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# Physiology of the thermophilic acetogen *Moorella thermoacetica*

Harold L. Drake and Steven L. Daniel

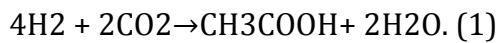
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## Abstract

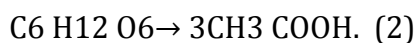
*Moorella thermoacetica* (originally isolated as *Clostridium thermoaceticum*) has served as the primary acetogenic bacterium for the resolution of the acetyl coenzyme A (acetyl-CoA) or Wood–Ljungdahl pathway, a metabolic pathway that (i) autotrophically assimilates CO<sub>2</sub> and (ii) is centrally important to the turnover of carbon in many habitats. The purpose of this article is to highlight the diverse physiological features of this model acetogen and to examine some of the consequences of its metabolic capabilities.

## 1. Historical perspectives

Acetogenesis, the metabolic process by which two molecules of CO<sub>2</sub> are reduced to acetate, was first reported in 1932, when Fischer and co-workers [40] demonstrated that unknown microorganisms in wastewater could convert H<sub>2</sub>–CO<sub>2</sub> to acetate according to the following stoichiometry:



In 1932, this reaction constituted a novel CO<sub>2</sub>-fixing process. Ten years later, Fontaine and co-workers [41] discovered a thermophilic bacterium, *Clostridium thermoaceticum* (Fig. 1A), that catalyzed the near stoichiometric conversion of glucose to acetate:



Although reactions (1) and (2) do not appear to be similar, they in fact have a common underlying feature: the reduction of CO<sub>2</sub> to acetate via a metabolic process that is now referred to as the acetyl-CoA pathway (Fig. 2). This pathway, which will be described below, is also referred to as the Wood–Ljungdahl pathway in recognition of the two biochemists (Fig. 1B and 1C) who, together with their co-workers and *C. thermoaceticum*, resolved the enzymological features of the pathway [29,32,35,36,98,128]. *C. thermoaceticum* is therefore the most historically important acetogen relative to the resolution of this pathway [32,75,128]. Indeed, the first <sup>14</sup>C-tracer experiments in biology were performed with *C. thermoaceticum* to demonstrate that CO<sub>2</sub> was reduced to acetate via an unknown autotrophic process [5,55]. Ironically, although we now know that this pathway constitutes a major autotrophic process that is centrally important to the cycling of carbon in various ecosystems, its enzymological features were resolved from an organism that was originally thought to be an obligate heterotroph; it was only after most of the components of the acetyl-CoA pathway had been purified and studied that it was

discovered that *C. thermoaceticum* contained hydrogenase [31] and was capable of chemolithoautotrophic growth on H<sub>2</sub>-CO<sub>2</sub> or CO-CO<sub>2</sub> [19].

*C. thermoaceticum* was reclassified as *Moorella thermoacetica* in the mid-1990s when the taxonomy of the genus *Clostridium* was restructured [16]. The organism will be referred to as *Moorella thermoacetica* throughout the remainder of this article.

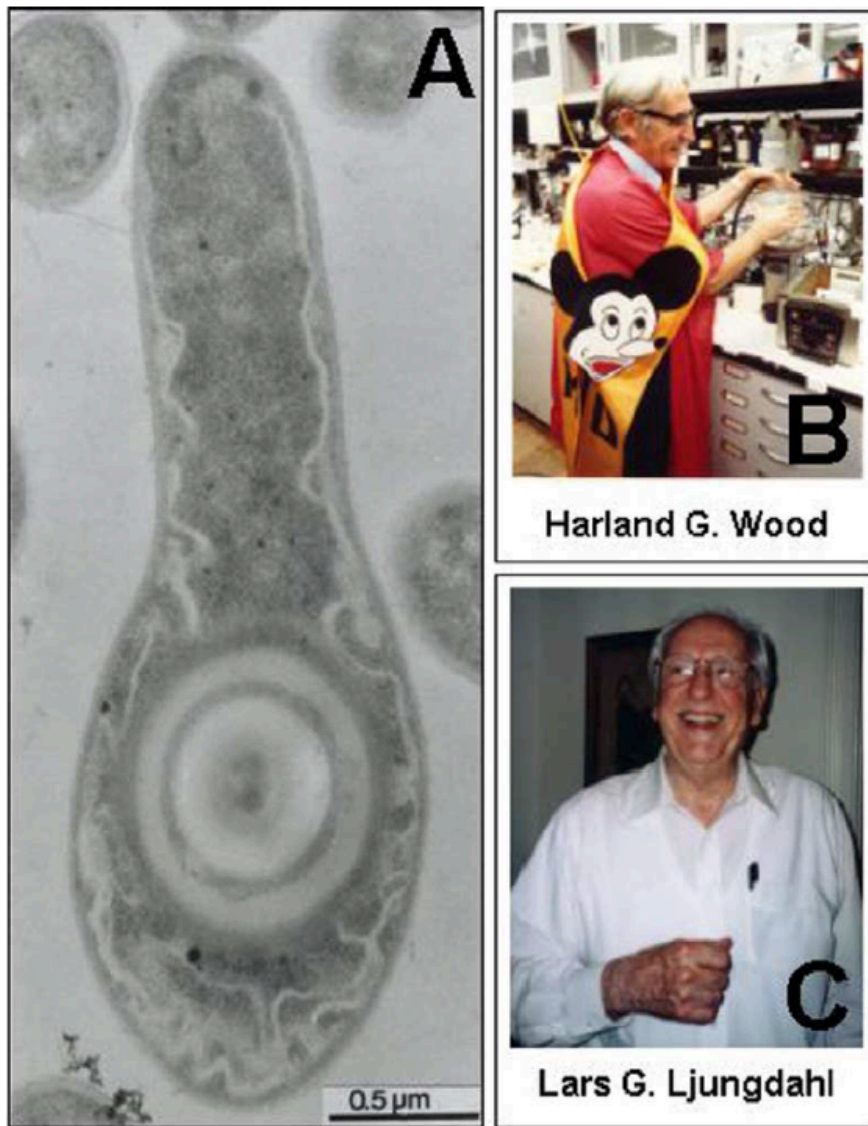


Fig. 1. (A) An electron micrograph of a sporulated cell of *Clostridium thermoaceticum* (ATCC 39073), which was reclassified as *Moorella thermoacetica* [16] (used with permission from [32]). (B, C) The two biochemists who were primarily responsible for resolving the enzymological features of the acetyl-CoA or Wood-Ljungdahl pathway in *M. thermoacetica*.

Acetogenesis was viewed as a somewhat obscure process for many decades until it

was realized that the acetyl-CoA pathway is widely distributed in nature. It is now known that acetogens are common inhabitants of diverse environments [32,36] and that non-acetogenic bacteria (e.g., sulfatereducing bacteria) and members of the domain Archaea (e.g., methanogenic bacteria) use metabolic pathways that are very similar to the acetyl-CoA pathway for either the autotrophic assimilation of CO<sub>2</sub> into biomass or the oxidation of acetate [32,43,44].

There are now 21 genera that contain approximately 100 acetogenic species, i.e., organisms that use the acetyl-CoA pathway [36]. Although numerous acetogens have been isolated, *M. thermoacetica* has served, and continues to serve, as the primary model acetogen in laboratory studies.

## 2. Physiology

Acetogens have been thought of as being metabolically limited and thermodynamically disadvantaged, but just the opposite is true. They can utilize a wide variety of electron donors and electron acceptors, and *M. thermoacetica* is no exception. Indeed, *M. thermoacetica* is the most metabolically diverse acetogen thus far characterized. This bacterium utilizes very diverse substrates (Table 1), grows both autotrophically and heterotrophically [19], and has only one organic nutritional requirement, nicotinic acid [78].

