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MINIREVIEW

Microbial Degradation of Monohydroxybenzoic Acids

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Hydroxybenzoic acids are the most important intermediates in the degradative pathways of various aromatic compounds. Microorganisms catabolize aromatic compounds by converting them to hydroxylated intermediates and then cleave the benzene nucleus with ring dioxygenases. Hydroxylation of the benzene nucleus of an aromatic compound is an essential step for the initiation and subsequent disintegration of the benzene ring. The incorporation of two hydroxyl groups is essential for the labilization of the benzene nucleus. Monohydroxybenzoic acids such as 2-hydroxybenzoic acid, 3-hydroxybenzoic acid, and 4-hydroxybenzoic acid through hydroxylation yield terminal aromatic intermediates like catechol, protocatechuic acid, gentisic acid, or pyrocatechuic acid that are susceptible for subsequent oxygenative cleavage of the benzene ring. These terminal aromatic intermediates are further degraded to cellular components through *ortho*-and /or *meta*-cleavage pathways and finally lead to the formation of constituents of the TCA cycle. Many groups of microorganisms have been isolated as degraders of hydroxybenzoic acids with diverse degradative routes and specific enzymes involved in their metabolic pathways. Various microorganisms carry out unusual non-oxidative decarboxylation of aromatic acids and convert them to respective phenols which have been documented. Further, *Pseudomonas* and *Bacillus* spp. are the most ubiquitous microorganisms, being the principal components of microflora of most soil and water environments.

Key words: Aromatic compounds, hydroxybenzoic acids, degradation, microorganisms, salicylic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, catechol, gentisic acid, protocatechuic acid

Aromatic compounds are abundant in the biosphere as components of plant material, complex polymer lignin and as environmental pollutants introduced by human beings. Pollutants include various kinds of pesticides and halogenated aromatic constitutents, many of which are toxic. The hydroxybenzoic acid are the most important intermediate metabolites in the microbial degradative pathways of various aromatic hydrocarbons, aromatic dicarboxylic acids, and phenolic compounds. The variety of dimeric lignin compounds are also converted into hydroxybenzoic acids. The biochemical basis for the metabolism of many diverse aromatic compounds has been studied to gain an understanding of natural biodegradation and to provide models for the degradation of xenobiotics (74). The dramatic advancement of this fascinating field in its historical perspective has been best understood by numerous reports, reviews, and monographs by several authors (2, 16, 17, 18, 24, 31, 76). The studies on microbial degradation of hydroxybenzoic acids over the past few decades has provided a wealth of knowledge on the metabolism of these compounds (9, 25, 27, 35, 37, 54, 83, 84).

The studies on the microbial metabolism of hydroxybenzoic acids are mainly concerned with the isolation and identification of microbial strains capable of utilizing these compounds and the elucidation of intermediary degradative pathways which lead to the mineralization of these compounds (49). The microbial strains are normally identified on the basis of their morphological and physiological charactristics by using various cultural and biochemical criteria. Many experimental approaches have been employed to investigate the degradative mechanism adopted by the microorganisms in the catabolic sequences of these compounds. These experiments are mainly concerned with the isolation and characterization of intermediary metabolites and also in the identification of the

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microbial enzymes involved in the degradative processes by employing different physico-chemical methods. A powerful tool that also enables us to unravel the metabolic pathways is the demonstration of the sequential induction of enzymes to oxidise a specific substrate and the intermediatary metabolites. Further, insight into the degradative pathway is also provided by assaying the probable key enzymes involved in various metabolic reaction sequences (8, 66).

A survey of the studies reveals the underlying unity as well as the diversity in the microbial metabolism of hydroxybenzoic acids as shown in Table 1. It may be observed that a relatively large class of compounds are metabolised through similar pathways by different microbial species, and also that a small class of compounds are metabolised through different pathways by a single species.

