ELSEVIER

FEMS Microbiology Reviews 22 (1999) 439–458

FEMS MICROBIOLOGY REVIEWS

Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway

Caroline S. Harwood [a], Gerhard Burchhardt [b], Heidrun Herrmann [b], Georg Fuchs [c,*]

[a] *Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA*
[b] *Institut für Genetik und Biochemie, Universität Greifswald, Jahnstr. 15a, D-17487 Greifswald, Germany*
[c] *Institut für Biologie II, Mikrobiologie, Universität Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany*

Received 1 September 1998; accepted 22 September 1998

Abstract

Aromatic compounds are important growth substrates for microorganisms. They form a large group of diverse compounds including lignin monomers, amino acids, quinones, and flavonoids. Aerobic aromatic metabolism is characterized by the extensive use of molecular oxygen which is essential for the hydroxylation and cleavage of aromatic ring structures. The anaerobic metabolism of low molecular mass soluble aromatic compounds requires, of necessity, a quite different strategy. In most known cases, aromaticity is broken by reduction and the ring is subsequently opened hydrolytically. A small number of different central aromatic intermediates can be reduced, the most common of which is benzoyl-CoA, a compound that is formed as a central intermediate in the degradation of a large number of aromatic growth substrates. This review concentrates on the anaerobic aromatic metabolism via the benzoyl-CoA pathway. The peripheral pathways that transform growth substrates to benzoyl-CoA include various types of novel reactions, for example carboxylation of phenolic compounds, reductive elimination of ring substituents like hydroxyl or amino groups, oxidation of methyl substituents, *O*-demethylation reactions and shortening of aliphatic side chains. The central benzoyl-CoA pathway differs in several aspects in the denitrifying, phototrophic and fermenting bacteria studied. In denitrifying and phototrophic bacteria it starts with the two-electron reduction of benzoyl-CoA to a cyclic dienoyl-CoA driven by the hydrolysis of two molecules of ATP to ADP+P_i. This ring reduction is catalyzed by benzoyl-CoA reductase and requires a low-potential ferredoxin as an electron donor. In *Rhodopseudomonas palustris* the cyclic diene is further reduced to cyclohex-1-ene-1-carboxyl-CoA. In the denitrifying species *Thauera aromatica*, the cyclic diene is hydrated to give 6-hydroxycyclohex-1-ene-1-carboxyl-CoA. Subsequent β-oxidation results in the formation of a cyclic β-oxo compound, followed by hydrolytic carbon ring opening yielding 3-hydroxypimelyl-CoA in the case of *T. aromatica* and pimelyl-CoA in the case of *R. palustris*. These intermediates are further β-oxidized via glutaryl-CoA; final products are 3 acetyl-CoA and 1 CO_2. In fermenting bacteria benzoyl-CoA may possibly be reduced to the level of cyclohex-1-ene-1-carboxyl-CoA in an ATP-independent reaction. The genes coding for the enzymes of the central benzoyl-CoA pathway have been cloned and sequenced from *R. palustris, T. aromatica,* and *Azoarcus evansii*. Sequence analyses of the genes support the concept that phototrophic and denitrifying bacteria use two slightly different pathways to metabolize benzoyl-CoA. The gene sequences have in some cases been very helpful for the identification of possible catalytic mechanisms that were not obvious from initial characterizations of purified enzymes.

* Corresponding author. Tel.: +49 (761) 203 2649; Fax: +49 (761) 203 2626; E-mail: fuchsgeo@ruf.uni-freiburg.de

0168-6445/99/$19.00 © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V.
PII: S0168-6445(98)00026-6

Keywords: Anaerobic aromatic metabolism; Benzoyl-CoA pathway; *Thauera aromatica*; *Rhodopseudomonas palustris*; *Azoarcus evansii*

Contents

1. Introduction

Aromatic compounds are ubiquitous growth substrates for microorganisms. They have in common with aliphatic hydrocarbons a chemical inertness that makes them 'difficult' substrates. Aerobic organisms that use aromatic compounds as carbon and energy sources first have to introduce hydroxyl groups into these substrates using molecular oxygen as obligatory cosubstrate before they can be utilized. The subsequent cleavage of aromatic carbon-carbon bonds also requires oxygen. Consequently, nature has evolved a huge variety of mono- and di-oxygenase enzymes to attack aromatic compounds [1]. The aerobic processes by which these substrates are oxidized to CO_2 have been well studied and will not be considered here.

The anaerobic metabolism of aromatic compounds, i.e. metabolism without molecular oxygen, has been studied in much less detail, and only in the last decade, based on pioneering studies of Evans and associates among others [2–5]. Some reasons for our relative lack of knowledge include the fact that standard laboratory microorganisms like *Escherichia coli* K12 are unable to metabolize aromatic compounds anaerobically and the relative difficulties of working with anaerobes and conducting biochemical studies with oxygen-sensitive enzymes. In fact, the groundwork for effective study of the problem is just now being laid.

In 1934 Buswell and associates [6] reported on the conversion of benzoate to biogas under methanogenic conditions. So far no eukaryotic organism has been shown to metabolize aromatic compounds in the absence of oxygen. The occurrence of complete degradation of soluble low molecular mass compounds under anaerobic conditions can be deduced from the lack of accumulation of such substrates in anoxic zones like sediments. The aromatic polymer lignin, which is relatively insoluble and cannot be taken up by cells, remains virtually unchanged in the absence of molecular oxygen, however.

Two groups of model microorganisms have been studied in some detail. The phototrophic proteobacterium (α-subgroup) *Rhodopseudomonas palustris* uses various aromatic substrates as the sole sources of cell carbon during anoxic photosynthetic growth [7]. The denitrifying bacteria *Thauera aromatica* and *Azoarcus evansii* and related species that belong to the β-subgroup of proteobacteria can grow with aromatic substrates as sole sources of cell carbon and energy [8–16]. Anaerobic aromatic degradation has also been documented in a variety of sulfate reducing bacteria [17–23] as well as in iron-reducing bacteria

[24–26] and fermentative bacteria [27–32]. Fermentation of aromatic compounds normally requires a syntrophic coculture of an aromatic fermenting bacterium, that produces mainly acetate, CO_2 and hydrogen gas, and a methanogenic or sulfate reducing bacterium that grows on these fermentation products and thus keeps the concentration of metabolic products low [27–32]. Examples of benzoate fermenting bacteria are *Syntrophus* species. Purple non-sulfur phototrophic bacteria and denitrifying bacteria are facultative aerobes that can oxidize many aromatic compounds both anaerobically and aerobically via two completely different pathways, with the aerobic pathways requiring oxygen.

The outlines of the peripheral and central parts of anaerobic pathways for the metabolism of a considerable number of aromatic substrates have been elucidated. Peripheral pathways channel different aromatic substrates into a few central aromatic intermediates: benzoyl-CoA, resorcinol (1,3-dihydroxybenzene), phloroglucinol (1,3,5-trihydroxybenzene), hydroxyhydroquinone (1,3,4-trihydroxybenzene), and possibly others. These intermediates become reduced to alicyclic compounds via a few central pathways. Recent reviews that partly also cover the resorcinol and phloroglucinol pathways are available [33–37]. As one of possibly several exceptions, hydroxyhydroquinone is initially oxidized to hydroxyquinone [38,39]. Ring opening proceeds in a hydrolytic reaction in all cases studied.

This paper deals only with the complete anaerobic metabolism of aromatic substrates that are metabolized via the benzoyl-CoA pathway. Transformations of aromatic compounds where the aromatic structure remains complete will not be covered although many of the reactions involved in incomplete metabolism may be similar to those found in the peripheral pathways discussed below.

2. Biochemistry of peripheral and central pathways

This chapter includes a short outline of the pathways that transform some substrates to benzoyl-CoA, and a description of how benzoyl-CoA is first reduced and how the products are then oxidized to acetyl-CoA. Acetyl-CoA oxidation to CO_2 in anaerobes may not necessarily be identical to what is known in aerobic organisms. The same holds true for the use of acetyl-CoA and CO_2 as sole carbon precursors for biosynthetic building blocks [36,37]. As will become clear, the energetic constraints of strictly anaerobic bacteria are likely to demand biochemical reactions that differ from those found in respiring chemotrophic or in phototrophic bacteria.

2.1. Peripheral pathways leading to benzoyl-CoA

The peripheral pathways by which denitrifying and phototrophic bacteria transform a selection of aromatic substrates to benzoyl-CoA are shown in Fig. 1. This is just a selection of reactions, and many variants of these pathways are to be expected. Under natural conditions, the complete degradation of a given substrate may be more complex and involve other competing microorganisms. This can be illustrated by comparing the fate of the aromatic amino acid tyrosine under different conditions. In enrichment cultures tyrosine is almost quantitatively converted to phenol and a C_3 unit by a group of microorganisms [40]; this reaction is catalyzed by tyrosine phenol lyase. These bacteria live at the expense of the C_3 unit leaving behind phenol, which is later metabolized by denitrifiers such as *Thauera aromatica*. However, in pure culture denitrifiers appear to metabolize tyrosine via 4-hydroxyphenylacetate and 4-hydroxybenzoyl-CoA to benzoyl-CoA, rather than via phenol [41]. Similar arguments may hold true for tryptophan and many other compounds that under natural conditions are degraded stepwise by an interplay of different microorganisms rather than by a single bacterium.

Aromatic amino acids can be oxidized to the corresponding arylacetic acids via conventional reactions [40,41]. Phenylacetate and its ring-hydroxylated analogues are also produced from flavonoids ([42]; M. Blaut, personal communication). Formation of benzoyl-CoA from phenylacetate necessitates the oxidation of the carboxymethyl side chain in the α-position [43,44] after the α-C-H bond has been activated by CoA esterification of the adjacent carboxyl group [45]. Oxidation of phenylacetyl-CoA to phenylglyoxylate and CoA, a four-electron redox reaction, is catalyzed by a newly characterized membrane-bound molybdoprotein, phenylacetyl-CoA:

