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Abstract: Acetogens utilize the acetyl-CoA Wood-Ljungdahl pathway as a terminal electron-accepting, energy-conserving, CO_2-fixing process. The decades of research to resolve the enzymology of this pathway (1) preceded studies demonstrating that acetogens not only harbor a novel CO_2-fixing pathway, but are also ecologically important, and (2) overshadowed the novel microbiological discoveries of acetogens and acetogenesis. The first acetogen to be isolated, *Clostridium aceticum*, was reported by Klaas Tammo Wieringa in 1936, but was subsequently lost. The second acetogen to be isolated, *Clostridium thermoaceticum*, was isolated by Francis Ephraim Fontaine and co-workers in 1942. *C. thermoaceticum* became the most extensively studied acetogen and was used to resolve the enzymology of the acetyl-CoA pathway in the laboratories of Harland Goff Wood and Lars Gerhard Ljungdahl. Although acetogenesis initially intrigued few scientists, this novel process fostered several scientific milestones, including the first ^{14}C-tracer studies in biology and the discovery that tungsten is a biologically active metal. The acetyl-CoA pathway is now recognized as a fundamental component of the global carbon cycle and essential to the metabolic potentials of many different prokaryotes. The acetyl-CoA pathway and variants thereof appear to be important to primary production in certain habitats and may have been the first autotrophic process on earth and important to the evolution of life. The purpose of this article is to (1) pay tribute to those who discovered acetogens and acetogenesis, and to those who resolved the acetyl-CoA pathway, and (2) highlight the ecology and physiology of acetogens within the framework of their scientific roots.

The Discoverers of Acetogens and Acetogenesis

Acetogenic bacteria, acetogens for short, are anaerobes that use the acetyl-CoA pathway for the reduction of CO_2 to the acetyl moiety of acetyl-coenzyme A (CoA), for the conservation of energy, and for the assimilation of CO_2 into cell carbon. Initially studied primarily for their novel CO_2-fixing properties, these prokaryotes are now known to be much more metabolically, ecologically, and phylogenetically diverse than once thought. Indeed, over 100 acetogenic species, representing 22 genera, have been isolated to date from a variety of habitats (e.g., soils, sediments, sludge, and the intestinal tracts of many animals, including humans and termites) (Table 1). Of the 22 different genera, *Acetobacterium* and *Clostridium* harbor the most known acetogenic species. Overall, acetogens as a group differ widely in their morphological, nutritional, and physiological properties. Furthermore, the acetyl-CoA pathway itself is widely distributed in nature, occurring in various forms in such microbial groups as methanogens and sulfate-reducing bacteria. These nonacetogens make use of metabolic pathways that are similar to the acetyl-CoA pathway for the assimilation of CO_2 into biomass or the oxidation of acetate.[1–3]

Table 1. Acetogenic bacteria isolated to date

Acetogen	Source of isolate	Gram type[b]	Cell morphology	Growth temperature[c]	Date of isolation; investigator
Acetitomaculum ruminis	Rumen fluid, steer	+	Rod	Mesophilic	1989; Greening and Leedle[183]
Acetoanaerobium noterae	Sediment	−	Rod	Mesophilic	1985; Sleat et al.[184]
"*Acetoanaerobium romashkovii*"	Oil field	+	Rod	Mesophilic	1992; Davydova-Charakhchyan et al.[185]
Acetobacterium bakii	Wastewater sediment	+	Rod	Psychrotrophic	1995; Kotsyurbenko et al.[186]
Acetobacterium carbinolicum	Freshwater sediment	+	Rod	Mesophilic	1984; Eichler and Schink[187]
"*Acetobacterium dehalogenans*"	Sewage digester sludge	+	Coccus	Mesophilic	1991; Traunecker et al.[166]
Acetobacterium fimetarium	Digested cattle manure	+	Rod	Psychrotrophic	1995; Kotsyurbenko et al.[186]
Acetobacterium malicum	Freshwater sediment	+	Rod	Mesophilic	1988; Tanaka and Pfennig[188]
Acetobacterium paludosum	Fen sediment	+	Rod	Psychrotrophic	1995; Kotsyurbenko et al.[186]
"*Acetobacterium psammolithicum*"	Subsurface sandstone	−	Rod	Mesophilic	1999; Krumholz et al.[95]
Acetobacterium tundrae	Tundra soil	+	Rod	Psychrotrophic	2000; Simankova et al.[92]
Acetobacterium wieringae	Sewage digester	+	Rod	Mesophilic	1982; Braun and Gottschalk[189]
Acetobacterium woodii	Marine sediment	+	Rod	Mesophilic	1977; Balch et al.[90]
Acetobacterium sp. AmMan1	Freshwater sediment	+	Rod	Mesophilic	1991; Dörner and Schink[190]
Acetobacterium sp. B10	Wastewater pond	+	Rod	Mesophilic	1989; Sembiring and Winter[191,192]
Acetobacterium sp. HA1	Sewage sludge	+	Rod	Mesophilic	1991; Schramm and Schink[193]
Acetobacterium sp. HP4	Lake sediment	+	Rod	Psychrotrophic	1989; Conrad et al.[194]
Acetobacterium sp. KoB58	Sewage sludge	+	Rod	Mesophilic	1988; Wagener and Schink[195]
Acetobacterium sp. LuPhet1	Sewage sludge	+	Rod	Mesophilic	1994; Frings and Schink[196]
Acetobacterium sp. LuTria3	Sewage sludge	+	Rod	Mesophilic	1994; Frings et al.[197]
Acetobacterium sp. MrTac1	Marine sediment	+	Rod	Mesophilic	1987; Emde and Schink[198]
Acetobacterium sp. OyTac1	Freshwater sediment	+	Rod	Mesophilic	1987; Emde and Schink[198]
Acetobacterium sp. RMMac1	Marine sediment	−	Rod	Mesophilic	1990; Schuppert and Schink[199]
Acetobacterium sp. 69	Sea sediment	+	Rod	Mesophilic	1992; Inoue et al.[200]

Acetobacterium sp.	Tundra wetland soil	+	Rod	Psychrotrophic	1996; Kotsyurbenko et al.[201]
Acetohalobium arabaticum	Saline lagoon	−	Rod	Mesophilic	1990; Zhilina and Zavarzin[202]
Acetonema longum	Wood-eating termite, gut	−	Rod	Mesophilic	1991; Kane and Breznak[203]
Bryantella formatexigens	Human feces	+	Rod	Mesophilic	2003; Wolin et al.[98]
"*Butyribacterium methylotrophicum*"	Sewage digester	+	Rod	Mesophilic	1980; Zeikus et al.[204]
Caloramator fervidus (?)	Hot spring	−	Rod	Thermophilic	1987; Patel et al.[205]
Clostridium aceticum	Soil	−	Rod	Mesophilic	1936; Wieringa,[5] Adamse,[9] Braun et al.[10]
"*Clostridium autoethanogenum*" (?)	Rabbit feces	+	Rod	Mesophilic	1994; Abrini et al.[158]
Clostridium carboxidivorans	Lagoon sediment	+	Rod	Mesophilic	2005; Liou et al.[89]
Clostridium coccoides	Mice feces, human feces	+	Coccoid rod	NR[d]	1976; Kaneuchi et al.,[206] Kamlage et al.[86]
Clostridium difficile AA1	Rumen, newborn lamb	+	Rod	Mesophilic	1998; Rieu-Lesme et al.[207]
Clostridium drakei	Coal mine pond sediment	+	Rod	Mesophilic	2000; Küsel et al.,[47] Liou et al.[89]
Clostridium formicaceticum	Sewage	−	Rod	Mesophilic	1967; El Ghazzawi,[17] Andreesen et al.[18]
Clostridium glycolicum 22	Sewage	+	Rod	Mesophilic	1977; Ohwaki and Hungate[88]
Clostridium glycolicum RD-1	Sea-grass roots	+	Rod	Mesophilic	2001; Küsel et al.[87]
Clostridium ljungdahlii	Chicken waste	+	Rod	Mesophilic	1988; Barik et al.,[101] Tanner et al.[83]
Clostridium magnum	Freshwater sediment	−	Rod	Mesophilic	1984; Schink[208]
Clostridium mayombei	Soil-feeding termite, gut	+	Rod	Mesophilic	1991; Kane et al.[203]
Clostridium methoxybenzovorans	Olive oil mill wastewater	+	Rod	Mesophilic	1999; Mechichi et al.[209]
Clostridium scatologenes	Soil, coal mine pond sediment	+	Rod	Mesophilic	1927; Weinberg and Ginsbourg,[46] Küsel et al.[47]
Clostridium ultunense	Swine manure digester	+	Rod	Mesophilic	1996; Schnürer et al.[210]
Clostridium sp. CV-AA1	Sewage sludge	−	Rod	Mesophilic	1982; Adamse and Velzeboer[211]
Clostridium sp. M5a3	Human feces	+	Rod	NR	1996; Bernalier et al.,[127] Leclerc et al.[212,213]
Clostridium sp. F5a15	Human feces	+	Rod	NR	1996; Bernalier et al.,[127] Leclerc et al.[212,213]
Clostridium sp. Ag4f2	Human feces	+	Rod	NR	1996; Bernalier et al.[127]
Clostridium sp. TLN2	Human feces	+	Coccobacillus	NR	1996; Bernalier et al.[127]
Eubacterium aggregans	Olive oil mill Wastewater	+	Rod	Mesophilic	1998; Mechichi et al.[209]
Eubacterium limosum	Rumen fluid, sheep	+	Rod	Mesophilic	1981; Sharak Genthner et al.[214]
Holophaga foetida	Freshwater ditch mud	−	Rod	Mesophilic	1992; Bak et al.,[134] Liesack et al.[84]

