

Review Article

Insights into the unique carboxylation reactions in the metabolism of propylene and acetone

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Alkenes and ketones are two classes of ubiquitous, toxic organic compounds in natural environments produced in several biological and anthropogenic processes. In spite of their toxicity, these compounds are utilized as primary carbon and energy sources or are generated as intermediate metabolites in the metabolism of other compounds by many diverse bacteria. The aerobic metabolism of some of the smallest and most volatile of these compounds (propylene, acetone, isopropanol) involves novel carboxylation reactions resulting in a common product acetoacetate. Propylene is metabolized in a four-step pathway involving five enzymes where the penultimate step is a carboxylation reaction catalyzed by a unique disulfide oxidoreductase that couples reductive cleavage of a thioether linkage with carboxylation to produce acetoacetate. The carboxylation of isopropanol begins with conversion to acetone via an alcohol dehydrogenase. Acetone is converted to acetoacetate in a single step by an acetone carboxylase which couples the hydrolysis of MgATP to the activation of both acetone and bicarbonate, generating highly reactive intermediates that are condensed into acetoacetate at a Mn²⁺ containing the active site. Acetoacetate is then utilized in central metabolism where it is readily converted to acetyl-coenzyme A and subsequently converted into biomass or utilized in energy metabolism via the tricarboxylic acid cycle. This review summarizes recent structural and biochemical findings that have contributed significant insights into the mechanism of these two unique carboxylating enzymes.

Alkene and ketone metabolism

Considerable attention has been focused on the microbial metabolism of xenobiotic compounds. Two classes of organic compounds of interest in this regard are alkenes and ketones, which are formed by both biogenic and anthropogenic processes. In spite of their toxicity, these compounds are utilized as primary carbon and energy sources by some bacteria and are generated as intermediate metabolites in the metabolism of other compounds by many diverse bacteria.

Alkenes

Alkenes are unsaturated hydrocarbons that contain a carbon–carbon double bond. Many organisms (plant, insect, cyanobacteria) synthesize alkenes naturally for protection against environmental threats [1–3]. Several gaseous and volatile alkenes are produced naturally. The most predominant of these compounds are associated with plants, including ethene [4,5], isoprene [6], and various monoterpenes. Alkenes have also been reported to be synthesized by various microorganisms [7,8]. For example, the synthesis of alkenes is widespread in cyanobacteria [9] and are thought to contribute significantly to the hydrocarbon cycle of the upper ocean [10]. Alkenes are additionally an important part of our everyday lives, having numerous industrial uses. They can be used as liquid transportation fuels and as precursors for plastics, where they are obtained by cracking of crude oils [11]. Many unsaturated hydrocarbons, especially the smaller, gaseous alkenes ethene, propene, 1,3-butadiene, and butene, are

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produced chemically on a large scale [12]. Alkenes serve as a feedstock for the petrochemical industry because they can participate in a wide variety of reactions, predominantly polymerization and alkylation. Due to their reactivity, unbranched aliphatic alkenes (e.g. ethylene, propylene, butene) are of particular concern as potential human health hazards [13,14].

Many organisms synthesize alkenes naturally for protection against environmental threats [1–3]. Several microbial alkene biosynthetic pathways that convert free fatty acids or fatty acids derivatives to alkenes have been identified or reconstructed [15,16]: (a) decarbonylation of fatty aldehydes (AAR-ADO pathway), (b) decarboxylation of fatty acids (OLET_{JE} pathway), (c) head-to-head' (i.e. C–C bond forming) hydrocarbon biosynthesis pathway (OleABCD), and (d) polyketide synthase (PKS) pathway (Ols) (Figure 1). The AAR-ADO pathway involves two candidate enzymes: an acyl-ACP reductase (AAR) and an aldehyde-deformylating oxygenase (ADO) [3,17]. In this AAR-ADO pathway, an acyl-ACP in the acyl–acyl carrier protein is reduced to an aldehyde by AAR and then the aldehyde is converted to an alkene with the formation of formate as a co-product by ADO (reaction a, Figure 1). The key enzyme to convert fatty acids to terminal alkenes is named OLET_{JE}. The fatty acid decarboxylase OLET_{JE}, isolated and purified from *Jeotgalicoccus* sp. ATCC 8456, is a P450 peroxygenase from the cyp152 family, which directly decarboxylate long-chain FFAs (C16–C20) to α -alkenes using H₂O₂ as its main source of electrons. It is indicated that OLET_{JE} also includes fatty acid hydroxylase activity and His85 in OLET_{JE} plays an important role in catalysis (reaction b, Figure 1) [18]. The 'head-to-head' pathway has been reported to take part in condensation reactions of fatty acid derivatives to generate long-chain alkenes and to require four protein families (OleABCD) (reaction c, Figure 1) [19]. The OleABCD protein families were defined within thiolase, α /β-hydrolase, AMP-dependent ligase/synthase, and short-chain dehydrogenase superfamilies, respectively [20]. The PKS pathway involves a large multi-domain type I polyketide synthase (Ole) (reaction d, Figure 1). Acyl-ACP is loaded to the ACP1 domain by the loading domain (LD), then two carbons from malonyl-CoA are added to the acyl-substrate by the central extension

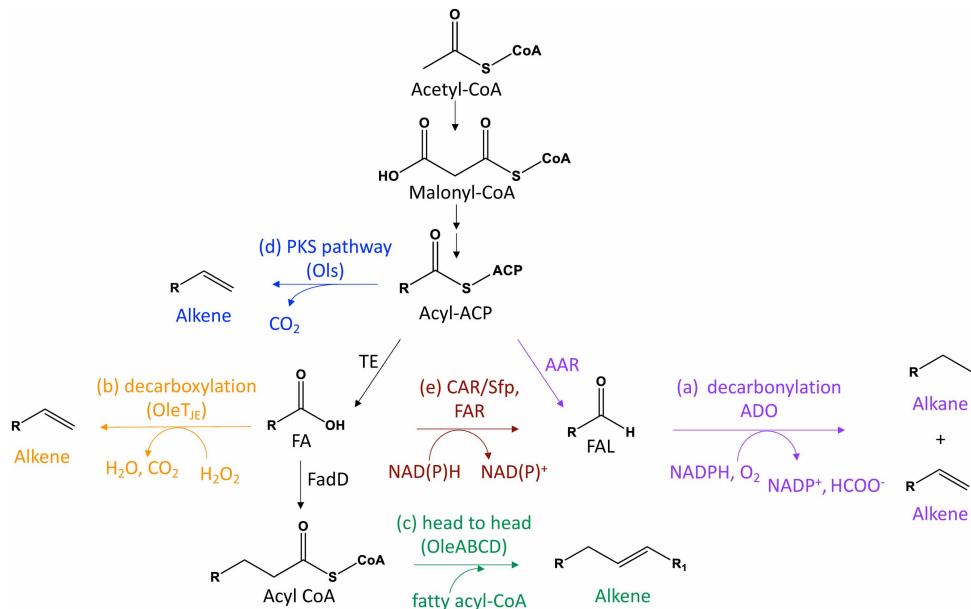


Figure 1. Pathways for fatty acid-based alkene biosynthesis.

Alkene biosynthetic pathways are shown in different colors: (a) decarbonylation of fatty aldehydes (AAR-ADO pathway) is shown in magenta, (b) decarboxylation of fatty acids (OLET_{JE} pathway) is shown in orange, (c) head-to-head' (i.e. C–C bond forming) hydrocarbon biosynthesis pathway (OleABCD) is shown in green, and (d) polyketide synthase (PKS) pathway (Ols) is shown in blue, and (e) reduction in fatty acids to alkenes through fatty aldehydes is shown in brown. A type I polyketide synthase (Ols), thioesterase (TE), fatty acid (FA), a cytochrome P450 enzyme that reduces fatty acids to alkenes (OLET_{JE}), carboxylic acid reductase (CAR), a phosphopantetheinyl transferase (Sfp), fatty acid reductase (FAR), fatty aldehyde (FAL), acyl-CoA synthase (FadD), a four protein families for long-chain olefin biosynthesis (OleABCD), acyl-ACP (acyl carrier protein) reductase (AAR), aldehyde-deformylating oxygenase (ADO).

module including ketosynthase, acyltransferase, ketoreductase, and the ACP2 domain and the β -keto group is reduced to a β -hydroxyl. The sulfotransferase domain activates the β -hydroxyl group via sulfonation. Subsequent dehydration and decarboxylation reactions could be catalyzed by the C-terminal thioesterase (TE) domain [21]. Recently, artificial alkene biosynthetic pathways were designed and implemented for the production of alkenes in *Escherichia coli*. The carboxylic acid reductase (CAR) from *Mycobacterium marinum* together with the phosphopantetheinyl transferase Sfp from *Bacillus subtilis* [22] and fatty acid reductase (FAR) complex encoded by the genes *luxC*, *luxE*, and *luxD* from *Photorhabdus luminescens* can catalyze the formation of fatty aldehydes from fatty acids [23,24]. Coupled with ADO from cyanobacteria, fatty aldehydes can be converted further to alkenes (reaction e, Figure 1).

