Acetogenic Bacteria

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Advanced article

Article Contents

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Acetogenic bacteria are a specialised group of strictly anaerobic bacteria that are ubiquitous in nature. Together with the methane-forming archaea they constitute the last limbs in the anaerobic food web that leads to the production of methane from polymers in the absence of oxygen. Acetogens are characterised by a unique pathway, the Wood–Ljungdahl pathway of carbon dioxide reduction with the acetyl-CoA synthase as the key enzyme. This pathway also allows chemolithoautotrophic growth on hydrogen and carbon dioxide and it is the only pathway known that combines carbon dioxide fixation with adenosine triphosphate (ATP) synthesis. Thus, it is considered the first biochemical pathway on earth. ATP is synthesised by a chemiosmotic mechanism with Na^+ or H^+ as coupling ion, depending on the organism. In cytochrome-free acetogens, energy is conserved by ferredoxin reduction followed by ferredoxin-dependent Na^+ (or H^+) translocation across the membrane (Rnf complex). Acetogens may represent ancestors of the first bioenergetically active cells in evolution.

Introduction

Acetogenic bacteria are a specialised group of strictly anaerobic bacteria that produce acetate as the main product of their metabolism. A characteristic of acetogenic bacteria that distinguishes them from all other bacteria that produce acetate as a byproduct in anaerobic metabolism is the de-novo synthesis of acetate from two mole of CO_2. The pathway of CO_2 reduction to acetate is termed 'Wood–Ljungdahl' pathway in honour of Harland G. Wood and Lars G. Ljungdahl, who elucidated this unique pathway, or acetyl-CoA pathway after the key intermediate acetyl-CoA; the key enzyme in the pathway is the acetyl-CoA synthase (ACS), an enzyme that was originally discovered as a CO dehydrogenase (CO-DH), which is the basis for a third synonym of the pathway, the CO-DH pathway. **See also**: Bacterial Fermentation

Acetogenic bacteria or acetogens are among the metabolically most versatile anaerobic organisms. Most of them are able to grow chemoorganoheterotrophically on a variety of different organic substrates, including sugars, C_1 compounds (CO, CO_2, methyl groups, formate, etc.), methoxylated aromatic compounds, dicarboxylic acids and alcohols. Oxidation of these compounds is coupled to the reduction of CO_2 in the Wood–Ljungdahl pathway. Under certain conditions, for example, during the fermentation of hexoses, acetate is the sole end product formed by acetogens. Thus, the term 'homoacetogens' was introduced to denote this capability. However, this term should no longer be used for the group of organisms but only for this type of fermentation (homoacetogenesis). Furthermore, the Wood–Ljungdahl pathway allows acetogens to grow autotrophically on $H_2 + CO_2$. Thus, this pathway serves as a way for CO_2 fixation and energy conservation. Acetogenesis from $H_2 + CO_2$ is considered the oldest biochemical pathway and the first life-sustaining process on earth since it is the only pathway of carbon dioxide fixation that also yields adenosine triphosphate (ATP).

Although the reduction of CO_2 to acetate is the main characteristic of the metabolism of acetogenic bacteria, this capability is not conditional. Under certain conditions, less than 3 mol of acetate per hexose or even no acetate is produced during the growth of an acetogen, depending on the organism, growth conditions and substrate utilised. For a definition of chemoorganoheterotrophy and chemolithoautotrophy **See also**: Bacterial Ecology

Phylogeny and genera of acetogens

The unifying physiological feature of acetogens is the organism's ability to reduce CO_2 to acetate via the acetyl-CoA pathway. However, phylogenetically acetogens are rather diverse. They are exclusively found in the domain *Bacteria*. Currently, there are 23 bacterial genera (**Table 1**) that contain more than 100 reported acetogenic species.

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Table 1 Genera of acetogenic bacteria. The first species of each genus to be classified as an acetogen are displayed. Note that the quotation marks indicate names that still need to be validated

Acetitomaculum ruminis (ATCC 43876^T)
Acetoanaerobium noterae (ATCC 35199^T)
Acetobacterium woodii (ATCC 29683^T)
Acetohalobium arabaticum (DSM 5501^T)
Acetonema longum (DSM 6540^T)
Marvinbryantia formatexigens (DSM 14469^T)
"*Butyribacterium methylotrophicum*" (ATCC 33266^T)
Caloramator fervidus (ATCC 43204^T)
Clostridium aceticum (DSM 1496^T)
Eubacterium limosum (ATCC 8486^T)
"*Fuchsiella alkaliacetigena*" (DSM 24880^T)
Holophaga foetida (DSM 6591^T)
Moorella thermoacetica (ATCC 35608^T)
Natroniella acetigena (DSM 9952^T)
Natronincola histidinovorans (DSM 11416^T)
Oxobacter pfennigii (DSM 3222^T)
Blautia productus (ATCC 35244^T)
Sporomusa ovata (DSM 2662^T)
Syntrophococcus sucromutans (DSM 3224^T)
Tindallia californiensis (DSM 14871^T)
Thermoacetogenium phaeum (DSM 12270^T)
Thermoanaerobacter kivui (ATCC 33488^T)
Treponema primitia (DSM 12427^T)

The most described acteogenic species are Gram-positive. In some genera, for example, *Acetobacterium* and *Sporomusa*, the members are exclusively acetogenic. However, many acetogens can be found in the genera containing both acetogenic and nonacetogenic bacteria (e.g. *Clostridium*, *Blautia*, *Eubacterium*, *Thermoanaerobacter* and *Treponema*).