Moorella glycerini	Hot spring sediment	+	Rod	Thermophilic	1997; Slobodkin et al.[118]
Moorella mulderi	Bioreactor	+	Rod	Thermophilic	2003; Balk et al.[215]
Moorella thermoacetica	Horse manure, soil	+/–	Rod	Thermophilic	1942; Fontaine et al.,[13] Gößner et al.[14–16]
Moorella thermoautotrophica	Hot spring	+/–	Rod	Thermophilic	1981; Wiegel et al.[216]
Moorella sp. F21	Soil	+	Rod	Thermophilic	2003; Karita et al.[133]
Moorella sp. HUC22–1	Mud	+	Rod	Thermophilic	2004; Sakai et al.[217]
Natroniella acetigena	Soda lake deposits	–	Rod	Mesophilic	1996; Zhilina et al.[97]
Natronincola histidinovorans	Soda lake deposits	+	Rod	Mesophilic	1998; Zhilina et al.[218]
Oxobacter pfennigii	Rumen fluid, steer	+	Rod	Mesophilic	1985; Krumholz and Bryant[219]
Ruminococcus hydrogenotrophicus	Human feces	+	Coccobacillus	Mesophilic	1996; Bernalier et al.[220,221]
Ruminococcus productus U1	Sewage digester	+	Coccus	Mesophilic	1984; Lorowitz and Bryant[222]
Ruminococcus productus Marburg	Sewage digester	+	Coccus	Mesophilic	1987; Geerligs et al.[223]
Ruminococcus schinkii	Rumen, 3-day-old lamb	+	Coccoid rod	Mesophilic	1996; Rieu-Lesme et al.[224]
Ruminococcus sp. TLF1	Human feces	+	Coccobacillus	NR	1996; Bernalier et al.[127]
Sporomusa acidovorans	Distillation waste water	–	Rod	Mesophilic	1985; Ollivier et al.[225]
Sporomusa aerivorans	Soil-eating termite, gut	–	Rod	Mesophilic	2003; Boga et al.[226]
Sporomusa malonica	Freshwater sediment	–	Rod	Mesophilic	1989; Dehning et al.[227]
Sporomusa ovata	Silage	–	Rod	Mesophilic	1984; Möller et al.[228]
Sporomusa paucivorans	Lake sediment	–	Rod	Mesophilic	1987; Hermann et al.[229]
Sporomusa silvacetica	Beech forest soil	+	Rod	Mesophilic	1997; Kuhner et al.[93]
Sporomusa sphaeroides	River mud	–	Rod	Mesophilic	1984; Möller et al.[228]
Sporomusa termitida	Wood-eating termite, gut	–	Rod	Mesophilic	1988; Breznak et al.[230]
Sporomusa sp. DR6	Rice field soil	+	Rod	NR	1999; Rosencrantz et al.[231]
Sporomusa sp. DR1/8	Rice field soil	+	Rod	NR	1999; Rosencrantz et al.[231]
Syntrophococcus sucromutans	Rumen fluid, steer	–	Coccus	Mesophilic	1986; Krumholz and Bryant[232]
Thermoacetogenium phaeum	Pulp wastewater reactor	+	Rod	Thermophilic	2000; Hattori et al.[233]
Thermoanaerobacter kivui	Lake sediment	–	Rod	Thermophilic	1981; Leigh et al.[234]
Tindallia californiensis	Alkaline lake sediment	+	Rod	Mesophilic	2003; Pikuta et al.[85]
Treponema azotonutricium	Termite, hindgut	NR	Spirochete	Mesophilic	1999; Leadbetter et al.,[129] Graber et al.[100]
Treponema primitia	Termite, hindgut	NR	Spirochete	Mesophilic	1999; Leadbetter et al.,[129] Graber et al.[100]
Unclassified					
AG (?)	Granular reactor sludge	+	Rod	Thermophilic	1996; Davidova and Stams[235]

AOR	Thermophilic digester	+	Rod	Thermophilic	1988; Lee and Zinder[106]
CS1Van	Human feces	+	Rod	Mesophilic	1993; Wolin and Miller[236]
CS3Glu	Human feces	+	Coccoid rod	Mesophilic	1993; Wolin and Miller[236]
CS7H	Human feces	+	Rod	Mesophilic	1993; Wolin and Miller[236]
D	Rumen fluid, deer	−	Rod	Mesophilic	1995; Rieu-Lesme et al.[237]
DMG58	River mud	−	Rod	Mesophilic	1984; Möller et al.[228]
EE121	Granular reactor sludge	+	Rod	NR	1990; Plugge et al.[238]
HA	Horse feces	−	Coccobacillus	NR	1995; Miller and Wolin[161]
I52	Human feces	−	Coccoid rod	Mesophilic	1994; Wolin and Miller[239]
S5a2	Human feces	+	Coccus	NR	1996; Bernalier et al.,[127] Leclerc et al.[212,213]
Ser8	Rumen, newborn lamb	NR	NR	NR	1995; Chaucheyras et al.[240]
SS1	406-m-deep sediment	+	Oval rod	Mesophilic	1993; Liu and Suflita[96]
TH-001	Sewage sludge	−	Rod	Mesophilic	1985; Frazer and Young[241]
VK64	Human feces	+	Coccus	NR	1996; Bernalier et al.[127]
X-8	Vegetable wastewater	−	Rod	Mesophilic	1982; Samain et al.[242]
ZT	Tundra soil	+	Rod	Psychrophilic	1992; Kotsyurbenko et al.,[243] Nozhevnikova et al.[244]
417/2	Oil field	−	Rod	Mesophilic	1992; Davydova-Charakhchyan et al.[185]
417/5	Oil field	−	Rod	Mesophilic	1992; Davydova-Charakhchyan et al.[185]
"New acetogenic bacterium"	Rumen, 15-hour-old lamb	+	Coccoid rod	Mesophilic	1996; Rieu-Lesme et al.[245]

[a]Bacteria listed appear to use the acetyl-CoA pathway for the synthesis of acetate and growth (modified from Drake,[246] Drake,[3] and Drake et al.[41,42]). If the acetogenic nature of an organism is uncertain, a question mark occurs after the name of the organism. Organisms not having validated names are enclosed in quotation marks.

[b]Gram type is based on electron microscopic analyses of the cell-wall structure, if reported. Otherwise, gram type is based on the gram stain reaction. (NOTE: Results of the gram stain reaction are not always in agreement with the electron microscopic analysis of the cell wall.) +/− indicates the gram type is variable.

[c]General temperature preference: psychrophilic (5–10°C), psychrotrophic (16–20°C), mesophilic (31–34°C), and thermophilic (58–62°C).

[d]NR, not reported.