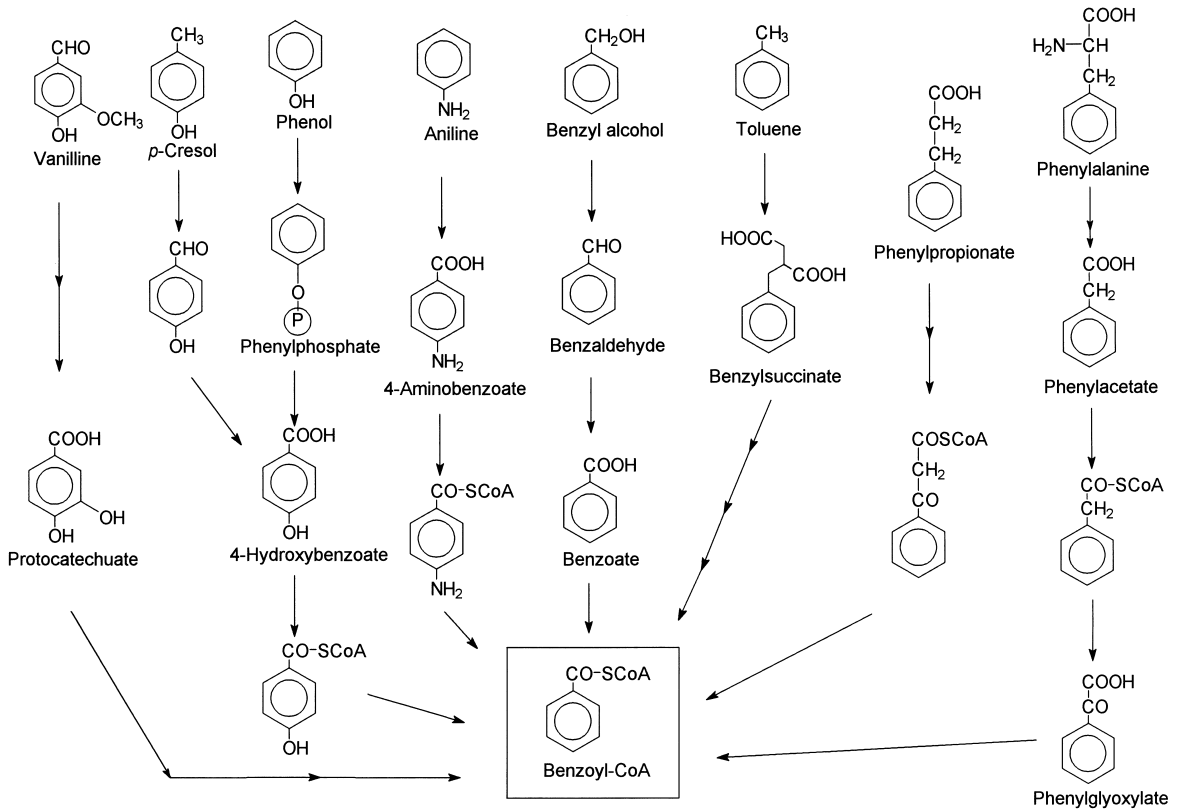

Fig. 1. Peripheral anaerobic pathways for the transformation of some aromatic growth substrates to the central intermediate benzoyl-CoA.

acceptor oxidoreductase ([46]; S. Rhee, G. Fuchs, unpublished). The electron acceptor may be a quinone. Oxidation of phenylglyoxylate to benzoyl-CoA and CO_2 is catalyzed by phenylglyoxylate:acceptor oxidoreductase [47]. The corresponding genes were cloned and expression of the CoA ligase and the phenylglyoxylate:acceptor oxidoreductase was observed in the heterologous host *E. coli* under anaerobic conditions (S. Haas, H. Herrmann, G. Burchhardt, unpublished). Benzoate, like most aromatic acids, is converted to benzoyl-CoA by a CoA ligase [48–51]. Other phenylalkane compounds or phenyl fatty acids may be oxidized by normal β-oxidation to benzoyl-CoA or phenylacetyl-CoA [52].

Different problems have to be overcome when phenol is converted to benzoyl-CoA [17,53]. This process requires three intermediates: phenylphosphate, 4-hydroxybenzoate, and 4-hydroxybenzoyl-CoA. *para*-Carboxylation of phenol to 4-hydroxybenzoate, known in chemistry as the Kolbe-Schmitt reaction, would be endergonic given the low ambient substrate concentrations. Thus, phenol is phosphorylated to phenylphosphate in an exergonic reaction; this requires an as yet unidentified energy-rich phosphoryl donor [54]. This is followed by an exergonic Mn^{2+}-dependent carboxylation of phenylphosphate to 4-hydroxybenzoate which is extremely oxygen-sensitive [55–57]. The whole process is thought to involve several proteins, as indicated from a search for genes coding for phenol-induced proteins. A cluster of genes was found, two of which are similar to phosphoenol-pyruvate synthase and may code for the phosphorylating enzyme. Four other genes have similarity with genes coding for enzymes of ubiquinone biosynthesis in *E. coli* that catalyze the decarboxylation of a 4-hydroxybenzoate derivative. These latter genes are thought to be involved in the carboxylation step (S. Breinig, H. Schägger, E. Schiltz, G.

Fuchs, unpublished). 4-Hydroxybenzoate is converted to its CoA thioester by a specific CoA ligase [58,59], and 4-hydroxybenzoyl-CoA is reductively dehydroxylated to benzoyl-CoA by a new molybdo-flavo-iron-sulfur enzyme, 4-hydroxybenzoyl-CoA reductase, with reduced ferredoxin serving as an electron donor [60–63]. Both carboxylation and reductive elimination of phenolic hydroxyl groups are common reactions in the metabolism of other phenolic and anilinic compounds [64–70]. Fermenting bacteria may also decarboxylate 4-hydroxybenzoate and derivatives to the phenolic compound and CO_2 by enzymes such as 4-hydroxybenzoate decarboxylase [57,71–74]. This simple enzyme may also bring about the carboxylation reaction at high concentrations of phenol and CO_2 and provided that 4-hydroxybenzoate is efficiently removed.

An interesting case is the oxidation of methyl substituents of the aromatic ring to carboxyl groups (H. Heider et al., this volume). Toluene is oxidized to benzoyl-CoA, *m*-xylene (1,3-dimethylbenzene) to 3-methylbenzoate (or its CoA ester), and *m*-cresol (3-methylphenol) to 3-hydroxybenzoate (or its CoA ester) [75]. In the case of toluene, this seemingly simple six-electron oxidation process requires a battery of different enzymes and intermediates. The initial attack is the addition of the methyl group of toluene to the double bond of fumarate, producing benzylsuccinate. This reaction requires a radical mechanism and a radical generating enzyme (Heider et al., this volume). Benzylsuccinate is further oxidized by β-oxidation, yielding benzoyl-CoA and succinyl-CoA. Similar pathways may be envisaged for the oxidation of the methyl group of *m*-xylene and *m*-cresol. The fate of the products, 3-hydroxybenzoate and 3-methylbenzoate, is still unknown. The oxidation of *p*-cresol (4-methylphenol) to 4-hydroxybenzoate proceeds, as in aerobic bacteria [71,76], by a *p*-cresol methylhydroxylase forming first, the alcohol, and then the aldehyde. 4-Hydroxybenzaldehyde is oxidized by an aldehyde dehydrogenase to 4-hydroxybenzoate. In contrast, *o*-cresol appears to be first carboxylated at the position *para* to the phenolic hydroxyl group [71].

Many aromatic compounds contain phenolic hydroxyl groups that are protected from undesired reaction by methyl ether formation. Aromatic methyl ether bonds are cleaved by vitamin B_{12}-dependent enzymes in which Co(I) represents a supernucleophile that is able to accept a methyl cation and to transfer it to tetrahydrofolate forming N^5-methyltetrahydrofolate. This process is known as *O*-demethylation [77–85].

2.2. Whole cell regulation of peripheral pathways

The whole cell regulation of peripheral pathways has been studied only in a limited number of bacteria and with a limited number of substrates. It is to be expected that regulation patterns and mechanisms differ in different organisms even if the set of enzymes used is similar. The organization of the genes will also differ (see below). A representative example of how peripheral pathways are regulated is the conversion of phenol to benzoyl-CoA by *Thauera aromatica* [86,87]. Phenol appears to be the inducer of a set of genes required for phenol phosphorylation and carboxylation to 4-hydroxybenzoate. 4-Hydroxybenzoate seems to induce 4-hydroxybenzoate-CoA ligase, and 4-hydroxybenzoyl-CoA probably induces 4-hydroxybenzoyl-CoA reductase. Note that 4-hydroxybenzoate is a branching point where *p*-cresol metabolism feeds in, and 4-hydroxybenzoyl-CoA is also formed from 4-hydroxyphenylacetate (derived from tyrosine). This suggests that probably three inducers are required for the regulation of three operons, which are not in close proximity to each other. In addition, a ferredoxin is required which is encoded by a gene lying on an additional operon which contains all enzymes of the central benzoyl-CoA pathway; this ferredoxin serves for both reductive dehydroxylation and for ring reduction. Finally, benzoyl-CoA may induce the central benzoyl-CoA pathway that transforms benzoyl-CoA to 3-hydroxypimelyl-CoA. The anaerobic pathways are repressed under aerobic conditions indicating a strict oxygen regulation. Whether nitrate, nitrite, NO, or N_2O exert a regulatory role in denitrifying bacteria is unknown. *T. aromatica* is able to thrive on aromatic substrates and N_2O, suggesting that nitrate is not an obligatory inducer. Catabolite repression probably also plays a role. Similar general patterns of regulation in response to oxygen and carbon source are also seen in *R. palustris*. The transcriptional regulation of the benzoyl-CoA reductase operon is discussed below.

2.3. Central benzoyl-CoA pathway: variants and common parts

T. aromatica and *R. palustris* metabolize benzoyl-CoA by two slightly different pathways. The variants of the central benzoyl-CoA pathway are shown in Fig. 2. Benzoyl-CoA is well suited to become reduced due to the CoA-thioesterified carboxyl group directly adjacent to the ring. The enzymatic aromatic ring reduction has been studied in some detail in *T. aromatica*. It is thought to proceed in alternating electron transfer and protonation steps, as observed in chemical Birch reduction of the aromatic ring. The redox potential of the first electron transfer is lowered by the COSCoA group ($E_0' = -1.8$ V) by 1.3 V compared to the unsubstituted benzene ring ($E_0' = -3.1$ V) (M. Boll, T. Mittelberger, J. Heinze, W. Eisenreich, A. Bacher, G. Fuchs, unpublished).

The reduction of benzoyl-CoA is catalyzed by the iron-sulfur protein benzoyl-CoA reductase [88,89]. The enzyme from *T. aromatica* catalyzes a two-electron reduction and requires 2 ATP which are hydrolyzed to ADP and inorganic phosphate. The product is cyclohex-1,5-diene-1-carboxy-CoA ([90,91], M. Boll, T. Mittelberger, J. Heinze, W. Eisenreich, A. Bacher, G. Fuchs, unpublished). The electron donor is a ferredoxin which contains two 4Fe/4S centers with a midpoint potential at pH 7 below that of the hydrogen electrode [92]. How this ferredoxin is reduced is unknown. The ATP requirement has been confirmed for benzoyl-CoA reducing enzymes from other sources (G. Fuchs, unpublished), although the exact ATP stoichiometry is not yet known. The nature of the ring reduction product may differ. In *R. palustris* the ring reducing enzyme may possibly reduce the diene intermediate [93] further to cyclohex-1-ene-1-carboxyl-CoA, but this has not been demonstrated.

Benzoyl-CoA reductase also reduces hydroxylamine and azide in an ATP-dependent two-electron reduction. Products should be ammonia plus water and ammonia plus nitrogen gas, respectively. Benzoyl-CoA reductase is generally specific for benzoyl-CoA but also reduces some benzoyl-CoA analogues, while phenylacetyl-CoA or free benzoate are not reduced [90]. A reaction mechanism has been proposed that is based on homologies between the deduced amino acid sequences of the four subunits of benzoyl-CoA reductase with the three subunits of the 2-hydroxyglutaryl-CoA hydratase system of *Acidaminococcus fermentans* [94,95]. The hydratase consists of a two-subunit enzyme that dehydrates the 2-hydroxyacyl-CoA compound to glutaconyl-CoA, and an activase protein that activates the dehydratase in a reaction that requires ATP and a reductant [96–98]. Activation probably involves the generation of an enzyme radical that represents the active form of the dehydratase and a radical mechanism has been proposed (Fig. 3). Similarly, benzoyl-CoA reduction has been proposed to proceed via radical intermediates [99]. In the dehydration reaction catalyzed by 2-hydroxyglutaryl-CoA hydratase, a one-electron reduction is proposed to be compensated for by an oxidation during the same catalytic cycle. Thus, only catalytic amounts of ATP are required to generate the radical stage of the enzyme once. The ring reduction reaction, in contrast, requires stoichiometric amounts of ATP to accomplish a net transfer of electrons to the ring.