### 2.1. The acetyl-CoA pathway

The acetyl-CoA pathway can be shown as a cyclic process relative to the electron carriers and cofactors that cycle between different states in the pathway [98,128]. However, as shown in Fig. 3, the acetyl-CoA pathway is a linear “one-carbon” process that does not involve a recycled multi-carbon intermediate to which CO<sub>2</sub> is fixed. In contrast, the Calvin cycle, the reductive tricarboxylic acid cycle, and the hydroxypropionate cycle are all CO<sub>2</sub>-fixing processes that are dependent upon the recycled intermediates ribulose biphosphate, oxaloacetate, and acetyl-CoA, respectively, for the initial fixation of CO<sub>2</sub>. Thus, the mechanism by which CO<sub>2</sub> is fixed in the acetyl-CoA pathway is quite distinct from cyclic CO<sub>2</sub>-fixing processes.

The acetyl-CoA pathway is a terminal electron-accepting process that can be coupled to the conservation of energy and also be utilized for the assimilation of CO<sub>2</sub> into cellular carbon (Fig. 3). The latter function is the basis for the autotrophic growth of *M. thermoacetica*. The methyl branch of the pathway reduces CO<sub>2</sub> to the methyl level via several tetrahydrofolate-dependent reactions, and the carbonyl branch reduces CO<sub>2</sub> to the carbonyl level via acetyl-CoA synthase. Acetyl-CoA synthase can also oxidize CO to CO<sub>2</sub>; this reaction can be used to assay acetyl-CoA synthase and is the basis for also referring to the enzyme as CO dehydrogenase. Acetyl-CoA synthase therefore plays a central role in the pathway, and recent studies have shown that the two subunits of this enzyme from *M. thermoacetica* display both open and closed conformations as well as a Ni-Ni-[Fe<sub>4</sub>-S<sub>4</sub>] cluster at the active site [23]. A description of each of the enzymes of the acetyl-CoA pathway is beyond the scope of this article. Their general properties are described in several

reviews [27,30,32,35,36,77,96–98,128]. The reader is directed to recent publications on some of the enzymes centrally important in the acetyl-CoA pathway that provide access to more recent literature on the enzymology of the pathway [23,68,70,71,82,95,99–101].

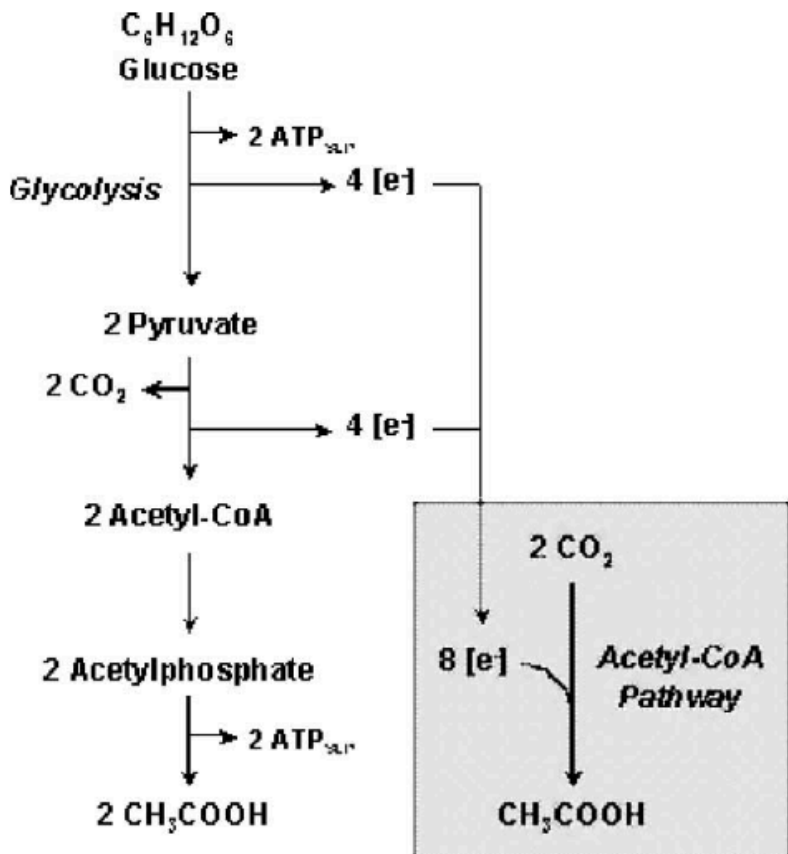


Fig. 2. Homoacetogenic conversion of glucose to acetate. The two molecules of  $CO_2$  that are reduced to acetate in the acetyl-CoA pathway can be derived from exogenous  $CO_2$  rather than the  $CO_2$  that is produced via the decarboxylation of pyruvate (see text). Abbreviations:  $ATP_{SLP}$ , ATP that is produced by substrate-level phosphorylation;  $[e^-]$ , reducing equivalent. Modified from [32].

The standard redox potential of the  $CO_2$ /acetate half-cell reaction approximates  $-290$  mV, and the change in Gibbs free energy for the  $H_2$ -dependent conversion of 2  $CO_2$  to acetate (see reaction (1) above) approximates  $-95$  kJ mol $^{-1}$  of acetate synthesized. The redox potential of the  $CO_2$ /acetate half-cell reaction is one of the lowest redox potentials for a terminal electron-accepting process in biology and is often cited as a reason for acetogens being thermodynamically disadvantaged. However, this view overlooks the other metabolic features of acetogens that likely contribute to their overall competitiveness under in situ conditions.

The growth of *M. thermoacetica* and other acetogens at the expense of  $CO$ , methanol, O-methyl groups of aromatic compounds, and even glucose can be significantly impaired when exogenous  $CO_2$  is not readily available [2,52,81,102]. Thus, even

though some acetogenic reactions do not contain CO<sub>2</sub> as a substrate (e.g., reaction (2)), the recycling of reduced electron carriers can be dependent upon exogenous CO<sub>2</sub>. Indeed, as illustrated in Fig. 2, approximately one-third of the carbon from uniformly labeled [14C]glucose is recovered as <sup>14</sup>CO<sub>2</sub>/carbonates (the percent relative distribution of recovered <sup>14</sup>C in products approximates 31% <sup>14</sup>CO<sub>2</sub>/carbonates, 62% [14C]acetate, and 6% [14C]biomass (Martin and Drake, unpublished data). In addition, substantial amounts of exogenous CO<sub>2</sub> are reduced to acetate when *M. thermoacetica* is cultivated on glucose [80]. Carbonic anhydrase, which catalyzes the interconversion of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>, might optimize the availability of intracellular CO<sub>2</sub> [10]. The ability of *M. thermoacetica* to obtain growth-supportive CO<sub>2</sub>-equivalents via the decarboxylation of carboxylated aromatic compounds accentuates not only the importance of CO<sub>2</sub>, but also the ability of acetogens to optimize the availability of CO<sub>2</sub> for acetogenesis [52,53].

## 2.2. Conservation of energy via substrate-level phosphorylation and electron transport phosphorylation

When acetogens are cultivated on sugars, the main function of the acetyl-CoA pathway is the oxidation of reduced electron carriers (e.g., NADH or reduced ferredoxin). Overall, 4 ATP are produced by substrate-level phosphorylation (ATPSLP) per hexose converted to 3 acetates (Fig. 2). Thus, hexose-coupled acetogenesis yields more ATPSLP per hexose than any other fermentative metabolism (e.g., ethanol and butyrate fermentations yield 2 and 3 ATPSLP per hexose, respectively). Although the acetyl-CoA pathway (i.e., reduction of 2 molecules of CO<sub>2</sub> to acetate) does not increase the net gain in ATPSLP (1 ATP is consumed in the activation of formate, and 1 ATP is produced when acetylphosphate is converted to acetate (Fig. 3)), *M. thermoacetica* conserves additional energy by chemiosmotic processes that generate a proton gradient that can drive energy-dependent processes (e.g., transport) or be coupled to the production of ATP by proton-dependent ATPase (Fig. 4) [24–27,29,77]. Protons can be translocated by an electron transport system that contains different cytochromes and menaquinones, and membrane-associated oxidoreductases (e.g., hydrogenase) can likewise generate a proton gradient (Fig. 4). The ability to conserve energy by a chemiosmotic process is essential for growth under chemolithoautotrophic conditions. Some acetogens lack electron transport systems and the conservation of energy is coupled to the generation of sodium-ion gradients and to sodium-ion-dependent ATPase [83,85]. For such acetogens, growth and the synthesis of acetate can be strictly dependent upon the availability of sodium ions [45,51,84,130]; the methyltransferase reaction at the terminal stage of the methyl branch of the acetyl-CoA pathway appears to be centrally important to the translocation of sodium ions [85,86]. In contrast, sodium ions are not required for either the heterotrophic or autotrophic growth of *M. thermoacetica* [130]. However, it cannot be excluded that *M. thermoacetica* translocates sodium ions. Indeed, under certain conditions, sodium-proton antiporters may be involved in the formation of electrochemical gradients by *M. thermoacetica* (Fig. 4) [116].