Hydroxylation of Benzene Nucleus

Microorganisms that catabolize aromatic compounds convert them to hydroxylated intermediates and then cleave the benzene rings with ring-fission dioxygenases. Hydroxylation of the benzene nucleus of an aromatic compound

Table 1. Degradation of hydroxybenzoic acids by various microorganisms

Compound	Microorganism	Intermediate	Cleavage of Intermediate	Reference
2-Hydroxy-benzoic acid	*Lignobacter* sp.	Gentisic acid	*meta*	Buswell *et al.*, 1980
	Micrococcus sp. strain 12B	Gentisic acid	*meta*	Eaton and Ribbons, 1982
	Micrococcus sp.	Gentisic acid	*meta*	Haribabu *et al.*, 1984
	Pseudomonas spp.	Catechol	*ortho*	Dagley, 1971; Shamsuzzamann and Barnsley, 1974; Manohar and Karegoudar, 1995
	Rhodococcus sp. strain B4	Gentisic acid	*meta*	Grund *et al.*, 1992
	Rhodococcus erythropolis S1	Gentisic acid	*meta*	Suemori *et al.*, 1995
	Amycolatopsis sp. DSM 43387	Catechol	*ortho*	Grund *et al.*, 1990
	Amycolatopsis sp. DSM 43388	Catechol	*ortho*	Grund *et al.*, 1990
	Streptomyces olivaceisceroticus DSM 415595	Gentisic acid	*meta*	Grund *et al.*, 1990
	Streptomyces niger DSM 40302	Catechol	*ortho*	Grund *et al.*, 1990
	Streptomyces umbirinus DSM 40278	Gentisic acid	*meta*	Grund *et al.*, 1990
	Bacillus sp.	Gentisic acid	*meta*	Mashetty *et al.*, 1995
3-Hydroxy-benzoic acid	*Micrococcus* sp. strain 12B	Protocatechuic acid	*ortho*	Eaton and Ribbons, 1982
	Micrococcus sp.	Protocatechuic acid	*ortho*	Haribabu *et al.*, 1984
	Amycolatopsis strain DSM 43387	Protocatechuic acid	*ortho*	Grund *et al.*, 1990
	Amycolatopsis strain DSM 43388	Gentisic acid	*meta*	Grund *et al.*, 1990
	Streptomyces spp.	Gentisic acid	*meta*	Sutherland *et al.*, 1981; Grund *et al.*, 1990
	Klebsiella pneumoniae	Gentisic acid	*meta*	Jones and Cooper, 1990
	Salmonella typhimurium	Gentisic acid	*meta*	Goetz and Harmuth, 1992
	Pseudomonas spp.	Gentisic acid	*meta*	Groseclose *et al.*, 1973; Harpel and Lipscomb, 1990;
	Pseudomonas testosteroni	Protocatechuic acid	*meta*	Michalover *et al.*, 1973
	Rhodococcus erythropolis	Gentisic acid	*meta*	Suemori *et al.*, 1995
	Bacillus spp.	Gentisic acid	*meta*	Grawford, 1975b; Mashetty *et al.*, 1996
	Pseudomonas putida BS893	2,3-Dihydroxy-benzoic acid	*ortho*	Strovoytov *et al.*, 1985
4-Hydroxy-benzoic acid	*Micrococcus* sp. strain 12B	Protocatechuic acid	*ortho*	Eaton and Ribbons, 1982
	Micrococcus sp.	Protocatechuic acid	*ortho*	Haribabu *et al.*, 1984
	Pseudomonas putida	Protocatechuic acid	*ortho*	Hosakawa and Stanier, 1996
	Pseudomonas fluorescens	Protocatechuic acid	*ortho*	van Berkel and Muller, 1991
	Pseudomonas sp. DJ12	Protocatechuic acid	*ortho*	Karegoudar *et al.*, 1991
	Streptomyces spp	Protocatechuic acid	*ortho*	Sutherland *et al.*, 1981; Grund *et al.*, 1990
	Micrococcus sp.	Protocatechuic acid	*ortho*	Haribabu *et al.*, 1984
	Amycolatopsis spp.	Protocatechuic acid	*ortho*	Suemori *et al.*, 1995
	Bacillus sp.	Protocatechuic acid	*ortho*	Mashetty *et al.*, 1995
	Bacillus brevis	Protocatechuic acid	*ortho*	Crawford, 1976
	Bacillus circulans	Protocatechuic acid	*meta*	Crawford, 1976
	Caulobacter crescents	Protocatechuic acid	*ortho*	Chatterjee and Bourquin, 1987