Cyclohex-1,5-diene-1-carboxy-CoA, the product of the ring reduction reaction in *T. aromatica*, is hydrated by a specific dienoyl-CoA hydratase to give 6-hydroxycyclohex-1-ene-1-carboxy-CoA [100]. This is then followed by NAD^+-dependent oxidation of the 6-hydroxy group to the 6-oxo group by a specific β-hydroxyacyl-CoA dehydrogenase. 6-Oxocyclohex-1-ene-1-carboxy-CoA is converted to 3-hydroxypimelyl-CoA by a hydrolytic enzyme that has been purified (D. Laempe and G. Fuchs, unpublished). This conversion must necessarily also include the addition of water at some point. At present it is not known whether water is added to the double bond prior to (which is more plausible), or after, the ring is cleaved, or how this is accomplished. Although the ring hydrolase appears to be pure, trace contamination by a very active enoyl-CoA hydratase cannot be excluded.

In *R. palustris* the sequence of reactions that occurs following ring reduction includes a specific enoyl-CoA hydratase that converts cyclohex-1-ene-1-carboxy-CoA to the 2-hydroxy compound [94], followed by oxidation with an NAD^+-dependent alcohol dehydrogenase to yield the 2-oxo compound ([101]; Pelletier and Harwood, unpublished). The β-oxoacyl-CoA compound is then cleaved by a ring hydrolyzing enzyme to produce pimelyl-CoA [102], which

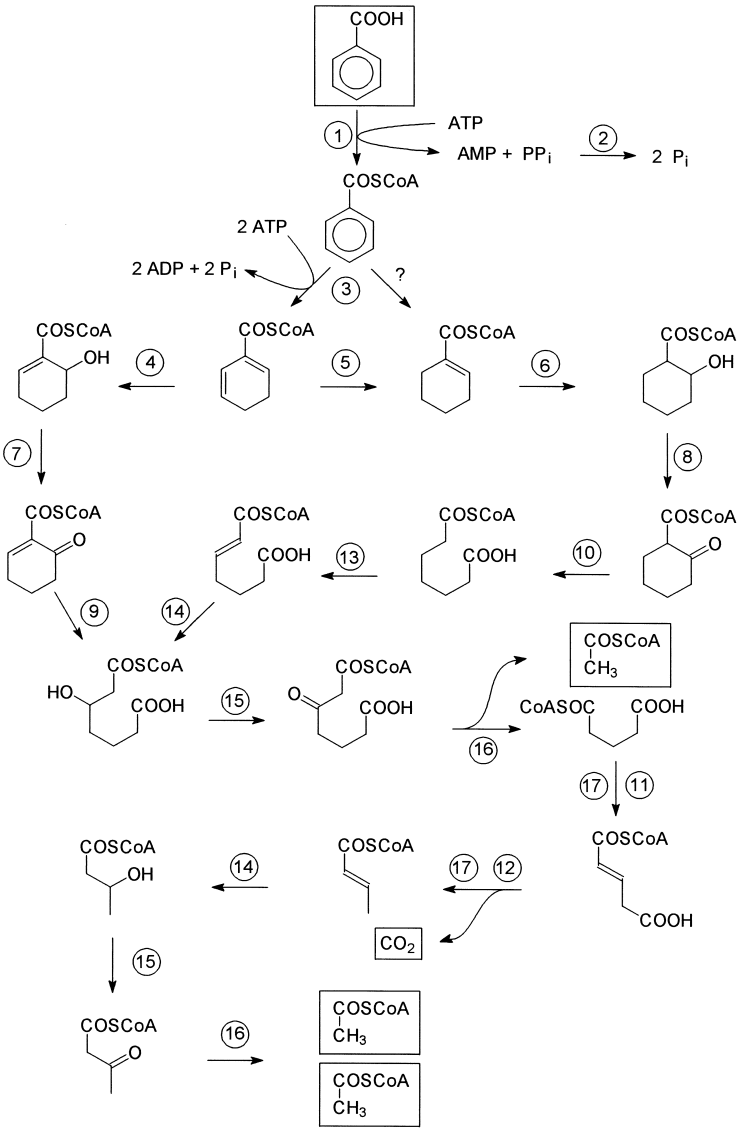

Fig. 2. Variants of the central benzoyl-CoA pathway in different bacteria (*Thauera aromatica*, *Rhodopseudomonas palustris*, *Syntrophus gentianae*). 1: Benzoate-CoA ligase (AMP-forming); 2: pyrophosphatase (in fermenting bacteria a proton-transducing membrane protein); 3: benzoyl-CoA reductase (hydrolyzing two ATP; in fermenting bacteria probably ATP-independent); 4: cyclic dienoyl-CoA hydratase; 5: dienoyl-CoA reducing enzyme activity; 6: cyclic enoyl-CoA hydratase; 7: β-hydroxyacyl-CoA dehydrogenase acting on cyclic 6-hydroxy-1-ene compound; 8: β-hydroxyacyl-CoA dehydrogenase acting on cyclic 2-hydroxy compound; 9: ring hydrolase presumably acting on cyclic 6-oxo-1-ene compound; 10: ring hydrolase acting on cyclic 2-oxo compound; 11: membrane-bound glutaryl-CoA dehydrogenase; 12: glutaconyl-CoA decarboxylase (membrane-bound); 13: acyl-CoA dehydrogenase; 14: enoyl-CoA hydratase; 15: 3-hydroxyacyl-CoA dehydrogenase; 16: β-ketothiolase; 17: soluble glutaryl-CoA dehydrogenase (decarboxylating). Reactions 1, 3, 4, 7, and 9 leading to 3-hydroxypimelyl-CoA have been demonstrated in *T. aromatica*. Reactions 1, 3, 6, 8, and 10 leading to pimelyl-CoA have been demonstrated in *R. palustris*. Reaction 17 has also been demonstrated in *T. aromatica* and *R. palustris*. Reactions 1, 2 and 11, 12 have been demonstrated in *S. gentianae*.

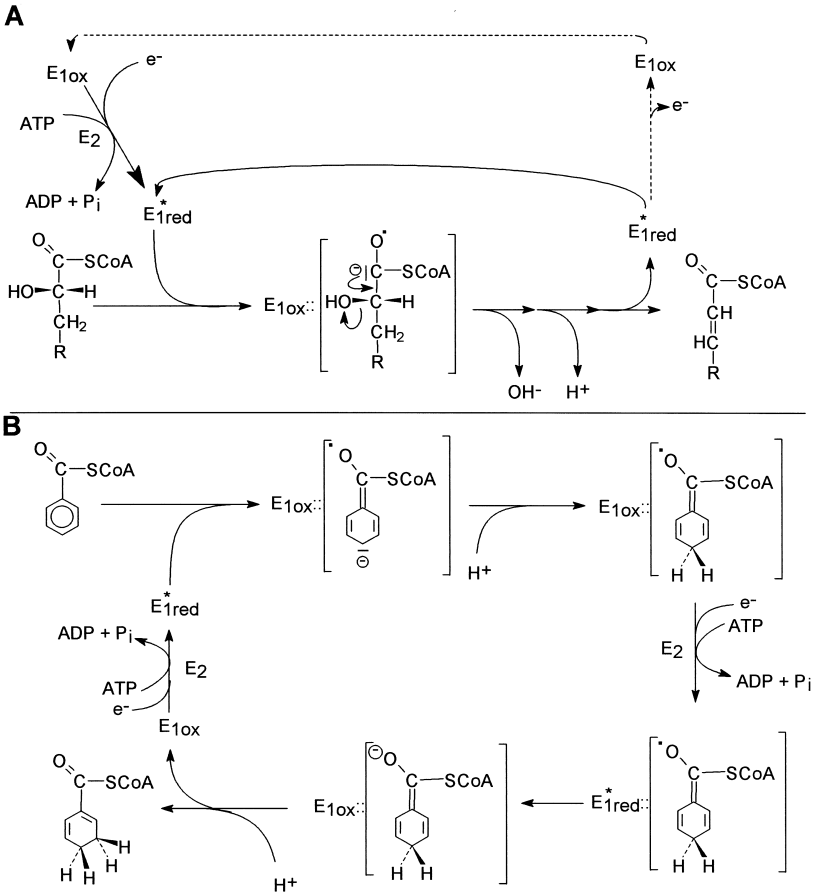

Fig. 3. Proposed reaction mechanism of (A) 2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans* and (B) ATP-dependent benzoyl-CoA reductase. Upper part: E_1 dehydratase, E_2 activase. Lower part: E_1 two-enzyme subunits of benzoyl-CoA reductase that are homologous to dehydratase; E_2 two-enzyme subunits of benzoyl-CoA reductase that are homologous to activase. E_1* indicates an activated state of the enzyme.

is subsequently oxidized via 3-hydroxypimelyl-CoA, as in *T. aromatica*.

β-Oxidation of (3-hydroxy)pimelyl-CoA first yields glutaryl-CoA and one molecule of acetyl-CoA. The intermediate glutaryl-CoA is then oxidized to glutaconyl-CoA and decarboxylated to crotonyl-CoA by one soluble flavo-enzyme, glutaryl-CoA dehydrogenase (decarboxylating) [103]. Crotonyl-CoA is oxidized to two molecules of acetyl-CoA. Hence, the final products of benzoyl-CoA metabolism are 3 acetyl-CoA, 1 CO_2 and 6 reducing equivalents. The reducing equivalents used in ring reduction are regained. Acetyl-CoA is finally oxidized to CO_2.

Energy is conserved by electron transport phosphorylation in anaerobic respiration or photosynthesis. The complete oxidation of benzoate in denitrifiers is described by the two equations

$$C_6H_5COO^- + H^+ + 12\ H_2O \rightarrow 7\ CO_2 + 30\ [H]$$

$$30\ [H] + 6\ HNO_3 \rightarrow 18\ H_2O + 3\ N_2.$$

Phototrophs and denitrifiers use acetyl-CoA for the synthesis of cell building blocks.