Many microorganisms, especially bacteria, fungi and yeast, utilize alkenes as sources of carbon and energy. Catabolism of alkenes has traditionally been considered as a strictly oxygen-dependent process. Studies of bacterial alkene-oxidizing metabolism have revealed the involvement of previously unrecognized metabolic pathways, new and surprising types of enzymes, and atypical cofactors [25]. Diverse bacteria, including various strains of *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Xanthobacter*, *Alcaligenes*, *Pseudomonas*, and *Corynebacterium*, are capable of aerobic growth with short-chain aliphatic alkenes (C2 to C6, e.g. propylene, ethene), aromatic alkenes (e.g. toluene), and chlorinated alkene (vinyl chloride, cis-, trans-1, 2-dichloroethylene, and trichloroethylene) as the sole added carbon source [26]. While some alkene-oxidizing bacteria are restricted to growth on a single alkene, e.g. ethylene or isoprene (2-methylbutadiene), others exhibit less selectivity for the growth substrate. In aerobic, alkene-utilizing bacteria, the initial attack of alkenes always requires molecular oxygen as a co-substrate. The first enzymes in the metabolic pathways of alkanes are monooxygenases, while aromatic hydrocarbons are oxygenated by either mono- or dioxygenases. These enzymes incorporate hydroxyl groups, derived from molecular oxygen, into the aliphatic chain or the aromatic ring. The alcohols formed from aliphatic hydrocarbons are then oxidized to the corresponding acids; the phenolic compounds generated by ring hydroxylation of aromatic hydrocarbons are direct precursors for oxidative ring cleavage [27,28].

More recently, several alkenes have also been demonstrated to be anaerobically utilized as substrates by several species of denitrifying, ferric iron-reducing, and sulfate-reducing bacteria [28]. Anaerobic degradation of straight-chain alkenes by enrichment cultures and sulfate-reducing bacteria has been reported; however, the initial reactions of these unsaturated hydrocarbons in anaerobic bacteria are still unknown. Recent studies reported anaerobic degradation of 1-alkenes by the sulfate-reducing bacterium *Desulfatibacillum aliphaticivorans* strain CV2803 isolated from a polluted marine sediment [29]. This strain has been shown to oxidize alkenes into fatty acids at the C-1 double bond (via a primary alcohol), and by the addition of an undefined carbon unit(s) at C-2 and C-3 and at the subterminal carbon of the saturated end of the molecule [C – (ω – 1)] [29]. Finally, the fourth group of anaerobic bacteria, the ‘proton-reducing’ hydrocarbon-degrading bacteria, utilize various alkenes and have been found in syntrophic association with methanogenic archaea [30,31]. These convert alkenes to CO₂, H₂, and acetate. This disproportionation is thermodynamically feasible only if the steady-state concentration of hydrogen (and possibly that of acetate) is kept at a low level and is achieved by hydrogen and acetate consumption by methanogens.

Ketones

Ketones are organic compounds that contain a carbonyl group and two aliphatic or aromatic substituents. The substituents may be saturated or unsaturated, linear or branched alkyl groups, or aromatic residues. They may be the same or different. Moreover, the alkyl or aryl substituents can also contain heteroatoms. More than one carbonyl group may be present in a particular molecule [32]. They are widespread in nature including plants, microorganisms, animals, and human, often combined with other functional groups. They are found in several sugars and in natural steroid hormones. Only a few ketones are manufactured on a large scale in the industry. They can be synthesized by a wide variety of methods, and because of their ease of preparation, relative stability, and high reactivity, they are nearly ideal chemical intermediates. Industrially, many complex organic compounds are synthesized using ketones as building blocks in hydrogenation, reductive condensation, reductive amination, oxidation to peroxides, ketal formation, and condensation reactions [32]. Ketones are most widely used as solvents, especially in industries manufacturing explosives, lacquers, paints, and textiles. Ketones are also used in tanning, as preservatives, and in hydraulic fluids. Most commonly used ketones are sugar (ketoses) and the industrial solvent acetone. Acetone is one of the most important industrial solvents used in such familiar products as paints, coatings, and nail-polish removers. They constitute a significant groundwater, air, water,

and soil contaminant. Acetone can also be produced biologically by the fermentative metabolism of certain anaerobic bacteria and from ketone body breakdown in mammals [33,34].

Many anaerobic bacteria, such as those in the genus *Clostridium*, produce acetone as a major fermentation end product [33]. In these bacteria, acetone is formed from the decarboxylation of acetoacetate in reactions catalyzed by acetoacetate decarboxylases (AAD) (reaction a, Figure 2) [35]. In some hydrocarbon-oxidizing bacteria of the genus *Mycobacterium*, the pathway of propane metabolism involves an initial hydroxylation reaction producing isopropanol, which is subsequently oxidized to acetone (reaction b, Figure 2) [36,37]. In the actinomycete *Gordonia* sp. strain TY-5, propane is oxidized to isopropanol by a monooxygenase (encoded by *prnABC*), and then isopropanol is further metabolized to acetone by three distinct NAD⁺-dependent secondary alcohol dehydrogenases (encoded by *adh1*, *adh2*, *adh3*) [38]. Epoxypropane, formed in certain bacteria by monooxygenation of propene, can undergo isomerization to acetone (reaction c, Figure 2) [39]. Various members of the *Vibrio* family possess a multistep pathway of leucine catabolism that produces 3-hydroxy-3-methylglutaryl-coenzyme A [40], which decomposes to yield acetone (reaction d, Figure 2). Finally, other microbes oxidize 2-nitropropane (reaction e, Figure 2), atrazine (reaction f, Figure 2), or other compounds to produce acetone [41,42].

In addition to being formed as an intermediate metabolite or product of bacterial metabolic pathways, acetone can serve as a growth-supporting substrate for many diverse bacteria. Previous studies on bacterial acetone metabolism both *in vivo* and *in vitro* suggested that acetone can be metabolized in two ways: an oxygen-dependent monooxygenase-catalyzed oxidation to produce acetol (hydroxyacetone), or a CO₂-dependent carboxylase-catalyzed carboxylation to produce acetoacetate. Most aerobic bacteria employ the hydroxyacetone pathway, although the corresponding enzyme system is not known [36,37,43,44]. In the propane-utilizing bacterium *Gordonia* sp. strain TY-5, however, acetone degradation has been shown to be initiated by a Baeyer–Villiger monooxygenase (BVMO) encoded by *acmA* to produce methyl acetate (reaction

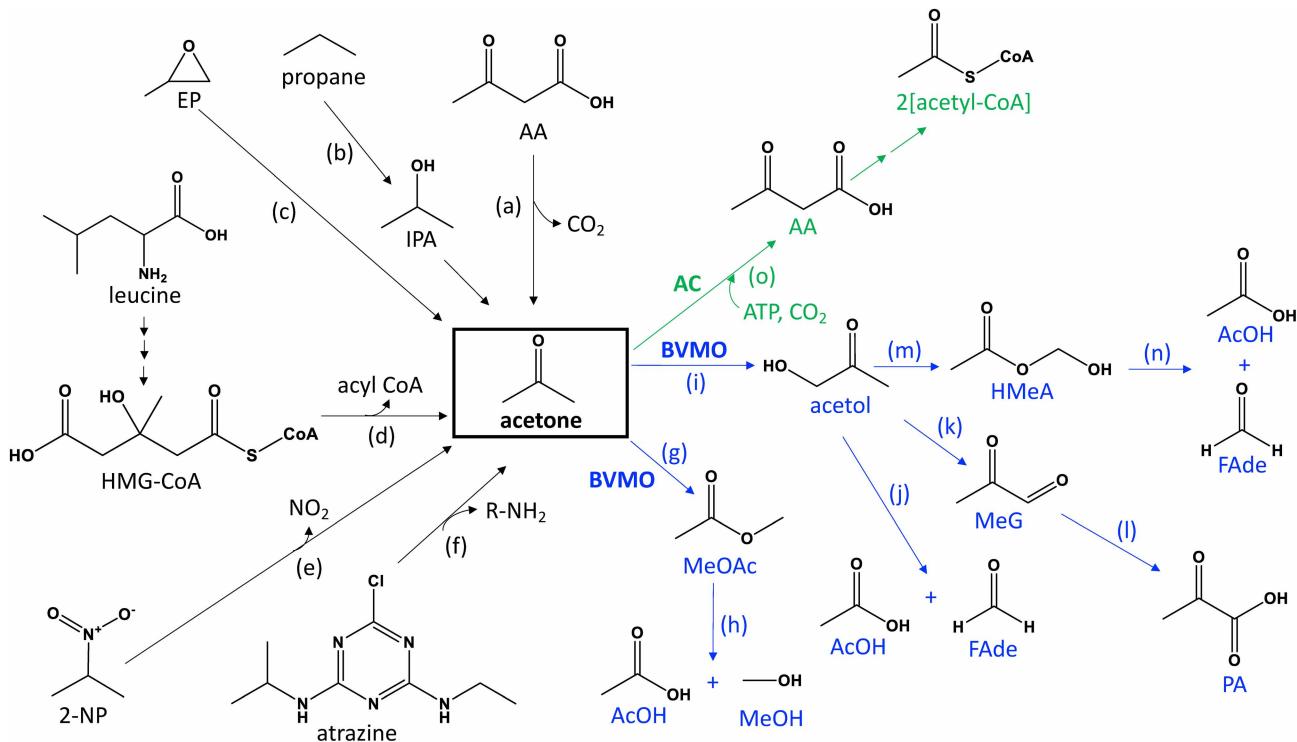


Figure 2. Overview of bacterial acetone metabolism.