Table 1  
An overview of the cellular and metabolic properties of *M. thermoacetica*

Property	<i>M. thermoacetica</i>	Reference
Classification	Low G + C, Gram-positive bacteria, <i>Clostridium</i> , cluster VI, <i>Bacteria</i> , <i>Firmicutes</i> , <i>Clostridia</i> , <i>Thermoanaerobacteriales</i> , <i>Thermoanaerobacteriaceae</i> , <i>Moorella</i> group, <i>Moorella</i> (type strain of genus)	[16]
Habitat	Soils	[46–48]
Colony morphology	Circular, smooth, opaque, beige	[41]
Cell		
Morphology	Rod-shaped	[41]
Typical size	0.4 × 2.8 μm	[41]
Arrangement	Single, pairs, and chains	[41]
Gram reaction	+	[41]
G + C content (mol %)	54	[14]
Spore		
Shape	Spherical	[41]
Location	Subterminal with bulged sporangium	[41]
Heat tolerance (D value at 121 °C)	83–111 min	[13]
Flagellum		
Flagellation pattern	Peritrichous	[41]
Motility	–	[41]
Nutritional requirement	Nicotinic acid and trace metals (e.g., Ni, Se, Zn, Mo, W, Co, Fe)	[3,61,78]
Temperature (°C)		
Optimum	55–60	[41]
Range	45–65	[41]
pH		
Optimum	6.8	[75]
Range	5.7–7.7	[13]
Electron donor <sup>a</sup>		
Sugars	Glucose, fructose, xylose	[3,41]
Gases	H <sub>2</sub> , CO	[19,59]
Alcohols	Methanol, ethanol, <i>n</i> -propanol, <i>n</i> -butanol	[7,19,123]
Organic acids	Formate, oxalate, glyoxylate, glycolate, pyruvate, lactate	[21,46,107,114]
Methoxylated aromatic compounds	Vanillate, vanillin, syringate, ferulate, sinapate, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 5-hydroxyvanillate, isovanillate, 2,3-dimethoxybenzoate, 2-methoxybenzoate, 3-methoxycatechol, 2,6-dimethoxyphenol, 2,3-dimethoxyphenol, 3-methoxyphenol, 2-methoxyphenol, 1,2,3-trimethoxybenzene, 1,4-dimethoxybenzene, 1,3-dimethoxybenzene, 1,2-dimethoxybenzene	[18,20,39,87,129]
Electron acceptor <sup>b</sup>		
C-based	CO <sub>2</sub> , decarboxylated carboxyl groups of aromatic compounds	[5,52,53]
N-based	Nitrate, nitrite	[4,42,106,108,109]
S-based	Thiosulfate, dimethylsulfoxide	[7]
Metabolic transformation <sup>c</sup>		
Cystine	→ 2 cysteine,	[62]
Glutamate	→ 5-aminolevulinic acid,	[60,88,89]
2,4,6-trinitrotoluene	→ hydroxyl-amino-nitrotoluenes,	[54,94]
CCl <sub>4</sub>	→ CH <sub>2</sub> Cl <sub>2</sub> + CH <sub>3</sub> Cl,	[38]
O <sub>2</sub> + 4[H]	→ 2H <sub>2</sub> O,	[57]
H <sub>2</sub> O <sub>2</sub> + XH <sub>2</sub>	→ X + 2H <sub>2</sub> O,	[57]
CdCl <sub>2</sub> + cysteine	→ CdS,	[17]
inorganic pyrophosphate	→ inorganic phosphate	[50]

a All are growth-supportive, acetogenic substrates except for ethanol, *n*-propanol, and *n*-butanol which require thiosulfate, dimethylsulfoxide, or nitrate as an electron acceptor [7,42]. Cellulose has recently been described as a substrate for an organism that is phylogenically closely related to *M. thermoacetica* [56].

b Nitrite can serve an electron acceptor during glyoxylate-dependent growth [109]. Pyruvate appears to also be reduced to lactate, since lactate can be produced when levels of available hexose-derived reductant is high (Fröstl and Drake, unpublished data; see text).

c None of these metabolic transformations alone are growth supportive for *M.*

thermoacetica. (Note: general reaction schemes are shown in the table, see indicated references for more detailed information.)