is an essential step for the initiation and subsequent disintegration of the benzene nucleus. Hydroxylation of the benzene ring is accomplished through the insertion of oxygen by the influence of hydroxylase enzymes in the presence of cofactors. The incorporation of two hydroxyl groups is essential for the labilization of the benzene nucleus. The peripheral aromatic compounds already possessing a hydroxyl group are metabolised by the introduction of another hydroxyl group most often at the *ortho* position or sometimes at the *para* position under the influence of flavin linked monooxygenase enzymes in the presence of cofactors. The examples of monohydroxylation reactions are found in the reports of several investigators (11, 13, 35, 54, 82, 83). The monohydroxybenzoic acids such as 2-hydroxybenzoic acid, 3-hydroxybenzoic acid, and 4-hydroxybenzoic acid on hydroxylation yield dihydroxylated compounds like catechol, protocatechuic acid, gentisic acid, or pyrocatechuic acid. The hydroxylation pathways adopted during the metabolism of these monohydroxybenzoic acids by different microorganisms are depicted in Fig. 1. The hydroxylation pathways of monohydroxybenzoic acids are the most frequently encountered ones which invariably involve the formation of dihydroxy compounds as the terminal intermediates that are susceptible for the subsequent oxygenative cleavage of the benzene nucleus.

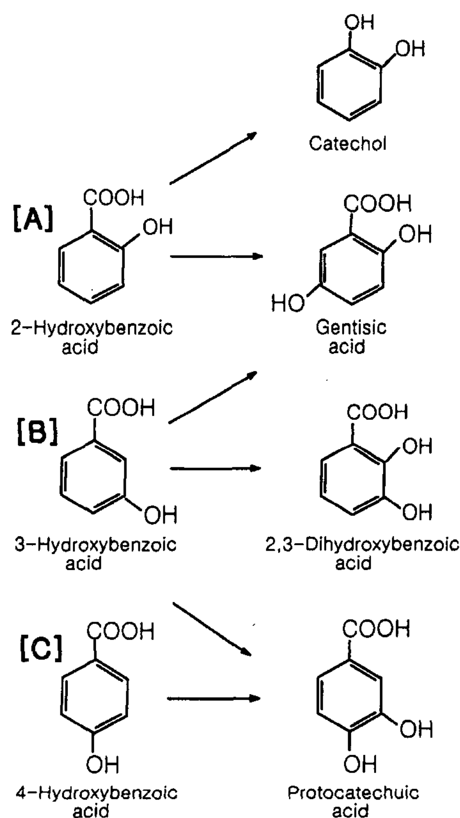

Fig. 1. Degradation of monohydroxybenzoic acids: A. 2-Hydroxybenzoic acid B. 3- Hydroxybenzoic acid, and C. 4-Hydroxybenzoic acid.