Different reactions that generate and degrade acetone are illustrated (for details see description in the text). Isopropanol (IPA), epoxypropane (EP), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), 2-nitropropane (2-NP), Baeyer–Villiger monooxygenase (BVMO), methylacetate (MeOAc), acetic acid (AcOH), methanol (MeOH), hydroxymethylene acetate (HMeA), formaldehyde (FAdE), methylglyoxal (MeG), pyruvic acid (PA), acetone carboxylase (AC), acetoacetate (AA).

g, Figure 2) [38,45]. A hydrolase (encoded by *acmB*) then splits the ester into acetic acid and methanol (reaction h, Figure 2). BVMOs belong to the class of oxidoreductases and catalyze the insertion of a single oxygen atom between the keto group and the adjacent carbon by a reaction equivalent to a chemical Baeyer–Villiger reaction [46]. BVMOs are flavin (mostly FAD)-dependent and require NAD(P)H to catalyze this reaction. Other types of monooxygenases may be capable of catalyzing terminal hydroxylation of acetone to acetol (reaction i, Figure 2). Acetol cleavage to form acetaldehyde and formaldehyde has been proposed (reaction j, Figure 2) [47]; however, characterization of an enzymatic activity has not been reported. Although acetol dehydrogenase (reaction k, Figure 2) and methylglyoxal dehydrogenase (reaction l, Figure 2) reactions, which would convert acetol to pyruvate, have been characterized [48]. Finally, an NADPH-dependent acetol monooxygenase catalyzing Baeyer–Villiger chemistry (reaction m, Figure 2) in *Mycobacterium* sp. has been reported to form hydroxymethylene acetate, which spontaneously decomposes to yield acetic acid and formaldehyde (reaction n, Figure 2) [26]. These BVMOs can be found in a wide range of bacteria where they were also reported to be involved in the catabolism of cycloketones [49–55], aromatic ketones [56,57], or aliphatic open-chain ketones [58,59]. Acetone undergoes CO₂-dependent carboxylation, yielding acetoacetate, which is subsequently converted through multiple steps to form two molecules of acetyl-CoA (reaction o, Figure 2) [60,61].

Aerobic propylene and acetone metabolism in *Xanthobacter autotrophicus* Py2

Many bacteria have been isolated that are capable of growing with a variety of alkenes and ketones as carbon sources. For example, *Xanthobacter* sp. [26,62] can utilize propylene, epoxypropane, isopropanol, and acetone as growth substrates. Convergent pathways have been characterized in which both epoxide derivatives of alkenes (epoxypropane) and ketones (acetone) are converted to acetoacetate, which then through the activity of a thiolase results in the formation of acetyl-coenzyme A (acetyl-CoA), a central metabolite. These two convergent pathways involved in the metabolism of these important xenobiotic compounds require, as terminal reactions, unique carboxylations with CO₂ as a co-substrate.

The most extensively studied pathway of aliphatic alkene oxidation is that of propylene metabolism in *X. autotrophicus* Py2 and *Rhodococcus rhodochrous* B276 [63–69]. The pathway is initiated by an alkene monooxygenase which catalyzes the NADH-dependent conversion of propylene to an enantiomeric mixture of R- and S-epoxypropane. The conversion of both epoxides to a β-ketoacid (acetoacetate) occurs by a three-step process requiring four enzymes (EaCoMT; R-HPCDH; S-HPCDH; 2-KPCC), CO₂, NAD⁺, NADPH, and the small organic cofactor coenzyme M (CoM; 2-mercaptopethanesulfonic acid) [63,70] (Figure 3A). The discovery that CoM is the cofactor for this process was somewhat of a surprise; previously, CoM had only been identified as having a role in methanogenic Archaea where a methyl group is transferred to CoM to form a methyl thioether

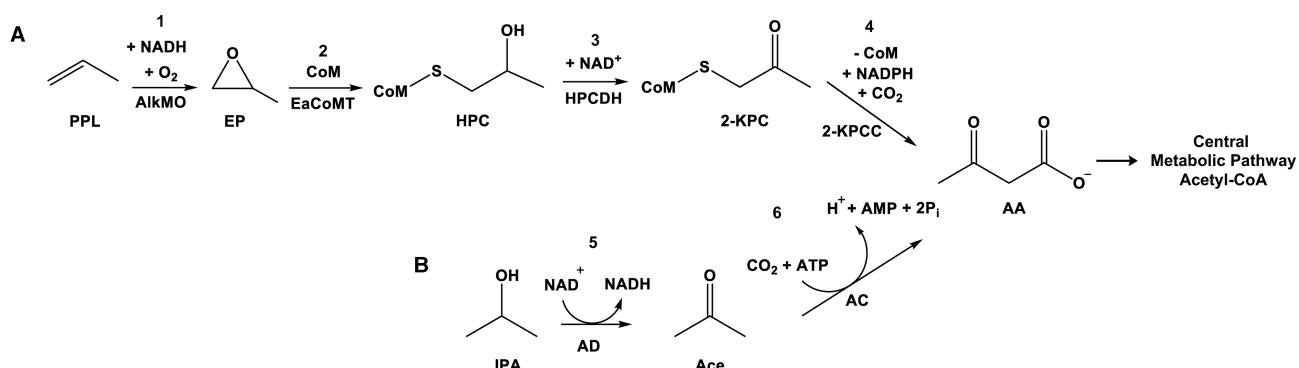


Figure 3. Metabolic pathways of propylene/epoxypropane and isopropanol/acetone in *X. autotrophicus*.

The convergent pathway of propylene/epoxypropane (A) and isopropanol/acetone (B) metabolism with acetyl-CoA as an end product elucidated for the aerobic microorganism *X. autotrophicus*. Propylene (PPL), alkene monooxygenase (AlkMO), (R,S)-epoxypropane (EP), coenzyme M (CoM), epoxyalkane Coenzyme M transferase (EaCoMT), (R,S)-β-hydroxythioether (HPC), (R,S)-hydroxypropyl-CoM dehydrogenase (HPCDH), β-ketothioether (2-KPC), 2-ketopropyl-coenzyme M carboxylase (2-KPCC), isopropanol (IPA), alcohol dehydrogenase (AD), acetone (Ace), acetone carboxylase (AC), acetoacetate (AA).

that is subsequently reduced to methane [71–75]. Interestingly, bacterial CoM is synthesized via a different biosynthetic pathway than in methanogenic archaea, representing a fairly profound case of convergent evolution. The bacterial CoM biosynthetic pathway requires new roles for members of the aspartase/fumarase and pyridoxal phosphate-dependent enzymes superfamilies [76].

Epoxyalkane Coenzyme M transferase (EaCoMT) is a zinc-dependent transferase that catalyzes the nucleophilic attack of CoM on short-chain epoxides resulting in ring opening and the formation of hydroxyalkyl-CoM thioether conjugates (Figure 3A). R- and S-hydroxypropyl-CoM dehydrogenases (R- and S-HPCDH) are short-chain dehydrogenases that catalyze the NAD⁺-dependent oxidation of 2-R- or 2-S-hydroxypropyl CoM (R- and S-HDC) to the achiral product 2-ketopropyl CoM (2-KPC). In this reaction, it was demonstrated through structural characterization and modeling of substrate-bound states of each dehydrogenase that CoM plays a critical role in the appropriate orientation of enantiomers in each enzyme for stereo-selective hydride abstraction. The final and most intriguing step of the pathway, from the point of view of mechanistic enzymology, is catalyzed by 2-ketopropyl-CoM oxidoreductase/carboxylase (2-KPCC) (Figure 3A). 2-KPCC is a unique member of the disulfide oxidoreductase (DSOR) enzyme family, which catalyzes the reductive cleavage and carboxylation of 2-KPC to produce acetoacetate and CoM and will be discussed in more detail mechanistically below.

X. autotrophicus Py2 can also utilize either isopropanol or acetone as growth substrates. Isopropanol is converted to acetone by the activity of a secondary alcohol dehydrogenase (Figure 3B). Acetone, either as a growth substrate or produced by the activity of alcohol dehydrogenase, is converted directly to the central metabolite acetoacetate through the activity of a novel ATP-dependent acetone carboxylase (AC) enzyme [77], and then converted into acetyl-CoA that can be utilized in central metabolic pathways (Figure 3B), as mentioned above.

The 2-ketopropyl-CoM oxidoreductase/carboxylase (2-KPCC) and acetone carboxylase (AC) enzymes

Carboxylases are enzymes that catalyze the incorporation of CO₂ into organic substrates. In propylene and acetone metabolism, converting 3-carbon substrates into 4-carbon substrates is required for their assimilation into central metabolism through acetyl-CoA [78]. To form the C–C bond, carboxylation enzymes must catalyze nucleophilic activation of substrates and electrophilic activation of CO₂. The final carboxylation step of both propylene and acetone metabolism demonstrates the use of high energy intermediates to overcome the activation energy of bond formation. These intermediates create enolacetone as a strong nucleophile and encapsulate CO₂ to prevent side reactions. Structural studies of both 2-KPCC and AC have demonstrated how enolacetone forms and is protected from solvent exposure.