Genomes of acetogens

The genomes of several acetogens have been sequenced so far, including *Acetobacterium woodii*, *Morella thermoacetica*, *Thermoacetogenium phaeum*, *Eubacterium limosum*, *Acetohalobium arabaticum*, *Blautia hydrogenothrophicuss* and *Clostridium ljungdahlii*. The genome sizes vary between 2.4 and 6 Mb with a GC content (mol% guanine and cytosine bases) ranging from 31% to 55%. Because acetogensis is a physiological trait acetogenic bacteria have to be identified by metabolic marker genes like the formyltetrahydrofolate synthetase gene (*fhs*) or the CO-DH/ACS gene complex (*acs*AB). Current studies aim to identify markers for the molecular identification and quantification of acetogenic populations in environmental studies. More importantly, recent developments established a genetic modification system in Clostridiales, which enables the genetic transformation of Gram-positive organisms and will give new insights on genome function, biochemical properties and regulation in acetogens.

Occurence of acetogenic bacteria

The metabolic versatility of acetogens allows the colonisation of a vast variety of habitats, reaching from gastrointestinal, terrestrial surface and subsurface, and aquatic ecosystems. The mammalian gastrointestinal tract harbours numerous H_2-utilising acetogenic bacteria. Recent studies have confirmed that some of the acetate formed in the human intestine is synthesised by microorganisms engaging the acetyl-CoA pathway. The microbially produced acetate is absorbed by the intestine wall and serves as carbon and energy source for the mammalian host. Albeit methanogenesis is predominant in the gastrointestinal system of ruminants, numerous ruminal acetogens have been isolated.

Acetogenic bacteria are an essential constituent of the termites gut system. Cellulolytic protozoa convert wood polysaccharides (e.g. cellulose) to acetate, H_2 and CO_2. In turn, H_2 and CO_2 are converted to further acetate by the acetogenic bacteria. In addition, acetogens can grow by oxidation of aldehydes and methoxy groups derived from aromatic compounds originating from the degradation of lignin. Thus, acetogens in the hindgut contribute approximately one-third of microbially produced acetate, which serves as the main source of carbon and energy for the termite.

Numerous H_2- and CO-utilising acetogenic bacteria have been isolated from soil (Küsel and Drake, 1995). Acetate is a dominant compound in soil solution, and many observations indicate an important role of acetate in the flux of carbon in soil. Approximately 10% of the 10^{13} kg acetate produced and further metabolised in terrestrial soils and sediments per annum are attributed to the acetogenic bacteria and the acetyl-CoA pathway.

In marine habitats the reduction of sulfate is the predominant anaerobic terminal electron-accepting process. However, under sulfate depleted conditions the flow of carbon and reductants shifts towards methanogenesis, but also acetogenesis at comparable high rates during the transitional phase. Thus, it is not surprising that countless H_2-utilising acetogenic bacteria have been isolated from marine sediments. Additionally, the occurrence of acetogens in hypersaline habitats and subsurface aquifers has been reported, underlining the ability of the organisms to colonise diverse ecological niches.

Metabolism of Acetogens

Acetogens can use a variety of different compounds as an energy and carbon source (**Table 2**). Historically, the first acetogens isolated were enriched with $H_2 + CO_2$ or hexoses. Hexoses are converted according to the reaction

$$C_6H_{12}O_6 + 4ADP + 4Pi \rightarrow 2CH_3COOH + 2CO_2 + 8[H] + 4ATP \quad [I]$$

First, the hexoses are oxidised by way of the Embden–Meyerhof–Parnas pathway (glycolysis) to yield 2 mol

Table 2 Metabolic capabilities of homoacetogenic bacteria

Acetate formation from $H_2 + CO_2$	1932
Hexose fermentation to three acetate units	1942
Utilisation of methanol	1969
Utilisation of carbon monoxide	1978
Demethylation of phenyl methyl ether	1981
Reduction of phenyl acryl acid derivatives	1981
Incomplete oxidation of primary aliphatic alcohols	1985
Energy-conserving reduction of caffeate	1988
Incomplete oxidation of mandelate	1991
Utilisation of oxalate and glycolate	1993
Energy-conserving reduction of nitrate	1993
Oxidation of aromatic aldehydes	1998

pyruvate, which are then split by the pyruvate:ferredoxin-oxidoreductase to 2 mol acetyl-CoA, reduced ferredoxin and CO_2 (**Figure 1**). Acetyl-CoA is further converted to acetate by phosphotransacetylase and acetate kinase. This pathway yields 4 mol of ATP by substrate level phosphorylation, the highest possible ATP yield in a fermenting bacterium. **See also**: Bacterial Fermentation

Surprisingly, stoichiometric analyses revealed that a third mole of acetate is formed from the CO_2 produced according to the reaction

$$CO_2 + 8[H] \rightarrow CH_3COOH + 2H_2O \qquad [II]$$

In total, the oxidation of hexoses yields three moles of acetate:

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad [III]$$

Because this type of fermentation yields only acetate, it is also referred to as homoacetogenesis. However, as mentioned above 'acetogens' can also grow 'non-acetogenically'.