2.4. The special case of fermenting bacteria

A third variant of the central benzoyl-CoA pathway seems to exist in fermenting bacteria and possibly in other strict anaerobes [31,32], as has been previously postulated [33,36,90]. The Gram-negative

bacterium *Syntrophus gentianae* degrades benzoate only when cocultured with acetate and hydrogen consuming methanogenic or sulfate reducing bacteria. Recent studies indicate that benzoate is activated to its CoA ester by an AMP- and pyrophosphate-forming CoA ligase and that pyrophosphate is hydrolyzed by a membrane-bound pyrophosphatase. This reaction is coupled to translocation of probably a single proton which is equivalent to one-third of an ATP. Reduction of benzoyl-CoA may generate cyclohex-1-ene-1-carboxyl-CoA. Thermodynamic calculations indicate that such a four-electron reduction would not necessarily require the hydrolysis of ATP. Further β-oxidation may proceed with the same intermediates as in *R. palustris*. However, there are enzymatic differences. Oxidation of glutaryl-CoA requires two enzymes, glutaryl-CoA dehydrogenase and a sodium ion-dependent, membrane-bound biotin-enzyme, glutaconyl-CoA decarboxylase. Decarboxylation is thought to translocate one sodium ion generating the equivalent of one-third of an ATP. Two oxidation steps, oxidation of pimelyl-CoA and of glutaryl-CoA, are probably membrane-bound and a proton-gradient-dependent reversed electron transport is required in order to release electrons from these 'high-potential' substrates as molecular hydrogen at the prevailing hydrogen partial pressure of 10^{-5} bar. These reactions are thought to consume two-thirds of an ATP each.

Benzoate fermentation results in the formation of 3 acetyl-CoA molecules which are used for ATP synthesis by substrate level phosphorylation via phosphotransacetylase and acetate kinase. The overall stoichiometry is as follows:

$$C_6H_5COO^- + H^+ + 6\ H_2O \rightarrow 3\ CH_3COO^- + 3\ H^+ + CO_2 + 3\ H_2.$$

The standard free energy change of this reaction at pH 7 is +47 kJ/mol benzoate. If one takes into account the physiological concentrations of the reactants in the order of 10^{-3} M and a hydrogen partial pressure of 10^{-5} bar the G′ value becomes −90 kJ/mol benzoate. If an output of 3 2/3 ATP per benzoate (assuming that the benzoyl-CoA reduction reaction does not require ATP) is compared with an input of 3 1/3 ATP, this leaves just one-third of an ATP for the fermentation. Such an energy yield is the absolute minimum necessary for an energy metabolism. This account does not consider a possible energy-driven benzoate uptake nor the possibility of the co-translocation of 3 H^+ together with 3 molecules of acetate that are excreted and then consumed by the syntrophic partner organism, e.g. by methanogens:

$$3\ CH_3COO^- + 3\ H^+ + CO_2 + 3\ H_2 \rightarrow 3.75\ CH_4 + 3.25\ CO_2.$$

The proton gradient created may allow ATP synthesis via a chemiosmotic mechanism. If the benzoyl-CoA reductase of *Syntrophus* sp. is in fact an ATP-independent enzyme, then it will be interesting to compare it with the ATP-dependent enzyme from other sources.

3. Molecular biology of the central benzoyl-CoA pathway

3.1. Gene organization and functional assignments

Clusters of genes involved in anaerobic benzoate degradation have been cloned and sequenced from the two denitrifiers, *T. aromatica* and *A. evansii*, and from the phototroph, *R. palustris* (Fig. 4) ([94,95,104]; K. Mohr, H. Herrmann and G. Burchhardt, unpublished). Firm functional assignments have been made for most of the *T. aromatica* genes based on comparison of their deduced amino acid sequences with the experimentally determined N-terminal amino acid sequences of enzymes purified from this bacterium (Fig. 5). In the case of *R. palustris*, the gene products indicated in Fig. 5, with the exception of benzoyl-CoA reductase, have been expressed in *E. coli* and shown to have the activity indicated. The benzoyl-CoA reductase genes, *badDEFG*, were initially identified based on the phenotypes of two mutants that were constructed by reverse genetic approaches. These mutants (*badE*::km and *badF*::km) were unable to grow on benzoate, but grew at wild-type rates on the free acid versions of several proposed benzoate pathway intermediates including cyclohex-1,5-diene-1-carboxylate [94]. Subsequently it was found that the deduced amino acid

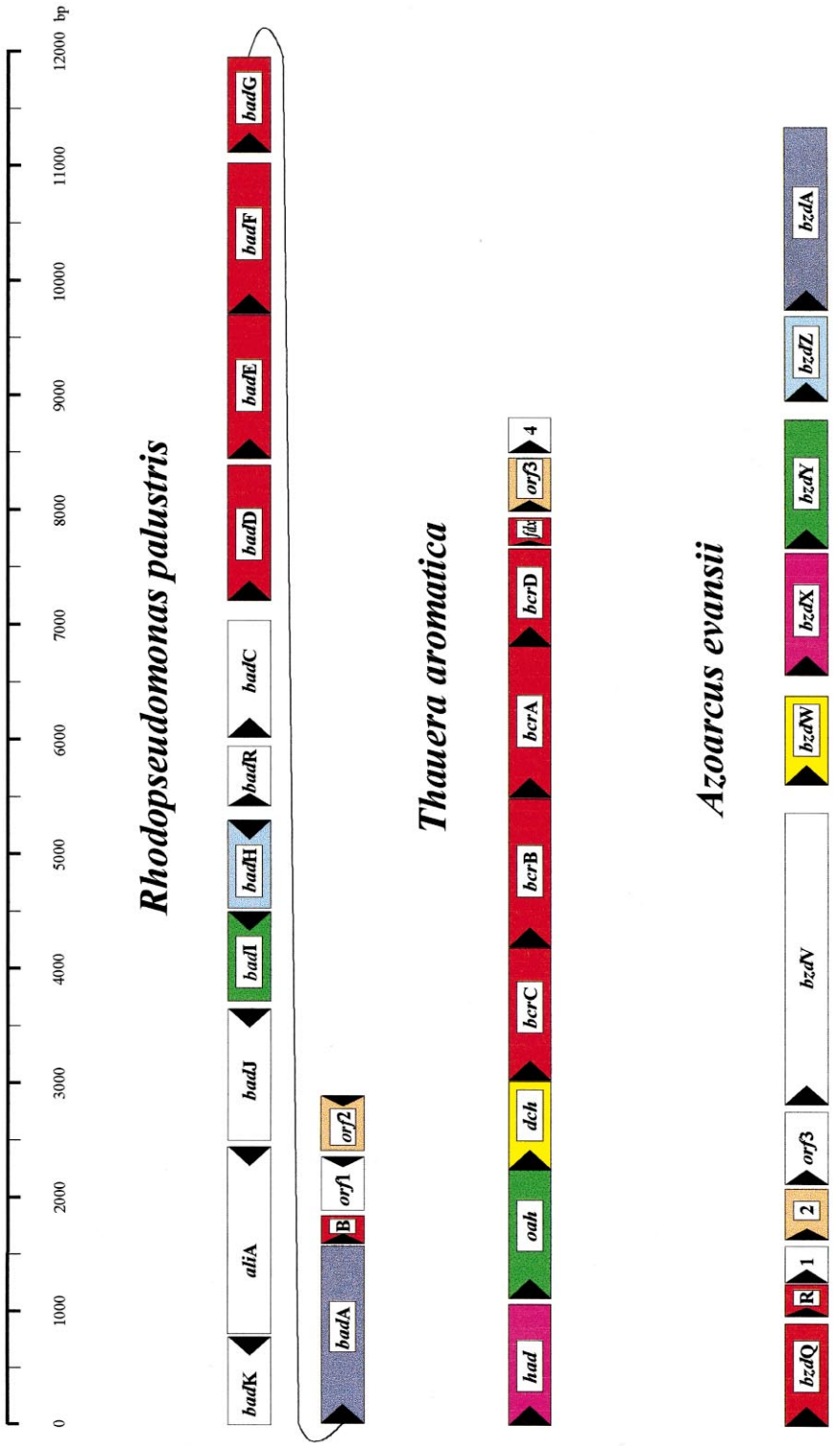

Fig. 4. Maps of benzoyl-CoA degradation gene clusters from *R. palustris*, *T. aromatica* and *A. evansii*. Arrows indicate direction of transcription. Genes in different clusters that have the same pattern are homologous. The genes from *R. palustris* and *T. aromatica* encode enzymes of benzoate degradation as indicated in Fig. 5. Other gene assignments are discussed in the text and in Table 1.

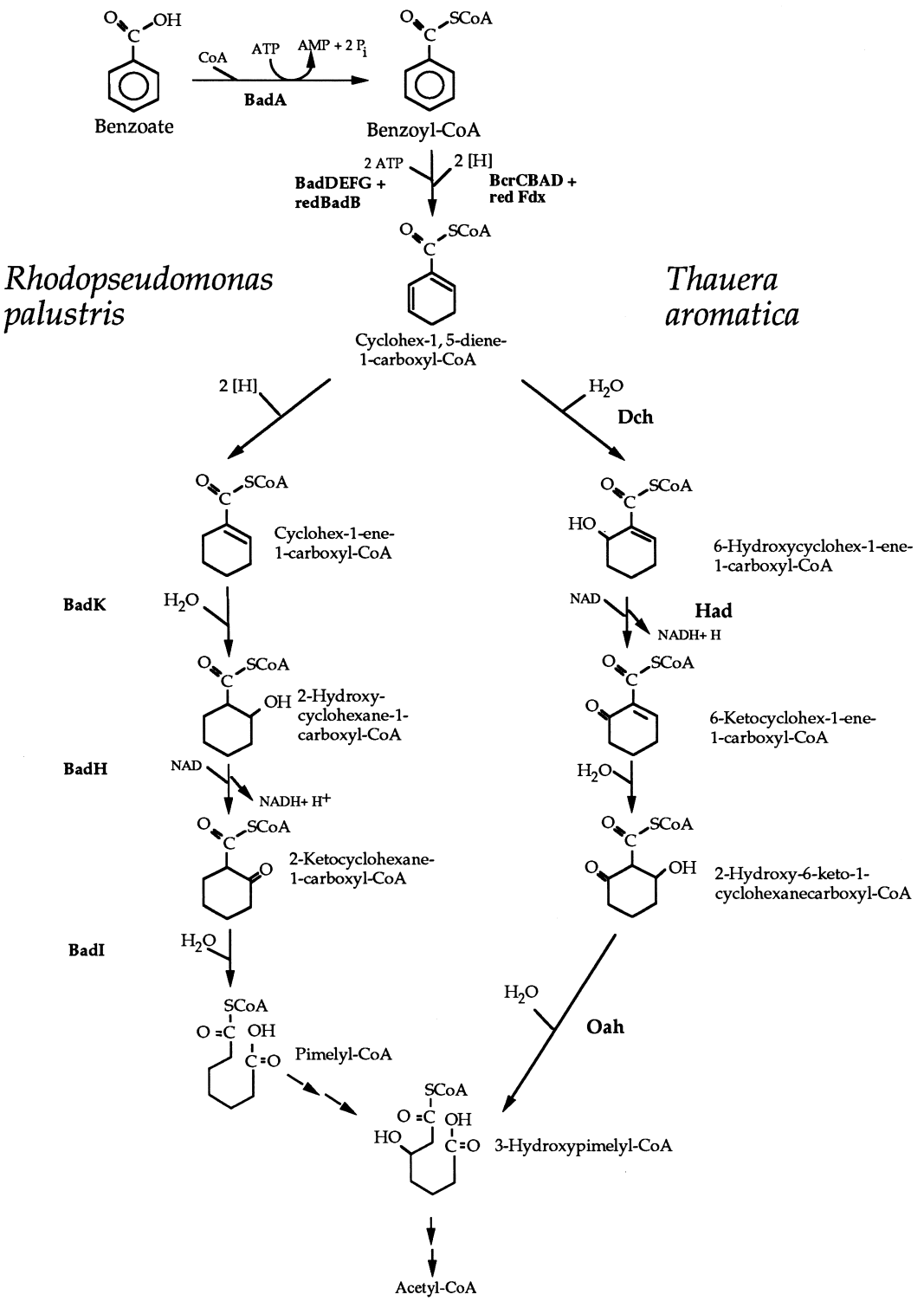

Fig. 5. Comparison of anaerobic benzoate degradation by *R. palustris* and *T. aromatica*.

