metabolised to methyl salicylates and methylcatechols. In the alternate pathway, the methyl group is oxidised to form naphthoic acids[134]. Treccani and Fiecchi identified 1,2-dihydroxy-1,2-dihyro- 7-methylnaphthalene as an intermediate in the oxidation of 2-methylnaphthalene by *Pseudomonas desmolyticum*[135].

Various pseudomonads isolated from oil-polluted estuarine waters have the ability to grow on 1- and 2-methyl naphthalene as the sole source of carbon and energy. Resting cell suspensions oxidized 1-methylnapthalene to 3-methyl salicylate[136]. In *P. putida* NCIB 9816, the catabolism of 2-methyl naphthalene is initiated by double hydroxylation of the unsubstituted aromatic ring. The methyl naphthalene dihydrodiol formed is further oxidised by a route similar to that found in case of the naphthalene catabolic pathway. The end product of the upper pathway is 4-methylcatechol which is further metabolised by the *meta* pathway. The enzyme system responsible for the oxidation of naphthalene may act on 2-methylnaphthalene nonspecifically. Interestingly, this organ-

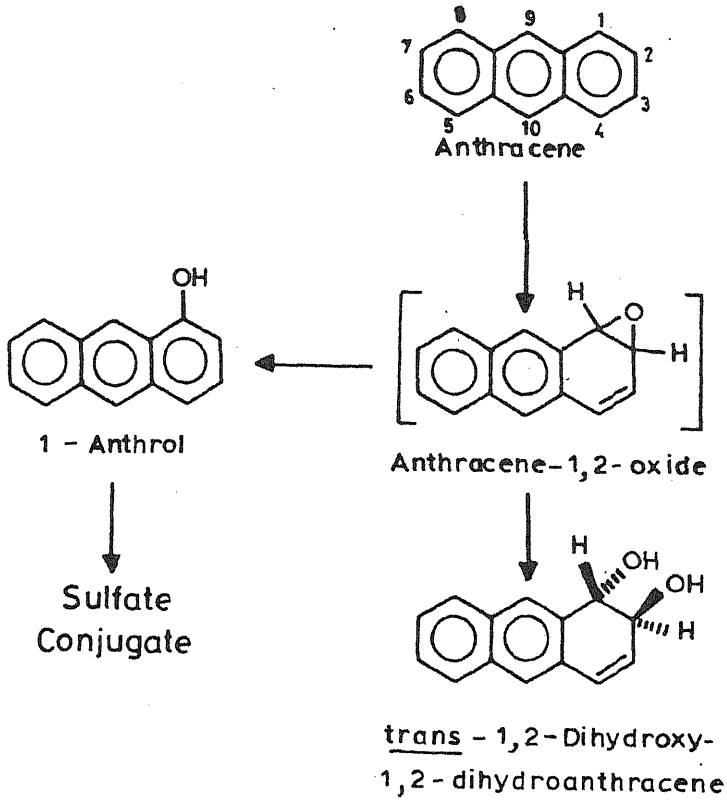


FIG. 11. Proposed reactions involved in the oxidation of anthracene by fungi.

ism failed to grow on 1-methylnapthalene[90]. However, *P. putida* strain NCIB 9816-3 had to be adapted to grow on 2-methylnapthalene. During adaptation, a spontaneous mutation has been reported to occur by a small deletion of a 1.2kb DNA fragment between the *nahG* and *nahH* genes on pWW60 plasmid. In this organism, the upper pathway enzymes are common to both naphthalene and 2-methylnapthalene[127].

The soil isolate *Pseudomonas putida* CSV86, isolated in our laboratory, has the ability to utilise naphthalene, 1-methylnaphthalene and 2-methylnaphthalene as the sole source of carbon and energy. The pathways for degradation of methylnaphthalenes in this organism were demonstrated by isolation and identification of metabolites from the spent medium, oxygen uptake in presence of various metabolic intermediates by the whole cells, and demonstrating enzyme activities in the cell extract[41,137].