The surprising finding of a homoacetogenic fermentation of hexoses could not be explained by the pathways known at that time and stimulated biochemical analyses that led to the discovery of the acetyl-CoA pathway by Harland G. Wood and Lars G. Ljungdahl. This pathway also enables the acetogens to grow chemolithoautotrophically according to the reaction

$$CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \qquad [IV]$$

Chemolithoautotrophic growth requires that the pathway is connected to an energy-conserving mechanism and indeed acetogenesis from $H_2 + CO_2$ is coupled to both substrate level phosphorylation and ion gradient-driven phosphorylation.

The acetyl-CoA pathway

As outlined above, the common physiological feature of acetogenic bacteria is the acetyl-CoA pathway. It has both dissimilatory and assimilatory functions during the growth of an acetogen: it is the terminal electron-accepting

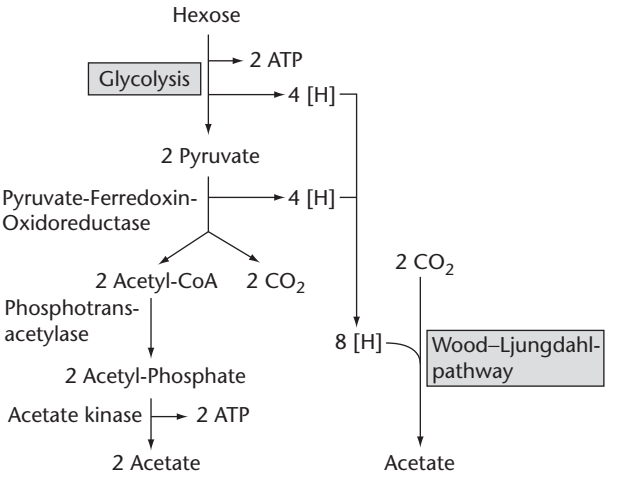

Figure 1 Fermentation of hexoses to acetate by acetogenic bacteria. The fermentation yields only acetate according to eqn [III]. This fermentation is referred to as homoacetogenesis.

process, reduces CO_2 to acetate, and provides the cell with a mechanism for the fixation of CO_2 and other C_1-compounds (e.g. formate and methanol), as acetyl-CoA, an intermediate of the pathway, is assimilated into cellular carbon.

The acetyl-CoA pathway comprises two reductive branches (Ragsdale and Pierce, 2008; **Figure 2**). On the methyl branch, CO_2 is reduced to the methyl level, whereas on the carbonyl branch, CO_2 is reduced to the carbonyl level. The initial step on the methyl branch is the reduction of CO_2 to formate by formate dehydrogenase (**Figure 2**). This reaction is reversible. The NADPH-dependent enzyme from *M. thermoacetica* is an $\alpha_2\beta_2$ tetramer containing tungsten, selenium and iron sulfur centres. Subsequently, formate is activated and bound to tetrahydrofolate (H_4F) by formyl-H_4F synthase. The formation of 10-formyl-H_4F is endergonic and requires the hydrolysis of ATP. In the next step, water is split off by 5,10-methenyl-H_4F-cyclohydrolase. The resulting methenyl group is reduced via methylene-H_4F to methyl-H_4F. The methyl group is then transferred to the enzyme ACS by a methyltransferase. The initial methyl group acceptor is the corrinoid/iron sulfur protein (Co/FeS-P) that contains iron sulfur clusters and a corrinoid cofactor with a supernucleophile Co(I) (a corrinoid is a compound such as cobalamin that contains a polyaromatic ring system (the corrin) similar to tetrapyrrole with cobalt coordinated by the inner four nitrogen atoms). With its free electron pairs, the Co(I) attacks the methyl group and abstracts a methyl cation, resulting in the formation of methyl-Co(III). Then the methyl group of the methylated Co/FeS-P is transferred to ACS.

The ACS is the key enzyme of the acetyl-CoA pathway. It was historically discovered as an oxidoreductase that can oxidise CO and is, therefore, also referred to as CO-DH/ACS to emphasise the bifunctional character of the enzymes. The enzyme has been purified from *M. thermoacetica* and *A.*