Table 1
Comparisons of deduced amino acid sequences of benzoate degradation genes

Enzyme	Sequence identity/similarity (%)		
	T. aromatica to *A. evansii*	*T. aromatica* to *R. palustris*	*R. palustris* to *A. evansii*
Benzoate-CoA ligase (BadA/BzdA)	NI	NI	57/72
Ferredoxin (Fdx/BadB/BzdR)	24/42	67/81	20/32
Benzoyl-CoA reductase			
α-subunit (BcrA/BadF)	NI	69/82	NI
β-subunit (BcrB/BadE)	NI	76/90	NI
γ-subunit (BcrC/BadD)	NI	67/81	NI
δ-subunit (BcrD/BadG/BzdQ)	33/54	70/79	30/53
Dienoyl-CoA hydratase (Dch/BzdW)	39/57	NC	NC
6-Hydroxycyclohex-1-ene-1-carboxyl-CoA dehydrogenase (Had/BzdX)	46/65	NC	NC
Alicyclic acid-CoA hydrolase (Oah/BadI/BzdY)	49/66	31/52	31/51

NI: one gene in the pair not yet identified.
NC: no corresponding enzyme present in the *R. palustris* pathway.

sequences of the BadDEFG proteins have a high degree of identity to the BcrCBAD subunits of the *T. aromatica* benzoyl-CoA reductase described above. In *A. evansii*, a gene cluster that included the *bzdA* gene, encoding benzoate-CoA ligase, was identified by hybridization with degenerate oligonucleotides designed from the N-terminal amino acid sequence of benzoate-CoA ligase purified from anaerobically-grown cells (G. Burchhardt and H. Herrmann, unpublished). Direct experimental evidence for the involvement of other genes in the *A. evansii* cluster in anaerobic benzoate degradation has not yet been obtained, but can be inferred in some cases based on comparisons with *T. aromatica* benzoate-degradation genes.

Analyses of the nucleotide sequences of the *R. palustris* and *T. aromatica* benzoate degradation genes (Fig. 4) support the concept that these two organisms metabolize benzoyl-CoA by two different routes. They have very similar benzoyl-CoA reductases with an overall amino acid sequence identity of the order of 70% (Table 1). Once benzoyl-CoA is reduced, however, the two pathways diverge, with the next step in *T. aromatica* being a hydration and the next probable step in *R. palustris* being a second 2-electron reduction. Two slightly different sets of reactions ensue which lead to the formation of ring cleavage substrates that have different structures. Differences in ring-cleavage substrates are reflected in differences in genes. The *R. palustris* and *T. aromatica* ring-cleavage genes are very different in size; the *T. aromatica oah* gene is 40% longer than the *R. palustris badI* gene, and the deduced amino acid sequences of BadI and Oah share only 30% identity.

Other genes in the clusters encode enoyl-CoA hydratases and alcohol dehydrogenases that are specific to either the *R. palustris* or the *T. aromatica* pathways. The *dch* and *badK* genes each encode enoyl-CoA hydratases. However, the two encoded enzymes are active with different substrates and catalyze reactions specific to either the *T. aromatica* (in the case of Dch) or *R. palustris* (in the case of BadK) pathways (Fig. 5). Consistent with this, these two genes share just 25/33% amino acid identity/similarity and are no more similar to each other than they are to other enoyl-CoA hydratases from mammalian and bacterial sources. Similarly Had from *T. aromatica* and BadH from *R. palustris* each catalyze NAD-dependent dehydrogenation reactions, but again with different substrates. In accordance with these differences, the Had sequence indicates membership in the family of long-chain zinc-containing alcohol dehydrogenases, whereas BadH is a short chain alcohol dehydrogenase. The two proteins show no significant amino acid sequence similarity.

Nucleotide sequence analysis indicates that the benzoate degradation by *A. evansii* probably follows a route similar to that used by *T. aromatica*, although physiological studies will be required to

confirm this. As indicated in Table 1, the *A. evansii* cluster includes genes that have reasonably high, though not strikingly so, identities at the amino acid level to the *dch*, *had* and *oah* genes. It also contains a gene, *bzdZ*, that is very similar to the *R. palustris badH* gene. At this point it is not clear where *bzdZ* functions in the benzoate degradation pathway, assuming that the pathway is similar to that of *T. aromatica*.

A puzzling feature of the *A. evansii* gene cluster is the presence of a gene (*bzdQ*) that is related to the benzoyl-CoA reductase subunit genes *badG* (*R. palustris*) and *bcrD* (*T. aromatica*), but much more distantly so than the *R. palustris* and *T. aromatica* genes are to each other. If the *A. evansii* BzdQ protein is in fact a subunit of benzoyl-CoA reductase, then it will be interesting to eventually compare the catalytic properties of benzoyl-CoA reductases to see if the *A. evansii* enzyme differs in some significant way. Another formal possibility is that *bzdQ* and associated genes that are presumably adjacent, but that have not yet been cloned, encode an enzyme that catalyzes a reaction other than benzoyl-CoA reduction.

3.2. Insights gained from gene sequences

The deduced amino acid sequences of benzoyl-CoA reductase and the ring cleavage enzymes, BadI (from *R. palustris*) and Oah/BzdY (from the denitrifiers) have been particularly informative because they have directed investigators to think about possible catalytic mechanisms that were not obvious from initial characterizations of purified enzymes. Hydrolytic enzymes that cleave carbon-carbon bonds are unusual and since cofactors or prosthetic groups were not found to be associated with purified 2-oxocylohexanecarboxyl-CoA hydrolase (BadI), it was difficult to draw inferences about key mechanistic features of the enzyme. Thus it was very helpful to know from the deduced amino acid sequence of BadI that it is homologous to members of the crotonase superfamily [102,105]. BadI is ca. 45, 35 and 25% identical at the amino acid level to naphthoate synthases, enoyl-CoA hydratases and 4-chlorobenzoyl-CoA dehalogenases, respectively. Oah/BzdY shows slightly less, but still striking similarity to these three types of enzymes. Although enzymes of the crotonase superfamily catalyze seemingly different reactions, all have been proposed to form a thioester-enolate intermediate during catalysis [106]. This is also likely to be a central feature of the ring cleavage hydrolases and should serve as a useful guide for future work, which might include, as one aspect, studies of specific site mutants. The close relationship of BadI and Oah/BzdY to naphthoate synthases is particularly intriguing, because these catalyze a ring closure reaction, which is formally the reverse of the ring cleavage reaction catalyzed by BadI and Oah.

The stoichiometric consumption of ATP that accompanies the reduction reaction catalyzed by benzoyl-CoA reductase is a feature that is shared by just one other well-studied enzyme, nitrogenase. This uniquely prokaryotic enzyme resembles benzoyl-CoA reductase in that it catalyzes a reduction reaction that requires a high activation energy. Examination of the benzoyl-CoA reductase gene sequences showed, however, that this enzyme is not similar to nitrogenase, but instead is related to 2-hydroxyglutaryl-CoA dehydratase from the anaerobe *Acidaminococcus fermentans* [94,95,97,98]. Proposed mechanisms of the dehydratase and benzoyl-CoA reductase reactions are compared and shown in Fig. 3 and are described above. 2-Hydroxyglutaryl-CoA dehydratase is a three-subunit enzyme comprised of the two catalytic subunits HgdA and HgdB which share amino acid similarity with BadE/BcrB and BadD/BcrC, respectively. The third 'activase' subunit, HgdC, is needed only in catalytic amounts, relative to HgdA and B, and is similar in amino acid sequence to BadF/BcrA and BadG/BcrD/BzdQ. This suggests that BadF/BcrA and BadG/BcrD are the subunits that are involved in ATP binding and hydrolysis. In fact, when considered as a unit, an ATPase binding domain can be found in the amino acid sequences of these proteins. There is an adenosine binding domain and one phosphate binding motif in BadF/BcrA and a phosphate binding domain on BadG/BcrD [107]. The predicted amino acid sequence of the enzymes together with studies with the purified 2-hydroxyglutaryl-CoA dehydratase and benzoyl-CoA reductase all point to the idea that, as a consequence of ATP hydrolysis, electrons are energized to a redox potential that is sufficiently low to accomplish difficult reduction reactions.

Benzoyl-CoA reductase and 2-hydroxyglutaryl-

CoA dehydratase appear to be founding members of a new family of enzymes. Open reading frames homologous to the genes encoding these proteins have been sequenced from *Archaeoglobus fulgidus*, *Methanopyrus kandleri*, *Methanococcus jannaschii* and *Escherichia coli*. The functions of the enzymes that are presumably encoded by these open reading frames are not yet known.

3.3. Missing genes

Some expected genes are missing from the sequenced gene clusters shown in Fig. 4. These include regulatory genes, none of which appear to be present in either the *T. aromatica* or *A. evansii* clusters, and a benzoate-CoA ligase gene, missing from the *T. aromatica* cluster. In *R. palustris* a gene that specifies the conversion of cyclohexadienecarboxyl-CoA to cyclohex-1-ene-1-carboxyl-CoA has not been identified. It is possible that *badC*, predicted to encode a long-chain alcohol dehydrogenase, plays this role, but this has not been proven. Another formal possibility is that the *R. palustris* benzoyl-CoA reductase catalyzes a four-electron reduction of benzoyl-CoA directly to cyclohex-1-ene-carboxyl-CoA. This idea has yet to be explored experimentally, however.

3.4. Genes unrelated to benzoyl-CoA metabolism

The *R. palustris* cluster includes two 'extra' genes whose functions are known. These are *aliA* which encodes cyclohexanecarboxylate-CoA ligase and *badJ* which encodes cyclohexanecarboxyl-CoA dehydrogenase, a flavin-containing enzyme. These enzymes function to feed the alicyclic acid cyclohexanecarboxylate, a growth substrate for *R. palustris*, into the benzoate pathway by converting it to cyclohex-1-ene-1-carboxyl-CoA [108,109]. In fact, the *badHIJaliAbadJK* operon encodes all five enzymes necessary for the degradation of cyclohexanecarboxylate to pimelyl-CoA. Three of these, *badH*, *badI* and *badK*, are involved in catalyzing reactions that are shared by the anaerobic benzoate and cyclohexanecarboxylate degradation pathways. This highlights a major difference between the pathways of the phototrophs and the denitrifiers. Whereas in *R. palustris* cyclohexanecarboxylate degradation and benzoate degradation are intimately associated and share common enzymes, this does not appear to be the case for the denitrifiers.

3.5. Genes with unknown functions

The three gene clusters each include a few extra genes whose functions are not known. Homologous versions of one gene (*orfs 2* in *A. evansii* and *R. palustris* and *orf3* in *T. aromatica*) are found in all three organisms, suggesting that they may play a role in anaerobic benzoate degradation. What this role may be is an open question because these genes do not strongly resemble any other genes in the databases. The deduced amino acid sequence of the *A. evansii* gene designated *bzdV* has some similarity to formate dehydrogenases, hydrogenases and glutamine synthases. The significance, if any, of this gene for anaerobic benzoate degradation is not known.