So, how did we get to this point relative to our understanding of acetogens and the acetyl-CoA pathway? Needless to say, the journey has been a long one, spanning some 60 years, with many stops and passengers along the way. The journey actually started in 1932 with the discovery of acetogenesis (i.e., the metabolic process by which two molecules of CO_2 are reduced to acetate) by F. Fischer and associates.[4] Their work demonstrated that microbial populations in sewage were competent in the formation of acetate from the H_2-dependent reduction of CO_2. Four years later, the Dutch microbiologist K. T. Wieringa (Fig. 1A) documented the isolation of the first acetogen, *Clostridium aceticum* (Fig. 1B and 1C; Table 1), from soil (i.e., ditch mud).[5] This spore-forming, mesophilic bacterium was shown to grow at the expense of H_2-CO_2 according to the following stoichiometry[5–7]:

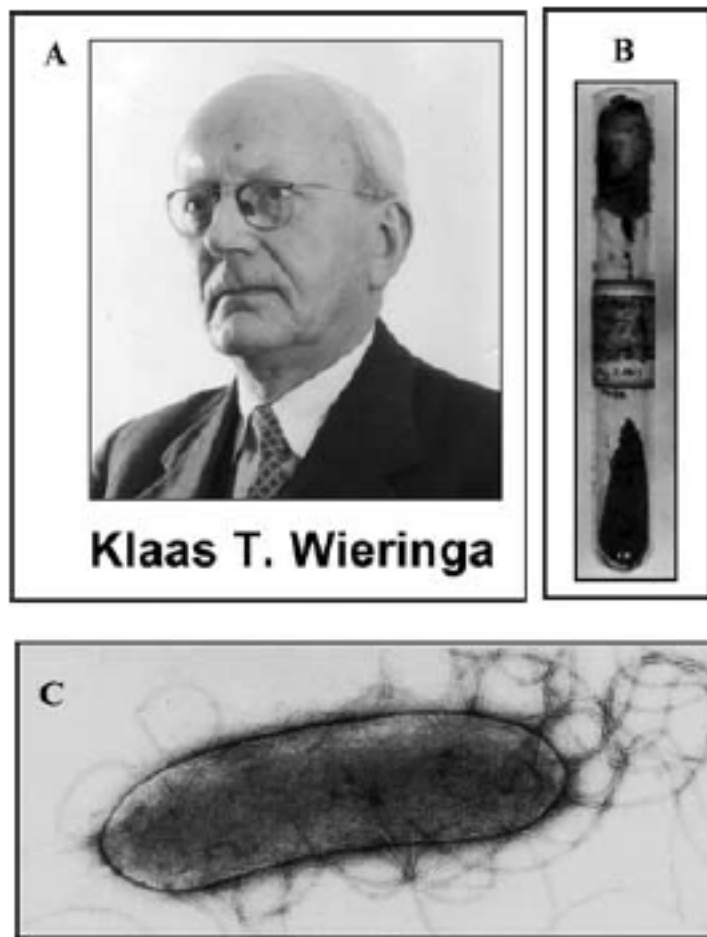$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$

Figure 1

Figure 1. (**A**) Klaas Tammo Wieringa, who isolated the first acetogen, *Clostridium aceticum*, in 1936.[5] (**B**) A culture tube, dated 7 May 1947, containing spores of *C. aceticum* in dried soil. The tube was obtained from H. A. Barker and contained spores derived from Wieringa's culture of *C. aceticum*. These spores were used to revive the organism, as reported by Adamse in 1980[9] and Braun *et al.* in 1981.[10] (**C**) Electron micrograph of a peritrichiously flagellated cell of *C. aceticum*.[10] (Parts (B) and (C) are from Drake *et al.*[42] and are used with the kind permission of Springer.)

In 1936, this reaction constituted a unique mechanism for the fixation of CO_2. Unfortunately, the culture of *C. aceticum* was later lost and no further work, except for one study to define its nutritional requirements,[8] was done with this acetogen until it was reisolated in the early 1980s (Fig. 1).[9–11] The life and career of K. T. Wieringa is encapsulated in the following paragraph and is largely based on information kindly provided by Wouter Middelhoven (Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands).

K. T. Wieringa was born into a well-to-do farmer's family on September 19, 1891 in Noordhorn, a village in the northeastern part of the Netherlands. He received his diploma at the University of Wageningen in 1916, spent some time at an agricultural research station in Groningen, and then returned to Wageningen in October 1918, when he was recruited by N. L. Söhngen [Professor of Microbiology and student of

M. W. Beijerinck (Delft; Söhngen's dissertation is dated July 1906)][12] to join him at the Landbouwhogeschool (Agricultural University). Upon arrival in Wageningen, Wieringa participated in designing and constructing the Laboratory of Microbiology that opened in 1922. Wieringa received his doctoral degree from Wageningen in 1928 and taught both laboratory and lecture courses on a variety of subjects, including plant diseases, nitrogen fixation, and soil fertility, areas that were representative of his research interests. He remained active in the laboratory for many years subsequent to his retirement in 1956. Wieringa was a devoted member of the Mennonite church and passed away on July 28, 1980 in Wageningen.

Ironically, the discovery of an old culture tube containing spores of *C. aceticum* (Fig. 1B) led to the revival of Wieringa's culture of *C. aceticum* close to the time of his death.[9–11] The discovery of the first organism known to be capable of acetogenic autotrophy was most certainly Wieringa's greatest scientific accomplishment, work likely inspired by Söhngen's keen interest in methanogenesis and the microbial conversions of gases. Wieringa's collaboratrion with Söhngen is acknowledged by Wieringa in the introductory comments of his early works on *C. aceticium*[5–7] and is attested to by Wieringa's obituary on Söhngen, who passed away on December 24, 1934.[12] Indeed, it was H_2-CO_2 methanogenic enrichments that Söhngen and Wieringa had set up before Söhngen's death that yielded subtle hints toward H_2-dependent acetogenesis. A slight discrepancy in the amount of carbon recovered during the H_2-dependent formation of methane led Wieringa to conclude that transfers of H_2-CO_2 methanogenic enrichments must also contain a physiologically new type of organism:

Now it has been found that subcultures of this type sometimes may absorb large amounts of hydrogen without any formation of CH_4. Microscopical examination of such cultures shows the presence of club-shaped spore-bearing bacilli.[6]

With a specially constructed gas-absorption apparatus that encouraged the growth of this novel, nonmethanogenic, H_2-consuming, spore-forming autotroph, Wieringa was able to enrich and isolate *C. aceticum*.[5] Despite the subsequent loss of *C. aceticum* for many decades, it was within this historical context that the first acetogen was isolated.

In 1942, F. E. Fontaine (Fig. 2A) and co-workers isolated the second acetogen, *Clostridium thermoaceticum* (Fig. 2B), a spore-forming, thermophilic bacterium that catalyzed the near stoichiometric conversion of glucose to acetate (Fig. 2C)[13]:

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH$$
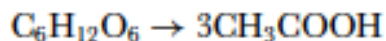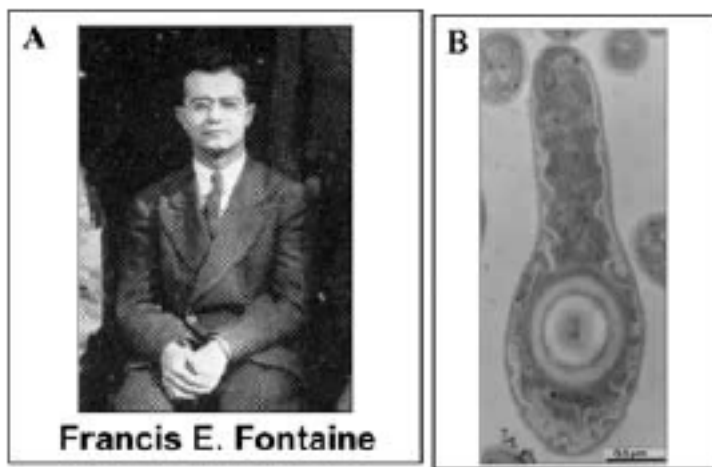
TABLE 7
Summary of glucose fermentations by C. thermoaceticum

NUMBER	ORIGINAL GLUCOSE	GLUCOSE FERMENTED	ACETIC ACID	RATIO: ACETIC/GLUCOSE
	mM/100 mL	mM/100 mL	mM/100 mL	
177-1	15.69	3.88	10.12	2.51
154-1	14.38	3.96	10.40	2.63
177-2	15.69	8.05	21.00	2.61
70-2a	12.22	8.19	21.00	2.57
56-304	13.60	9.37	23.50	2.51
70-4a	12.22	9.46	23.90	2.53
70-4b	12.22	10.20	26.40	2.59
180-128	12.24	10.29	26.20	2.54
300-155	12.15	11.26	28.40	2.53
300-156	12.15	11.54	30.05	2.60
Average of 21 fermentations....		8.64	22.06	2.55

Figure 2. (**A***)* Francis Ephraim Fontaine, who isolated the second acetogen, *Clostridium thermoaceticum*, in 1942.[13] This picture was taken in the 1930s when Fontaine was a graduate student at the University of Wisconsin- Madison. (**B**) Electron micrograph of a sporulated cell of *C. thermoaceticum* ATCC 39073, which was reclassified in 1994 as *Moorella thermoacetica* (Collins *et al.*[48]).(From Drake[3] and used with kind permission of Springer.) (**C**) Original data from Fontaine *et al.*[13] These data document the near stoichiometric conversion of 1 mole of glucose to 3 moles of acetate by *C. thermoaceticum*. (Used with kind permission of the American Society of Microbiology.)