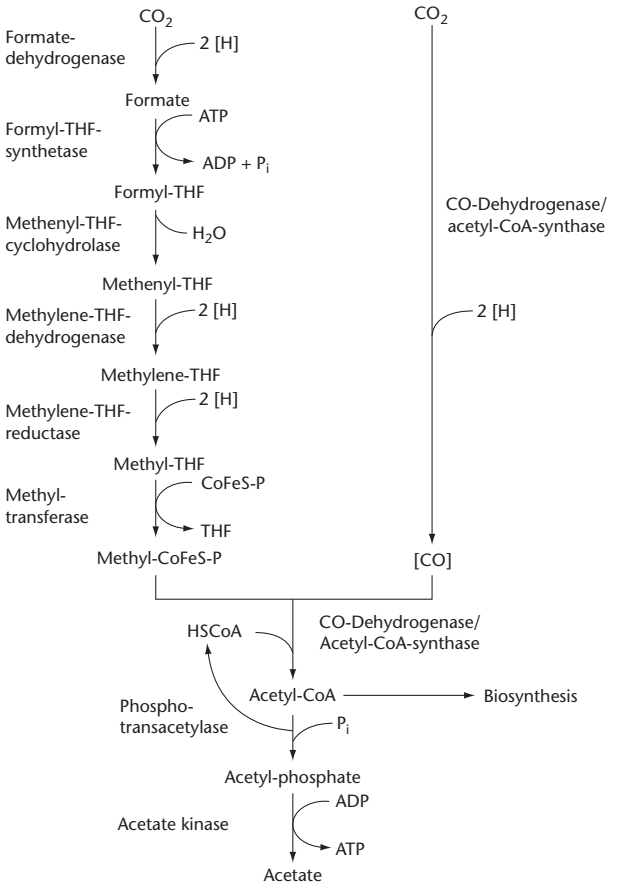

Figure 2 The Wood–Ljungdahl pathway. Abbreviations: THF, tetrahydrofolate; HSCoA, coenzyme A; P_i, inorganic phosphate; e^-, electron; CoFe/S-P, corrinoid-iron sulfur protein; ATP, adenosine 5′-triphosphate. 2[H], reducing equivalents (NADPH for the first reaction in *M. thermoacetica*, NADH for the second and third reaction and reduced ferredoxin for the CO-DH reaction).

woodii, and is an $\alpha_2\beta_2$ tetramer. The acetyl-CoA reaction is catalysed by the α subunits, whereas the CO-DH reaction is catalysed by the β subunits (Grahame, 2003). The reactive site of each α subunit is the so-called 'A cluster' comprising an Fe_4S_4 cluster and Ni. They catalyse the synthesis of acetyl-CoA from CO, CoA and methylated Co/FeS-P. The 'A clusters' are connected with a narrow channel that has two branches to the β subunits 'C cluster'. The channel is postulated to concentrate the CO and prevents the poisonous gas from interacting with other cellular constituents. The 'C clusters' catalyse the reversible reduction of CO_2 to CO. In subsequent steps, acetyl-CoA, synthesised by the ACS/CO-DH is then converted to acetate by phosphotransacetylase and acetate kinase.

The acetyl-CoA pathway was first discovered in acetogens. There, it serves a catabolic function because it conserves energy in the form of ATP, but it also serves an anabolic function. Carbon fixation is via the actetyl-CoA pathway to the level of acetyl-CoA, which is then carboxylated to yield pyruvate. Oxaloacetate is built from pyruvate or phosphoenolpyruvate by a second carboxylation. The acetyl-CoA pathway is the most widespread pathway for CO_2 fixation in anaerobes and found in acetogenic and sulfate reducing bacteria and in sulfate reducing archaea and methanogenic archaea. Furthermore, it also serves as a catabolic pathway in a slightly modified form in methanogenesis by methanogenic archaea (Fuchs, 2011).

Bioenergetics of Acetogenesis

During fermentation of sugars to acetyl-CoA, reduced ferredoxin and CO_2 according to eqn [I], four mol of ATP are gained by substrate level phosphorylation. That is the maximum of ATP an organism can get by substrate level phosphorylation during hexose fermentation. Furthermore, from the ability of acetogens to grow on $H_2 + CO_2$ it is evident that some more energy must be conserved by acetogens in the acetyl-CoA pathway. The pathway is also coupled to substrate level phosphorylation, but the net synthesis of ATP formed via substrate-level phosphorylation is zero: one mole of ATP is gained in the acetate kinase reaction but the activation of formate, an intermediate of the pathway, requires one mole of ATP. It is now well established that the reduction of CO_2 to acetate is coupled to a chemiosmotic mechanism of energy conservation. Two mechanisms of energy conservation in the acetyl-CoA pathway have to be distinguished that are used by acetogens, both resulting in the generation of a transmembrane gradient, which in turn is used for ATP synthesis via an F_1F_O ATP synthase. One mechanism involves a ferredoxin:NAD^+ oxidoreductase, the Rnf complex, the other involves cytochromes. *A. woodii* and *M. thermoacetica* have served as model organisms for studying the two energy-conserving processes, respectively.

Energy conservation in cytochrome-containing acetogens

The ability to conserve energy via a chemiosmotic mechanism is dependent on the presence of membrane-bound electron carriers. Menaquinone MK-7 (2-methyl-3-heptaprenyl-1,4-naphtoquinone; $E_0' = -74$ mV) and two *b*-type cytochromes (cyt b_{559}, $E_0' = -215$ mV) have been identified as membrane-integral electron carriers in *M. thermoacetica* and the closely related *Moorella thermoautotrophica* (formerly: *Clostridium thermautotrophicum*). In subsequent studies, a flavoprotein was copurified with cyt b_{559}. All these membraneous components are very likely involved in electron transport processes serving the conservation of energy via a proton-dependent, chemiosmotic mechanism. However, the nature of the electron donor and acceptor systems is currently unknown. Some of the enzymes of the acetyl-CoA pathway are at least loosely associated with the cytoplasmic membrane. These include potential electron donors, that is, reducing equivalents-generating enzymes, like hydrogenase, CO-DH and NADH dehydrogenase, as well as potential electron acceptors, like methylene-H_4F reductase.