2-ketopropyl-CoM oxidoreductase/carboxylase (2-KPCC)

2-KPCC is a homodimeric protein composed of 57 kDa subunits, each containing one molecule of FAD [79]. The deduced amino acid sequence of 2-KPCC indicates that the enzyme is a member of the DSOR family of enzymes [80–82]. Enzymes within this family catalyze diverse reactions involving the two-electron reduction in a substrate. They contain a flavin adenine dinucleotide (FAD) cofactor and a redox-active cysteine pair which participate in the reduction in the substrate molecule. The prototype members of the DSOR family, which have been extensively characterized mechanistically and structurally, are dihydrolipoamide dehydrogenase, glutathione reductase, and mercuric reductase [82]. Members of this family, with the exception of mercuric reductase, cleave a disulfide bond followed by protonation of the leaving group. 2-KPCC, however, contains key differences, which allow it to carry out reductive cleavage and carboxylation at a C–S bond.

Biochemical, mechanistic, and structural studies of 2-KPCC have led to the proposal of a unique catalytic cycle for the enzyme [79,83–85]. The reaction scheme (Figure 4A) and the solved structure of 2-KPCC (Figure 4B) are shown in Figure 4. Like DSORs, the catalytic cycle is initiated when NADPH reduces the enzyme FAD, which then reduces the oxidized cysteine pair (Cys82 and Cys87 in the primary sequence of 2-KPCC). The substrate 2-KPC then binds, followed by a nucleophilic attack of the interchange cysteine thiol on the substrate thioether sulfur, resulting in heterolytic cleavage of the S–C bond. This step represents a fundamentally novel reaction with respect to the DSOR family, in that the cysteine attacks a thioether rather than a disulfide, as in all members of the family except mercuric reductase. The cleavage of the thioether results in the formation of a stabilized carbanion of acetone and a mixed disulfide of cysteine and CoM, both of which are unique to the mechanism of 2-KPCC. Enolacetone serves as the nucleophile for an attack on CO₂ in the

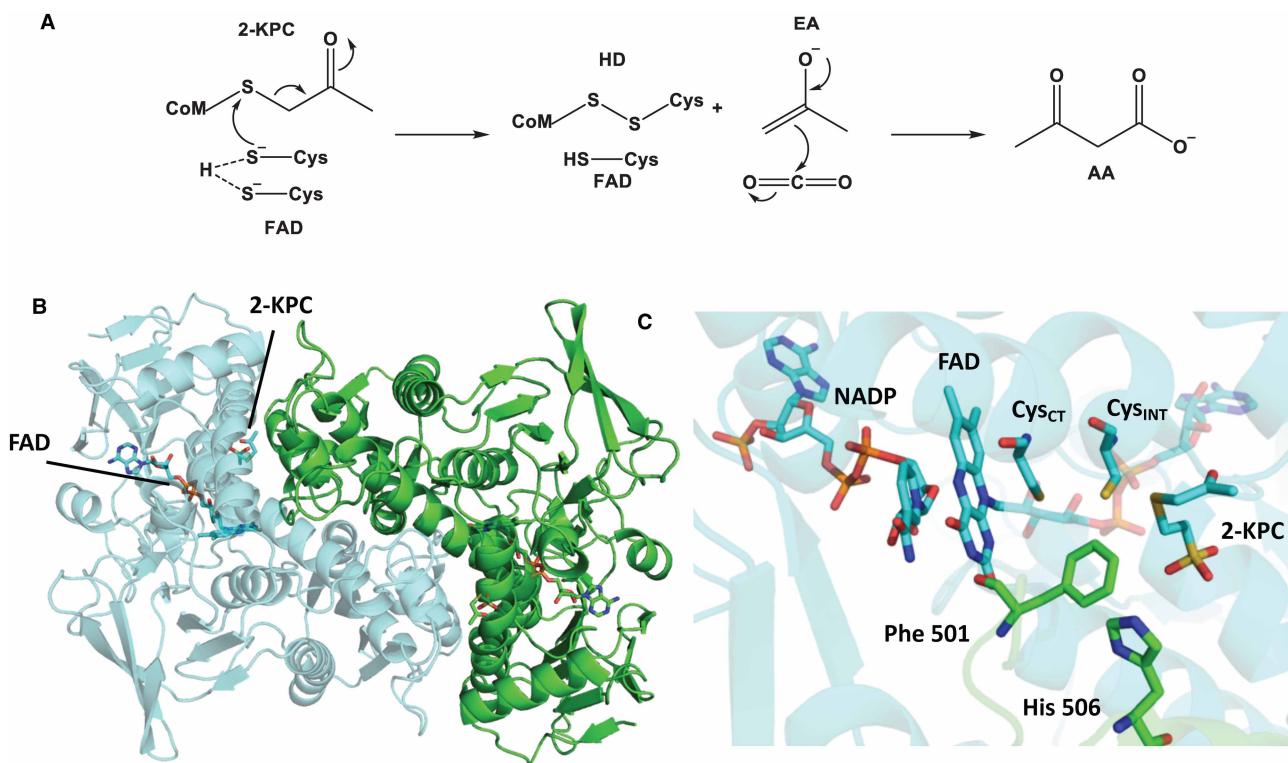


Figure 4. Enzymatic reaction and structure of 2-KPCC.

(A) Scheme of proposed carboxylation reaction in 2-KPCC. The S–C bond of 2-KPC is cleaved, forming the enolacetone (EA) intermediate and a disulfide bond between Cys_{INT} and CoM. Enolacetone can react with CO₂ to form acetoacetate (AA). (B) Ribbon diagram of 2-KPCC structure with one monomer in cyan (transparent) and the other in green showing positions of bound FAD and 2-KPC. (C) Active site of 2-KPCC showing bound 2-KPC and the hydrophobic dyad FXXXXH (F-Phe501 and H-His506) unique to 2-KPCC. Hydrophobic dyad residues are located on the adjacent monomer (green monomer in B) and interact with the active site residues of neighboring monomer (cyan monomer in B). Residues, cofactors and products of the reaction are shown in sticks. Atomic coloring scheme: carbon, cyan or green; nitrogen, blue; oxygen, red; sulfur, yellow.

β -ketothioether (2-KPC), hydrophobic dyad (HD), flavin adenine dinucleotide (FAD), enolacetone (EA), acetoacetate (AA).

carboxylation step, forming acetoacetate. In the absence of CO₂, enolacetone will be protonated to form side product acetone. The catalytic cycle is completed by reduction in the mixed disulfide of CoM and the interchange thiol, forming free CoM and the mixed disulfide cysteine pair.

Structural characterization of 2-KPCC with and without substrate has also provided further insight into how 2-KPCC directs carboxylation [79,84,86]. The structure of the substrate-bound state clearly indicates that the substrate binds the active site through the strong electrostatic interactions of two arginine side chains with the sulfonate group of the substrate. This provides the proper alignment of the substrate with the redox-active disulfide for facile cleavage. Furthermore, a histidine-oriented water molecule could interact with the substrate keto group to stabilize the enolate intermediate [79]. A comparison of the structures in the native and substrate-bound states indicated that 2-KPC induces a conformational change that results in the collapse of the substrate access channel. A cis-proline flanked loop region, which is stabilized by a divalent metal ion, acts as a shield for the substrate [84]. This encapsulation of the substrate is unique to 2-KPCC, whereas the DSOR members maintain an open structure, permitting substrate access for protonation.

2-KPCC also promotes the unusual carboxylation of a C–S bond. DSORs contain a conserved HXXXXE catalytic dyad; however, 2-KPCC contains a FXXXXH dyad (Figure 4C). It has been proposed that this modification excludes an acid–base from the active site to aid in promoting carboxylation in 2-KPCC [87,88]. Mutagenesis of 2-KPCC's active site dyad to mimic that of DSORs shifted the product formation to favor protonation of the substrate to acetone, suggesting the specificity of this residue in the active site (native phenylalanine (Phe) vs histidine (His)) indeed plays a role in product identity [87]. In addition to providing a