C. thermoaceticum was isolated from horse manure. In retrospect, the mammalian gastrointestinal tract (as we know it) can hardly be considered a habitat that is suitable for colonization by thermophilic microorganisms. It is therefore more likely that *C. thermoaceticum*, as a transient organism voided from the horse's gut or as an organism introduced into the manure from the surrounding soil, was able to grow due to the elevated temperatures provided by the composting manure. Indeed, *C. thermoaceticum* has been isolated from Kansas prairie soils and Egyptian garden soils (Fig. 3),[14-16] and, thus, is widely distributed in soils that experience elevated (thermophilic) temperatures.
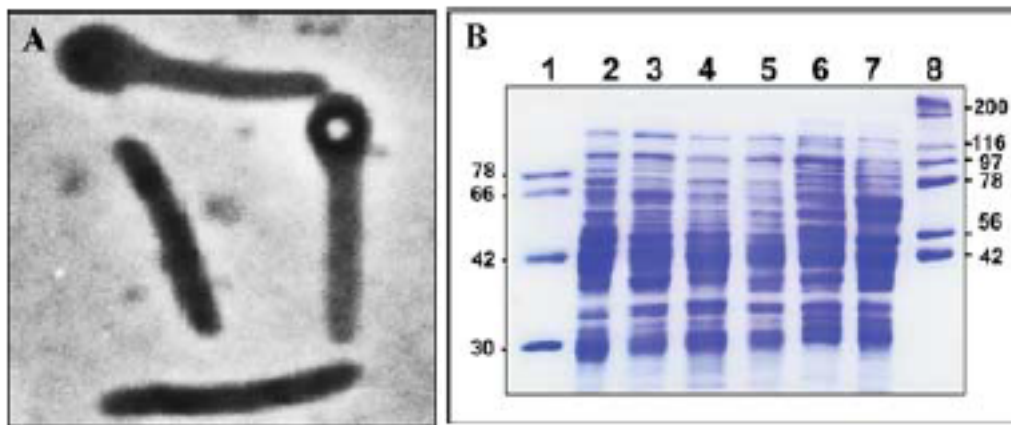
Figure 3. (**A**) *M. thermoacetica* PT1 (DSM 12993) obtained from Kansas prairie soil. (**B**) Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis of *M. thermoacetica* isolates obtained from Kansas and Egyptian soils. Cells were cultivated on fructose; *Lanes 2–7* are protein profiles of the different isolates of *M. thermoacetica,* while *Lanes 1 and 8* are molecular-weight standards. All isolates have nearly identical metabolic capabilities to *M. thermoacetica* ATCC 39073 and grow chemolithoautotrophically at the expense of H_2-CO_2 or CO-CO_2 (Daniel *et al.*[43]). (Parts (A) and (B) are from Drake and Daniel[94] and are used with the kind permission of Elsevier.)

Given the abundance of acetogens in a wide variety of habitats (Table 1), it seems ironic that nearly three decades would pass after the isolation of *C. thermoaceticum* before J. R. Andreesen (Fig. 4A) and associates validated the isolation of *Clostridium formicoaceticum*, which was first reported by E. El Ghazzawi in 1967 and constituted the third published acetogen (Fig. 4B and 4C).[17,18] This spore-forming, mesophilic bacterium was isolated from sewage sludge and produced both formate and acetate during glucose-dependent fermentation.[17,18] In addition, C. *formicoaceticum* possesses other diverse metabolic potentials including the ability to (1) fix dinitrogen gas, (2) utilize the reductant derived from the oxidation of aromatic aldehyde groups for growth and acetate synthesis, and (3) dissimilate fumarate to acetate and succinate without engaging the acetyl-CoA pathway.[19-22] Surprisingly, with all of its metabolic capabilities relative to carbon and reductant flow, *C. formicoaceticum* is unable to grow at the expense of H_2-CO_2.[18,23]
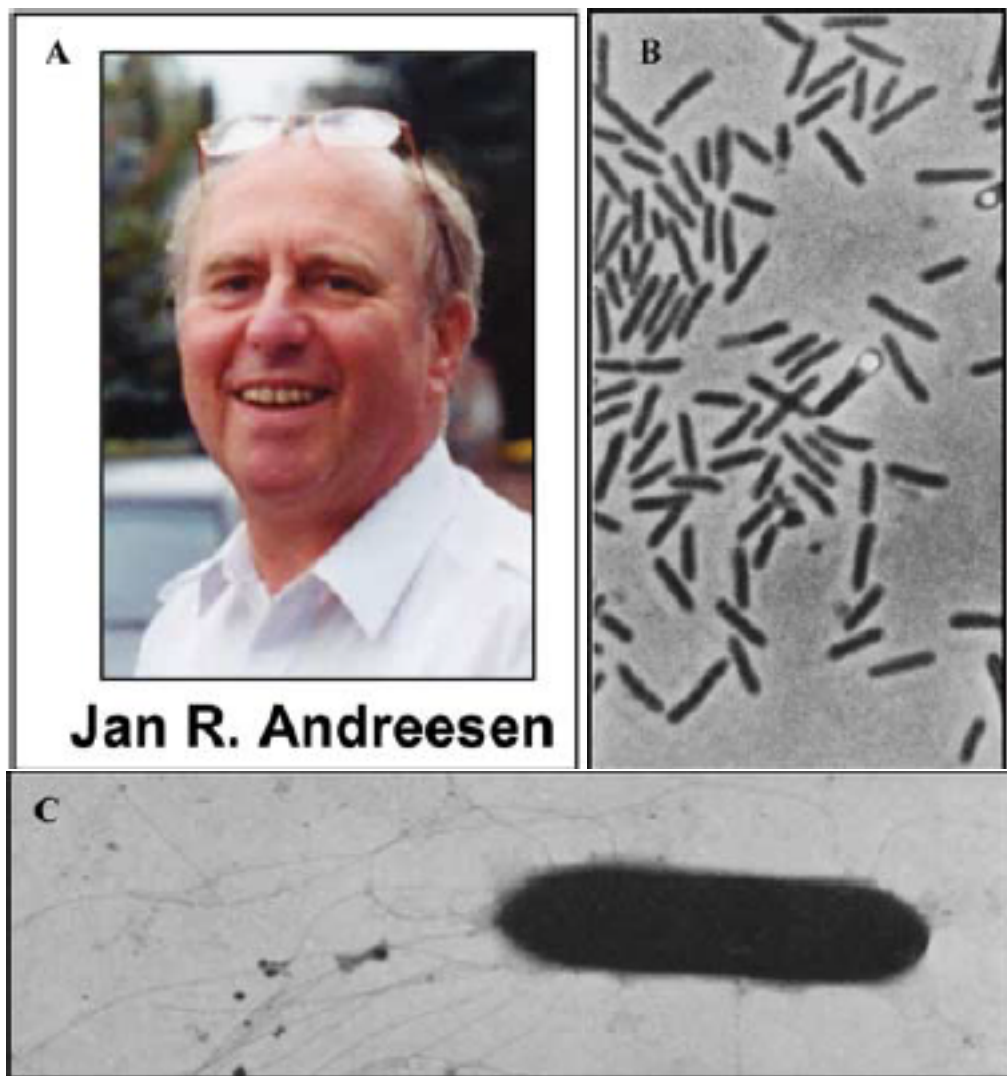
Figure 4. (**A**) Jan Remmer Andreesen, who in 1970 validated the isolation of the third acetogen, *Clostridium formicoaceticum*.[17,18] (**B**) *C. formicoaceticum* A1 obtained from an agar slant culture. (**C**) Electron micrograph showing the peritrichous flagellation of *C. formicoaceticum* A1. (Parts (B) and (C) are from Andreesen *et al*.[18] and are used with the kind permission of Springer.)

With the loss of the *C. aceticum* culture and the absence of *C. formicoaceticum*, the isolation of *C. thermoaceticum* by Fontaine proved to be important and timely, given that this organism was the only acetogen available for laboratory study. This circumstance, in turn, ultimately set the stage for *C. thermoaceticum* to become the most historically important acetogen relative to the resolution of the acetyl-CoA pathway (Table 2).[3,24,25] Nonetheless, when *C. thermoaceticum* was isolated, it was unclear as to the nature of the metabolic process that could account for the formation of nearly 3 moles of acetate per mole of glucose (Fig. 2C). At that time, carbohydrate-based fermentations were usually known to yield such end-products as lactate, ethanol, or mixtures of one- and two-carbon products. The isolation and characterization of *C. thermoaceticum* constituted a portion of Fontaine's doctoral research in the Department of Biochemistry at the University of Wisconsin-Madison, and this matter was addressed by Fontaine on page 53 of his dissertation (dated

May 19, 1941) entitled "The Fermentation of Cellulose and Glucose by Thermophilic Bacteria":

Table 2. Milestones that led to resolving the acetyl-CoA Wood–Ljungdahl pathway and chemolithoautotrophic abilities of the model acetogen _Clostridium thermoaceticum[a]_