Degradative Pathways of Hydroxybenzoic Acids

Degradation of 2-hydroxybenzoic acid

It has been well documented that 2-hydroxybenzoic acid (salicylic acid) oxidatively decarboxylate to produce catechol by salicylate hydroxylase in the *Pseudomonas* spp. (16, 53, 73). The hydroxylation of salicylic acid at the C5 position to yield gentisic acid has also been observed in *Rhodococcus* (35, 83), *Lignobacter* (5) and *Micrococcus* spp. (25, 37) as shown in Fig. 1A. Grund *et al.* (35) demonstrated the existence of two different routes within the genera *Streptmyces and Amycolatopsis. Streptomyces olivaceiscleroticus* DSM415595, *Streptomyces niger* DSM 40302 and *Amycolatopsis sp.* DSM 43387 and 43888 converted salicylic acid to catechol (Fig. 1A). However, *Streptomyces umbvinus* DSM 40278 converted salicylic acid to gentisic acid. The enzyme salicylate hydroxylase has also been purified and characterized from many microorganisms by different authors (81, 92). Salicylate 1-hydroxylase has been extensively studied and is one of the model enzymes for flavin containing monooxygenases (85, 86, 88). Another enzyme salicylate 5-hydroxylase which forms gentisic acid, requires unusual cofactors CoA and ATP (32). Suemori *et al.* (82) purified and characterized three types of monohydroxybenzoate oxygenases, Salicylate 5-hydroxylase, 3-hydroxybenzoate 6-hydroxyalse and 4-hydroxybenzoate 3-hydroxylase from *Rhodococcus erythropolis* S1. The effects of various growth substrates on the induction of enzymes involved in the degradation pathways of three monohydroxybenzoic acids were studied.

Degradation of 3-hydroxybenzoic acid

3-Hydroxybenzoic acid may be degraded through protocatechuic acid by 3-hydroxybenzoate 4-hydroxylase (6, 35, 56, 77) or gentisic acid by 3-hydroxybenzoate 5-hydroxylase (12, 25, 26, 32, 35, 37, 43) or via 2,3-dihydroxybenzoic acid by 3-hydroxybenzoate 2-hydroxylase (70, 79). The degradative pathways of 3-hydroxybenzoic acid is shown in Fig. 1A. Grund *et al.* (35) showed that within the genus *Amycolatopsis,* strain DSM 43387 metabolized 3-hydroxybenzoic acid via protocatechuic acid and detected 3-hydroxybenzoate 4-hydroxylase and protocatechuate 3,4-dioxygenase activities. Further, another *Amycolatopsis* strain, DSM 43388, converted 3-hydroxybenzoic acid to gentisic acid. 3-Hydroxybenzoate hydroxylase was purified and characterized from *Rhodococcus ertythropolis* S1 (81) and *Pseudomonas aeruginosa* (33, 58).

Degradation of 4-hydroxybenzoic acid

It has been demonstrated that 4-hydroxybenzoic acid is degraded to protocatechuic acid by 4-hydroxybenzoate 3-

hydroxylase (14, 41, 45, 55, 84) as shown in Fig. 1C. 4-Hydroxybenzoate hydroxylase is a member of the class of flavin-dependent monooxygenases. This enzyme catalyzes the conversion of 4-hydroxybenzoic acid to protocatechuic acid, an intermediate step in the degradation of aromatic compounds in soil bacteria. This enzyme has been purified from different bacterial systems by various investigators (75, 72, 89). The enzyme from *Pseudomonas fluorescens* is one of the most extensively studied flavoprotein aromatic monohydroxylase. The catalytic and biophysical properties of this enzyme have been investigated in detail (28). This enzyme shows narrow specificity. The structure and mechanism of 4-hydroxybenzoate hydroxylase from *Pseudomonas fluorescens* and *Pseudomonas* sp. CBS3 has also been studied in greater detail (30, 89). The structure of this enzyme is unusual because there is no well-defined binding site for NADPH coenzyme. It was observed that a gratuitous induction of 4-hydroxybenzoate hydroxylase on all substrates that catabolized via protocatechuic acid.

Disintegration of Benzene Nucleus

Dissimilation of the benzene is a crucial step in the microbial catabolism of aromatic compounds. Benzene nucleus of a terminal aromatic metabolite is cleaved by the incorporation of both the atoms of oxygen into the substrate under the influence of dioxygenase enzyme to yields aliphatic fragments. The disintegration of the benzene nucleus during the degradation of aromatic substrates by different microorganisms is known to occur generally by two modes of ring cleavages, namely the *ortho*- cleavage and *meta*-cleavage which are referred as intradiol and extradiol fissions, respectively (51, 63).