The degradative pathways for 1-methylnaphthalene in *P. putida* CSV86 are shown in Fig. 12. In one of the pathways, the unsubstituted aromatic ring of 1-methylnaphthalene is double hydroxylated, resulting in the formation of 1,2-dihyro-1,2-dihydroxy-8-methyl naphthalene. This dihydrodiol is further oxidised to 3-methylsalicylate and 3-methylcatechol. The generated methylcatechol is oxidised by *meta* pathway. Thus, this

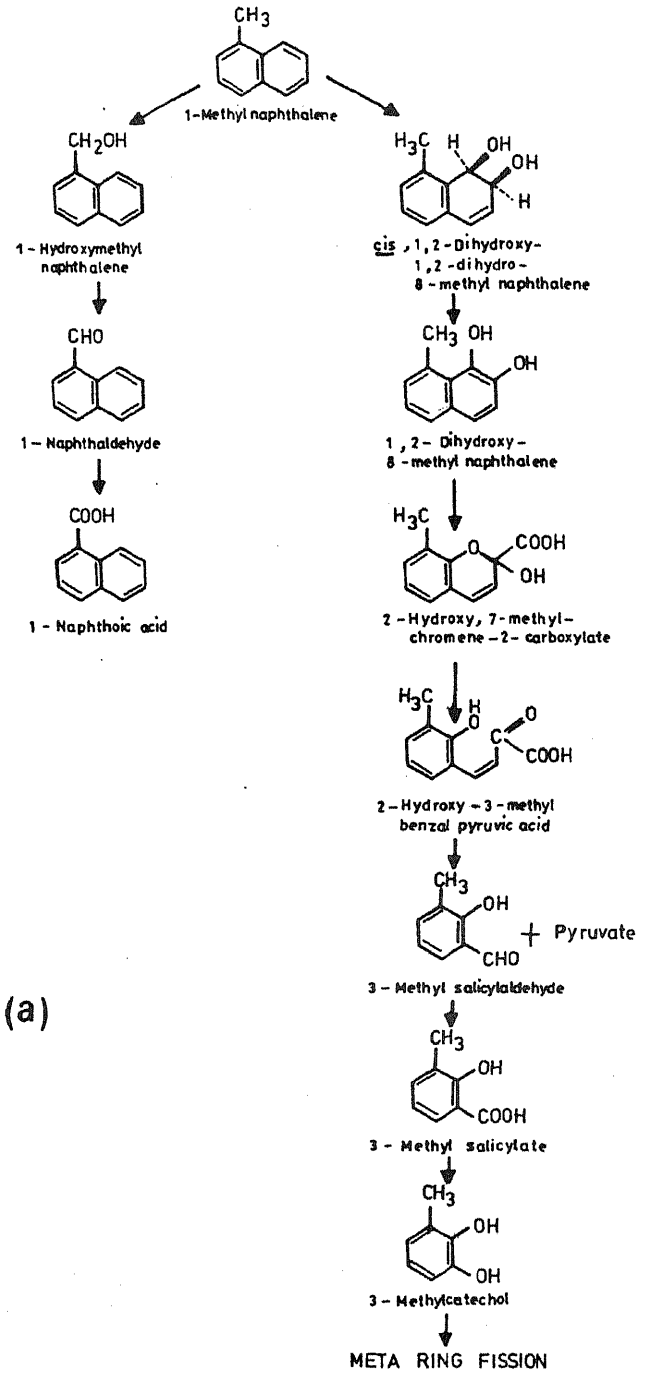


FIG. 12. Pathways for the metabolism of 1-methylnaphthalene by *P. putida* CSB86.

path way serves as the sole source of carbon and energy. Isolation and identification of 1-hydromethylnapthalene, 1-naphthaldehyde, and 1-naphthoic acid from the spent medium of the 1-methylnaphthalene grown culture suggested the occurrence of an alternative pathway for 1-methylnaphthalene degradation. The cells grown on naphthalene or 1-methylnaphthalene failed to respire on 1-methylnaphthalene, 1-naphthaldehyde and 1-naphthoic acid. Further, no metabolites of 1-naphthoic acid were detected in the spent medium. Also, the organism failed to grow on 1-naphthoic acid. These resutls suggest that, 1-methylnaphthalene is transformed by side chain hydroxylation to 1-naphthoic acid and thrown out into the medium[137].