Year	Event
	Events prior to the isolation of _Clostridium thermoaceticum_
1927	Isolation of _Clostridium scatologenes_ (Weinberg and Ginsbourg[46]); shown to be an acetogen in 2000 (Küsel et al.[47])
1932	H_2-dependent conversion of CO_2 to acetate in sewage sludge (Fischer et al.[4])
1936	Discovery of first acetogen, _Clostridium aceticum_; total synthesis of acetate from H_2-CO_2 (Wieringa[5–7]) (NOTE: Culture was lost.)
1942	Discovery of second acetogen, _Clostridium thermoaceticum_; conversion of glucose to 3 acetate (Fontaine et al.[13])
1944	Acetogenic conversion of pyruvate to acetate (Barker[247])
1945–1952	Synthesis of acetate from $^{14}CO_2$ (Barker and Kamen[33]) or $^{13}CO_2$ (Wood[34,132])
1955	Formate as a methyl-group precursor (Lentz and Wood[248])
1964	Methylcobalamin as methyl-group precursor (Poston et al.[249])
1965	Autotrophic synthesis of cell-carbon precursors from CO_2 (Ljungdahl and Wood[250])
1966–1969	Proposal of one-carbon pathway for the tetrahydrofolate/corrinoid-mediated synthesis of acetate from CO_2 (Ljungdahl et al.,[251] Ljungdahl and Wood[252])
1973–1976	Discovery that tungsten is a biologically active metal in formate dehydrogenase (Andreesen and Ljungdahl,[35] Ljungdahl and Andreesen,[37] Ljungdahl[36])
1973–1986	Resolution of the tetrahydrofolate pathway (reviewed in Ljungdahl[25])
1978–1980	Discovery of CO dehydrogenase as a nickel-containing enzyme (Diekert and Thauer,[60] Drake et al.[62])
1981	Resolution of enzmes required for synthesis of acetyl-CoA from pyruvate and methyltetrahydrofolate (Drake et al.[253])
1981–1982	Demonstration that CO replaces the carboxyl-group of pyruvate and undergoes an exchange reaction with acetyl-CoA (Drake et al.,[254] Hu et al.[61])
1982	Discovery of hydrogenase (Drake[137])
1983	Purification of CO dehydrogenase (Diekert and Ritter,[59] Ragsdale et al.[255])
1983	Use of H_2 and CO under organotrophic conditions (Kerby and Zeikus[256])
1984	Resolution of nutritional requirements (Lundie and Drake[257])
1984	Enzyme system for H_2-dependent synthesis of acetyl-CoA (Pezacka and Wood[258])
1984–1986	CO dehydrogenase is acetyl-CoA synthase (Pezacka and Wood,[258,259] Ragsdale and Wood[260]), and CO is the carbonyl precursor in the acetyl-CoA pathway under growth conditions (Diekert et al.,[58] Martin et al.[113])
1985–1991	Catalytic mechanism of acetyl-CoA synthase (reviewed in Ragsdale[64])
1986–1990	H_2- and CO-dependent electron-transport system coupled to the synthesis of ATP (Ivey and Ljungdahl,[261] Hugenholtz and Ljungdahl,[262,263] Das et al.[264])
1990	Chemolithoautotrophic growth on H_2-CO_2 and CO-CO_2 (Daniel et al.[43])
1991	Integrated model for catabolic, anabolic, and bioenergetic features of the acetyl-CoA Wood–Ljungdahl pathway (Wood and Ljungdahl[24])

SOURCE: Modified from Drake et al.[41,42]
[a]_Clostridium thermoaceticum_ was reclassified to _Moorella thermoacetica_ in 1994 (Collins et al.[48]).

Since, in this fermentation, 2.5 mols of a two-carbon compound (acetic acid) are obtained from 1 mol of glucose it seems probable that either there is some primary cleavage of glucose other than the classical 3–3 split, or that a one-carbon compound is being reabsorbed. Of these two possibilities, the recent work on carbon dioxide uptake makes the latter seem more likely. Although the carbon analyses show that there is no net gain or net loss of carbon dioxide, it is nevertheless possible that carbon dioxide is produced and then reabsorbed.[26]

The phrase "recent work on carbon dioxide uptake" was referring to various studies that assessed the uptake of carbon dioxide into organic carbon.[4,27–31] After completing his doctoral studies at Wisconsin, Fontaine went on to work at American Cyanamid's Lederle Laboratories division in Pearl River, New York. Fontaine was born in 1916 in Sheboygan, Wisconsin, and passed away in 1983 in Shohola, Pennsylvania.

The fixation of CO_2 proposed by Fontaine was demonstrated experimentally a few years later in 1945 with the landmark ^{14}C-studies of Barker and Kamen.[32,33] These studies were the first in biology to make use of carbon-14 and demonstrated that *C. thermoaceticum* incorporated $^{14}CO_2$ equally into both carbons of acetate.[32,33] That *C. thermoaceticum* was capable of synthesizing acetate from two molecules of CO_2 was later confirmed in 1952 by H. G. Wood using $^{13}CO_2$ and mass spectrometry.[34] On a collective basis, these early physiological and isotopic ('tracer') studies by Wieringa, Fontaine, Barker, Kamen, and Wood made it clear that acetogens possessed a new autotrophic mechanism for the fixation of CO_2. However, it would take nearly 40 more years of sustained research to determine the exact nature of the metabolic process by which acetogens convert 2 moles of CO_2 into 1 mole of acetate. *C. thermoaceticum* gave birth to many scientific milestones en route to resolving the acetyl-CoA pathway, including the discovery by Andreesen and Ljungdahl that tungsten is a biologically active metal.[35–37]

By the mid-to-late 1980s, the individual steps involved in the acetyl-CoA pathway were elucidated. This pathway is also referred to as the Wood–Ljungdahl pathway in recognition of the two biochemists H. G. Wood and L. G. Ljungdahl, who, together with their co-workers, resolved the chemical and enzymological features of the pathway using *C. thermoaceticum* as their model acetogen (Fig. 5).[3,24,25,38–42] Ironically, although we now know that the acetyl-CoA pathway represents a major autotrophic process that is central to carbon flow in various ecosystems, its biochemical details were resolved with *C. thermoaceticum*, an organism initially considered to be an obligate heterotroph. Interestingly, it was well after the enzymological details of the pathway were more or less established that autotrophic growth by *C. thermoaceticum* at the expense of H_2-CO_2 and CO-CO_2 was demonstrated.[43] Milestones leading up to the resolution of the acetyl-CoA pathway and autotrophic capabilities of *C. thermoaceticum* can be found in Table 2 and in numerous review articles.[3,24,25,38,41,42,44,45] Ironies are dispersed among the milestones. One rather large irony is that in 1927, M. Weinberg and B. Ginsbourg isolated a bacterium, *Clostridium scatologenes*, but did not discover a certain physiological novelty that it harbors.[46] In 2000, K. Küsel and co-workers[47] discovered that *C. scatologenes* is an acetogen, meaning that an acetogen had already been isolated 9 years prior to the isolation of *C. aceticum* by Wieringa.

In 1994, *C. thermoaceticum* was reclassified as *Moorella thermoacetica* when the taxonomy of the genus *Clostridium* was reorganized.[48] The organism will be referred to as *Moorella thermoacetica* throughout the remainder of this article.

Acetyl-CoA Wood–Lungdahl Pathway: Evolution, Fixation of Carbon, Bioenergetics

The acetyl-CoA pathway is a reductive, linear, "one-carbon" process (Fig. 5), and is thus in marked contrast to cyclic CO_2-fixing processes (i.e., the Calvin cycle, the reductive tricarboxylic acid cycle, and the hydroxypropionate cycle) that are dependent upon recycled intermediates (i.e., ribulose bisphosphate, oxaloacetate, and acetyl-CoA, respectively) for the initial fixation of CO_2.[24,49] The relatively simple features of the linear acetyl-CoA pathway and the catalytic mechanisms by which carbon is chemically fixed in this pathway have been cited as reasons why the acetyl-CoA pathway might have been important in the evolution of life (i.e., the so-called primordial soup that existed on earth at the time the evolution of life was initiated might have fostered chemical processes indicative of those reflected in the key catalytic steps in the acetyl-CoA pathway by which organic molecules were initially formed).[50–53] These concepts were well conceived many years ago.[49] For example:

It is becoming apparent that the Acetyl-CoA Pathway plays a significant role in the carbon cycle. The direct combination of two CO_2 to form acetate may have been used by the earliest life forms rather than the more complicated cyclic mechanisms of autotrophism.[49]

The usage of acetyl-CoA synthase for a variety of processes in nonacetogens, such as methanogens, sulphate reducers, hydrogenogens, and possibly anammox bacteria, illustrates how widely various features of the acetyl-CoA pathway are distributed among evolutionarily diverse functional groups of prokaryotes.[1,2,54–57]