3.6. Transcriptional regulation of the benzoyl-CoA pathway

Physiological studies have shown that *T. aromatica*, *A. evansii* and *R. palustris* each modulate levels of benzoate degradation enzymes in response to the environmental cues of oxygen and carbon source [86,87,101,110]. A *badE::'lacZ* chromosomal fusion strain of *R. palustris* has been used to show that for the *badDEFG* operon, at least, much of this regulation occurs at the level of transcription. Levels of *badE::lacZ* expression were 100-fold higher in cells grown anaerobically with benzoate as compared to cells grown aerobically on succinate. Two proteins, AadR and BadR, regulate *badE* expression in response to oxygen and benzoate, respectively ([107,111,112]; Egland and Harwood, unpublished).

The *aadR* gene maps to a region of the *R. palustris* chromosome outside the benzoate degradation gene cluster. *R. palustris aadR* mutants grow extremely slowly on benzoate and other aromatic compounds under anaerobic conditions [111]. AadR is a member of the Fnr family of regulators, of which Fnr from *E. coli* is the best studied ([113,114]; see also P. Kiley, H. Beinert, this volume). Fnr-type proteins regulate gene expression in response to oxygen. In the case of *E. coli*, Fnr activates genes for anaerobic respiration.

The active form of Fnr contains a 4Fe/4S center that disassembles upon exposure to oxygen, rendering the protein inactive [115]. Thus the iron-sulfur center acts as an oxygen sensor. Many Fnr family members, including AadR, have three conserved cysteine residues at the N-terminus and one in the middle of the protein [116]. It is likely that some, or all, of these participate in iron-sulfur center formation. The amino acid similarities of AadR to Fnr suggest that AadR functions to activate expression of benzoate degradation genes in response to anaerobiosis. Consistent with this, an *aadR* mutation causes expression of the *badE::'lacZ* fusion to be dramatically reduced in cells grown anaerobically on either benzoate or succinate. Benzoate still, however, induces *badE* expression, although to lower final levels than are seen in wild-type cells.

The *badR* gene, present in the benzoate gene cluster, is predicted to encode a protein belonging to the MarR family of transcriptional regulators [107,117]. The best characterized MarR family members are regulators of antibiotic resistance. Several of these have been shown to regulate gene expression in response to the aromatic acid salicylate. A *badR* mutant of *R. palustris* grows slowly on benzoate, but normally on succinate. Furthermore, in an anaerobically grown *badE::'lacZ badR* strain, levels of LacZ activity do not vary depending on whether or not benzoate is present. Thus it is likely that BadR regulates benzoyl-CoA reductase gene expression in response to benzoate or benzoyl-CoA.

4. Conclusions

Critical features of peripheral aromatic degradation pathways that converge at benzoyl-CoA as a common intermediate continue to be worked out and a more detailed picture of pathways already under study is to be expected in the near term. It is likely that increasing numbers of structurally diverse compounds will become the targets of investigation as new species of aromatic-degrading anaerobes representing every type of energy-yielding metabolism continue to be isolated and described. The application of coordinated biochemical and molecular approaches to two organisms, *T. aromatica*, a denitrifier of the β-subgroup of proteobacteria and *R. palustris*, a phototroph belonging to the α-subgroup, has revealed that anaerobic benzoyl-CoA degradation includes a ring reduction reaction followed by a series of conventional hydration and dehydrogenation reactions of the type found in fatty acid β-oxidation, culminating in hydrolytic ring cleavage. The ring reduction and ring cleavage reactions are catalyzed by two new kinds of enzymes. The first of these, benzoyl-CoA reductase, will be an intriguing target for future studies aimed at the fundamental question of how a protein can convert chemical energy (ATP hydrolysis) to electrochemical energy (e.g. generation of an electron donor of a sufficiently low redox potential to accomplish ring reduction). The ring hydrolases expand the range of reactions carried out by members of the crotonase superfamily and their further study should contribute to the development of a unified mechanistic model for how members of this superfamily function. A surprising finding that has emerged from studies of *T. aromatica* and *R. palustris* is that the two organisms use two slightly different pathways to metabolize benzoyl-CoA. At present it is not clear what advantage, if any, a particular pathway may afford a given organism. In the case of fermentative bacteria it is clear that the details of benzoyl-CoA metabolism will be influenced by the energetic constraints to which this group must adhere. More studies will be required, however, to determine the precise features of a third variant of the benzoyl-CoA pathway, as it exists in the fermentative bacteria. Such studies as well as studies of benzoyl-CoA metabolism by sulfate-reducing and iron-reducing bacteria will allow the development of a comprehensive view of aromatic compound degradation in the absence of oxygen that may still yield surprises.

Acknowledgments

C.S.H. is grateful for support from the US Department of Energy, Division of Energy Biosciences (Grant DE-FG02-95ER20184) and the US Army Research Office (Grant DAAG55-98-1-0188). G.B. and H.H. acknowledge the continuous financial support of their work by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. G.F. acknowledges the generous financial support of

his work by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

References

[1] Dagley, S. (1971) Catabolism of aromatic compounds by micro-organisms. Adv. Microb. Physiol. 6, 1–46.
[2] Dutton, P.L. and Evans, W.C. (1968) The metabolism of aromatic compounds by *Rhodopseudomonas palustris*. Biochem. J. 113, 525–535.
[3] Evans, W.C. (1977) Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature 270, 17–22.
[4] Young, L.Y. (1984) Anaerobic degradation of aromatic compounds. In: Microbial Degradation of Organic Compounds (Gibson, D.T., Ed.), pp. 487–523. Marcel Dekker, New York, NY.
[5] Evans, W.C. and Fuchs, G. (1988) Anaerobic degradation of aromatic compounds. Annu. Rev. Microbiol. 42, 289–317.
[6] Tarvin, D. and Buswell, A.M. (1934) The methane fermentation of organic acids and carbohydrates. J. Am. Chem. Soc. 56, 1751–1755.
[7] Harwood, C.S. and Gibson, J. (1988) Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. 54, 712–717.
[8] Schennen, U., Braun, K. and Knackmuss, H.-J. (1985) Anaerobic degradation of 2-fluorobenzoate by benzoate-degrading, denitrifying bacteria. J. Bacteriol. 161, 321–325.
[9] Nozawa, T. and Maruyama, Y. (1988) Anaerobic metabolism of phthalate and other aromatic compounds by a denitrifying bacterium. J. Bacteriol. 170, 5778–5784.
[10] Anders, A., Kaetzke, A., Kämpfer, P., Ludwig, W. and Fuchs, G. (1995) Taxonomic position of aromatic degrading denitrifying pseudomonad strains K172 and KB740 and their description as new members of the genera *Thauera*, *T. aromatica* sp. nov., and *Azoarcus*, *A. evansii* sp. nov., respectively, members of the beta subclass of Proteobacteria. Int. J. Syst. Bacteriol. 45, 327–333.
[11] Springer, N., Ludwig, W., Philipp, B. and Schink, B. (1998) *Azoarcus anaerobius* sp. nov., a resorcinol-degrading, strictly anaerobic, denitrifying bacterium. Int. J. Syst. Bacteriol. 48, 953–956.
[12] Song, B., Young, L.Y. and Palleroni, N.J. (1998) Identification of denitrifier strain T1 as *Thauera aromatica* and proposal for emendation of the genus *Thauera* definition. Int. J. Syst. Bacteriol. 48, 889–894.
[13] Rhee, S.K., Lee, G.M., Yoon, J.H., Park, Y.H., Bae, H.S. and Lee, S.T. (1997) Anaerobic and aerobic degradation of pyridine by a newly isolated denitrifying bacterium. Appl. Environ. Microbiol. 63, 2578–2585.
[14] Van Schie, P.M. and Young, L.Y. (1998) Isolation and characterization of phenol-degrading denitrifying bacteria. Appl. Environ. Microbiol. 64, 2432–2438.
[15] Zhou, J., Fries, M.R., Chee-Sanford, J.C. and Tiedje, J.M. (1995) Phylogenetic analysis of a new group of denitrifiers capable of anaerobic growth on toluene and description of *Azoarcus tolulyticus* sp. nov. Int. J. Syst. Bacteriol. 45, 500–506.
[16] Blake, C.K. and Hegeman, D.G. (1987) Plasmid pCBI carries genes for anaerobic benzoate catabolism in *Alcaligenes xylosoxidans* subsp. *denitrificans* PN-1. J. Bacteriol. 169, 4878–4883.
[17] Bak, F. and Widdel, F. (1986) Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* gen. nov., sp. nov. Arch. Microbiol. 146, 177–180.
[18] Szewzyk, U. and Pfennig, N. (1987) Complete oxidation of catechol by the strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. Arch. Microbiol. 147, 163–168.
[19] Widdel, F. and Bak, F. (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: The Prokaryotes (Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.-H., Eds.), pp. 3352–3378. Springer, New York, NY.
[20] Gorny, N. and Schink, B. (1994) Anaerobic degradation of catechol by *Desulfobacterium* sp. strain Cat2 proceeds via carboxylation to protocatechuate. Appl. Environ. Microbiol. 60, 3396–3400.
[21] Küver, J., Kulmer, J., Jannsen, S., Fischer, U. and Blotevogel, K.-H. (1993) Isolation and characterization of a new spore-forming sulfate-reducing bacterium growing by complete oxidation of catechol. Arch. Microbiol. 159, 282–288.
[22] Rabus, R., Nordhaus, R., Ludwig, W. and Widdel, F. (1993) Complete oxidation of toluene under strictly anaerobic conditions by a new sulfate-reducing bacterium. Appl. Environ. Microbiol. 59, 1444–1451.
[23] Beller, H.R., Spormann, A.M., Sharma, P.K., Cole, J.R. and Reinhard, M. (1996) Isolation and characterisation of a novel toluene-degrading sulfate-reducing bacterium. Appl. Environ. Microbiol. 62, 1188–1196.
[24] Lovley, D.R., Giovannoni, S.J., White, D.C., Champine, J.E., Phillips, E.J.P., Gorby, Y.A. and Goodwin, S. (1993) *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch. Microbiol. 159, 336–344.
[25] Lovley, D.R. and Lonergan, D.J. (1990) Anaerobic oxidation of toluene, phenol and *p*-cresol by the dissimilatory iron reducing organism GS-15. Appl. Environ. Microbiol. 56, 1858–1864.
[26] Lonergan, D.J., Jenter, H.L., Coates, J.D., Phillips, E.J., Schmidt, T.M. and Lovley, D.R. (1996) Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J. Bacteriol. 178, 2402–2408.
[27] Mountfort, D.O., Brulla, W.J., Krumholz, L.R. and Bryant, M.P. (1984) *Syntrophus buswelli* gen. nov., sp. nov.: a benzoate catabolizer from methanogenic ecosystems. Int. J. Syst. Bacteriol. 34, 216–217.
[28] Hopkins, B.T., McInerney, M.J. and Warikoo, V. (1995) Evidence for anaerobic syntrophic benzoate degradation threshold and isolation of the syntrophic benzoate degrader. Appl. Environ. Microbiol. 61, 526–530.
[29] Auburger, G. and Winter, J. (1996) Activation and degrada-