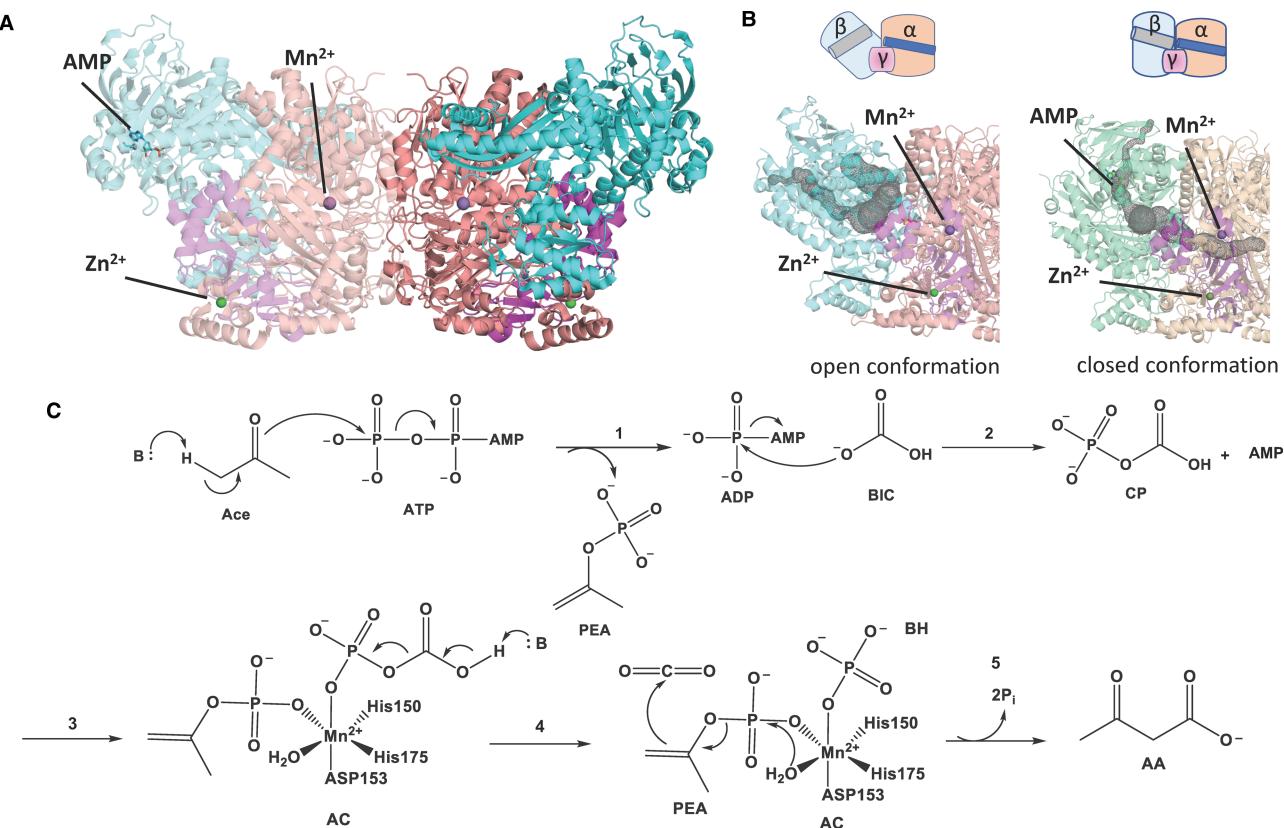


Figure 5. Structure and mechanism of acetone carboxylase (AC).

(A) Ribbon diagram of AC, a heterohexameric ($\alpha\beta\gamma$)₂ enzyme with the α -subunit (dark red) forming the dimer interface and containing the Mn^{2+} active site. The nucleotide-binding site is in the β -subunit (cyan), which shares an interface with the α -subunit and the γ -subunit (magenta). (B) The conformational shift of 11 Å between the ligand-free structure (left, open conformation) and the AMP bound structure (right, close conformation) shows the encapsulation of the substrate to the enzyme core allowing access to the Mn^{2+} active site. (C) Scheme of the mechanism of AC. First in the nucleotide-binding site acetone (Ace) is phosphorylated by the γ -phosphoryl group of ATP to form phosphoenolacetone (PEA). Then the β -phosphoryl group of ATP phosphorylates bicarbonate (BIC) to carboxyphosphate (CP). The intermediates diffuse through the substrate tunnel to the Mn^{2+} active site and co-ordinate with Mn^{2+} where carboxyphosphate is decarboxylated to form CO_2 allowing the enolacetone to bond with the carbon dioxide after hydrolysis of the phosphate group. Atomic coloring scheme: carbon, cyan; dark red or green; manganese, dark blue; zinc, green. Acetone (Ace), phosphoenolacetone (PEA), bicarbonate (BIC), carboxyphosphate (CP), acetone carboxylase (AC), acetoacetate (AA).

source of protons in DSORs, the His lowers the pKa of the Cys_{INT} such that it can reduce its substrate. With the replacement with Phe in 2-KPCC, its Cys_{INT} pKa is not lowered but rather Cys_{INT} and Cys_{CT} share a proton, protecting the enolacetone intermediate from free protons and rendering the Cys_{CT} an internal acid-base (Figure 4A) [88]. Such proton sharing between two cysteines stabilize a reactive thiolate anion as observed in thioredoxin [89]. In thioredoxin, the reactive thiolate also resides in a hydrophobic environment, much like 2-KPCC. Proton sharing between the reactive cysteines in 2-KPCC, thereby, is thought to stabilize the cysteine (Cys_{INT}) which interacts with substrate, enabling the splitting of the strong C–S bond.

Acetone carboxylase (AC)

Acetone metabolism has been biochemically characterized from multiple proteobacteria classes, with the most extensive focus on the α -proteobacteria *X. autotrophicus* Py2 and β -proteobacteria *Aromatoleum aromaticum*. While the majority of the biochemical and structural work has been done in *X. autotrophicus* Py2, reports from *A. aromaticum* show fundamental differences in the reported reaction stoichiometry and metal content. *A. aromaticum* AC was described as hydrolyzing two ATP to two AMP and four inorganic phosphates (P_i) for each molecule of acetone carboxylated [90]. This is in contrast with AC from *X. autotrophicus* Py2, which only

requires a single molecule of ATP hydrolyzed to AMP and two P_i per acetone carboxylated [59]. AC from *A. aromaticum* has been reported to contain Fe at its active site instead of Mn²⁺ present in the AC from *X. autotrophicus* Py2 [60,68,90].

While the active site of AC has not been characterized to the extent of 2-KPCC, structural studies have allowed a mechanism to be proposed for *X. autotrophicus* Py2 AC [91]. AC has two heterotrimeric $\alpha\beta\gamma$ subunits joined by the interacting α -subunits to form a dimeric core. The α -subunits contain a catalytic Mn²⁺ site while the β -subunits contain the nucleotide-binding site (Figure 5A). The structure of AC was determined in multiple conformational states, demonstrating significant conformational changes that occur during catalytic turnover [91]. The structure shows the nucleotide and Mn²⁺ active sites located more than 40 Å away from each other. The distance between the active sites can be rationalized by comparing the conformational states. The changes in these states show that nucleotide-binding communicates with the Mn²⁺ active site through an extensive 11–18 Å rotation of the β subunit (Figure 5B). It has been proposed that the rotation encapsulates the substrates ATP, HCO₃[−], and acetone at the nucleotide-binding site. Acetone is phosphorylated through a concerted reaction involving the abstraction of an α -proton with the hydrolysis of the γ -phosphoryl group of ATP to the carbonyl oxygen of acetone, generating the phosphoenolacetone intermediate. The HCO₃[−] is proposed to be phosphorylated by the β -phosphoryl group of ATP, creating AMP and carboxyphosphate (Figure 5C). These reactive intermediates are protected from solvent hydrolysis by the substrate tunnel and can freely diffuse inside the enzyme. During the conformational shift upon AMP binding, α -Glu89 co-ordinates to the Mn²⁺ active site, causing the proximal helix to become disordered. This disordering of the proximal helix allows for access of the phosphorylated intermediates to the Mn²⁺ active site. The intermediates will then co-ordinate with the Mn²⁺ and displace α -Glu89, subsequently closing off access to the Mn²⁺ active site. The coordination of the phosphorylated intermediates has been proposed to facilitate two reaction steps: (1) carboxyphosphate decay to yield electrophilic CO₂ and (2) the creation of enolacetone through the dephosphorylation of phosphoenolacetone (Figure 5C). This *in situ* creation of enolacetone and CO₂, which combine to form acetooacetate, is similar to the proposed the 2-KPCC-catalyzed reaction. The formation of the product and release of inorganic phosphates allows the enzyme to reset. The conformational states and proposed mechanism show a cyclic nature to the activity of the enzyme, where substrate binding causes rotation of the β -subunit, encapsulating the reactive intermediates. The rotation opens access to the Mn²⁺ active site where the intermediates can diffuse to co-ordinate with the Mn²⁺. Once the intermediates displace α -Glu89, the tunnel closes and the cycle can start again.

Conclusions

Studies on propylene and acetone metabolism in bacteria have identified a central role for CO₂ and specifically CO₂ fixation reactions in these processes. The CO₂ fixing enzymes of the pathways, 2-KPCC and AC, are distinct carboxylases with unique molecular properties and cofactor requirements. Characterization of these novel metabolic pathways coupled with advancement in metabolic engineering of microorganisms can provide a blueprint to promote coupling the metabolism of potentially deleterious compounds to CO₂ fixation.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

2-KPC, 2-ketopropyl CoM; AAR, acyl-ACP reductase; AC, acetone carboxylase; BVMO, Baeyer–Villiger monooxygenase; DSOR, oxidoreductase; PKS, polyketide synthase.