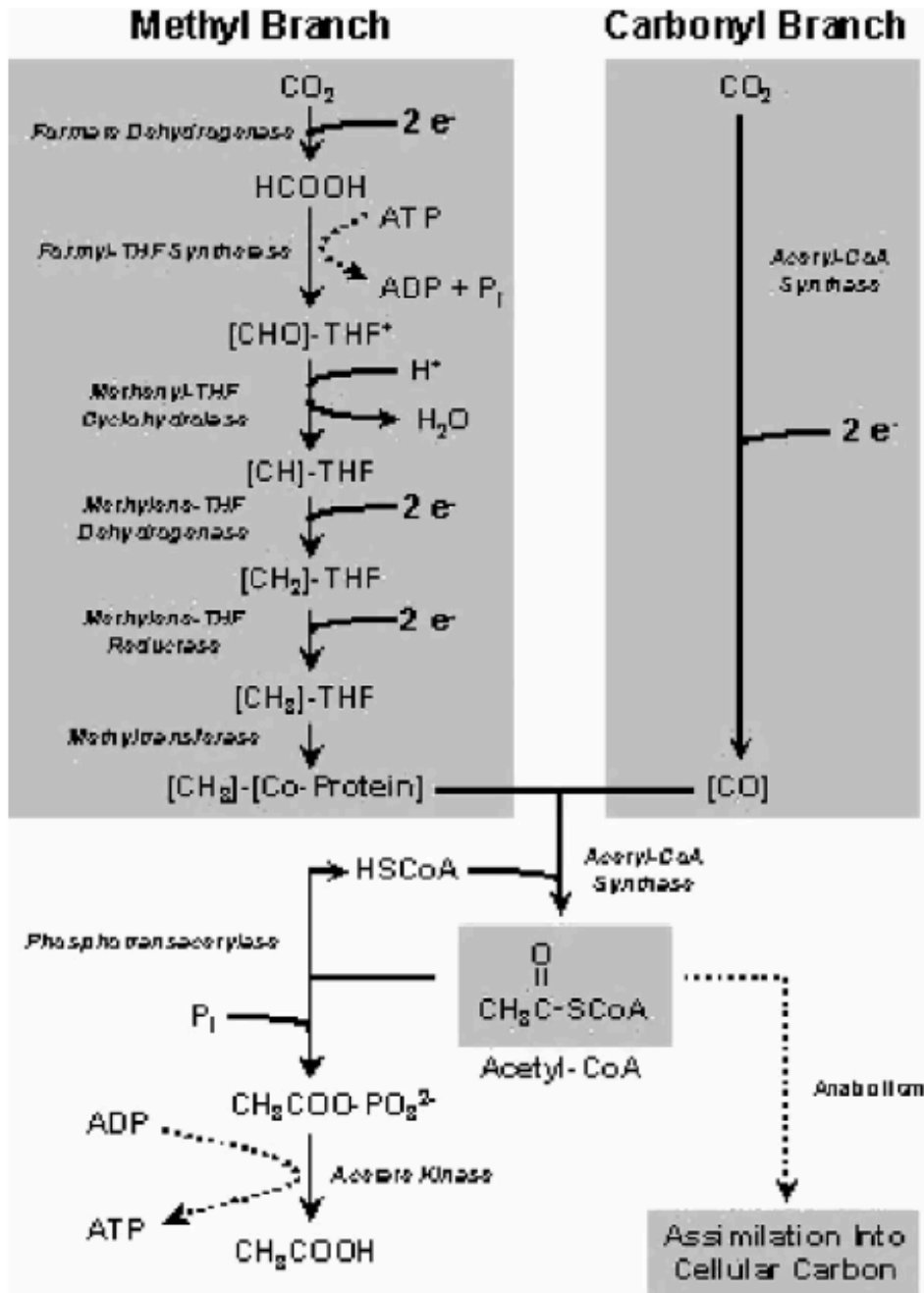


Fig. 3. The acetyl-CoA pathway as resolved from *M. thermoacetica*. Abbreviations: THF, tetrahydrofolate; HSCoA, coenzyme A;  $\text{P}_i$ , inorganic phosphate;  $[e^-]$ , reducing equivalent; Co-Protein, corrinoid enzyme. Used with permission from [86].

### 2.3. Electron donors



H<sub>2</sub> is the simplest electron donor utilized by biological systems, and, as illustrated in reaction (1), this molecule is oxidized by acetogens. Indeed, H<sub>2</sub> is the only known noncarbonaceous source of growth-supportive reductant for acetogens. *M. thermoacetica* contains multiple hydrogenases, and their expression is dependent on growth conditions [31,58]. Hydrogenase in *M. thermoacetica* is constitutive, but the activity of hydrogenase in H<sub>2</sub>-cultivated cells is 15- and 18-fold greater than in glucose- and CO-cultivated cells, respectively [19]. Despite the importance of this capability to lithotrophic growth, information on hydrogenases of acetogens is scant. Information on the ability of acetogens to metabolize complex, high-molecular-weight macromolecules (e.g., cellulose, starch, lignin, pectin, and gelatin) is likewise scant. However, very few efforts to isolate polymerdegrading acetogens have been reported in the literature. It is therefore of particular note that the recent isolation of what appears to be a cellulose-degrading strain of *M. thermoacetica* [56] suggests that some strains of this acetogen may have the ability to hydrolyze and use complex polymers. “*Bryantella formatexigens*” (quotation marks indicate that this name has not yet been validated), an acetogen recently isolated from human feces, used amorphous cellulose for growth when first isolated but lost this capability after prolonged cultivation [126]. These observations on the ability of acetogens to utilize cellulose can be considered breakthrough discoveries if they can be verified.

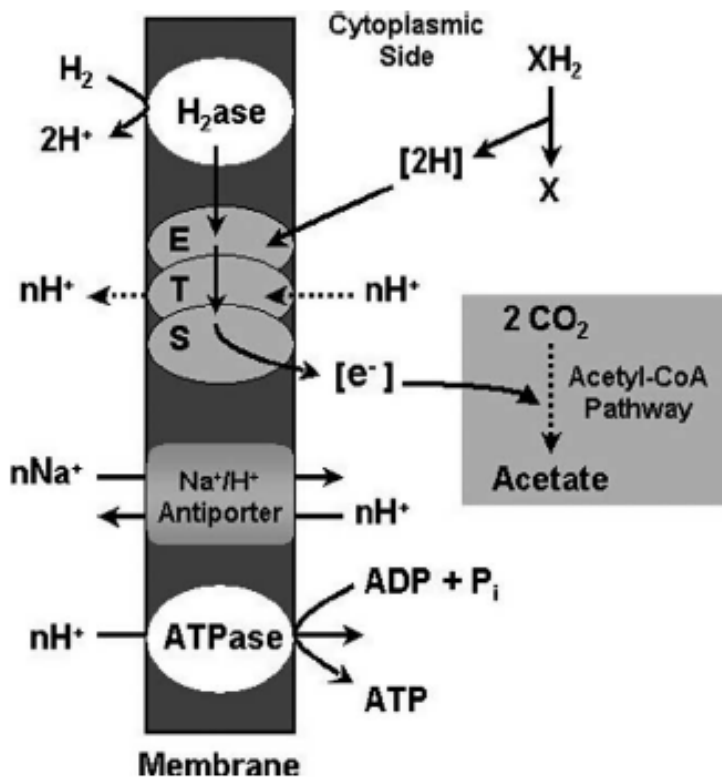
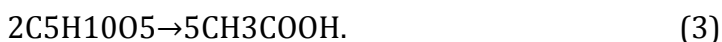


Fig. 4. Mechanisms for the formation of a proton gradient and the chemiosmotic conservation of energy by *M. thermoacetica*. Abbreviations: H<sub>2</sub>ase, hydrogenase; ETS, electron transport system; ATPase, ATP synthase; e<sup>-</sup>, reducing equivalent. See

[29,77] for more detailed information.

The utilization of various monosaccharides for growth and acetate synthesis is a common feature among most acetogens [36,73,103]. With *M. thermoacetica* (Table 1), the hexoses glucose and fructose and the pentose xylose are growth-supportive sugars [3,41]. As illustrated in Fig. 2, the homoacetogenic conversion of glucose (or fructose) to 3 acetates begins with the oxidation of glucose to pyruvate via glycolysis. Radioisotopic studies have confirmed that the glycolytic pathway operating in *M. thermoacetica* is the Embden–Meyerhof–Parnas (EMP) pathway [127]; however, glyceraldehyde-phosphate dehydrogenase is the only enzyme of this pathway which has actually been documented in this acetogen [117]. Xylose is converted to acetate according to the following stoichiometry:



However, the pathway for xylose conversion to acetate has not been studied in *M. thermoacetica*. Regardless, pyruvate formed by glycolysis is subjected to oxidation by pyruvate ferredoxin oxidoreductase; this ultimately leads to the production of acetyl-CoA and the formation of 2 acetates (Fig. 2). The 8 reducing equivalents that are collectively generated during glycolysis and the oxidation of pyruvate are subsequently used to reduce CO<sub>2</sub> to acetate (the “third acetate”) via the acetyl-CoA pathway (Fig. 2). During growth at the expense of simple sugars, the cell is supplied with anabolic precursors (e.g., breakdown products of sugars) and ATP formed via substrate-level phosphorylation. Thus, the anabolic and catabolic functions (i.e., the assimilation of CO<sub>2</sub> and the conservation of energy via chemiosmotic coupling, respectively) of the acetyl-CoA pathway that are essential under autotrophic conditions appear to be of less significance during sugar-dependent growth; thus, under certain heterotrophic conditions, the primary function of the acetyl-CoA pathway appears to be the oxidation (i.e., recycling) of electron carriers that are reduced during the breakdown of organic compounds. This, however, is not the case when the cell is subjected to growth under chemolithoautotrophic conditions.

The nature of the acetyl-CoA pathway lends itself to the use of simple, one-carbon substrates, and *M. thermoacetica*, like most acetogens, uses various one-carbon substrates as a source of reductant and carbon (Table 1). One-carbon substrates are oxidized but also enter the pathway directly. For example, both formate and CO are oxidizable substrates but have different entry points into the pathway. Formate enters at the level of the formyltetrahydrofolate synthase reaction on the methyl branch, while CO is utilized as a carbonyl precursor on the carbonyl branch (Fig. 3). The initiation of CO-dependent acetogenesis occurs optimally when exogenous CO<sub>2</sub> is available, apparently because the oxidation of electron carriers that are reduced during the oxidation of CO does not occur unless an excess of CO<sub>2</sub> (the terminal electron acceptor during acetogenesis) is readily available [102]. The ability of acetogens to convert CO to acetate is a highly specialized biological process and occurs according to the following stoichiometry:



During CO-dependent acetogenesis, 3 molecules of CO are oxidized to CO<sub>2</sub>, yielding 6 reducing equivalents that are then used to reduce one molecule of CO<sub>2</sub> to the methyl level (i.e., methyltetrahydrofolate on the methyl branch of the acetyl-CoA pathway). CO enters carbonyl branch of the pathway as a preformed carbonyl-level molecule. Thus, during CO-dependent acetogenesis, CO is the origin of the carboxyl group of acetate, while the methyl group is derived from CO<sub>2</sub> [80]. The low redox potential of CO-derived electrons (-520 mV) can generate H<sub>2</sub> and CH<sub>4</sub> as trace products during growth [102]. Recent reviews outline many of the biochemical features of acetyl-CoA synthase, the enzyme primarily responsible for both the oxidation and utilization of CO during acetogenesis [70,101].