In the case of *ortho*-cleavage, the aromatic ring is cleaved by oxidative fission of the bond between the two consecutive carbon atoms bearing the hydroxyl groups. In the case of *meta*-cleavage, the aromatic ring is cleaved by oxidative fission of the bond between the two consecutive carbon atoms, one bearing a hydroxyl group and another bearing a substitutent group other than the hydroxyl group.

The fission products depending upon the mode of cleavage of the aromatic ring are further degraded through different pathways into simpler aliphatic compounds such as *cis, cis*-muconic acid, 3-ketoadipic acid, maleyl pyruvic acid or 2-hydroxymuconic semialdehyde by simple decarboxylation, hydrolysis, and isomerization reactions under the influence of decarboxylase, hydroxylase or isomerase, respectively, until the routes are finally lead to the formation of succinic acid, fumaric acid, maleic acid, citric acid or oxalic acid which are the constituents of the Krebs cycle.

Degradative Routes of Terminal Aromatic Metabolites

In many microbial groups, numerous aromatic compounds are ultimately degraded to one of the ortho-diphenolics, catechol, protocatechuic acid, gentisic acid or pyrocatechuic acid as the terminal aromatic metabolites which are further degraded to cellular components through *ortho*- and /or *meta*-cleavage pathways. The study on the degradation of these terminal metabolites in several microorganisms has been made in detail by many investigators. The degràdative pathways of some of these terminal metabolites are described in the following paragraphs.

Degradation of catechol

Catechol is a key metabolite in the degradation of many aromatic compounds. Bacteria can degrade the aromatic ring of catechol by *ortho*- or *meta*- fission. The nucleus of catechol in the case of *ortho*-fission is cleaved between the position C1 and C2 both bearing the hydroxyl groups by catechol 1,2-dioxygenase to yield *cis,cis* muconic acid (20. 39, 66) as shown in Fig. 2A. The muconic acid is finally transformed by the successive action of muconate lactonizing enzyme, muconolactone isomerase, and enol-lactone hydroxylase to 3-ketoadipic acid. The ketoadipic acid is eventually converted to succinic acid and acetic acid which are the constitutents of the TCA cycle. Catechol 1,2-dioxygenase was the first dioxygenase discovered. This enzyme has been purified, characterized and its

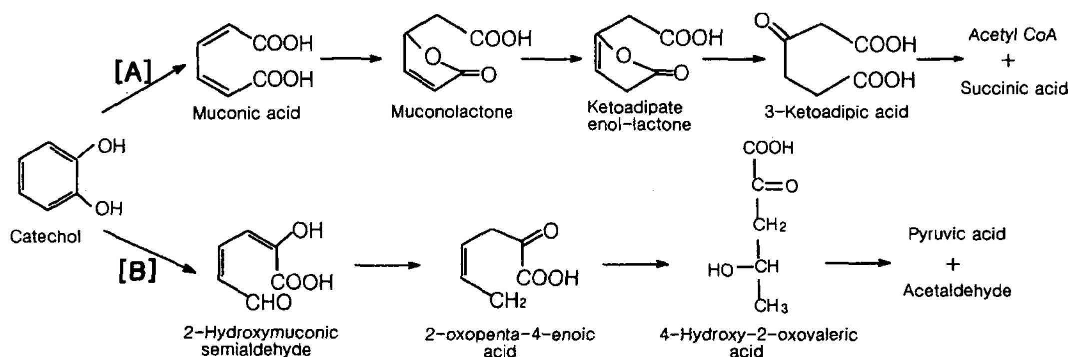

Fig. 2. Degradation of catechol: pathways initiated by A. catechol 1,2-dioxygenase, and B.catechol 2,3-dioxygenase.

spectral properties were made from several microbial sources (40, 46, 50).