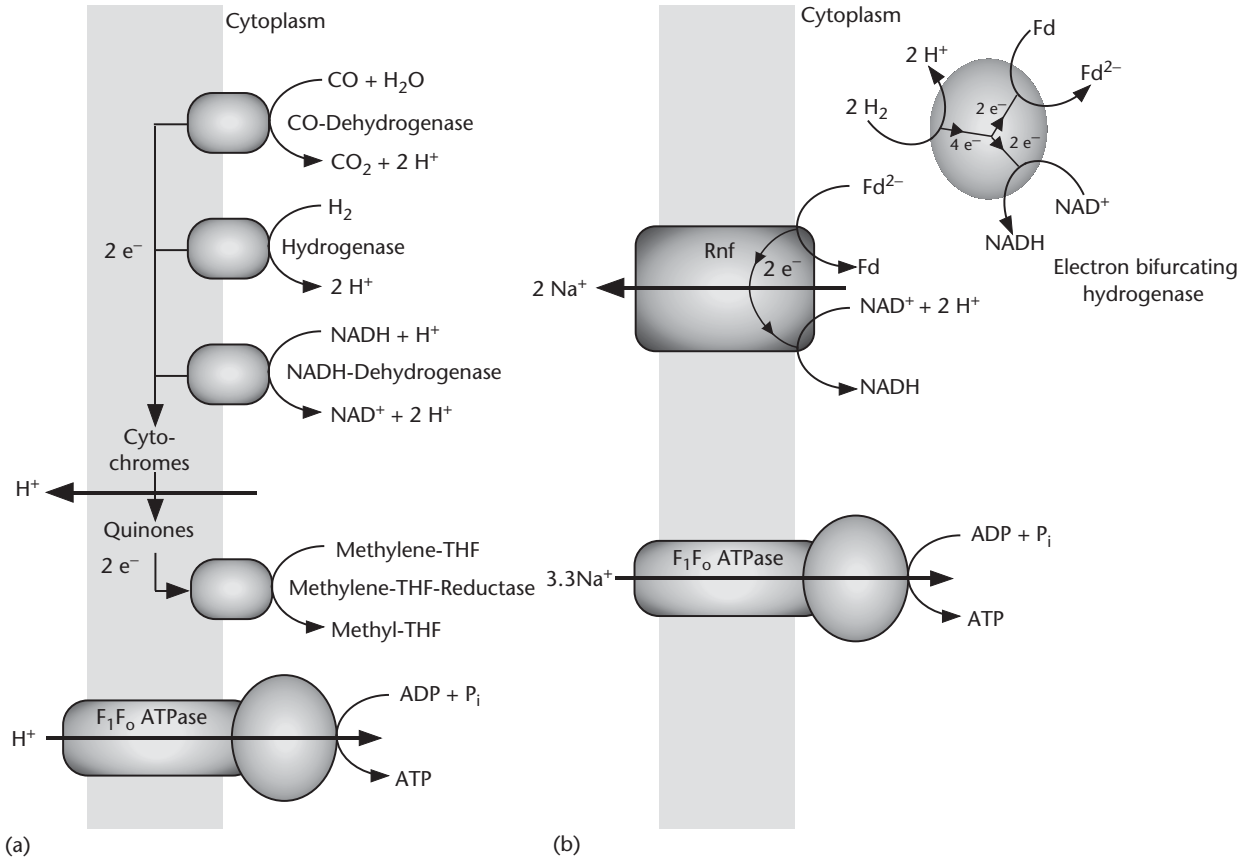

Figure 3 Chemiosmotic mechanisms of energy conservation in acetogens during lithoautotrophic growth. (a) Proton-dependent conservation of energy with the hypothetical involvement of different oxidoreductases that channel electrons into the electron transport chain. (b) Na^+-dependent conservation of energy by the Rnf complex that is fuelled by reduced ferredoxin generated by electron bifurcation. The NADH produced by the electron-bifurcating hydrogenase and the Rnf complex is used to reduce CO_2 to acetate.

Figure 3a displays a model based on the assumption that the reduction of methylene-H_4F might be the last step in a membraneous electron transport chain. The transfer of electrons deriving from hydrogenase, CO-DH or NADH dehydrogenase to methylene-H_4F reductase could result in the generation of a transmembrane proton potential. However, experimental evidence for this scenario is still missing. The electrochemical proton potential drives the synthesis of ATP via a H^+-F_1F_O-ATP synthase (Ivey and Ljungdahl, 1986).

Energy conservation in Rnf-containing acetogens

The earlier speculation that cytochrome-free acetogens such as *A. woodii* have a sodium ion-translocating methyltransferase could not be verified biochemically and was finally ruled out after the genome had been sequenced (Poehlein *et al.*, 2012). The entire carbon pathway in the acetyl-CoA pathway is localised in the cytoplasm. Instead, *A. woodii* has a membrane-bound ferredoxin:NAD^+ oxidoreductase that is encoded by the *rnf* genes and, therefore, also referred to as Rnf complex. The Rnf complex is composed of six subunits, three of which are polytopic membrane proteins, that have flavins and iron-sulfur centre as electron carriers. The complex catalyses exergonic electron transfer from reduced ferredoxin to NAD^+ and the energy set free is used to pump out Na^+ from the cytoplasm thus establishing an electrochemical sodium ion gradient across the membrane (Biegel and Müller, 2010; **Figure 3b**).

The transmembrane electrochemical Na^+ gradient in *A. woodii* is used by an Na^+-F_1F_O-ATP synthase for the synthesis of ATP. Biochemical and molecular analyses revealed that the enzyme is of the Na^+-F_1F_O type of ATP synthases but *A. woodii* is rather unusual in having several different gene copies that encode different subunits of the membrane-embedded rotor of this unique enzyme (Schmidt *et al.*, 2009). In addition to ATP synthesis, the electrochemical sodium ion gradient is used to drive flagella rotation and thus motility of *A. woodii*. Furthermore, because *A. woodii* can grow in the apparent absence of an electrochemical proton potential, it is also likely that secondary transport systems are not driven by H^+ but Na^+ symporter.