As illustrated in Figure 5, much of Ljungdahl's work focused on understanding how CO_2 was reduced to a bound methyl unit on the methyl branch of the pathway, while much of Wood's work focused on resolving how CO_2 was reduced to a carbonyl unit on the carbonyl branch of the pathway. One should note that the simplicity of the carbonyl branch on paper belies the complexity of the experiments needed to resolve it. The two branches of the acetyl-CoA pathway, and thus the joint efforts of Wood and Ljungdahl, merge at the synthesis of acetyl-CoA that is subsequently converted to either acetate or assimilated into biomass. Acetyl-CoA synthase not only catalyzes the reduction of CO_2 to CO and the synthesis of acetyl-CoA as shown in Figure 5, but can also oxidizes CO to CO_2; this latter reaction is the historical basis for referring to acetyl-CoA synthase as CO dehydrogenase.[58–62] It is now understood that the different subunits of acetyl-CoA synthase are capable of catalyzing different reactions. The enzymological features of the acetyl-CoA pathway that was resolved from *M. thermoacetica* can be found in several reviews and articles.[24,38–42,44,45,63–75]

Energy conservation occurs during the reductive synthesis of acetate by substrate-level phosphorylation and chemiosmotic processes. Four molecules of adenosine

triphosphate (ATP) are produced by substrate-level phosphorylation (ATP$_{SLP}$) for each hexose (e.g., glucose) that is converted to three acetates ([Fig. 6](#)), a number that is higher than the amount of ATP$_{SLP}$ formed by normal fermentations (e.g., homolactate fermentation yields two ATP$_{SLP}$ per glucose consumed). However, when the acetyl-CoA pathway operates under autotrophic conditions (e.g., during growth at the expense of H$_2$ and CO$_2$), there is no net gain in ATP$_{SLP}$ (1 ATP$_{SLP}$ is required for the activation of formate, and 1 ATP$_{SLP}$ is produced when acetylphosphate is converted to acetate by acetate kinase) ([Fig. 5](#)). Thus, growth of acetogens under autotrophic conditions is strictly dependent upon chemiosmotic, energy-conserving processes that are coupled to the translocation of protons or sodium ions.[44,76,77] Certain acetogens (e.g., *M. thermoacetica*) utilize membranous electron transport systems that translocate protons out of the cell. Such acetogens have membranous, proton-translocating electron transport systems than contain cytochromes, menaquinones, and various oxidoreductases (e.g., hydrogenase); the formation of a proton motive force subsequently drives the cytoplasmic formation of ATP by proton-dependent ATPases.[39,44,75] Other acetogens (e.g., *Acetobacterium woodii*) lack membranous electron transport systems and translocate sodium ions concomitant to membranous transmethylation processes during acetate synthesis. The methyltransferase reaction at the terminal stage of the methyl branch of the acetyl-CoA pathway serves a duel function and translocates sodium ions out of the cell; the subsequent gradient of sodium ions drives the formation of ATP by sodium-dependent ATPase.[76,77] Acetogens that utilize sodium pumping are dependent on sodium for growth at the expense of acetogenesis, while those acetogens that have proton-translocating electron transport systems do not require sodium for growth at the expense of acetogenesis.[78–80] Motility can likewise be dependent on sodium.[81] Some acetogens might also employ sodium-proton antiporters for generating electrochemical gradients.[82]

Lars G. Ljungdahl

Harland G. Wood

Methyl Branch

Carbonyl Branch

CO_2

$2\ e^-$

Formate
Dehydrogenase

HCOOH

Formyl-H_4F
Synthetase

ATP

ADP + P_i

$[CHO]$-H_4F^+

H^+

Methenyl-H_4F
Cyclohydrolase

H_2O

$[CH]$-H_4F

$2\ e^-$

Methylene-H_4F
Dehydrogenase

$[CH_2]$-H_4F

$2\ e^-$

Methylene-H_4F
Reductase

$[CH_3]$-H_4F

Methyltransferase

Acetyl-CoA Synthase

$[CH_3]$-$[Co$-Protein$]$

CO_2

Acetyl-CoA
Synthase

$2\ e^-$

$[CO]$

HSCoA

Phospho-
transacetylase

P_i

$$\begin{array}{c} O \\ \| \\ CH_3C\text{-}SCoA \end{array}$$

Anabolism

ADP

CH_3COO-PO_3^{2-}

Acetate
Kinase

ATP

**Assimilation Into
Cellular Carbon**

CH_3COOH

Figure 5. The acetyl-CoA Wood–Ljungdahl pathway. *Brackets* indicate that a particular C_1 unit is bound to a cofactor or structurally associated with an enzyme. Abbreviations: H_4F = tetrahydrofolate; HSCoA = coenzyme A; P_i= inorganic phosphate; e^-= electron; Co-Protein = corrinoid enzyme; ATP = adenosine 5′-triphosphate. (The pathway is from Müller *et al.*[77] and is used with the kind permission of Horizon Bioscience. The photographs of Harland Goff Wood and Lars Gerhard Ljungdahl are from Drake and Daniel[94] and are used with the kind permission of Elsevier.)
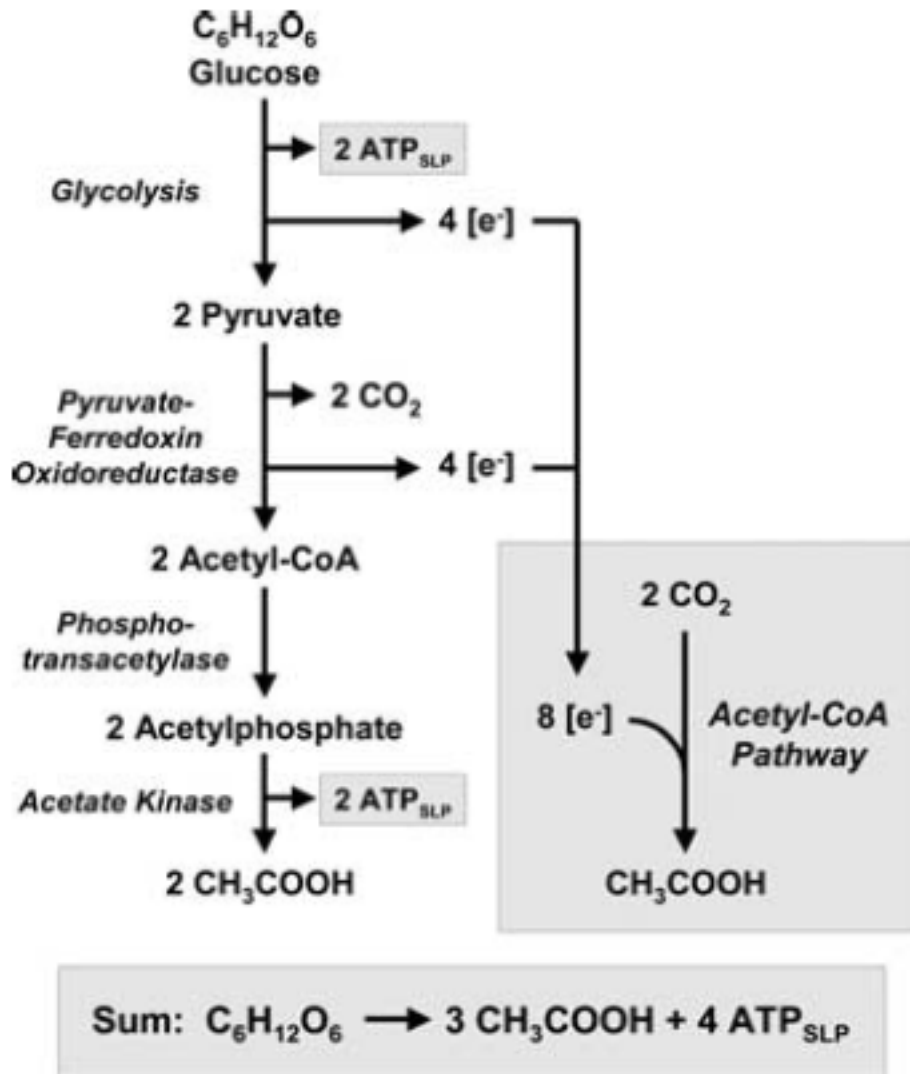
Figure 6. Homoacetogenic conversion of glucose to acetate. The two molecules of CO_2 that are reduced to acetate in the acetyl-CoA pathway can be derived from exogenous CO_2 rather than the CO_2 that is produced via the decarboxylation of pyruvate (see text). Abbreviations: ATP_{SLP}= ATP that is produced by substrate-level phosphorylation; $[e^-]$, reducing equivalent. (From Müller *et al.*[77] and used with the kind permission of Horizon Bioscience.)

Organismal Diversity of Acetogenic Prokaryotes

Acetogens are defined as anaerobes that use the acetyl-CoA pathway for the (1) reductive synthesis of the acetyl moiety of acetyl-CoA from CO_2, (2) conservation of energy, and (3) assimilation of CO_2 into biomass.[3,41,42] Although the production of acetate is the classic hallmark of acetogens, the production of acetate is not a part of this definition, mainly because an acetogen might not form acetate (i.e., acetate formation is conditional and dependent upon both the acetogen and growth environment). Nonetheless, the ability of an organism to form acetate as the sole reduced end-product is compelling evidence that it utilizes the acetyl-CoA pathway per the definition given earlier and is an acetogen. As explained in detail elsewhere,[3,41,42] referring to microorganisms as acetogens when they form acetate by processes that do not involve the reductive synthesis of acetate from CO_2 yields unfortunate confusion in the literature.