The nucleus of the catechol in the case of *meta*-fission is cleaved between positions C2 and C3 by the action of catechol 2,3-dioxygenase to yield 2-hydroxymuconic semialdehyde (59) as shown in the route of Fig. 2B. This semialdehyde is subsequently hydrolysed to form 4-hydroxy 2-ketovaleric acid which is transformed finally to acetaldehyde and pyruvic acid. The purification, characterization and substrate specificity of catechol 2,3-dioxygenase have been made by several investigators (48, 60, 62, 63, 71, 87). Some bacteria express a modified *ortho*-cleavage pathway that allows the mineralization of chlorocatechol. The catalytic properties of these enzymes from different bacteria exhibit different specificities (7). Some bacteria, especially members of the genus *Pseudomonas* have enzymes for both pathways, when the bacterium was grown on salicylic acid, benzoic acid and 4-chlorobenzoic acid (47).

Degradation of gentisic acid

Gentisic acid is a key intermediate and a focal point in aerobic metabolism of a large number of aromatic compounds. The microbial degradation of gentisic acid is mediated by gentisate 1,2-dioxygenase through oxygenative cleavage and insertion reaction as shown in Fig. 3. The formed maleyl pyruvic acid is converted to central metabolites either directly or following isomerization to fumaryl pyruvic acid, thus providing assimilatory carbon and energy for the bacteria exclusively from the degradation of gentisic acid. The gentisic acid degradation pathway has been identified in a variety of natural isolates such as *Bacillus* (10), fluorescent *Pseudomonas* spp. (3). Gentisate 1,2-dioxygenase has been purified from *Moraxella osloensis* (15), *Pseudomonas testosteroni* and *Pseudomonas acidovorans* (38), *Pseudomonas alcaligenes* and *Pseudomonas putida* (29). The spectroscopic studies of the nitrosyl

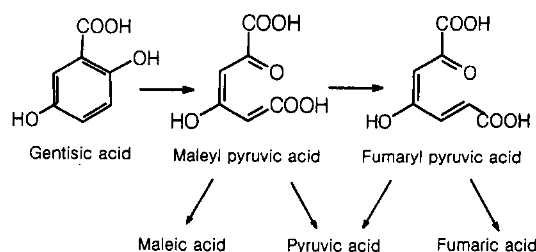

Fig. 3. Degradation of gentisic acid.

complex of the gentisate 1,2-dioxygenase strongly indicate the active centre iron and the molecular mechanism are most similar to those of the extradiol dioxygenase class. Several enzymes of the gentisic acid pathway in *Pseudomonas alcaligenes* and *Pseudomonas putida* were reported to possess broad specificities (29).

Degradation of protocatechuic acid

Protocatechuic acid is one of the key intermediates for the microbial catabolism of benzenoid molecules. It is known to be a substrate for three distinct ring fission dioxygenases. Protocatechuic acid may be cleaved by protocatechuate 3,4-dioxygenase, yielding 3-carboxy muconic acid or by protocatechuate 4,5-dioxygenase, yielding 4-carboxy 2-hydroxy muconic semialdehyde. Alternatively, the ring cleavage may be mediated by a protocatechuate 2,3-dioxygenase to yield 5-carboxy 2-hydroxymuconic semialdehyde. The degradative pathways of protocatechuic acid via *ortho*-cleavage by protocatechuate 3,4-dioxygenase in *Pseudomonas putida* (57, 66) and via *meta*-cleavage through C2-C3 fission by protocatechuate 2,3-dioxygenase in *Bacillus macerans* (14) and through C4-C5 fission by protocatechuate 4,5-dioxygenase in *Pseudomonas testosterone* (19, 22) are illustrated respectively in Fig. 4A, B, and C.