tion of benzoate, 3-phenylpropionate and crotonate by *Syntrophus buswellii* strain GA. Evidence for electron-transport phosphorylation during crotonate respiration. Appl. Microbiol. Biotechnol. 44, 807–815.
[30] Warikoo, V., McInerney, M.J., Robinson, J.A. and Suflita, J.M. (1996) Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl. Environ. Microbiol. 62, 26–32.
[31] Schöcke, L. (1997) Energetik des methanogenen Benzoatabbaus durch *Syntrophus gentianae*. Thesis, University of Konstanz.
[32] Schöcke, L. and Schink, B. (1997) Energetics of methanogenic benzoate degradation by *Syntrophus gentianae* in syntrophic coculture. Microbiology 143, 2345–2351.
[33] Schink, B., Brune, A. and Schnell, S. (1992) Anaerobic degradation of aromatic compounds. In: Microbial Degradation of Natural Products (Winkelmann, G., Ed.), pp. 219–242. VCH, Weinheim.
[34] Elder, D.J.E. and Kelly, D.J. (1994) The bacterial degradation of benzoic acid and benzenoid compounds under anaerobic conditions: unifying trends and new perspectives. FEMS Microbiol. Rev. 13, 441–468.
[35] Harwood, C.S. and Gibson, J. (1997) Shedding light on anaerobic benzene ring degradation: a process unique to prokaryotes? J. Bacteriol. 179, 301–309.
[36] Heider, J. and Fuchs, G. (1997) Microbial anaerobic aromatic metabolism. Anaerobe 3, 1–22.
[37] Heider, J. and Fuchs, G. (1997) Anaerobic metabolism of aromatic compounds. Eur. J. Biochem. 243, 577–596.
[38] Gallus, C. and Schink, B. (1998) Anaerobic degradation of alpha-resorcylate by *Thauera aromatica* strain AR-1 proceeds via oxidation and decarboxylation to hydroxyhydroquinone. Arch. Microbiol. 169, 333–338.
[39] Philipp, B. and Schink, B. (1998) Evidence of two oxidative steps initiating anaerobic degradation of resorcinol (1,3-dihydroxybenzene) by the denitrifying bacterium *Azoarcus anaerobius*. J. Bacteriol. 180, 3644–3649.
[40] Barker, H.A. (1981) Amino acid degradation by anaerobic bacteria. Annu. Rev. Biochem. 50, 23–40.
[41] Seyfried, B., Tschech, A. and Fuchs, G. (1991) Anaerobic degradation of phenylacetate and 4-hydroxyphenylacetate by denitrifying bacteria. Arch. Microbiol. 155, 249–255.
[42] Winter, J., Popoff, M.R., Grimont, P. and Bokkenheuser, V.D. (1991) *Clostridium orbiscindens* sp. nov., a human intestinal bacterium capable of cleaving the flavonoid C-ring. Int. J. Syst. Bacteriol. 41, 355–357.
[43] Sembiring, T. and Winter, J. (1989) Anaerobic degradation of phenylacetic acid by mixed and pure cultures. Appl. Microbiol. Biotechnol. 31, 84–88.
[44] Mohamed, M.E., Seyfried, B., Tschech, A. and Fuchs, G. (1993) Anaerobic oxidation of phenylacetate and 4-hydroxyphenylacetate to benzoyl-CoA and CO_2 in denitrifying *Pseudomonas* sp. Evidence for an a-oxidation mechanism. Arch. Microbiol. 159, 563–573.
[45] Mohamed, M.E. and Fuchs, G. (1993) Purification and characterisation of phenylacetate-coenzyme A ligase from a denitrifying *Pseudomonas* sp., an enzyme involved in the anaerobic degradation of phenylacetate. Arch. Microbiol. 159, 554–562.
[46] Schneider, S. and Fuchs, G. (1998) Phenylacetyl-CoA:acceptor oxidoreductase, a new alpha-oxidizing enzyme that produces phenylglyoxylate. Assay, membrane localization, and differential production in *Thauera aromatica*. Arch. Microbiol. 169, 509–516.
[47] Hirsch, W., Schägger, H. and Fuchs, G. (1998) Phenylglyoxylate:NAD^+ oxidoreductase (CoA benzoylating), a new enzyme of anaerobic phenylalanine metabolism in the denitrifying bacterium *Thauera aromatica*. Eur. J. Biochem. 251, 907–915.
[48] Hutber, G. and Ribbons, D.W. (1983) Involvement of coenzyme A esters in the metabolism of benzoate and cyclohexanecarboxylate by *Rhodopseudomonas palustris*. J. Gen. Microbiol. 129, 2413–2420.
[49] Geissler, J.A., Harwood, C.S. and Gibson, J. (1988) Purification and properties of benzoate-coenzyme A ligase, a *Rhodopseudomonas palustris* enzyme involved in the anaerobic degradation of benzoate. J. Bacteriol. 170, 1709–1714.
[50] Altenschmidt, U., Oswald, B. and Fuchs, G. (1991) Purification and characterisation of benzoate-coenzyme A ligase and 2-aminobenzoate-coenzyme A ligases from denitrifying *Pseudomonas* sp. J. Bacteriol. 173, 5494–5501.
[51] Auburger, G. and Winter, J. (1992) Purification and characterisation of benzoyl-CoA synthetase from a syntrophic, benzoate degrading, anaerobic mixed culture. Appl. Microbiol. Biotechnol. 37, 789–795.
[52] Elder, D.J.E., Morgan, P. and Kelly, D.J. (1992) Anaerobic degradation of *trans*-cinnamate and w-phenylalkane carboxylic acids by the photosynthetic bacterium *Rhodopseudomonas palustris*: evidence for a beta-oxidation mechanism. Arch. Microbiol. 157, 148–154.
[53] Tschech, A. and Fuchs, G. (1987) Anaerobic degradation of phenol by pure cultures of newly isolated denitrifying pseudomonads. Arch. Microbiol. 148, 213–217.
[54] Lack, A. and Fuchs, G. (1994) Evidence that phenol phosphorylation to phenylphosphate is the first step in anaerobic phenol metabolism in a denitrifying *Pseudomonas* sp. Arch. Microbiol. 161, 306–311.
[55] Lack, A., Tommasi, I., Aresta, M. and Fuchs, G. (1991) Catalytic properties of phenol carboxylase: in vitro study of CO_2: 4-hydroxybenzoate isotope exchange reaction. Eur. J. Biochem. 197, 473–479.
[56] Lack, A. and Fuchs, G. (1992) Carboxylation of phenylphosphate by phenol carboxylase, an enzyme system of anaerobic phenol metabolism. J. Bacteriol. 174, 3629–3636.
[57] Gallert, C., Knoll, G. and Winter, J. (1991) Anaerobic carboxylation of phenol to benzoate: use of deuterated phenols revealed carboxylation exclusively in the C4-position. Appl. Microbiol. Biotechnol. 36, 124–129.
[58] Biegert, T., Altenschmidt, U., Eckerskorn, C. and Fuchs, G. (1993) Enzymes of anaerobic metabolism of phenolic compounds. 4-Hydroxybenzoate-CoA ligase from a denitrifying *Pseudomonas* species. Eur. J. Biochem. 213, 555–561.
[59] Gibson, J., Dispensa, M., Fogg, G.C., Evans, D.T. and Harwood, C.S. (1994) 4-Hydroxybenzoate-coenzyme A-ligase

from *Rhodopseudomonas palustris*: purification, gene sequence, and role in anaerobic degradation. J. Bacteriol. 176, 634–641.

[60] Brackmann, R. and Fuchs, G. (1993) Enzymes of anaerobic metabolism of phenolic compounds. 4-Hydroxybenzoate-CoA reductase (dehydroxylating) from a denitrifying *Pseudomonas* species. Eur. J. Biochem. 213, 563–571.

[61] Gibson, J., Dispensa, M. and Harwood, C.S. (1997) 4-Hydroxybenzoyl coenzyme A reductase (dehydroxylating) is required for anaerobic degradation of 4-hydroxybenzoate by *Rhodopseudomonas palustris* and shares features with molybdenum-containing hydroxylases. J. Bacteriol. 179, 634–642.

[62] elKasmi, A.E., Brackmann, R., Fuchs, G. and Ragsdale, S.W. (1995) Hydroxybenzoyl-CoA reductase: coupling kinetics and electrochemistry to derive enzyme mechanisms. Biochemistry 34, 11668–11677.

[63] Breese, K. and Fuchs, G. (1998) 4-Hydroxybenzoyl-CoA reductase (dehydroxylating) from the denitrifying bacterium *Thauera aromatica*: prosthetic groups, electron donor, and genes of a member of the molybdenum-flavin-iron-sulfur proteins. Eur. J. Biochem. 251, 916–923.

[64] Roberts, D.J., Fedorak, P.M. and Hrudey, S.E. (1990) CO_2 incorporation and 4-hydroxy-2-methylbenzoic acid formation during anaerobic metabolism of *m*-cresol by a methanogenic consortium. Appl. Environ. Microbiol. 56, 472–478.

[65] Rudolphi, A., Tschech, A. and Fuchs, G. (1991) Anaerobic degradation of cresols by denitrifying bacteria. Arch. Microbiol. 155, 238–248.

[66] Schnell, S., Brune, A. and Schink, B. (1991) Degradation of hydroxyhydroquinone by the strictly anaerobic fermenting bacterium *Pelobacter massiliensis* sp. nov. Arch. Microbiol. 155, 511–516.

[67] Bisaillon, J.G., Lépine, F., Beaudet, R. and Sylvestre, M. (1991) Carboxylation of *o*-cresol by an anaerobic consortium under methanogenic conditions. Appl. Environ. Microbiol. 57, 2131–2134.

[68] Gorny, N. and Schink, B. (1994) Complete anaerobic oxidation of hydroquinone by *Desulfococcus* sp. strain Hy5: indications of hydroquinone carboxylation to gentisate. Arch. Microbiol. 162, 131–135.

[69] Schnell, S. and Schink, B. (1991) Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl-CoA in *Desulfobacterium anilini*. Arch. Microbiol. 155, 183–190.

[70] Gorny, N. and Schink, B. (1994) Hydroquinone degradation via reductive dehydroxylation of gentisyl-CoA by a strictly anaerobic fermenting bacterium. Arch. Microbiol. 161, 25–32.

[71] Kluge, C., Tschech, A. and Fuchs, G. (1990) Anaerobic metabolism of resorcylic acids (*m*-dihydroxybenzoic acids) and resorcinol (1,3-benzenediol) in a fermenting and in a denitrifying bacterium. Arch. Microbiol. 155, 68–74.

[72] He, Z. and Wiegel, J. (1995) Purification and characterisation of an oxygen-sensitive reversible 4-hydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum*. Eur. J. Biochem. 229, 77–82.

[73] He, Z. and Wiegel, J. (1996) Purification and characterisation of an oxygen-sensitive, reversible 3,4-dihydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum*. J. Bacteriol. 178, 3539–3543.

[74] Zhang, X. and Wiegel, J. (1994) Reversible conversion of 4-hydroxybenzoate and phenol by *Clostridium hydroxybenzoicum*. Appl. Environ. Microbiol. 60, 4182–4185.