Because the only chemiosmotic coupling site in *A. woodii* is 'fueled' by oxidation of a reduced electron acceptor with a very low redox potential ($E_0' \approx -500\,mV$), the entire metabolism is optimised to reduce ferredoxin. However, reduction of ferredoxin with H_2 ($E_0' = -414\,mV$) or NADH ($E_0' = -320\,mV$) is thermodynamically impossible. To overcome this thermodynamic barrier, *A. woodii* uses the mechanism of electron bifurcation: the electrons coming from a given donor are split, one goes energetically downhill and the energy set free is used to 'pump up' the other electron to the more electronegative acceptor. The iron-only hydrogenase of *A. woodii* oxidises hydrogen and requires both the electron acceptors, NAD^+ and ferredoxin, that are reduced simultaneously (Schuchmann and Müller, 2012). Whether or not the exergonic methylene–THF reductase is also coupled to ferredoxin reduction remains to be established.

The ion specificity for the Rnf complex has so far been proven experimentally only for *A. woodii*. Growth experiments did not reveal a Na^+ dependence of growth in the Rnf-containing acetogen *C. ljungdahlii*. In addition, inhibitor studies and the absence of a conserved Na^+-binding motif in ATP synthase suggest that the Rnf complex of *C. ljungdahlii* is not Na^+, but H^+ motive (Köpke *et al.*, 2010; Tremblay *et al.*, 2013). Thus, Rnf-containing acetogens can have a sodium ion or proton-based bioenergetics.

Alternative Electron Donors

The acetyl-CoA pathway serves as a way for the interconversion of the most oxidised C_1 compound, CO_2, and the most reduced C_1 compound, a methylated intermediate. The pathway is reversible and works in reductive direction during CO_2 reduction. In some organisms, the pathway is used to oxidise acetate to CO_2, and in acetogens the oxidative pathway is employed when methyl groups are used as carbon and energy source. Methanol is disproportionated by acetogens according to the reaction

$$CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O \qquad [V]$$

The methyl group is transferred to THF by action of two methyltransferases and a corrinoid protein. Methyltransferase I (MT I) abstracts the methyl group and transfers it to the corrinoid, from where it is donated to THF by action of MT II. One methyl group is oxidised to CO_2 yielding six electrons that are used to reduce 3 mol of CO_2 to CO. Therefore, this type of metabolism requires the presence of CO_2 in the medium.

Methanol was the first methyl-group-containing substrate shown to be metabolised by acetogens. Later on it was shown that the methyl group of glycine betaine or even the methoxy group of aromatic compounds can be used as carbon and energy source by acetogens. The latter group includes substrates such as trimethoxycinnamate, trimethoxybenzoate, vannilate and others (see **Figure 4**). The methyl group of these substrates is funnelled into the central pathway also by way of methyltransferases. Interestingly, the demethoxylated aromatic compound can no further be metabolised by acetogens.

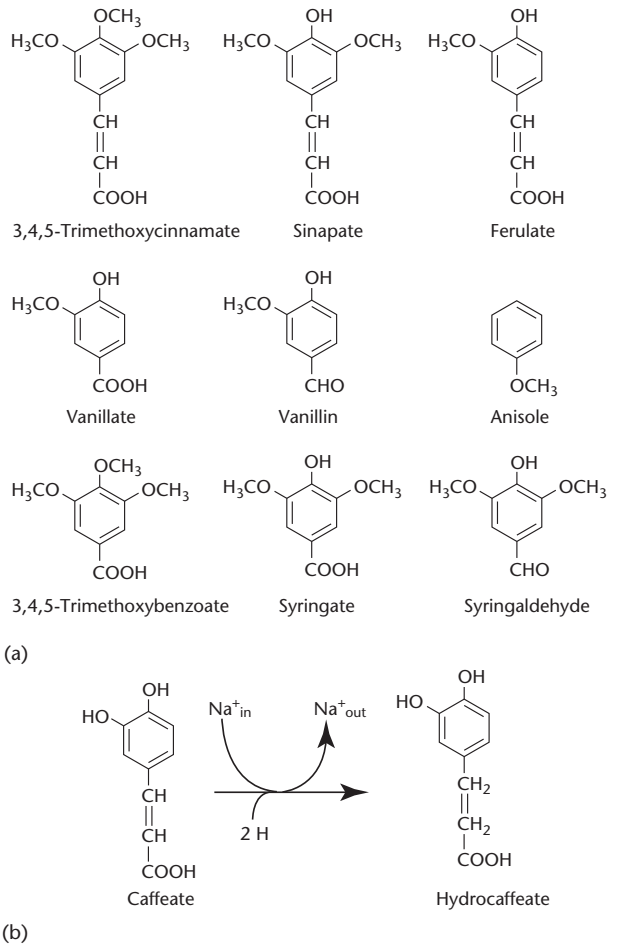

Figure 4 Aromatic substrates that are utilised by acetogens.

In addition to the methoxy group, some aromatic aldehydes serve as electron donors. *Clostridium formicoaceticum* can use hydroxyphenyl aldehydes as a sole source of reductants for acetate synthesis. Under these conditions, sugars are no longer oxidised indicating a regulatory event that favours aldehyde oxidation.