Protocatechuate 3,4-dioxygenase was first studied by

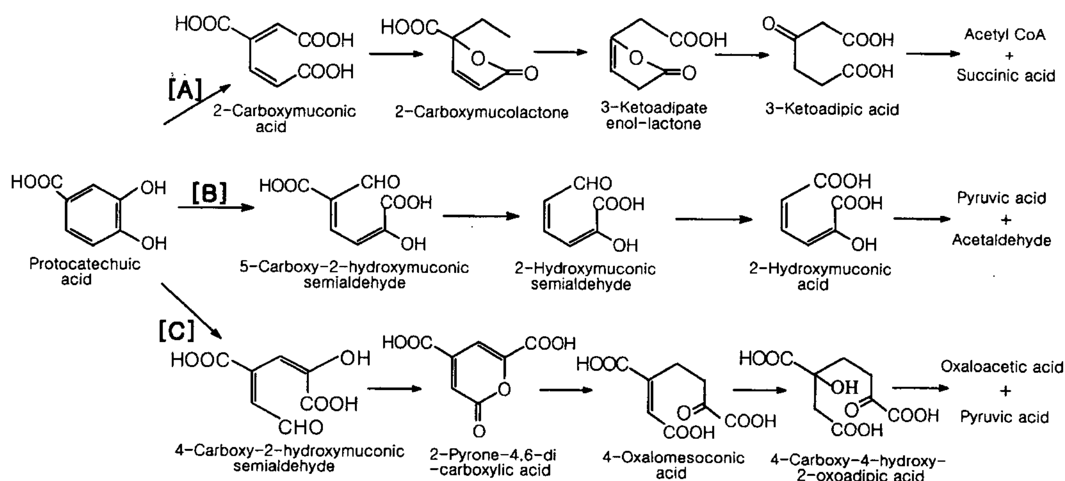

Fig. 4. Degradation of protocatechuic acid: pathways initiated by A. protocatechuate 3,4-dioxygenase, B. protocatechuate 2,3-dioxygenase, and C. protocatechuate 4,5-dioxygenase.

Stanier and Ingrahm (78) and later since then a number of investigators have described various methods for purifying this enzyme from different sources (4, 42, 80). Pujar and Ribbons (68) purified protocatechuate 3,4-dioxygenase from *Pseudomonas fluorescens* by affinity chromatography. Protocatechuate 3,4-dioxygenase from *Pseudomonas cepacia* DB01 has also been studied extensively by Ludwig *et al.* (52). This enzyme is composed of equimolar amounts of two nonidentical subunits of 23,000 Da (α-subunits) and 26,000 Da (β-subunits). The molecular size of the holoenzyme is 200,000 Da. There are four α and four β subunits that make up the intact enzyme. The holoenzyme contains four iron atoms. Crystals of protocatechuate dioxygenase have been obtained by Ludwig *et al.* (52). The structure of protocatechuate 3,4-dioxygenase from *Pseudomonas aeruginosa* has been determined by Ohlendorf *et al.* (64) and Vetting *et al.* (90).

Crawford *et al.* (13) purified protocatechuate 2,3-dioxygenase from *Bacillus macerans* to near homogeneity. The product of protocatechuate 2,3-dioxygenase, 5-carboxy 2-hydroxymuconic semialdehyde is labile and even at neutral pH readily decarboxylate to 2-hydroxymuconic semialdehyde. However, 5-carboxy 2-hydroxymuconic semialdehyde is transiently stable at pH 7.0 so that its formation and disappearance after 2,3-fission of protocatechuic acid can be observed spectrophotometrically. Wolgel *et al.* (91) purified protocatechuate 2,3-dioxygenase from *Bacillus macerans* to homogeneity and studied its physical and spectroscopic characterization. Protocatechuate 2,3-dioxygenase is the final member of the protocatechuate dioxygenase family.

Protocatechuate 4,5-dioxygenase, an enzyme catalyzing the ring cleavage of the aromatic ring of protocatechuic acid with the insertion of two atoms of the oxygen was first described by Dagley and Patel (21) This enzyme has been purified from *Pseudomonas* (65) and *Pseudomonas paucimobilis* (61) to a homogeneous state and its molecular weight determined to be approximately 150,000 Da. This enzyme contains one iron per molecule of the enzyme protein (1, 94).