Alternative pathways were also observed for the degradation of 2-methylnaphthalene in *P. putida*. In one of the pathways, the unsubstituted ring is oxidised giving rise to 1, 2-dihydro-1,2-dihydroxy-7-methylnapahthalene. This dihydrodiol is further metabolised to 4-methylsalicylate and 4-methylcatechol. Isolation of 2-hydroymethyl naphthalene, 2-naphthaladehydr, and 2-naphthoic acid from the spent medium suggested the oxidation of the methyl side chain of 2-methylnaphthalene. The cell suspension prepared from grown cells failed to respire on 2-naphthoic acid. Also, the organism failed to grow on 2-naphthoic acid. Interestingly, isolation and GC-MS identification of 7-hydroxymethylnaphthtoquinone,2-hydroxy-methy;-chrome-2- carboxylate, and 4-hydromethylsalicylaldehyde from the spent medium suggested that, the generated 2-hydroxymethylnaphthalene is further oxidised by hydroxylation of the unsubstituted ring. The overall pathways for the degradation of 2-methylnaphthalene by *P. putida* CSV86 are shown in Fig. 13[137].

Information on the metabolism of methylnaphthalenes by algae and fungi has come from the work done by Cerniglia and his coworkers[138,139]. Three cyanobacteria, *Agmēnellum quadruplicatum* strain PR-6, *Oscillatoria* sp. strain JCM and *Anabaena* sp. strain CA grown photoauto trophically in the presence of either 1- or 2-methyl naphthalene, oxidise both compounds at the methyl group to form 1-hydroxymethylnaphthalene and 2-hydromethylnaphthalene, respectively. These compounds have been isolated from the spent medium and their structures were determined by mass spectral analysis[138]. The fungus *Cunninghamella elegans* transforms 1- and 2-methylnaphthalene by side chain oxidation as well as by the oxidation of the aromatic ring. The major compounds isolated from the spent medium are 1- and 2-hydroxymethylnaphthalene. Other compounds isolated and identified from the spent medium are 1- and 2-naphthoic acid, 5-hydroxy-1-naphthaoic acid, 5-hydroxy-2-naphthoic acid, 6-hydroxy-2-naphthoic acid and phenolic derivatives of 1-and 2-methylnaphthalene[139].

Mammals also oxidise methylnaphthalenes by oxidation of the methyl group as well as by ring hydroxylation. Kaubisch and his colleagues reported the *in vitro* metabolism of 1- and 2- methylnaphthalene by guinea pig hepatic microsomes. oxidation occurs primarily at the methyl substituent to form 1- and 2- naphthoic acid, respectively. Dihydrodiols and methylnaphthols are minor products[140]. In contrast, Breger and his coworkers showed that hepatic microsomes of rats and rainbow trout metabolise 2-methylnaphthalene to three isomeric dihydrodiols. In addition, phenoic metabolites and their derivatives are also found[141].