[75] Bonting, C.F.C., Schneider, S., Schmidtberg, G. and Fuchs, G. (1995) Anaerobic degradation of *m*-cresol via methyl oxidation to 3-hydroxybenzoate by a denitrifying bacterium. Arch. Microbiol. 164, 63–69.

[76] Hopper, D.J., Bossert, I.D. and Rhodes-Roberts, M.E. (1991) *p*-Cresol-methylhydroxylase from a denitrifying bacterium involved in anaerobic degradation of *p*-cresol. J. Bacteriol. 173, 1298–1301.

[77] Bache, R. and Pfennig, N. (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch. Microbiol. 130, 255–261.

[78] DeWeerd, K.A., Saxena, A., Nagle, D.P. and Suflita, J.M. (1988) Metabolism of the ^{18}O-methoxy substituent of 3-methoxybenzoic acid and other unlabeled methoxybenzoic acids by anaerobic bacteria. Appl. Environ. Microbiol. 54, 1237–1242.

[79] Berman, M.H. and Frazer, A.C. (1992) Importance of tetrahydrofolate and ATP in the anaerobic *O*-demethylation reaction for phenylmethylethers. Appl. Environ. Microbiol. 58, 925–931.

[80] Liesack, W., Bak, F., Kreft, J.U. and Stackebrandt, E. (1994) *Holophaga foetida* gen. nov., sp. nov., a new homoacetogenic bacterium degrading methoxylated aromatic compounds. Arch. Microbiol. 162, 85–90.

[81] Kreft, J.U. and Schink, B. (1993) Demethylation and degradation of phenylmethylethers by the sulfide-methylating homoacetogenic bacterium strain TMBS4. Arch. Microbiol. 159, 308–315.

[82] Stupperich, E. and Konle, R. (1993) Corrinoid-dependent methyl transfer reactions are involved in methanol and 3,4-dimethoxybenzoate metabolism by *Sporomusa ovata*. Appl. Environ. Microbiol. 59, 3110–3116.

[83] Stupperich, E., Konle, R. and Eckerskorn, C. (1996) Anaerobic *O*-demethylation of methoxynaphtols, methoxyfuran and fluoroanisols by *Sporomusa ovata*. Biochem. Biophys. Res. Commun. 223, 770–777.

[84] elKasmi, A.E., Rajasekharan, S. and Ragsdale, S.W. (1994) Anaerobic pathway for conversion of methyl group of aromatic methyl ethers to acetic acid by *Clostridium thermoaceticum*. Biochemistry 33, 11217–11224.

[85] Kreft, J.U. and Schink, B. (1994) O-Demethylation by the homoacetogenic anaerobe *Holophaga foetida* studied by a new photometric methylation assay using electrochemically produced cob(I)alamin. Eur. J. Biochem. 226, 945–951.

[86] Dangel, W., Brackmann, R., Lack, A., Mohamed, M.E., Koch, J., Oswald, B., Seyfried, B., Tschech, A. and Fuchs, G. (1991) Differential expression of enzyme activities initiating anoxic metabolism of various aromatic compounds via benzoyl-CoA. Arch. Microbiol. 155, 256–262.

[87] Heider, J., Boll, M., Breese, K., Breinig, S., Ebenau-Jehle, C., Feil, U., Gadon, N., Laempe, D., Leuthner, B., Mohamed, M.E.S., Schneider, S., Burchhardt, G. and Fuchs, G. (1998)

Differential induction of enzymes involved in anaerobic metabolism of aromatic compounds in the denitrifying bacterium *Thauera aromatica*. Arch. Microbiol. 170, 120–131.

[88] Koch, J. and Fuchs, G. (1992) Enzymatic reduction of benzoyl-CoA to alicyclic compounds, a key reaction in anaerobic aromatic metabolism. Eur. J. Biochem. 205, 195–202.

[89] Koch, J., Eisenreich, W., Bacher, A. and Fuchs, G. (1993) Products of enzymatic reduction of benzoyl-CoA, a key reaction in anaerobic aromatic metabolism. Eur. J. Biochem. 211, 649–661.

[90] Boll, M. and Fuchs, G. (1995) Benzoyl-coenzyme A reductase (dearomatising), a key enzyme of anaerobic aromatic metabolism. ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. Eur. J. Biochem. 234, 921–933.

[91] Boll, M., Albracht, S.P.J. and Fuchs, G. (1995) Benzoyl-CoA reductase (dearomatising), a key enzyme of anaerobic aromatic metabolism. Study of ATPase activity, ATP stoichiometry of the reaction and EPR properties of the enzyme. Eur. J. Biochem. 244, 860–871.

[92] Boll, M. and Fuchs, G. (1998) Identification and characterization of the natural electron donor ferredoxin and of FAD as a possible prosthetic group of benzoyl-CoA reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. Eur. J. Biochem. 251, 946–954.

[93] Gibson, K.J. and Gibson, J. (1992) Potential early intermediates in anaerobic benzoate degradation by *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. 58, 696–698.

[94] Egland, P.G., Pelletier, D.A., Dispensa, M., Gibson, J. and Harwood, C.S. (1997) A cluster of bacterial genes for anaerobic benzene ring biodegradation. Proc. Natl. Acad. Sci. USA 94, 6484–6489.

[95] Breese, K., Boll, M., Alt-Mörbe, J., Schägger, H. and Fuchs, G. (1998) Genes coding for the benzoyl-CoA pathway of anaerobic aromatic metabolism in the bacterium *Thauera aromatica*. Eur. J. Biochem. 256, 148–154.

[96] Müller, U. and Buckel, W. (1995) Activation of (R)-2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans*. Eur. J. Biochem. 230, 698–704.

[97] Dutscho, R., Wohlfarth, G., Buckel, P. and Buckel, W. (1989) Cloning and sequencing of the genes of 2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans*. Eur. J. Biochem. 181, 741–746.

[98] Bendrat, K., Müller, U., Klees, A.G. and Buckel, W. (1993) Identification of the gene encoding the activator of (R)-2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans* by gene expression in *Escherichia coli*. FEBS Lett. 329, 329–331.

[99] Buckel, W. and Keese, R. (1995) Einelektronen-Redoxreaktionen von Coenzym-A-Estern in anaeroben Bakterien – ein Vorschlag für einen neuen Mechanismus. Angew. Chemie 107, 1595–1598.

[100] Laempe, D., Eisenreich, W., Bacher, A. and Fuchs, G. (1998) Cyclohexa-1,5-diene-1-carboxyl-CoA hydratase, an enzyme involved in anaerobic metabolism of benzoyl-CoA in the denitrifying bacterium *Thauera aromatica*. Eur. J. Biochem. 255, 618–627.

[101] Perrotta, J.A. and Harwood, C.S. (1994) Anaerobic metabolism of cyclohex-1-ene-1-carboxylate, a proposed intermediate of benzoate degradation, by *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. 60, 1775–1782.

[102] Pelletier, D.A. and Harwood, C.S. (1998) 2-Ketocyclohexanecarboxyl coenzyme A hydrolase, the ring cleavage enzyme required for anaerobic benzoate degradation by *Rhodopseudomonas palustris*. J. Bacteriol. 180, 2330–2336.

[103] Härtel, U., Ecker, E., Koch, J., Fuchs, G., Linder, D. and Buckel, W. (1993) Purification of glutaryl-CoA dehydrogenase from *Pseudomonas* sp., an enzyme involved in the anaerobic degradation of benzoate. Arch. Microbiol. 159, 174–181.

[104] Egland, P.G., Gibson, J. and Harwood, C.S. (1995) Benzoate-coenzyme A ligase, encoded by *badA*, is one of three ligases able to catalyze benzoyl-coenzyme A formation during anaerobic growth of *Rhodopseudomonas palustris* on benzoate. J. Bacteriol. 177, 6545–6551.

[105] Babbitt, P.C. and Gerlt, J.A. (1997) Understanding enzyme superfamilies. Chemistry as the fundamental determinant in the evolution of new catabolic activities. J. Biol. Chem. 272, 30591–30594.

[106] Dunaway-Mariano, D. and Babbitt, P.C. (1994) On the origins and functions of the enzymes of the 4-chlorobenzoate to 4-hydroxybenzoate converting pathway. Biodegradation 5, 259–276.

[107] Egland, P.G. (1997) The molecular basis of anaerobic benzoate degradation. Ph.D. Thesis, The University of Iowa.

[108] Küver, J., Xu, Y. and Gibson, J. (1995) Metabolism of cyclohexane carboxylic acid by the photosynthetic bacterium *Rhodopseudomonas palustris*. Arch. Microbiol. 164, 337–345.

[109] Emig, M.D., Pelletier, D.A. and Harwood, C.S. (1998) Identification of cyclohexanecarboxyl-CoA dehydrogenase, a flavin-containing enzyme encoded by the *badJ* gene from *Rhodopseudomonas palustris*, abstr. Q-86. In: Abstracts of the 98th General Meeting of the American Society for Microbiology 1998. American Society for Microbiology, Washington, DC.

[110] Kim, M.-K. and Harwood, C.S. (1991) Regulation of benzoate-CoA ligase in *Rhodopseudomonas palustris*. FEMS Microbiol. Lett. 83, 199–204.

[111] Dispensa, M., Thomas, C.T., Kim, M.K., Perrotta, J.A., Gibson, J. and Harwood, C.S. (1992) Anaerobic growth of *Rhodopseudomonas palustris* on 4-hydroxybenzoate is dependent on AadR, a member of the cyclic AMP receptor protein family of transcriptional regulators. J. Bacteriol. 174, 5803–6813.

[112] Egland, P.G. and Harwood, C.S. (1997) Regulation of *Rhodopseudomonas palustris* genes *badDEFG* encoding benzoyl-CoA reductase, abstr. K-38. In: Abstracts of the 97th General Meeting of the American Society for Microbiology 1997. American Society for Microbiology, Washington, DC.

[113] Unden, G. and Schirawski, J. (1997) The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signal and reactions. Mol. Microbiol. 25, 205–210.

[114] VanSpanning, R.J.M., DeBoer, A.P.N., Reijnders, W.N.M., Westerhoff, H.V., Stouthamer, A.H. and VanDerOost, J.

(1997) FnrP and NNR of *Paracoccus denitrificans* are both members of the FNR family of transcriptional activators but have distinct roles in respiratory adaptation in response to oxygen limitation. Mol. Microbiol. 25, 893–907.

[115] Khoroshilova, N., Popescu, C., Münck, E., Beinert, H., and Kiley, P.J. (1997) Iron-sulfur cluster disassembly in the FNR protein of *Escherichia coli* by O_2: [4Fe-4S] to [2Fe-2S] conversion with loss of biological activity. Proc. Natl. Acad. Sci. USA 94, 6087–6092.

[116] Melville, S.B. and Gunsalus, R.P. (1990) Mutations in *fnr* that alter anaerobic regulation of electron transport-associated genes in *Escherichia coli*. J. Biol. Chem. 265, 18733–18736.

[117] Sulavik, M.C., Gambino, L.F. and Miller, P.F. (1995) The MarR repressor of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli*: prototypic member of a family of bacterial regulatory proteins involved in sensing phenolic compounds. Mol. Med. 1, 436–446.