References

- Howard, R.W. and Blomquist, G.J. (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* **50**, 371–393
<https://doi.org/10.1146/annurev.ento.50.071803.130359>

- 2 Jetter, R. and Kunst, L. (2008) Plant surface lipid biosynthetic pathways and their utility for metabolic engineering of waxes and hydrocarbon biofuels. *Plant J.* **54**, 670–683 <https://doi.org/10.1111/j.1365-313X.2008.03467.x>
- 3 Schirmer, A., Rude, M.A., Li, X., Popova, E. and Del Cardayre, S.B. (2010) Microbial biosynthesis of alkanes. *Science* **329**, 559–562 <https://doi.org/10.1126/science.1187936>
- 4 Abeles, F.B. (1973) *Ethylene in Plant Biology*, Academic Press, Inc., New York, NY
- 5 Primrose, S.B. (1979) Ethylene in agriculture: the role of the microbe. *J. Appl. Bacteriol.* **46**, 1–25 <https://doi.org/10.1111/j.1365-2672.1979.tb02579.x>
- 6 Rasmussen, R.A. (1970) Isoprene: identified as a forest-type emission to the atmosphere. *Environ. Sci. Technol.* **4**, 667–671 <https://doi.org/10.1021/es60043a008>
- 7 Ladygina, N., Dedyukhina, E.G. and Vainshtein, M.B. (2006) A review on microbial synthesis of hydrocarbons. *Process Biochem.* **41**, 1001–1014 <https://doi.org/10.1016/j.procbio.2005.12.007>
- 8 Wang, W., Liu, X. and Lu, X. (2013) Engineering cyanobacteria to improve photosynthetic production of alka(e)nes. *Biotechnol. Biofuels* **6**, 1–9 <https://doi.org/10.1186/1754-6834-6-1>
- 9 Coates, R.C., Podell, S., Korobeynikov, A., Lapidus, A., Pevzner, P., Sherman, D.H. et al. (2014) Characterization of cyanobacterial hydrocarbon composition and distribution of biosynthetic pathways. *PLoS One* **9**, 1–12 <https://doi.org/10.1371/journal.pone.0085140>
- 10 Lea-Smith, D.J., Biller, S.J., Davey, M.P., Cotton, C.A.R., Perez Sepulveda, B.M., Turchyn, A.V. et al. (2015) Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle. *Proc. Acad. Natl. Acad. Sci. U.S.A.* **112**, 13591–13596 <https://doi.org/10.1073/pnas.1507274112>
- 11 Kissin, Y.V. (2001) Chemical mechanisms of catalytic cracking over solid acidic catalysts: alkanes and alkenes. *Catal. Rev.* **43**, 85–146 <https://doi.org/10.1081/CR-100104387>
- 12 van Ginkel, C.G., Welten, H.G.J. and de Bont, J.A.M. (1987) Oxidation of gaseous and volatile hydrocarbons by selected alkene-utilizing bacteria. *Appl. Environ. Microbiol.* **53**, 2903–2907 <https://doi.org/10.1128/AEM.53.12.2903-2907.1987>
- 13 An, Y.J. (2004) Toxicity of benzene, toluene, ethylbenzene, and xylene (BTEX) mixture to *Sorghum bicolor* and *Cucumis sativus*. *Bull. Environ. Contam. Toxicol.* **72**, 1006–1011 <https://doi.org/10.1007/s00128-004-0343-y>
- 14 Delgado-Saborit, J.M., Stark, C. and Harrison, R.M. (2011) Carcinogenic potential, levels and sources of polycyclic aromatic hydrocarbon mixture in indoor and outdoor environments and their implications for air quality standards. *Environ. Int.* **37**, 383–392 <https://doi.org/10.1016/j.envint.2010.10.011>
- 15 Wang, W. and Lu, X. (2013) Microbial synthesis of alka(e)nes. *Front. Bioeng. Biotechnol.* **1**, 1–5 <https://doi.org/10.3389/fbioe.2013.00010>
- 16 Kang, M.K. and Nielsen, J. (2017) Biobased production of alkanes and alkenes through metabolic engineering of microorganisms. *J. Ind. Microbiol. Biotechnol.* **44**, 613–622 <https://doi.org/10.1007/s10295-016-1814-y>
- 17 Li, N., Chang, W.C., Warui, D.M., Booker, S.J., Krebs, C. and Bollinger, J.M. (2012) Evidence for only oxygenative cleavage of aldehydes to alk(a)e(nes) and formate by cyanobacterial aldehyde decarbonylases. *Biochemistry* **51**, 7908–7916 <https://doi.org/10.1021/bi300912n>
- 18 Rude, M.A., Baron, T.S., Brubaker, S., Alibhai, M., Del Cardayre, S.B. and Schirmer, A. (2011) Terminal olefin (1-alkene) biosynthesis by a novel P450 fatty acid decarboxylase from *Jeotgalicoccus* species. *Appl. Environ. Microbiol.* **77**, 1718–1727 <https://doi.org/10.1128/AEM.02580-10>
- 19 Albro, P.W. and Dittmer, J.C. (1969) The biochemistry of long-chain, nonisoprenoid hydrocarbons. 3. The metabolic relationship of long-chain fatty acids and hydrocarbons and other aspects of hydrocarbon metabolism in *Sarcina lutea*. *Biochemistry* **8**, 1913–1918 <https://doi.org/10.1021/bi00833a022>
- 20 Sukovich, D.J., Seffernick, J.L., Richman, J.E., Gralnick, J.A. and Wackett, L.P. (2010) Widespread head-to-head hydrocarbon biosynthesis in bacteria and role of OleA. *Appl. Environ. Microbiol.* **76**, 3850–3862 <https://doi.org/10.1128/AEM.00436-10>
- 21 Mendez-Perez, D., Begemann, M.B. and Pfleger, B.F. (2011) Modular synthase-encoding gene involved in alpha-olefin biosynthesis in *Synechococcus* sp. strain PCC 7002. *Appl. Environ. Microbiol.* **77**, 4264–4267 <https://doi.org/10.1128/AEM.00467-11>
- 22 Quadri, L.E.N., Weinreb, P.H., Lei, M., Nakano, M.M., Zuber, P. and Walsh, C.T. (1998) Characterization of Sfp, a *Bacillus subtilis* phosphopantetheinyl transferase for peptidyl carrier protein domains in peptide synthetases. *Biochemistry* **37**, 1585–1595 <https://doi.org/10.1021/bi9719861>
- 23 Akhtar, M.K., Turner, N.J. and Jones, P.R. (2013) Carboxylic acid reductase is a versatile enzyme for the conversion of fatty acids into fuels and chemical commodities. *Proc. Acad. Natl. Acad. Sci. U.S.A.* **110**, 87–92 <https://doi.org/10.1073/pnas.1216516110>
- 24 Howard, T.P., Middelhaufe, S., Moore, K., Edner, C., Kolak, D.M., Taylor, G.N. et al. (2013) Synthesis of customized petroleum-replica fuel molecules by targeted modification of free fatty acid pools in *Escherichia coli*. *Proc. Acad. Natl. Acad. Sci. U.S.A.* **110**, 7636–7641 <https://doi.org/10.1073/pnas.1215966110>
- 25 Ensign, S.A. (2001) Microbial metabolism of aliphatic alkenes. *Biochemistry* **40**, 5845–5853 <https://doi.org/10.1021/bi015523d>
- 26 Hartmans, S. and de Bont, J.A.M. (1986) Acetol monooxygenase from *Mycobacterium pyl* cleaves acetol into acetate and formaldehyde. *FEMS Microbiol. Lett.* **36**, 155–158 <https://doi.org/10.1111/j.1574-6968.1986.tb01686.x>
- 27 Harwood, C.S. and Parales, R.E. (1996) The beta-ketoadipate pathway and the biology of self-identity. *Annu. Rev. Microbiol.* **50**, 553–590 <https://doi.org/10.1146/annurev.micro.50.1.553>
- 28 Heider, J., Spormann, A.M., Beller, A.R. and Widdel, F. (1998) Anaerobic bacterial metabolism of hydrocarbons. *FEMS Microbiol. Rev.* **22**, 459–473 <https://doi.org/10.1111/j.1574-6976.1998.tb00381.x>
- 29 Grossi, V., Cravo-Laureau, C., Méou, A., Raphael, D., Garzino, F. and Hirschler-Réa, A. (2007) Anaerobic 1-alkene metabolism by the alkane- and alkene-degrading sulfate reducer *Desulfatibacillus aliphaticivorans* strain CV2803 T. *Appl. Environ. Microbiol.* **73**, 882–7890 <https://doi.org/10.1128/AEM.01097-07>
- 30 Vogel, T.M. and Grbic-Galic, D. (1986) Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. *Appl. Environ. Microbiol.* **52**, 200–202 <https://doi.org/10.1128/AEM.52.1.200-202.1986>
- 31 Edwards, E.A. and Grbic-Galic, D. (1994) Anaerobic degradation of toluene and oxylene by a methanogenic consortium. *Appl. Environ. Microbiol.* **60**, 313–322 <https://doi.org/10.1128/AEM.60.1.313-322.1994>
- 32 Siegel, H. and Eggersdorfer, M. (2012) Ketones. *Ullman's Encycl. Chem.* **5**, 1–20 https://doi.org/10.1002/14356007.a15_077
- 33 Davies, R. and Stephenson, M. (1941) Studies on the acetone-butyl alcohol fermentation: Nutritional and other factors involved in the preparation of active suspensions of *Cl. acetobutylicum* (Weizmann). *Biochem. J.* **5**, 1320–1331 <https://doi.org/10.1042/bj0351320>
- 34 Argiles, J.M. (1986) Has acetone a role in the conversion of fat to carbohydrate in mammals? *Trends Biochem. Sci.* **11**, 61–63 [https://doi.org/10.1016/0968-0004\(86\)90256-2](https://doi.org/10.1016/0968-0004(86)90256-2)