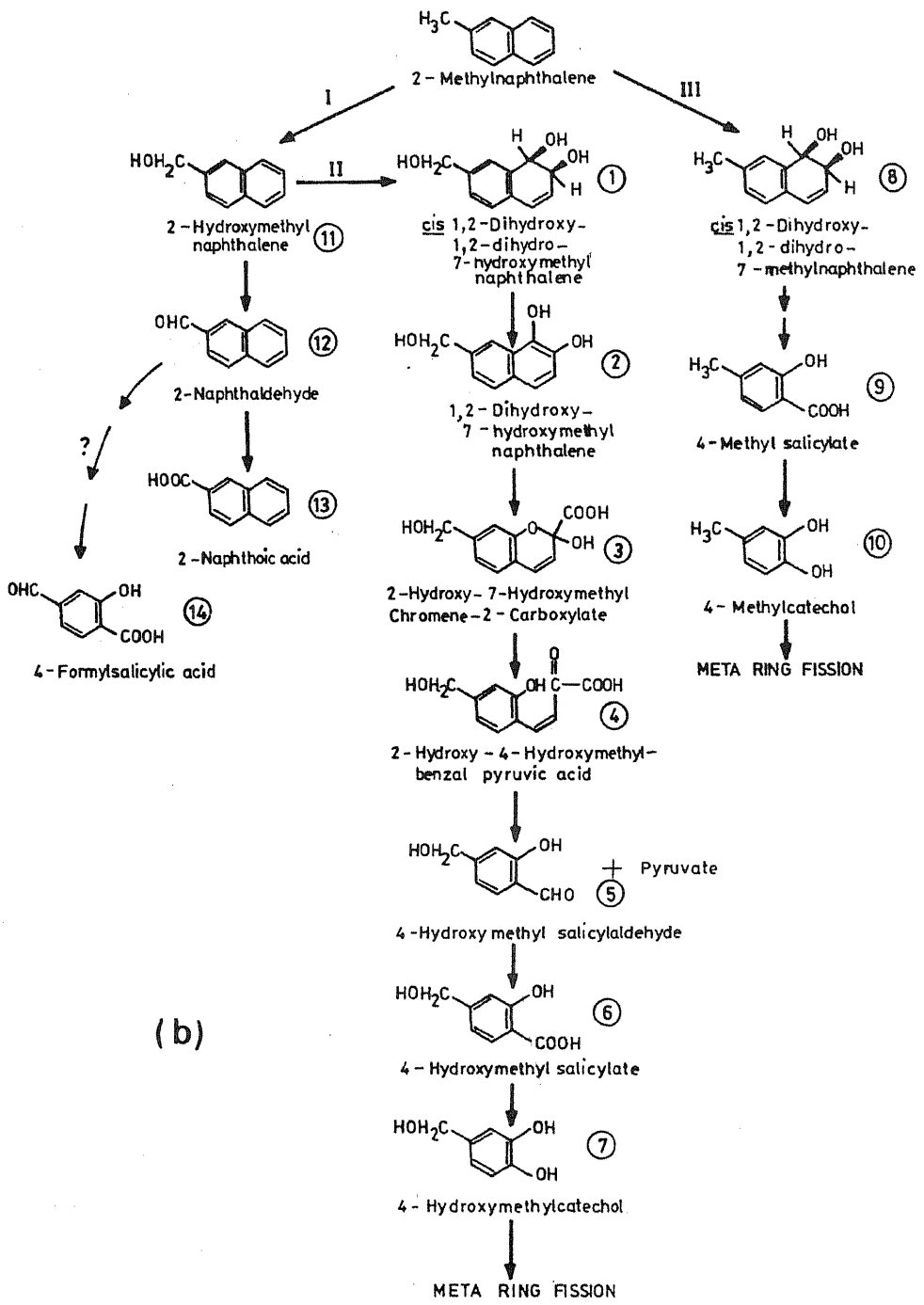


FIG. 13. Proposed pathways for the degradation of 2-methylnaphthalene by *P. putida* CSV86.

Dimethylnaphthalenes have also been reported to be susceptible to microbial degradation. Flavobacteria and *Alcaligenes* sp. strain D-59 have been shown to grow on 2,6-di methylnaphthalene as the sole source of carbon and energy[142,143]. In flavobacteria, catabolism of this compound is initiated by the sequential oxidation of one of the methyl groups to give rise to 6-methylnaphthalene2-carboxylic acid. At this stage, the aromatic ring is hydroxylated to form 1-hydroxy-6-methylnaphthalene-2-carboxylic acid which is converted to 1,2-dihyroxy-6-methylnaph-thalene. This compound is further metabolised by similar steps described for naphthalene, resulting in the formation of 4-methylnaphthalene. This compound is further metabolised by similar steps described for naphthalene, resulting in the forma-tion of 4-methylcatechol as the end product. Thus, in these fla-vobacteria, the first methyl group is sequentially oxidized to -COOH and then the aromatic ring is attacked in order to metabolise 2,6-dimethylnaphthalene completely[142].