The utilization of the acetyl-CoA pathway is the main unifying feature of acetogens. However, they display extreme genetic diversity, having genomic G + C contents that vary between 22 mol% (*Clostridium ljungdahlii*[83]) to 62 mol% (*Holophaga foetida*[84]). Acetogens have been assigned to 22 different bacterial genera[41,42]: *Acetitomaculum*, *Acetoanaerobium*, *Acetobacterium*, *Acetohalobium*, *Acetonema*, *Bryantella*, *"Butyribacterium," Caloramator, Clostridium, Eubacterium, Holophaga, Moorella, Natroniella, Natronincola, Oxobacter, Ruminococcus, Sporomusa, Syntrophococcus, Tindallia, Thermoacetogenium, Thermoanaerobacter, Treponema* (name in quotation marks has not been validated). In certain cases, the acetogenic nature of an organism that is characterized to be an acetogen is less than certain. For example, *Tindallia californiensis* appears to be an acetogen, in that it forms large amounts of acetate from various amino acids and pyrvuate, and cell extracts have hydrogenase and CO dehydrogenase activities.[85] However, (1) substrate/product stoichiometries have not been reported for this organism, (2) the occurrence of hydrogenase and CO dehydrogenase activities is not definitive evidence that an organism utilizes the acetyl-CoA pathway (e.g., cell extracts of *Clostridium pasteurianum* have both hydrogenase and CO dehydrogenase activities, but the organism is not an acetogen), and (3) the organism forms a mixture of products and is described as a fermentative organotroph. Thus, the acetogenic nature of this genus remains uncertain. Nonetheless, the potential occurrence of acetogenic strains in the genus *Tindallia* is noteworthy, as this genus is alkaliphilic.

Some acetogenic genera are monophyletic (i.e., all species of a genus that have been isolated to date are acetogens). *Moorella* and *Sporomusa* are examples of such genera. However, many acetogens are phylogenetically dispersed within genera that contain nonacetogenic species (Fig. 7). For example, *Clostridium* and *Ruminococcus* contain acetogens that are dispersed among closely related species that are not acetogens [e.g., the closest relative of the acetogen *C. formicoaceticum* is the nonacetogen *Clostridium felsineum* (99.3% 16S rRNA gene sequence similarity)]. Thus, the classification of new acetogens is sometimes problematic, in that the phylogenetic position of 16S rRNA gene sequences is inadequate for resolving the functional identity of a potential acetogen. In some cases, species that were not originally described as acetogens when first isolated are later discovered to be acetogens (e.g., *Clostridium coccoides*[86] and *C. scatologenes*[47]). In other cases, essentially identical organisms (i.e., organisms that have essentially identical 16S rRNA genes) may display opposite acetogenic capacities. For example, the type

strain of *C. glycolicum* does not display acetogenic capabilities, but *C. glycolicum* strain RD-1[87] and *C. glycolicum* strain 22[88] are acetogens. Prolonged cultivation under certain conditions in the laboratory might cause certain acetogens to lose the capacity to engage the acetyl-CoA pathway and grow acetogenically.[41,42] DNA-DNA hydridation of genomic material can differentiate species-level differences between acetogens and nonacetogens where 16S rRNA-level differences are inadequate. For example, *C. scatologenes* SL1 was initially characterized as an acetogenic strain of the type strain of this species[47] and later shown to be a unique species by DNA-DNA hybridization and reclassified as *Clostridum drakei*.[89]
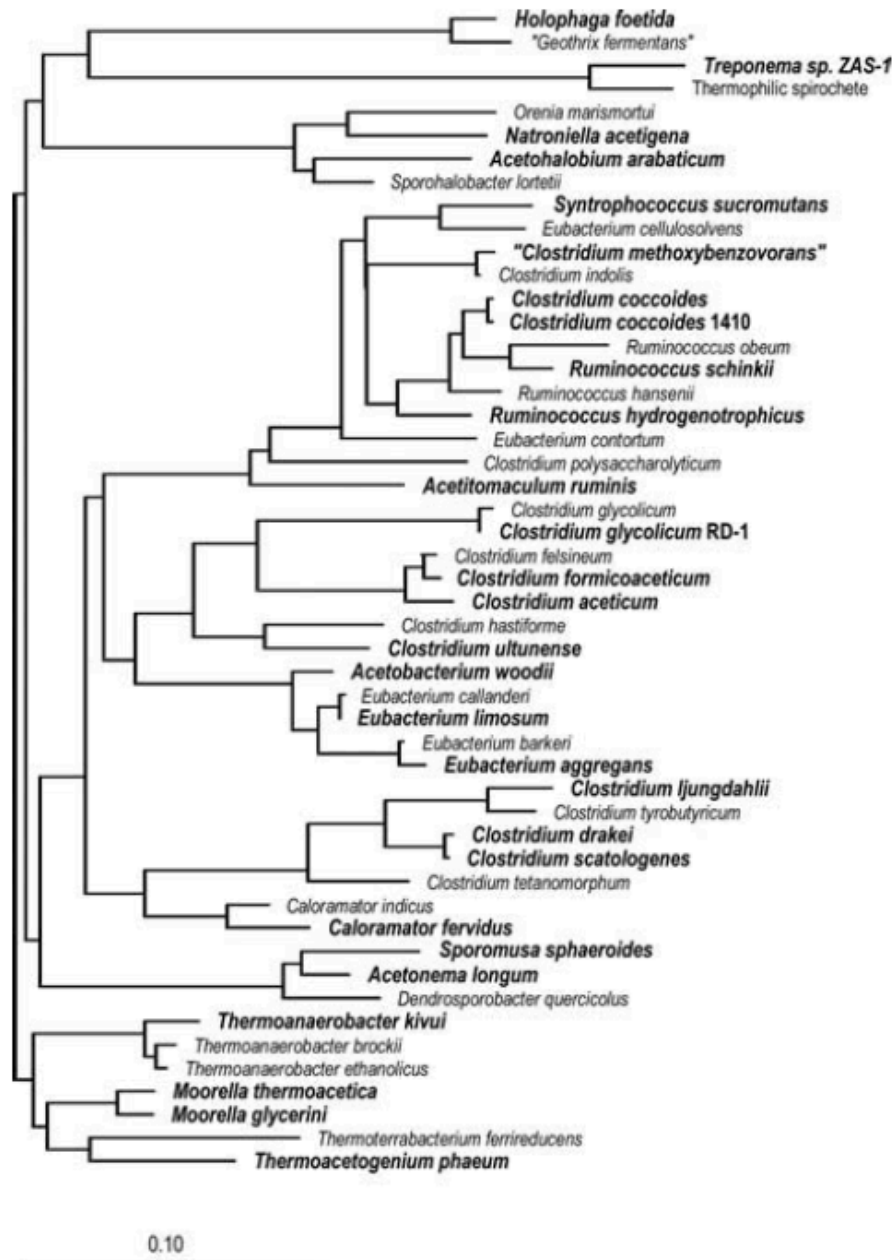
Figure 7. Parsimony tree of selected acetogenic bacteria (*bold font*) and their closest nonacetogenic relatives (*nonbold font*) based on full-length 16S rRNA sequences. *Bar* corresponds to 10 nucleotide substitutions per 100 sequence positions. (From Drake *et al*.[41,42] and used with the kind permission of Springer.)

Acetogens have been isolated from very diverse habitats ([Table 1](#)), including sediments (e.g., *A. woodii* from blackish sediment of a marine estuary[90] and *H. foetida* from freshwater sediment[84]), soils (e.g., psychrotrophic *Acetobacterium tundrae* from tundra soil and Antarctic surficial material,[91,92] the mesophile *Sporomusa silvacetica* from forest soil,[93] and the thermophile *M. thermoacetica* from Egyptian and Kansas soils that experience thermophilic temperatures[14–16,94]), the subsurface (e.g., strain SS1 and "*Acetobacterium psammolithicum*" from subsurface sediment and sandstone, respectively[95,96]), acidic coal mine ponds (e.g., *C. drakei*[47,89]), salt-lake soda deposits (e.g., *Natroniella acetigena* from the soda deposits at Lake Magadi, Kenya[97]), fecal material (e.g., *C. coccoides* and *Bryantella formatexigens* from human feces,[86,98]*Treponema primitia* from the termite hindgut,[99,100] and *C. ljungdahlii* from chicken manure/waste[83,101]), and estuarine and salt-marsh plants (e.g., *C. glycolicum* RD-1 from the roots of the sea grass *Halodule wrightii*[87] and *Sporomusa rhizae* from the roots of the needlerush *Juncus roemerianus*[102]).