- 35 Westheimer, F.H. (1969) Acetoacetate decarboxylase from *Clostridium acetobutylicum*. *Methods Enzymol.* **43**, 231–241 [https://doi.org/10.1016/S0076-6879\(69\)14045-8](https://doi.org/10.1016/S0076-6879(69)14045-8)
- 36 Lukins, H.B. and Foster, J.W. (1963) Methyl ketone metabolism in hydrocarbon-utilizing mycobacteria. *J. Bacteriol.* **85**, 1074–1087 <https://doi.org/10.1128/JB.85.5.1074-1087.1963>
- 37 Vestal, J.R. and Perry, J.J. (1969) Divergent metabolic pathways for propane and propionate utilization by a soil isolate. *J. Bacteriol.* **99**, 216–221 <https://doi.org/10.1128/JB.99.1.216-221.1969>
- 38 Kotani, T., Yurimoto, H., Kato, N. and Sakai, Y. (2007) Novel acetone metabolism in a propane-utilizing bacterium, *Gordonia* sp. strain TY-5. *J. Bacteriol.* **189**, 886–893 <https://doi.org/10.1128/JB.01054-06>
- 39 Small, F.J., Tilley, J.K. and Ensign, S.A. (1995) Characterization of a new pathway for epichlorohydrin degradation by whole cells of *Xanthobacter* strain Py2. *Appl. Environ. Microbiol.* **61**, 1507–1513 <https://doi.org/10.1128/AEM.61.4.1507-1513.1995>
- 40 Nemeczek-Marshall, M., Wojciechowski, C., Wagner, W.P. and Fall, R. (1999) Acetone formation in the vibrio family: a new pathway for bacterial leucine catabolism. *J. Bacteriol.* **181**, 7493–7499 <https://doi.org/10.1128/JB.181.24.7493-7499.1999>
- 41 Nagpal, A., Valley, M.P., Fitzpatrick, P.F. and Orville, A.M. (2006) Crystal structures of nitroalkane oxidase: insights into the reaction mechanism from a covalent complex of the flavoenzyme trapped during turnover. *Biochemistry* **45**, 1138–1150 <https://doi.org/10.1021/bi051966w>
- 42 Nagy, I., Compernolle, F., Ghys, K., Vanderleyden, J. and De Mot, R. (1995) A single cytochrome P-450 system is involved in degradation of the herbicides EPTC (S-ethyl dipropylthiocarbamate) and atrazine by *Rhodococcus* sp. strain NI86/21. *Appl. Environ. Microbiol.* **61**, 2056–2060 <https://doi.org/10.1128/AEM.61.5.2056-2060.1995>
- 43 Taylor, D.G., Trudgill, P.W., Cripps, R.E. and Harris, P.R. (1980) The microbial metabolism of acetone. *J. Gen. Microbiol.* **118**, 159–170 <https://doi.org/10.1099/00221287-118-1-159>
- 44 Coleman, J.P. and Perry, J.J. (1984) Fate of the C1 product of propane dissimilation in *Mycobacterium vaccae*. *J. Bacteriol.* **160**, 1163–1164 <https://doi.org/10.1128/JB.160.3.1163-1164.1984>
- 45 Hausinger, R.P. (2007) New insights into acetone metabolism. *J. Bacteriol.* **189**, 671–673 <https://doi.org/10.1128/JB.01578-06>
- 46 Mihovilovic, M.D., Mueller, B. and Stanetty, P. (2002) Monooxygenase-mediated Baeyer–Villiger oxidations. *Eur. J. Org. Chem.* **22**, 3711–3730 [https://doi.org/10.1002/1099-0690\(200211\)2002:22<3711::AID-EJOC3711>3.0.CO;2-5](https://doi.org/10.1002/1099-0690(200211)2002:22<3711::AID-EJOC3711>3.0.CO;2-5)
- 47 Ensign, S.A., Small, F.J., Allen, J.R. and Sluis, M.K. (1998) New roles for CO₂ in the microbial metabolism of aliphatic epoxides and ketones. *Arch. Microbiol.* **169**, 179–187 <https://doi.org/10.1007/s002030050558>
- 48 Cooper, R.A. (1984) Metabolism of methylglyoxal in microorganisms. *Annu. Rev. Microbiol.* **38**, 49–68 <https://doi.org/10.1146/annurev.mi.38.100184.000405>
- 49 Donoghue, N.A., Norris, D.B. and Trudgill, P.W. (1976) The purification and properties of cyclohexanone monooxygenase *Nocardia globerculata* CL1 and *Acinetobacter* NCIB 9871. *Eur. J. Biochem.* **63**, 175–192 <https://doi.org/10.1111/j.1432-1033.1976.tb10220.x>
- 50 Stewart, J.D. (1998) Cyclohexanone monooxygenase: a useful reagent for asymmetric Baeyer–Villiger reactions. *ChemInform* **29**, <https://doi.org/10.1002/chin.199844320>
- 51 Griffin, M. and Trudgill, P.W. (1976) Purification and properties of cyclo-pentanone oxygenase of *Pseudomonas* NCIB 9872. *Eur. J. Biochem.* **63**, 199–209 <https://doi.org/10.1111/j.1432-1033.1976.tb10222.x>
- 52 Brzostowicz, P.C., Walters, D.M., Thomas, S.M., Nagarajan, V. and Rouviere, P.E. (2003) mRNA differential display in a microbial enrichment culture: simultaneous identification of three cyclohexanone monooxygenases from three species. *Appl. Environ. Microbiol.* **69**, 334–342 <https://doi.org/10.1128/AEM.69.1.334-342.2003>
- 53 Kostichka, K., Thomas, S.M., Gibson, K.J., Nagarajan, V. and Cheng, Q. (2001) Cloning and characterization of a gene cluster for cyclododecanone oxidation in *Rhodococcus ruber* SC1. *J. Bacteriol.* **183**, 6478–6486 <https://doi.org/10.1128/JB.183.21.6478-6486.2001>
- 54 Kyte, B.G., Rouviere, P.E., Cheng, Q. and Stewart, J.D. (2004) Assessing the substrate selectivities and enantioselectivities of eight novel Baeyer–Villiger monooxygenases toward alkyl-substituted cyclohexanones. *J. Org. Chem.* **69**, 12–17 <https://doi.org/10.1021/jo0302531>
- 55 van Beilen, J.B., Mourlane, F., Seeger, M.A., Kovac, J., Li, Z., Smits, T.H.M. et al. (2003) Cloning of Baeyer–Villiger monooxygenases from *Comamonas*, *Xanthobacter* and *Rhodococcus* using polymerase chain reaction with highly degenerate primers. *Environ. Microbiol.* **5**, 174–182 <https://doi.org/10.1046/j.1462-2920.2003.00401.x>
- 56 Kamerbeek, N.M., Moen, M.J.H., van der Ven, J.G.M., van Berkel, W.J.H., Fraaije, M.W. and Janssen, D.B. (2001) 4-Hydroxyacetophenone mono-oxygenase from *Pseudomonas fluorescens* ACB. *Eur. J. Biochem.* **268**, 2547–2557 <https://doi.org/10.1046/j.1432-1327.2001.02137.x>
- 57 Fraaije, M.W., Wu, J., Heuts, D.P.H.M., van Hellemond, E.W., Spelberg, J.H.L. and Janssen, D.B. (2005) Discovery of a thermostable Baeyer–Villiger monooxygenase by genome mining. *Appl. Microbiol. Biotechnol.* **66**, 393–400 <https://doi.org/10.1007/s00253-004-1749-5>
- 58 Britton, L.N. and Markovetz, A.J. (1977) A novel ketone monooxygenase from *Pseudomonas cepacia*. *J. Biol. Chem.* **252**, 8561–8566 PMID: 925012
- 59 Fraaije, M.W., Kamerbeek, N.M., Heidekamp, A.J., Fortin, R. and Janssen, D.B. (2004) The prodrug activator EtaA from *Mycobacterium tuberculosis* is a Baeyer–Villiger monooxygenase. *J. Biol. Chem.* **279**, 3354–3360 <https://doi.org/10.1074/jbc.M307770200>
- 60 Boyd, J.M., Ellsworth, H. and Ensign, S.A. (2004) Bacterial acetone carboxylase is a manganese-dependent metalloenzyme. *J. Biol. Chem.* **279**, 46644–46651 <https://doi.org/10.1074/jbc.M407177200>
- 61 Sluis, M.K. and Ensign, S.A. (1997) Purification and characterization of acetone carboxylase from *Xanthobacter* strain Py2. *Proc. Acad. Natl. Acad. Sci. U.S.A.* **94**, 8456–8461 <https://doi.org/10.1073/pnas.94.16.8456>
- 62 van Ginkel, C.G. and de Bont, J.A.M. (1986) Isolation and characterization of alkeneutilizing *Xanthobacter* spp. *Arch. Microbiol.* **145**, 403–407 <https://doi.org/10.1007/BF00470879>
- 63 Allen, J.R., Clark, D.D., Krum, J.G. and Ensign, S.A. (1999) A role for coenzyme M (2-mercaptoethanesulfonic acid) in a bacterial pathway of aliphatic epoxide carboxylation. *Proc. Acad. Natl. Acad. Sci. U.S.A.* **96**, 8432–8437 <https://doi.org/10.1073/pnas.96.15.8432>
- 64 Allen, J.R. and Ensign, S.A. (1996) Carboxylation of epoxides to β-keto acids in cell extracts of *Xanthobacter* strain Py2. *J. Bacteriol.* **178**, 1469–1472 <https://doi.org/10.1128/JB.178.5.1469-1472.1996>
- 65 Allen, J.R. and Ensign, S.A. (1997) Characterization of three protein components required for functional reconstitution of the epoxide carboxylase multienzyme complex from *Xanthobacter* strain Py2. *J. Bacteriol.* **179**, 3110–3115 <https://doi.org/10.1128/JB.179.10.3110-3115.1997>