In *Alcaligenes* sp. strain D-87, Miyachi and his coworkers reported oxidationof 6-methylnaphthalene-2-carboxylic acid by sequential oxidation of the methyl group, giving rise to 2,6-naphthalene dicarboxylic acid. This result was supported by the isolation and identification of 2,6-naphthalene dicarboxylic acid from the spent medium. However, strain D-87 showed no oxygen uptake on 2,6-naphthalene dicarboxylic acid suggesting that 2,6-naphthalene dicarboxylic acid is a dead end product 143. The pathway for the degradationof 2,6-dimethylnapthalene is shown in Fig. 14. Besides the degradation of 2,6-di methylnaphthalene, the organism was able to metabolize 1,2-;1,4-; 2,6- and 2,7- dimethyl naphthalene to the corresponding methyl naphthalene carboxylic acids. Interestingly, the organism was also able to convert 2,3-dimethylnaphthalene to 2,3-naphthalene dicarboxylic acid.

## 8. Metabolism of naphthoic acids

Naphthoic acid, a carboxy substituted naphthalene, is excreted by various microorganisms as detoxification biotrasfor-mation product of methylnaphthalene. In algae and fungi, besides naphthoic acids, glucuronated and sulfated conjugates of naphthoic acids as well as various isomers of hydroxy substituted naphthoic acids were also detected[138,139]. Similar types of reactions were also reported in mammalian and hepatic microsomes of rats and rainbow trout[140,141]. Transformation of methylnaphthalenes to naphthoic acids is also reported in various bacterial systems[41,134,137]. In *P. putida* CSV86, 1-and 2-methylnaphthalene were converted to 1- and 2-naphthoic acid and these were excreted into the medium as dead end products. The cell suspension prepared from methylnaphthalene grown cells failed to show any oxygen uptake in presence of naphthoic acids. Also the cells failed to utilise 1- and 2-naphthoic acid as the sole source of carbon and energy[137].

In our laboratory, we have isolated a soil bacterium *P. malto-philia* CSV89, which utilises 1-naphthoic acid as the sole source of carbon and energy. In this bacterium, the naphthoic acid degradation pathway is initiated by the initial double hydroxylation of the unsubstituted ring of 1-naphthoic acid, resulting in the formationof 1,2-dihydroxy-1,2-dihydro-8-carboxy naphthalene. This dihydrodiol is further metabolised to 3-formyl sali-