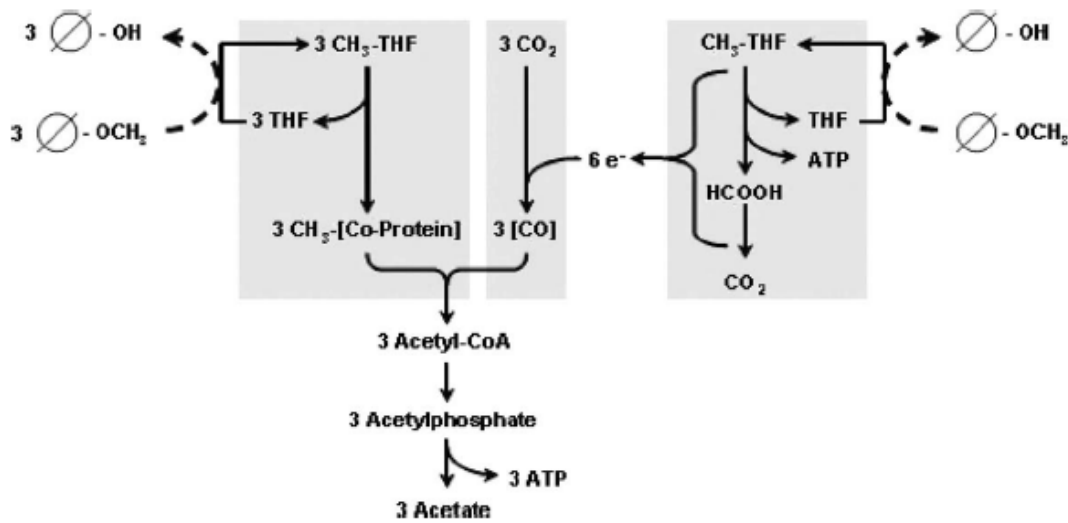
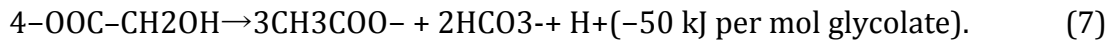
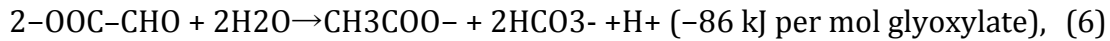


Fig. 5. Scheme illustrating hypothetical routes by which O-methyl groups from methoxylated aromatic compounds can be utilized by *M. thermoacetica*. Abbreviations: THF, tetrahydrofolate; CoA, coenzyme A; e<sup>-</sup>, reducing equivalent; Co-Protein, corrinoid enzyme. Modified from [34]. This figure is based on the work of numerous investigations with different acetogens; see [86] for more detailed information.

*M. thermoacetica* is specialized in utilizing substrates that have preformed methyl-level groups (Table 1). Methoxyl groups of an extensive number of aromatic compounds are subject to O-demethylation by *M. thermoacetica*, apparently by a broad-spectrum O-demethylase system [20]. When methyl-groups are utilized acetogenically, a disproportionation occurs (Fig. 5). The full oxidation of one methyl group yields 6 reducing equivalents that are used to reduce 3 molecules of CO<sub>2</sub> to CO, which are subsequently condensed with 3 methyl-groups (derived by the concerted action of demethylases and methyltransferases) in the terminal stages of the acetyl-CoA pathway to form 3 molecules of acetyl-CoA. This process theoretically yields one ATPSLP per methyl group utilized, is a common feature of many acetogens, and illustrates the metabolic versatility of the acetyl-CoA pathway.

The use of the two-carbon compounds oxalate, glyoxylate, and glycolate has not been widely demonstrated in obligately anaerobic bacteria, especially in thermophilic anaerobes. However, with *M. thermoacetica* (Table 1), these two-carbon compounds are readily used as a sole source of energy and are converted to acetate and CO<sub>2</sub> (shown below as HCO<sub>3</sub><sup>-</sup>) according to the following reactions (kJ per mol calculated from [118]) [21,107]:



The molar cell yields of glyoxylate-grown cells are slightly more than twice those of oxalate- or glycolate-grown cells [21,107]. The differences observed in cell yields reflect the differences in the standard changes in Gibbs free energy between glyoxylate-derived acetogenesis (reaction (6)) and oxalate- or glycolate-derived acetogenesis (reactions (5) and (7), respectively). Thus, relative to cellular energetics, glyoxylate appears to be more effective in supporting growth than oxalate or glycolate. When the efficiency of growth is based on the amount of biomass formed per theoretical pair of reducing equivalents consumed, glyoxylate- and oxalate-dependent growth are nearly the same, and both are approximately three times more than that of glycolate-dependent growth [21,107]. This result is surprising given that glycolate is a more reduced substrate than glyoxylate or oxalate.

Information on how different two-carbon carboxylates are metabolized by *M. thermoacetica* might help to shed some light on the nature of the differences observed in molar yields and cellular energetics. The enzyme system by which *M. thermoacetica* catabolizes oxalate requires a utilizable electron acceptor; CoA-level intermediates appear to not be involved [22]. Thus, oxalate appears to be catabolized via a novel reaction mechanism where oxalate is oxidatively decarboxylated to CO<sub>2</sub> and reductant, the latter being used in the acetyl-CoA pathway for the conservation of energy and biomass synthesis. Further evidence that oxalate, glyoxylate, and glycolate are metabolized via distinct mechanisms in *M. thermoacetica* has been obtained from observing the growth-supporting abilities of these substrates under nitrate-supplemented basal culture conditions. In the presence of nitrate, oxalate and glyoxylate, unlike glycolate and other substrates (H<sub>2</sub>, CO, and methoxyl groups), are growth supportive for *M. thermoacetica* [107–109]. Oxalate- and glyoxylate-grown cells appear able to bypass the repression of the acetyl-CoA pathway by rerouting reductant or carbon flow or both during growth under nitrate-supplemented conditions (see Section 2.4).

*M. thermoacetica* has different growth efficiencies on different substrates couple [19], which is based in part on the thermodynamic constraints of the acetyl-CoA

pathway or the amount of energy that can be conserved for a particular redox couple. For example, short-chain alcohols are not readily utilized by *M. thermoacetica* when CO<sub>2</sub> is used as an electron acceptor. However, ethanol and propanol become readily utilizable when nitrate is dissimilated (see Section 2.4) [42].

#### 2.4. Electron acceptors

*M. thermoacetica*'s ability to use CO<sub>2</sub> as a terminal electron acceptor via the acetyl-CoA pathway (Section 2.1) is the primary reason why this bacterium was studied so intensely for many decades following its isolation in 1942 [41]. Paradoxically, CO<sub>2</sub> is not the preferred terminal electron acceptor of *M. thermoacetica*. When this bacterium is cultivated in the presence of nitrate, the acetyl-CoA pathway is not utilized and reductant flow is channeled exclusively to the dissimilation of nitrate to ammonium [42,106,108]. As such, *M. thermoacetica* is a facultative nitrate dissimilator that engages the acetyl-CoA pathway when nitrate is not available. Ironically, if *M. thermoacetica* had been isolated in the presence of nitrate, its acetogenic nature might not have been discovered.