Many microorganisms carry out an unusual non-oxidative decarboxylation of aromatic acids and convert them to respective phenols (23, 67). In bacteria, this reaction has been reported for 4-hydroxybenzoic acid, protocatechuic acid (67), and 2,3-dihydroxybenzoic acid (44) which undergo similar decarboxylation. The enzyme 2,3-dihydroxybenzoate decarboxylase catalyzes the formation of catechol from dihydroxybenzoic acid without requiring any cofactors.

Concluding Remarks

Survey of the aerobic Pseudomonads showed that the mode of benzene ring cleavage of protocatechuic acid was taxonomically dependent. The *ortho*-cleavage of protocatechuic acid was characteristic of the entire fluorescent group. Whereas *meta*-cleavage was confined to the non-fluorescent organisms (70). It was observed that several bacteria capable of assimilating salicylic acid and 3-hydroxybenzoic acid via gentisic acid, which is degraded to pyruvic acid and fumaric acid through reactions from maleyl pyruvic acid to fumaryl pyruvic acid by a glutathione independent isomerase. It is of interest that many glutathione independent maleyl pyruvic acid isomerases have been observed in Gram-positive bacteria or *Actinomycetes*, while nearly all glutathione-dependent maleyl pyruvic acid isomerases have been found in Gram-negative bacteria (15, 32, 83).

The two genera of *Streptomyces* and *Amycolatopsis* resemble each other with respect to degradation routes. The differences appeared in only the degradation of salicylic acid and 3-hydroxybenzoic acid. The genus *Streptomyces* catabolized salicyclic acid by two different routes (35). All *Amycolatopsis* strains and two of the three *Streptomyces* can degrade salicylic acid via catechol, while, in *Streptomyces umbrins*, the gentisic acid pathway was induced. This shows that two different salicylate hydroxylases may exist, salicylate 1-hydroxylase leading to the formation of catechol and salicylate 5-hydroxylase that forms gentisic acid. Such situation is also know in the genus *Pseudomonas,* where two different pathways for the degradation of salicylic acid occur, one via catechol and a second via gentisic acid. The two different routes for the degradation of 3-hydroxybenzoic acid, either via gentisic acid or protocatechuic acid was noted in the genus *Amycolatopsis*. This reflects the identical situation in the genus *Pseudomonas*, where two different 3-hydroxybenzoate hydroxylases have been observed. The catabolic diversity for the degradation of monocyclic aromatic compounds within the genera *Streptomyces* and *Amycolatopsis* is quite similar to that observed within the *Pseudomonas* sp. (47).

Further, numerous microorganisms, in addition to *Bacilli* and Pseudomonads, degrade 4-hydroxybenzoic acid via the 3-ketoadipic acid pathway including the representatives of *Acinetobacter, Nocardia, Alcaligenes*, and *Azatobacter.* The catabolic pathway of 4-hydroxybenzoic acid via protocatechuic acid by protocatechuate 2,3-dioxygenase has been described thus far only among the strains of *Bacillus circulans* (13). Intradiol dioxygenase like protocatechuate 3,4-dioxygenase contains ferric ion and open the aromatic ring between the vicinyl hydroxyl groups. In contrast, extradiol dioxygenase such as protocatechuate 4,5-dioxygenase invariably contains ferrous ion and open the ring adjacent to one of the hydroxyl groups to form highly coloured muconic semialdehyde. The analogous set of reactions also occur in the oxidative cleavage of catechol. The intradiol and extradiol nomenclature can not be applied to enzymes that catalyze the ring cleavage of key intermediates such as gentisic acid, that do not have vicinyl hydroxyl groups. However, gentisate 1,2-dioxygenase contains ferrous ion

and behaves mechanistically like an extradiol dioxygenase.

It is observed that *Pseudomonas* and *Bacillus* spp. are the most ubiquitous microorganisms, being principal components of microflora of most soil and water environments. It is apparent that these group of organisms are probably important as degraders of aromatic compounds in natural environments. The gene analysis of microorganisms will be necessary to determine the modes of induction and expression of monohydroxybenzoic acid degradative pathways.

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