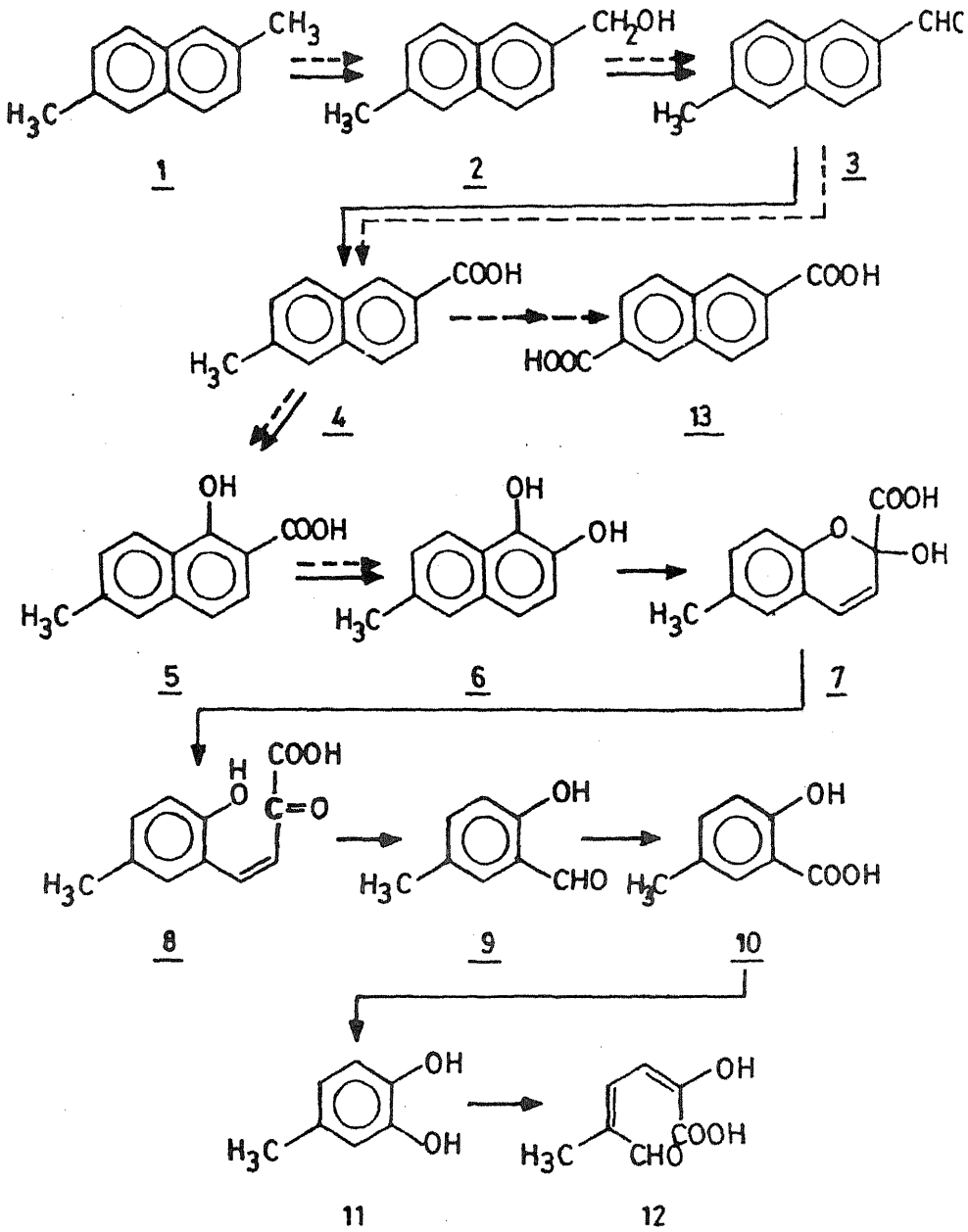


FIG. 14. The metabolic pathway for the degradation of 2,6-dimethylnaphthalene by *Flavobacteria* sp (——) and *Alcaligenes* sp (-----). 1. 2,6-Dimethylnaphthalene; 2. 2-Hydroxymethyl-6-methyl naphthalene; 3. 6-Methylnaphthalene-2-aldehyde; 4. 6-Methylnaphthalene-2-carboxylate; 5. 1-Hydroxy-6-methylnaphthalene-2-carboxylate; 6. 1,2-Dihydroxy-6-methylnaphthalene; 7. 2-Carboxy-2-hydroxy-6-methylchromene; 8. 2′-Hydroxy-5′-methyl benzalpyruvate; 9. 5-Methylsali-cylaldehyde; 10. 6-Methylsalicylate; 11. 4-Methylcatechol; 12. 2-Hydroxy-5-methyl muconic semialdehyde and 13. 2,6-Naphthalene dicarboxylic acid.