- 66 Allen, J.R. and Ensign, S.A. (1997) Purification to homogeneity and reconstitution of the individual components of the epoxide carboxylase multiprotein enzyme complex from *Xanthobacter* strain Py2. *J. Biol. Chem.* **272**, 32121–32128 <https://doi.org/10.1074/jbc.272.51.32121>
- 67 Allen, J.R. and Ensign, S.A. (1998) Identification and characterization of epoxide carboxylase activity in cell extracts of *Nocardia corallina* B276. *J. Bacteriol.* **180**, 2072–2078 <https://doi.org/10.1128/JB.180.8.2072-2078.1998>
- 68 Small, F.J. and Ensign, S.A. (1995) Carbon dioxide fixation in the metabolism of propylene and propylene oxide by *Xanthobacter* strain Py2. *J. Bacteriol.* **177**, 6170–6175 <https://doi.org/10.1128/jb.177.21.6170-6175.1995>
- 69 Small, F.J. and Ensign, S.A. (1997) Alkene monooxygenase from *Xanthobacter* strain Py2: Purification and characterization of a four-componenet system central to the bacterial metabolism of aliphatic alkenes. *J. Biol. Chem.* **272**, 24913–24920 <https://doi.org/10.1074/jbc.272.40.24913>
- 70 Ensign, S.A. and Allen, J.R. (2003) Aliphatic epoxide carboxylation. *Annu. Rev. Biochem.* **72**, 55–76 <https://doi.org/10.1146/annurev.biochem.72.121801.161820>
- 71 DiMarco, A.A., Bobik, T.A. and Wolfe, R.S. (1990) Unusual coenzymes of methanogenesis. *Annu. Rev. Biochem.* **59**, 355–394 <https://doi.org/10.1146/annurev.bi.59.070719.0002035>
- 72 Taylor, C.D. and Wolfe, R.S. (1974) A simplified assay for coenzyme M (HSCH₂CH₂SO₃). Resolution of methylcobalamin-coenzyme M methyltransferase anduse of sodium borohydride. *J. Biol. Chem.* **249**, 4886–4890 PMID: 4152560
- 73 Taylor, C.D. and Wolfe, R.S. (1974) Structure and methylation of coenzyme M (HSCH₂CH₂SO₃). *J. Biol. Chem.* **249**, 4879–4885 PMID: 4367810
- 74 Thauer, R.K. (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiology* **144**, 2377–2406 <https://doi.org/10.1099/00221287-144-9-2377>
- 75 Wolfe, R.S. (1991) My kind of biology. *Annu. Rev. Microbiol.* **45**, 1–35 <https://doi.org/10.1146/annurev.mi.45.100191.000245>
- 76 Partovi, S.E., Mus, F., Gutknecht, A.E., Martinez, H.A., Tripet, B.P., Lange, B.M. et al. (2018) Coenzyme M biosynthesis in bacteria involves phosphate elimination by a functionally distinct member of the aspartate/fumarate superfamily. *J. Biol. Chem.* **293**, 5236–5246 <https://doi.org/10.1074/jbc.RA117.001234>
- 77 Sluis, M.K., Small, F.J., Allen, J.R. and Ensign, S.A. (1996) Involvement of an ATP-dependent carboxylase in a CO₂-dependent pathway of acetone metabolism by *Xanthobacter* strain Py2. *J. Bacteriol.* **178**, 4020–4026 <https://doi.org/10.1128/JB.178.14.4020-4026.1996>
- 78 Small, K.J. and Ensign, S.A. (1995) Carbon dioxide fixation in the metabolism of propylene and propylene oxide by *Xanthobacter* Strain Py2. *J. Bacteriol.* **177**, 6170–6175 <https://doi.org/10.1128/JB.177.21.6170-6175.1995>
- 79 Pandey, A.S., Nocek, B., Clark, D.D., Ensign, S.A. and Peters, J.W. (2006) Mechanistic implications of the structure of the mixed-disulfide intermediate of the disulfide oxidoreductase, 2-ketopropyl-coenzyme M oxidoreductase/carboxylase. *Biochemistry* **45**, 113–120 <https://doi.org/10.1021/bi051518o>
- 80 Westphal, A.H., Swaving, J., Jacobs, L. and De Kok, A. (1998) Purification and characterization of a flavoprotein involved in the degradation of epoxyalkanes by *Xanthobacter* Py2. *Eur. J. Biochem.* **257**, 160–168 <https://doi.org/10.1046/j.1432-1327.1998.2570160.x>
- 81 Swaving, J., de Bont, J.A., Westphal, A. and de Kok, A. (1996) A novel type of pyridine nucleotide-disulfide oxidoreductase is essential for NAD⁺- and NADPH-dependent degradation of epoxyalkanes by *Xanthobacter* strain Py2. *J. Bacteriol.* **178**, 6644–6646 <https://doi.org/10.1128/JB.178.22.6644-6646.1996>
- 82 Williams, C.H.J. (1992) Lipoyl dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase: a family of flavoenzyme transhydrogenases. *Chem. Biochem. Flavoenzymes* **3**, 121–211
- 83 Clark, D.D., Allen, J.R. and Ensign, S.A. (2000) Characterization of five catalytic activities associated with the NADPH:2-ketopropyl-coenzyme M [2-(2-ketopropylthio)ethanesulfonate] oxidoreductase/carboxylase of the *Xanthobacter* strain Py2 epoxide carboxylase system. *Biochemistry* **39**, 1294–1304 <https://doi.org/10.1021/bi992282p>
- 84 Nocek, B., Jang, S.B., Jeong, M.S., Clark, D.D., Ensign, S.A. and Peters, J.W. (2002) Structural basis for CO₂ fixation by a novel member of the disulfide oxidoreductase family of enzymes, 2-ketopropyl-coenzyme M oxidoreductase/carboxylase. *Biochemistry* **41**, 12907–12913 <https://doi.org/10.1021/bi026580p>
- 85 Kofoed, M.A., Wampler, D.A., Pandey, A.S., Peters, J.W. and Ensign, S.A. (2011) Roles of the redox-active disulfide and histidine residues forming a catalytic dyad in reactions catalyzed by 2-ketopropyl coenzyme M oxidoreductase/carboxylase. *J. Bacteriol.* **193**, 4904–4913 <https://doi.org/10.1128/JB.05231-11>
- 86 Pandey, A.S., Mulder, D.W., Ensign, S.A. and Peters, J.W. (2011) Structural basis for carbon dioxide binding by 2-ketopropyl coenzyme M oxidoreductase/carboxylase. *FEBS Lett.* **585**, 459–464 <https://doi.org/10.1016/j.febslet.2010.12.035>
- 87 Prussia, G.A., Gauss, G.H., Mus, F., Conner, L., DuBois, J.L. and Peters, J.W. (2016) Substitution of a conserved catalytic dyad into 2-KPCC causes loss of carboxylation activity. *FEBS Lett.* **590**, 2991–2996 <https://doi.org/10.1002/1873-3468.12325>
- 88 Streit, B.R., Mattice, J.R., Prussia, G.A., Peters, J.W. and DuBois, J.L. (2019) The reactive form of a C-S bond-cleaving, CO₂-fixing flavoenzyme. *J. Biol. Chem.* **294**, 5137–5145 <https://doi.org/10.1074/jbc.RA118.005554>
- 89 Jeng, M.F., Holmgren, A. and Dyson, H.J. (1995) Proton sharing between cysteine thiols in *Escherichia coli* thioredoxin: implications for the mechanism of protein disulfide reduction. *Biochemistry* **34**, 10101–10105 <https://doi.org/10.1021/bi00032a001>
- 90 Schühle, K. and Heider, J. (2012) Acetone and butanone metabolism of the denitrifying bacterium "Aromatoleum aromaticum" demonstrates novel biochemical properties of an ATP-dependent aliphatic ketone carboxylase. *J. Bacteriol.* **194**, 131–141 <https://doi.org/10.1128/JB.05895-11>
- 91 Mus, F., Alleman, A.B., Kabasakal, B.V., Wells, J.N., Murray, J.W., Nocek, B.P. et al. (2017) Structural basis for the mechanism of ATP-dependent acetone carboxylation. *Sci. Rep.* **7**, 1–10 <https://doi.org/10.1038/s41598-016-0028-x>