In addition to one-carbon compounds such as formate and CO, alcohols like acetoin or glycerol, hexoses, pentoses, lactate and also dicarboxylic acids like oxalate and fumarate may be used by acetogens.

Alternative Terminal Electron Acceptors

As outlined above, CO_2 functions as a terminal electron acceptor during heterotrophic and autotrophic growth but many acetogenic bacteria can also use alternative terminal electron acceptors (Drake *et al.*, 2002, 2006). Under these

conditions, the acetyl-CoA pathway may be repressed and the 'acetogen' might not even produce acetate at all. The alternative terminal electron acceptors used by acetogenic bacteria are diverse and include aromatic acrylate groups, fumarate, nitrate, nitrite, thiosulfate and dimethylsulphoxide.

Morella thermoacetica and *M. thermoautotrophica* preferentially use nitrate as the terminal electron acceptor, which is reduced to nitrite and ammonium. During growth on different substrates such as vanillin, vanillate, methanol, glucose, CO and H_2, nitrate was preferentially used even in the presence of CO_2. No acetate was synthesised under these conditions. The same observations have been made when cells were supplemented with nitrite when grown on glyoxylate. Under CO_2-depleted conditions *M. thermoacetica* and *M. thermoautotrophica* are also able to use thiosulfate and dimethylsulfoxide as the alternative terminal electron acceptor.

Clostridium formicoaceticum grows with fumarate as the sole carbon and energy source. Fumarate is disproportionated as follows:

$$\text{Fumarate} + 2H_2O \rightarrow \text{Acetate} + 2CO_2 + 4[H] \quad \text{[VI]}$$

$$2\text{Fumarate} + 4[H] \rightarrow 2\text{Succinate} \quad \text{[VII]}$$

$$3\text{Fumarate} + 2H_2O \rightarrow {}_2\text{Succinate} + \text{Acetate} + 2CO_2 \quad \text{[VIII]}$$

The reducing equivalents that are generated during the oxidation of fumarate are not transferred to another acceptor, but are instead used for the reduction of additional fumarate. Under CO_2-limiting conditions *C. formicoaceticum* can utilise fumarate as an electron acceptor independent of dismutation when grown on methanol or vanillate. The same holds true for *Clostridium aceticum* that can transfer H_2-derived electrons to fumarate when CO_2 is scarce. Various phenyl acrylates, compounds derived from the degradation of lignin, can be used by *A. woodii* and several other acetogenic bacteria as alternative terminal electron acceptors. Caffeate is reduced to hydrocaffeate, which is no further metabolised.

The regulatory mechanisms that control the channelling of substrate-derived electrons away from the acetyl-CoA pathway and towards the alternative terminal electron acceptor remain obscure. In the presence of nitrate no acetate could be detected in cultures of *M. thermoacetica*. Thus, nitrate obviously represses the acetyl-CoA pathway. A first hint for this nitrate-dependent 'down regulation' was the observation that membranes of *M. thermoacetica* and *M. thermoautotrophica* grown in the presence of nitrate lacked cytochrome *b*, which is a crucial membranous electron carrier involved in the methyl branch of the acetyl-CoA pathway. Additionally, nitrate might influence the synthesis and activity of enzymes of the acetyl-CoA pathway.

The dismutation of fumarate and fumarate respiration are strictly regulated by CO_2 in *C. formicoaceticum*. In the presence of both fumarate and CO_2 substrate-derived electrons are preferentially channelled towards the acetyl-CoA pathway. H_2-dependent fumarate respiration in *C. aceticum* is repressed in the presence of CO_2 and acetogenesis is the preferred electron-accepting process.

The capability of using alternative terminal electron acceptors is not constitutive, but is induced by the compound itself. Genes coding for components of nitrate respiration in *M. thermoacetica* are induced by nitrate. The ability of *A. woodii* to reduce caffeate is induced by phenyl acrylate. However, the level of regulation is yet unknown.

Energy conservation coupled to the reduction of alternative terminal electron acceptors

The reduction of CO_2 to acetate in the acetyl-CoA pathway is coupled to the generation of either a transmembrane H^+ or Na^+ gradient, depending on the species, that is used for ATP synthesis. The use of an alternative terminal electron not only serves as an 'electron sink' to recycle reduced electron carriers, but is also coupled to the conservation of energy. Growth experiments with *M. thermoacetica* and *M. thermoautotrophica* demonstrated that the reduction of nitrate is coupled to an energy-conserving process. A fumarate reductase is located at the outer side of the cytoplasmic membrane in *C. formicoaceticum* and *b*-type cytochromes and menaquinone have been detected in this acetogen. These findings suggest that an energy-conserving mechanism similar to H_2-dependent fumarate respiration in *Wollinella succinogenes* and *Escherichia coli*.

Caffeate respiration in *A. woodii* is well understood. The Rnf complex is the only chemiosmotic coupling site and ferredoxin is reduced by hydrogen by the beforementioned multisubunit, electron-bifurcating hydrogenase (**Figure 5**). Caffeate is activated to caffeyl-CoA before its reduction by one of the two processes: initially, caffeate is activated at the expense of ATP by a caffeyl-CoA synthetase, but in the steady state of respiration, an energy neutral CoA transferase transfers the CoA from hydrocaffeyl-CoA to caffeate. Caffeyl-CoA is then reduced by a caffeyl-CoA reductase/Etf complex that uses electron bifurcation to ferredoxin followed by its reoxidation at the Rnf complex (**Figure 5**). Thus, altogether caffeate respiration yields 0.9 mol ATP/mol caffeate (Hess *et al.*, 2013; Bertsch *et al.*, 2013).