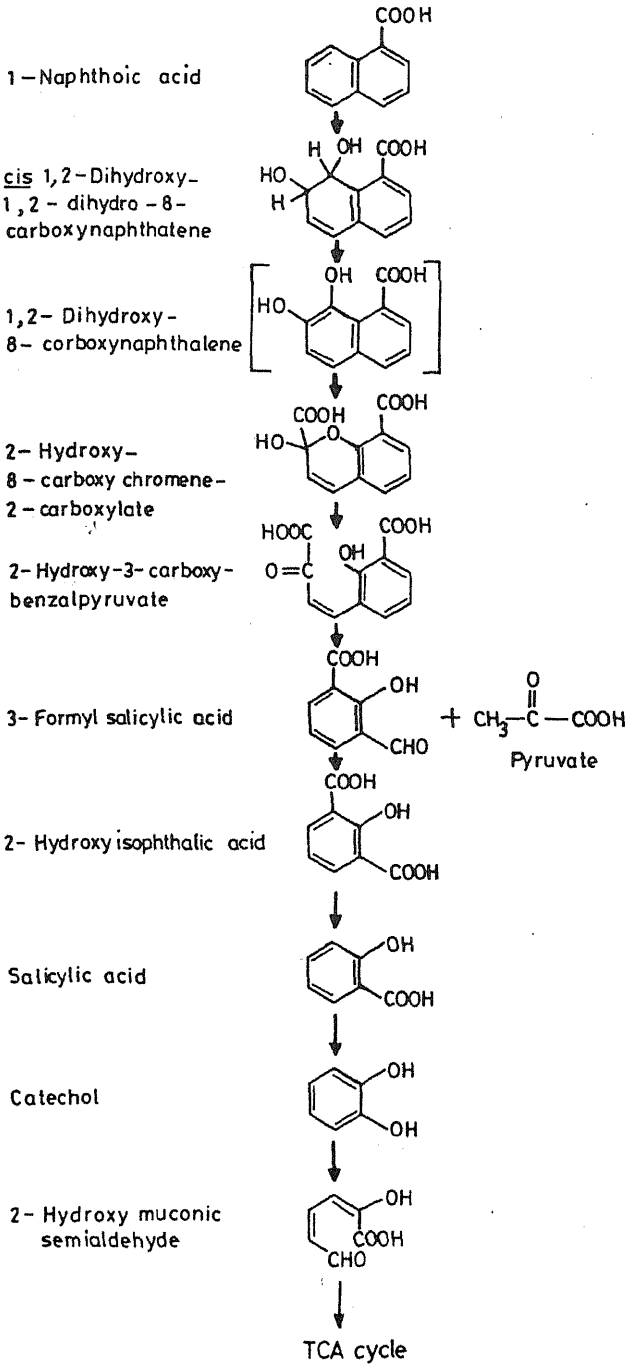


FIG. 15. Proposed pathway for the metabolism of 1-naphthoic acid by *P.* maltophilia CSV89.

cylate by a series of enzyme catalyzed reactions similar to that involved in the naphthalene/methylnaphthalene degradation. The generated 3-formylsalicylate is further oxidized to catechol *via* 2-hydroxyisophthalate and salicylate. The resultant catechol is further metabolised by *meta* pathway resulting in the formation of 2-hydroxy muconic semialdehyde[144,145]. The proposed pathway for the metabolism of 1-naphthoic acid is depicted in Fig. 15.

Various hydroxy substituted naphthoic acids have also been reported as metabolic intermediates in the degradation of anthracene and phenanthrene. In bacterial systems, 2-hydroxy-3-naphthoic acid is generated as a result of oxidation of one of the aromatic ring of anthracene. The generated 2-hydroxy-3-naphthoic acid is oxidatively decarboxylated to 2,3-dihydroxynaphthalene. However, further pathway for its metabolism is not clear. In phenanthrene metabolism, 1-hydroxy-2-naphthoic acid is reported as a metabolic intermediate. The generated hydroxynaphthoate is oxidatively decarboxy,ated to 1,2-dihydroxy naphthalene and subsequently oxidised to salicylate and catechol. In flavobacteria, 6-methy-1-2-carboxynaphthalene is reported as a metabolic intermediate in the degradative pathway of 2,6-dimethylnapthalene. The generated 6-methyl-2-carboxy naphthalene is metabolized to methylsalicylate and methylcatechol. However, in *Alcalegenes* sp. there are two pathways for the degradation of 6-methyl-1, 2-carboxynaphthalene. One of the pathways involves the oxidationof carboxy substituted aromatic ring and its subsequent oxidation to methylsalicylate and methylcatechol. However, in the other pathway 6-methy1-2-carboxynaphthalene is transformed to 2,6-naphthalene dicarbosylic acid, a dead end product, by the oxidati n of methy1 group (Fig. 14).

In conclusion, it may be pointed out that although extensive investigations have been carried out on the biochemical and genetic aspects of the bacterial degradation of naphthalene, there is an urgent need for a detailed study of the chemical pathways for the catabolism of substituted naphthalenes, anthracene and phenanthrene and the characterization of the plasmids associated with these transformations. The mechanism of the enzymatic reactions involved, the regulation of the activity and expression of the key enzymes are the other areas which are yet to be explored.

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