Both nitrate and nitrite are used by *M. thermoacetica* as energy conserving terminal electron acceptors [106,109]. The dissimilation of nitrate can significantly increase the growth efficiency of *M. thermoacetica*. For example, H<sub>2</sub>-dependent growth yields (i.e., the amount of biomass formed per H<sub>2</sub>-derived reducing equivalent consumed) are up to 8-fold higher when nitrate is dissimilated than when CO<sub>2</sub> is utilized as a terminal electron acceptor [42]. This improved growth efficiency is due in part to the improved thermodynamics of the higher redox potential of the nitrate/ ammonium half-cell reaction in comparison to the CO<sub>2</sub>/acetate half-cell reaction. The standard change in Gibbs free energy for H<sub>2</sub>-dependent acetogenesis is approximately -95 kJ per mol reaction, while that of the H<sub>2</sub>-dependent dissimilation of nitrate to ammonium is approximately -600 kJ per mol reaction [118].

As with other nitrate dissimilators, nitrate is reduced to nitrite, which is subsequently reduced to ammonia. When nitrate is present in the growth medium, a membrane-associated b-type cytochrome that is required on the methyl branch of the acetyl-CoA pathway is not synthesized, i.e., this cytochrome is not present in the membrane (Fig. 6). This metabolic defect disables the acetyl-CoA pathway. There are conflicting reports regarding the synthesis of the enzymes of the acetyl-CoA pathway when cells are dissimilating nitrate [4,42]. The acetyl-CoA pathway is linked to anabolism (Fig. 3), and the cell's inability to form acetyl-CoA from CO<sub>2</sub> (required for the assimilation of carbon when CO<sub>2</sub> is the sole source of carbon) during the dissimilation of nitrate is overcome with preformed methyl and carbonyl groups [42]. Thus, the ability of *M. thermoacetica* to synthesize acetyl-CoA via acetyl-CoA synthase is retained even when cells are dissimilating nitrate, as long as preformed methyl and carbonyl groups are available. This fact indicates that (a) the inability of the cell to assimilate CO<sub>2</sub> during nitrate dissimilation is not because

acetyl-CoA synthase is repressed and (b) the control of electron flow is the primary reason why the catabolic function of the acetyl-CoA pathway is repressed when cells dissimilate nitrate [33,35,36].

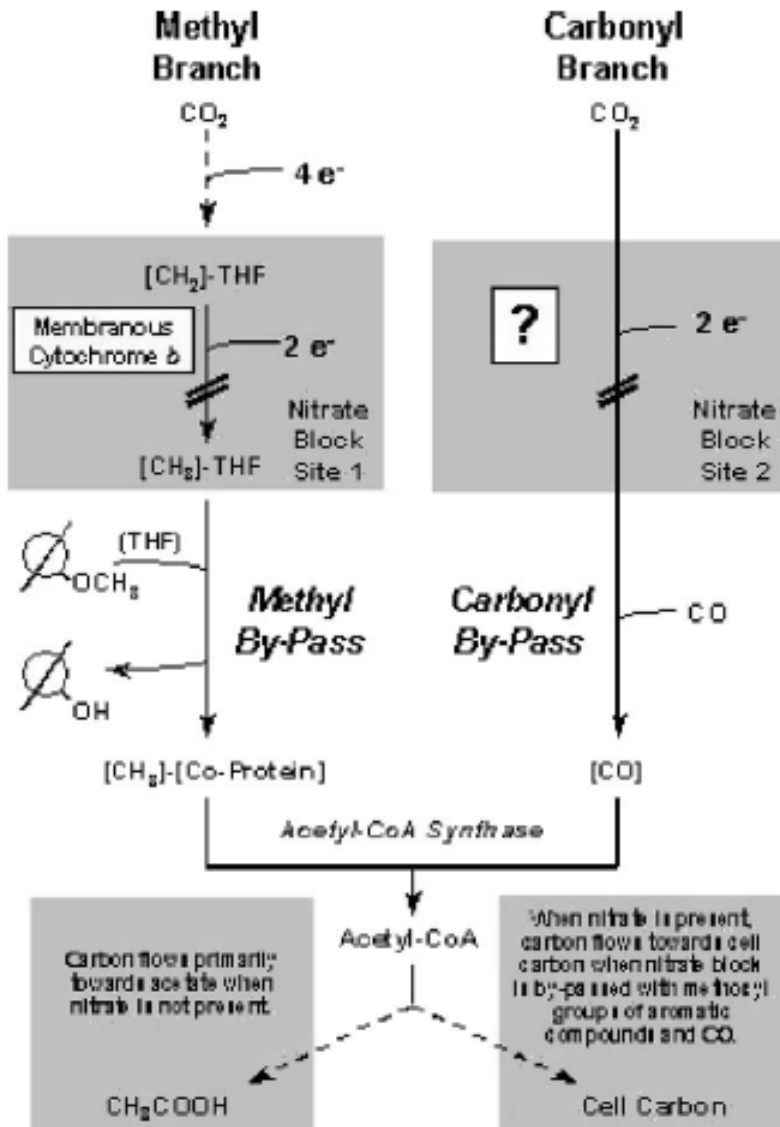


Fig. 6. Scheme illustrating where the acetyl-CoA pathway is blocked when nitrate is dissimilated to ammonium by *M. thermoacetica*. Abbreviations: THF, tetrahydrofolate; CoA, coenzyme-A; Co-Protein, corrinoid enzyme. The dissimilation of nitrite to ammonium appears to have the same affect [109]. Modified from [34].

The activity of hydrogenase [31] in nitrate-dissimilating cells is 14-fold lower than that of acetogenic cells [42], and this oxidoreductase might have an unknown essential function in the flow of reductant to the acetyl-CoA pathway even when exogenous  $\text{H}_2$  is not available as a substrate. As shown in Fig. 6, nitrate also affects

the flow of reductant to the carbonyl branch of the pathway, and hydrogenase might be involved in the flow of this reductant, not as a classic hydrogenase, but as an oxidoreductase that shuttles electrons to the carbonyl branch of the pathway, i.e., to the CO-synthesizing function of acetyl-CoA synthase. If this theory is true, the cell's ability to reduce CO<sub>2</sub> to CO would be compromised when the activity of this oxidoreductase (i.e., the enzyme that is assayed as a hydrogenase) is impaired. Consistent with this concept, the metabolic block on the carbonyl branch is overcome by supplying the cell with CO (Fig. 6) [42]. *M. thermoacetica* produces traces of H<sub>2</sub> under certain conditions, and the ability to produce H<sub>2</sub> is retained by resting cells [79]. Thus, an oxidoreductase that can be detected (i.e., assayed) as a hydrogenase might indeed be involved in reductant flow during acetogenesis. Very little is known about how *M. thermoacetica* utilizes other terminal electron acceptors. Thiosulfate and dimethylsulfoxide have been reported to serve as terminal electron acceptors for *M. thermoacetica* under certain conditions; these acceptors yield sulfide and dimethylsulfide as reduced end products [7]. *M. thermoacetica* produces lactate and formate when it is grown on glucose, CO, or H<sub>2</sub> under nitrate-dissimilating conditions [42]; (Fröstl and Drake, unpublished data), indicating that pyruvate and CO<sub>2</sub> can be reduced to lactate and formate, respectively; however, the levels of lactate and formate that are produced are low, and there is no indication that these processes are engaged for the conservation of energy. Lactate and formate are substrates for *M. thermoacetica*, and their production likely occurs via a reversal of processes that are normally engaged for their utilization. This reversal of catabolic processes also occurs in the case of H<sub>2</sub>, which is normally an acetogenic substrate but can be produced under certain conditions [19,79]. Ethanol and propanol are also substrates under nitrate-dissimilating conditions, and it can be projected that, like lactate, formate, and H<sub>2</sub>, they might be produced at trace levels when the cell is subjected to excess reductant.