Acetogenic Habitats and Competition with Other Microorganisms

In nonmarine environments, acetogens compete with methanogenic archaea for hydrogen and CO_2. The free energy change ($\Delta G_0'$) associated with methanogenesis ($-131\,kJ\,mol^{-1}$) is larger compared with acetogenesis ($-95\,kJ\,mol^{-1}$) and thus methanogens have a thermodynamic advantage. However, acetogens seem to be more robust and can grow at lower temperatures and seem to be more resistant to oxygen. Especially the latter may give

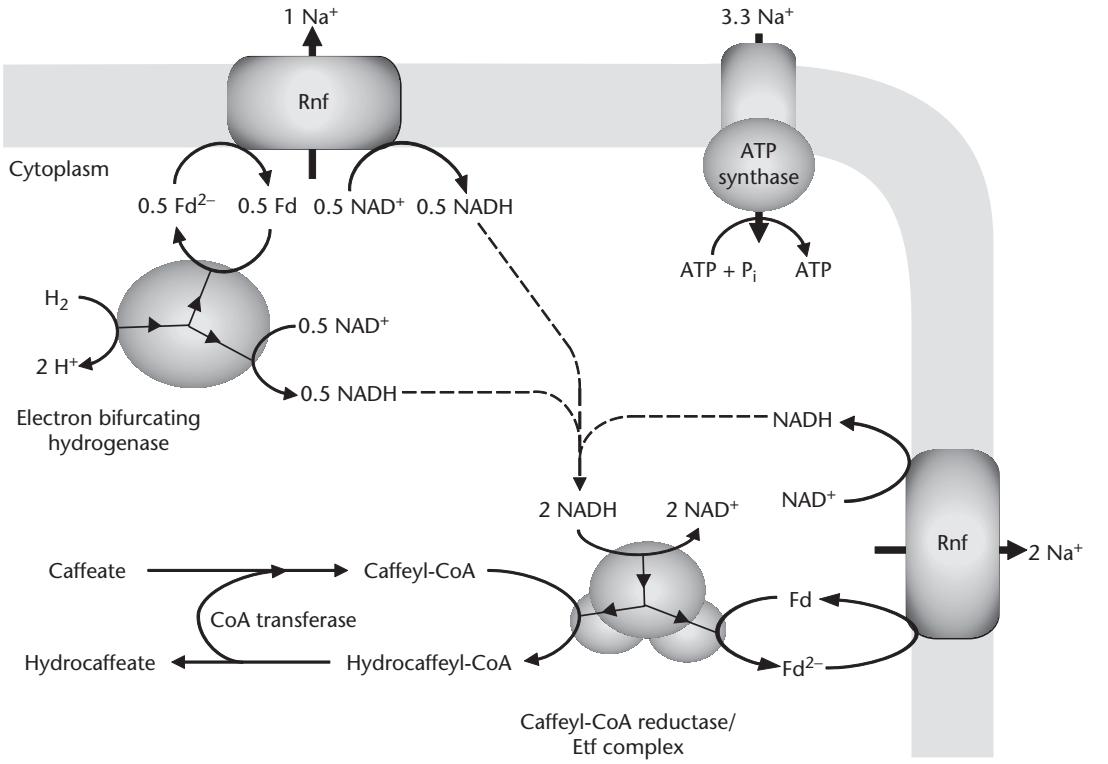

Figure 5 Biochemistry and bioenergetics of hydrogen-dependent caffeate respiration as carried out by *A. woodii*. Adapted from Bertsch *et al.* (2013). © The American Society for Biochemistry and Molecular Biology.

acetogens an advantage in certain environments. Furthermore, the metabolism of acetogens is more flexible and not restricted to a few compounds as in methanogens. They can grow autotrophically and heterotrophically as well as mixotrophically. This wide range of substrates used clearly gives them an advantage in anoxic environments such as soil. Their ability to grow at slightly acidic pH as well as to use substrates derived from cellulose or lignin makes them very competitive in soils.

In marine environments, methanogens and acetogens have to compete with sulfate reducers. Again, sulfate reduction is thermodynamically favoured but acetogens keep their ecological niche by using substrates not consumed by sulfate reducers.

Biotechnological Applications of Acetogenic Bacteria

Their ability to reduce CO_2 to acetyl-CoA has made acetogens prime candidates for the production of biocommodities from carbon dioxide. In addition, a number of acetogens can grow on and produce acetyl-CoA from carbon monoxide or syngas that contains a mixture of CO, H_2 and CO_2. Ethanol is already produced from syngas with *C. ljungdahlii* on an industrial scale. Other products of interest are, for example, acetate, butanediol or biodiesel. Electron and carbon flow can be directed to the desired product not only by classical changes in the fermentation conditions, but also by metabolic design as for some acetogens a genetic system to generate knockout strains and to express genes heterologously has been developed. The use of acetogens as a platform for carbon dioxide or syngas-based biotechnology has just been started and will certainly flourish in the next decade.

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