## 2.5. Response to O<sub>2</sub>

In redox-unstable environments, resident acetogens must be able to tolerate exposure to O<sub>2</sub> in order to survive. *M. thermoacetica* has three known strategies for survival when confronted with oxidative stress: (i) the expression of protective enzymes that can remove O<sub>2</sub> and its toxic byproducts (e.g., superoxide and peroxide); (ii) use of the alternative electron acceptor nitrate; and (iii) the formation of a symbiotic relationship with an O<sub>2</sub>-consuming facultative or microaerophilic microorganism (Fig. 7). Recent studies have shown that *M. thermoacetica* [57], as well as other acetogens [8,9,57,66], can grow in culture media containing O<sub>2</sub> and is able to consume small amounts of O<sub>2</sub>. *M. thermoacetica* possesses peroxidase and NADH-oxidase activities and the genes for rubredoxin oxidoreductase and rubrerythrin, proteins that have superoxide reductase and peroxidase activities, respectively [28,57,112]. Classic superoxide dismutase and catalase, both of which produce O<sub>2</sub>, have not been detected in *M. thermoacetica* [57]. For *M. thermoacetica* (an

acetogen found in oxic soils [see below]), the ability to metabolize  $O_2$  provides one strategy for the removal of small amounts of  $O_2$  that infuse into soil microsites colonized by this acetogen. In addition, its ability to dissimilate nitrate at positive redox potentials [42,106] (the standard redox potential of the nitrate/nitrite half-cell reaction (the first reaction in the dissimilation of nitrate) is 0.43 mV) rather than  $CO_2$ , provides a way for the cell to “switch off” the  $O_2$ -sensitive acetyl-CoA pathway when faced with unfavorable redox conditions. Finally, a close trophic relationship has been observed between *M. thermoacetica* and the fermentative microaerophilic bacterium *Thermicanus aegyptius* (see Section 3.3) [48].

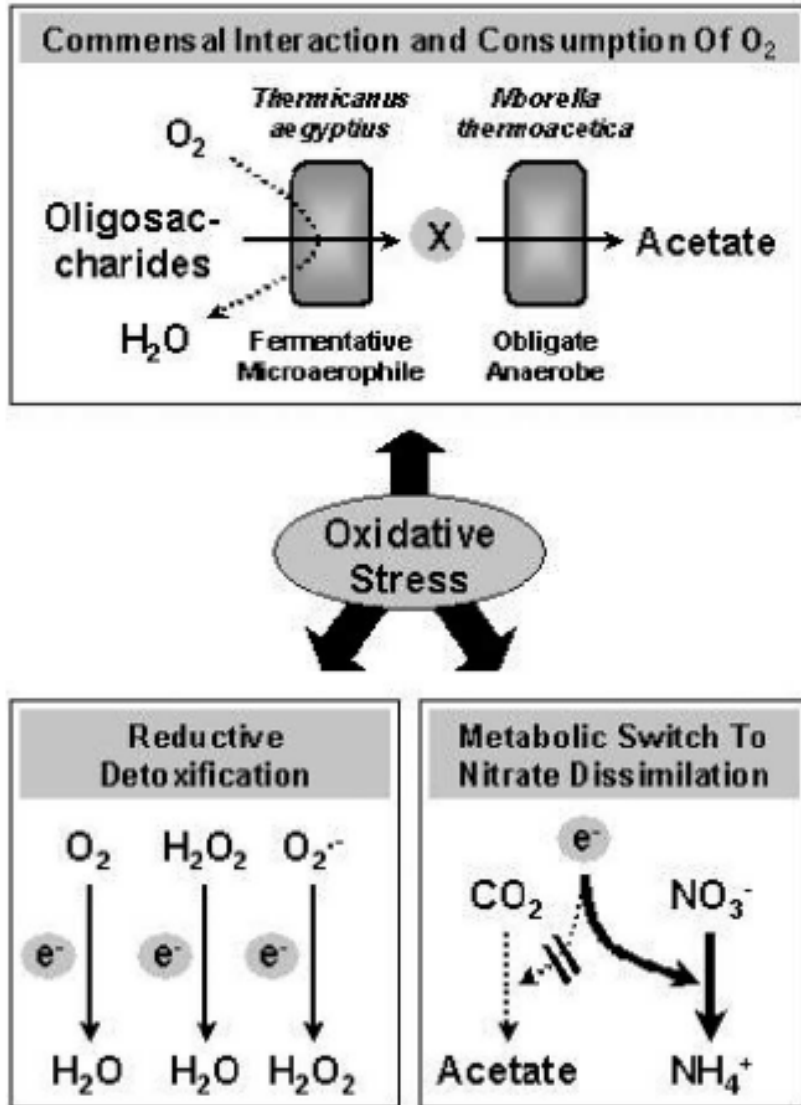


Fig. 7. Mechanisms by which *M. thermoacetica* copes with  $O_2$  and oxidative stress. Abbreviations: X, products (e.g.,  $H_2$ , formate, lactate) that are derived from the partial oxidation of oligosaccharides (e.g., stachyose); e<sup>-</sup>, electron. Modified from [86].



## 2.6. Commercialization of physiological abilities

The global, commercial production of acetic acid approximated 1010 kg in 2001, and numerous studies have evaluated the potential use of *M. thermoacetica* to commercially produce both acetic acid and calcium-magnesiumacetate (an environmentally safe road de-icer) [12,15,36,74,76,105,111, 124,125]. However, as is the case with all known acetogens, *M. thermoacetica* is inhibited by high concentrations of acetate and does not grow under acidic conditions, mainly because the cell cannot maintain a proton motive force ( $\Delta\text{pH}$ ) and transmembrane electrical potential ( $\Delta\Psi$ ) under such conditions [6]. These limitations have made it impossible to commercialize the acetogenic abilities of both wild-type and mutant strains of *M. thermoacetica* [125]. Immobilization of *M. thermoacetica* has also not circumvented these problems [122]. The conversions of synthesis gas ( $\text{H}_2$ , CO, and  $\text{CO}_2$ ) to acetic acid, ethanol, and butanol by acetogens have likewise been accessed [1,49,93,115].

The bioremediation potentials of acetogens have received little attention. Certain acetogens dehalogenate anthropogenic compounds [38,120], and acetyl-CoA synthase transforms 2,4,6-trinitrotoluene (TNT) [54,94]; however, an application of these catalytic activities has not been reported. *M. thermoacetica* sequesters cadmium [17]; whether it can be used to remediate materials contaminated with cadmium or other heavy metals is unknown. The potential to commercially produce fine chemicals (e.g., corrinoids, cysteine, 5-aminolevulinic acid, hydroxyl acids) and enzymes (e.g., acetate kinase) with *M. thermoacetica* and other acetogens has been evaluated [36,60,62,69,104]; however, a commercial-scale production of a fine chemical has not been achieved.

## 3. Ecological perspectives

The in situ activities of *M. thermoacetica* have not been investigated. Nonetheless, the theoretical considerations outlined in this section highlight features of *M. thermoacetica* that likely contribute to its ability to compete and survive in habitats where it is found.

### 3.1. Habitat and global distribution

*M. thermoacetica* was originally isolated from horse manure [41]. Although elevated temperatures in composting manure would favor the growth of thermophiles, a mammalian gastrointestinal tract can hardly be considered the habitat of *M. thermoacetica*. Given the origin of the original isolate, it is not surprising that *M. thermoacetica* has been isolated from prairie soils from Kansas and Egyptian garden soil (Fig. 8) [46–48]. It is therefore likely that *M. thermoacetica* is globally distributed in soils that periodically experience thermophilic temperatures. In

support of this postulation, the capacity of soils to form acetate from H<sub>2</sub>-CO<sub>2</sub> is enhanced by elevated temperatures [64,121].

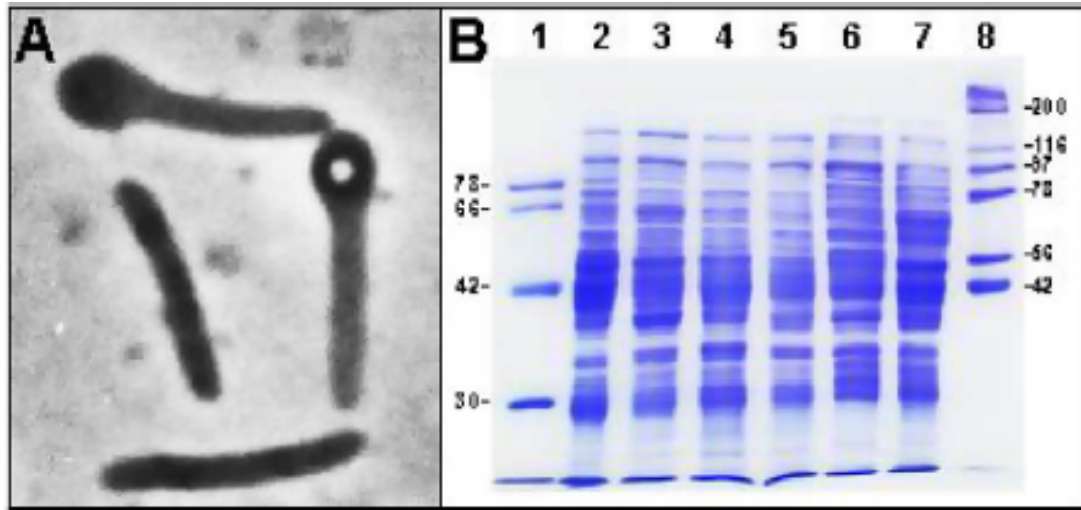


Fig. 8. (A) *M. thermoacetica* PT1 (DSM 12993; EMBL accession number for the 16S rRNA gene sequence is AJ633105; “PT” stands for prairie thermophile) obtained from Kansas prairie soil. (B) Lanes 2–7 are protein profiles of different strains of *M. thermoacetica* obtained from either Kansas soil or Egyptian soil; cells were cultivated on fructose. Lanes 1 and 8 are molecular weight standards. All isolates have nearly identical metabolic capabilities to *M. thermoacetica* ATCC 39073 [19] and grow chemolithoautotrophically at the expense of H<sub>2</sub>-CO<sub>2</sub> or CO-CO<sub>2</sub>.

Well-drained, aerated soils contain anoxic microzones [110,113,119] and have an enormous capacity to form acetate from endogenous organic matter under anoxic conditions (up to 15 g C-acetate per kg dry wt. soil) [64,91, 121]. The consumption of acetate that is formed anaerobically in soils is linked to oxidative processes (e.g., nitrate-, iron- and O<sub>2</sub>-dependent respiration) [64,65,67,121]. Numerous organisms are responsible for acetate formation in soils, but the rapid conversion of supplemental substrates (i.e., H<sub>2</sub> or CO) to acetate by soils under anoxic conditions and the occurrence of mesophilic acetogens in soils (e.g., *Sporomusa silvacetica* [63]) suggest that acetogens are active in anoxic microzones of terrestrial soils. Indeed, acetogens that inhabit soils are able to withstand periods of oxic drying [121] and constitute a significant group of the cultured anaerobes in litter and soils [65,90]. Although *M. thermoacetica* is likely of minor significance to the overall turnover of carbon in soils, the survival strategies it employs are also found in other acetogens, thus making the information gained with this model acetogen of general importance to understanding the activities of other acetogens in terrestrial habitats.

### 3.2. Durability and vigor

*M. thermoacetica* is a robust and hearty thermophile, and its metabolic diversity is likely the primary basis for the organism's ability to compete with other microbes for substrates. However, other factors also contribute to the survival strategies of this acetogen. For example, the spores of *M. thermoacetica* are among the most heat-resistant spores characterized. The decimal reduction time at 121 °C (i.e., the time required at 121 °C to achieve a 90% reduction of the viable population) for *M. thermoacetica* spores can be as long as 111 minutes [13]. For those who have worked with *M. thermoacetica*, it is well known that glassware used to cultivate the organism is difficult to sterilize, mainly because its spores can survive autoclaving [41]. Indeed, *M. thermoacetica* has been isolated as a contaminant from autoclaved media [13]. The ability of *M. thermoacetica* spores to survive high temperatures demonstrates that this anaerobic bacterium can also survive standard commercial canning procedures.

The ability of *M. thermoacetica* to use nitrate as an electron acceptor and to consume and tolerate small amounts of O<sub>2</sub> indicate that this bacterium can accommodate a wide range of redox conditions (see above). Likewise, its ability to utilize or transform a diverse number of molecules (Table 1) demonstrates that its trophic links are not restricted to a single metabolic feature. Mixotrophic conditions and cosubstrate utilization enhances the competitiveness of acetogens [72,92]. Its ability to couple the oxidation of organic substrates to the reduction of inorganic nitrogenous compounds indicates that *M. thermoacetica* facilitates an intercycle coupling between the carbon and nitrogen cycles in soils. Thus, the in situ durability and vigor of *M. thermoacetica* is likely determined by (i) the longevity of its spores, (ii) its ability to cope with changes in redox potential and utilize alternative electron acceptors, and (iii) the simultaneous use of different electron donors.

### 3.3. Commensal interaction

Although *M. thermoacetica* can tolerate small amounts of O<sub>2</sub>, the ability of *M. thermoacetica* to survive in habitats that are subject to fluctuations in O<sub>2</sub> would be enhanced by trophic interactions with microorganisms that could consume O<sub>2</sub>. In support of this concept, *M. thermoacetica* and the fermentative microaerophile *T. aegyptius* were coisolated as a commensal pair from well-drained, aerated Egyptian soil [48]. *M. thermoacetica* grows commensally on fermentation products (e.g., lactate) produced by *T. aegyptius* from oligosaccharides (e.g., stachyose) that *T. aegyptius*, but not *M. thermoacetica*, can utilize (Fig. 7). Under in situ conditions, the fermentative microaerophile *T. aegyptius* would theoretically minimize the level of O<sub>2</sub> in microzones inhabited by *M. thermoacetica*. Since the activity of any acetogen that inhabits an environment subject to fluctuations in O<sub>2</sub> (e.g., the gastrointestinal tract of termites [11,37]) would be enhanced by trophic links to microbes capable of consuming O<sub>2</sub>, the commensal interaction of *M. thermoacetica* and *T. aegyptius* is a general principle that can likely be extended to other acetogens and their microaerophilic partners endemic to such habitats.

## 4. Conclusions

*M. thermoacetica* is the keystone acetogen relative to our understanding of the physiological diversities and biochemistry of acetogens. With a wide range of capabilities from nitrate dissimilation to oxalate-dependent acetogenesis, this organism has a most robust metabolism. Perhaps for this reason, *M. thermoacetica* has been the basis for the careers of many investigators, and, in the process, has taught us many principles that appear to be applicable to most other acetogens. For those of us who have learned from this organism, we owe its discoverers, Fontaine, Peterson, McCoy, and Johnson many thanks. Little did they know that their *Clostridium thermoaceticum* (*M. thermoacetica*) would be the treasure chest from which the enzymology of a globally important form of autotrophy would be resolved. This treasure chest held many other gems as well. The organism with which the first published biological investigations with  $^{14}\text{C}$  were achieved has been and continues to be one of the most important models for understanding the biochemistry of folates and corrinoids, the bioinorganic chemistry of the trace metals cobalt, selenium, and nickel, the bioenergetics of obligate anaerobes, and the ecological importance of metabolic diversity.

On these closing notes, it is obligatory to recall that acetogens and the autotrophic fixation of  $\text{CO}_2$  to acetate (i.e., acetogenesis) are microbiological discoveries that continue to be the main basis upon which work, be it biochemical, cellular, or ecological, with acetogens is based. As we continue to improve our learning skills (i.e., methodologies), we can look forward to learning more about the fascinating talents of acetogens from *M. thermoacetica*.

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