The diverse habitat range of acetogens demonstrates that organisms that can utilize the acetyl-CoA pathway are adapted to a broad range of *in situ* conditions. Many acetogens are spore-formers, a feature that likely aids in their *in situ* survival. Indeed, spores of *M. thermoacetica* have a decimal reduction time (i.e., the time required to decrease the population of viable spores by 90%) of nearly 2 hours at 121°C.[103] Some acetogens have connecting filaments ([Fig. 8](#)), structures that might aid cells in remaining close to one another for structural or communication purposes.
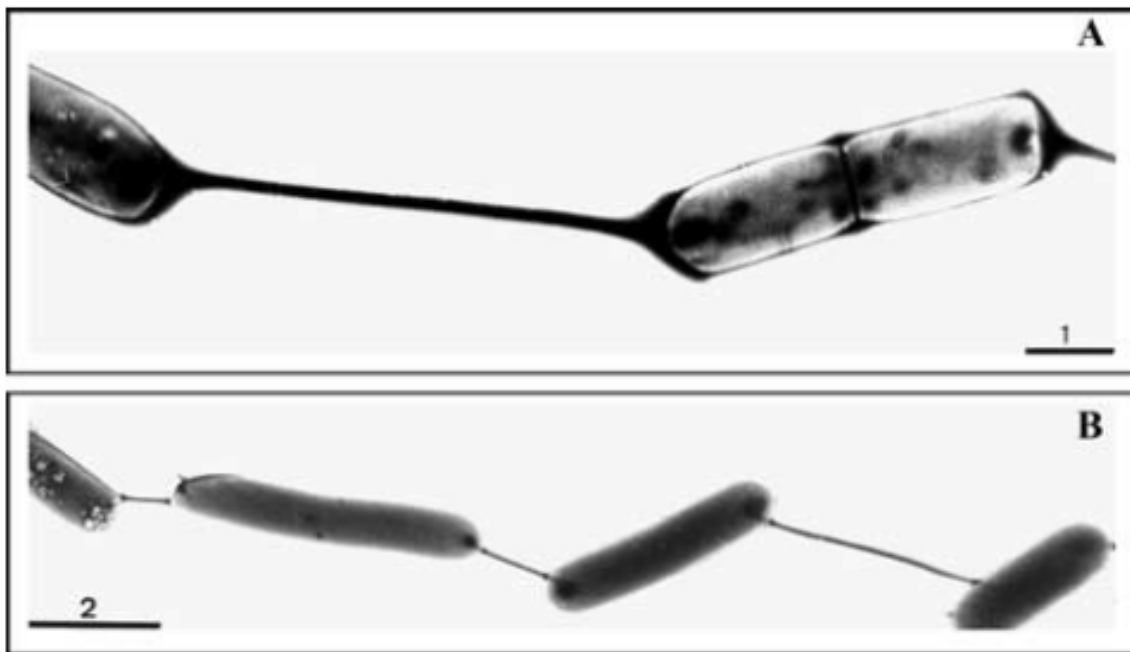
Figure 8. Electron micrographs of (**A**) the acetogen *Clostridium glycolicum* RD-1. (From Küsel *et al*.[87] and used with the kind permission of the American Society for Microbiology.) (**B**) The nitrogen-fixing bacterium *Clostridium akagii.* (From Drake *et al*.[41,42] and used with the kind permission of Springer.) Both organisms have the

potential to form connecting filaments that tether cells to each other. The ultrastructure of connecting filaments is shown.[87,181,182] *Bars* are in micrometers.

The usage of the acetyl-CoA pathway for autotrophic assimilation of carbon and acetate utilization by methanogens,[1,2,104,105] and the apparent reversibility of the pathway in some organisms (e.g., strain AOR[106] and *T. phaeum*[107]) makes it likely that Archaea exist that can grow via acetogenesis. Indeed, recent evidence demonstrates that the methanogenic archaeon *Methanosarcina acetivorans* C2A uses the acetyl-CoA pathway to convert carbon monoxide (CO) to both acetate and methane, that is, methane is not the sole reduced end-product of this methanogen under certain conditions (Fig. 9).[52,108] Similar observations have been made with the archaeon *Archaeoglobus fulgidus* VC16, that is, this archaea can grow via CO-dependent acetogenesis.[109] The capacity of anaerobic archaea to consume and oxidize CO has been known for decades,[110] and it is not without interest that this domain possesses organisms capable of engaging the acetyl-CoA pathway for the synthesis of acetate and the conservation of energy. Future studies must determine if these potentials occur under *in situ* environmental conditions, that is, are of ecological significance. The phylogenetic diversity of such organisms must likewise be determined.
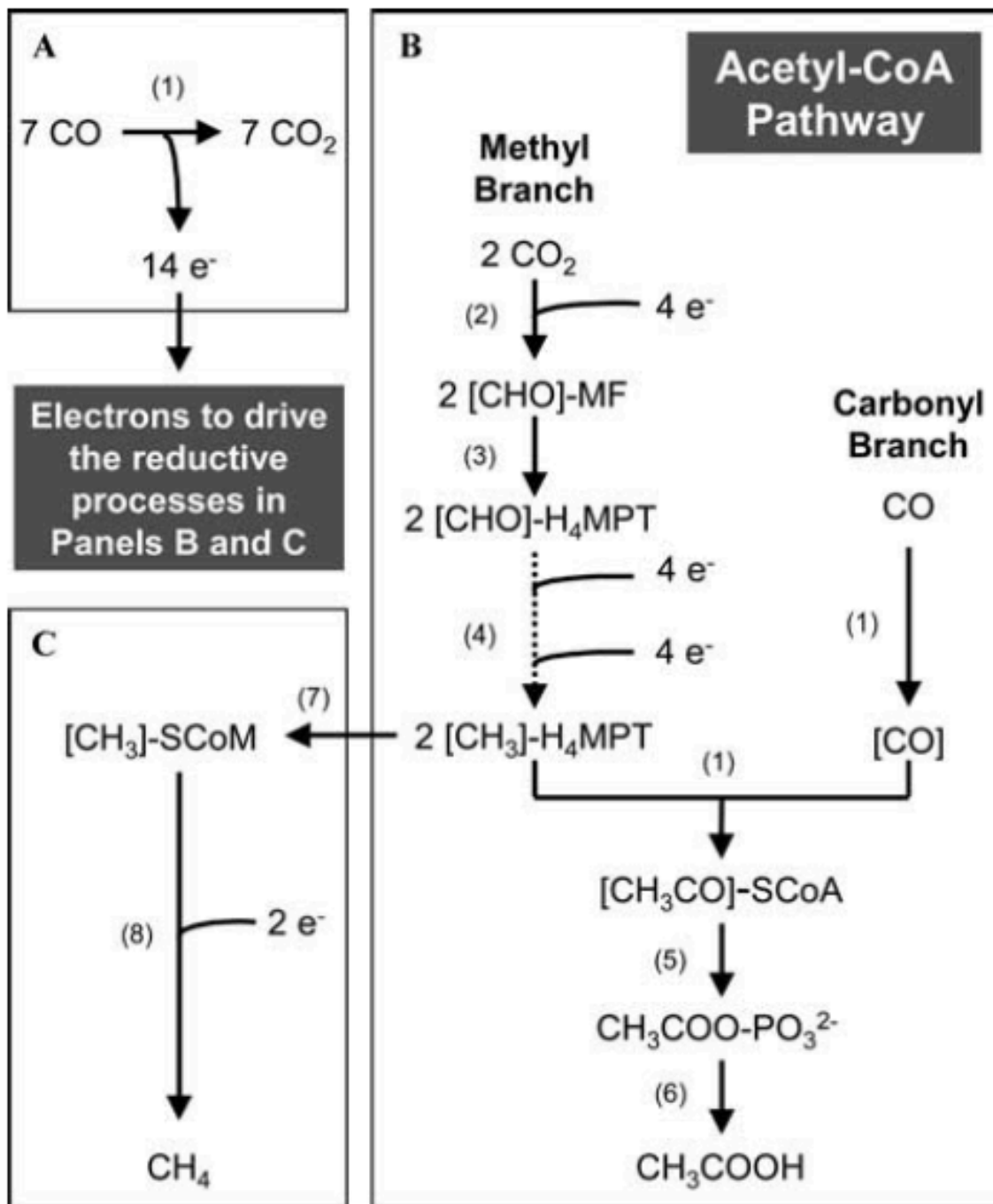
Figure 9. Metabolic processes by which carbon monoxide is utilized by *Methanosarcina acetivorans*. (**A**) Oxidation of carbon monoxide; (**B**) reductive synthesis of acetate via the acetyl-CoA pathway; (**C**) production of methane. *Parenthetical numbers* identify enzymes that catalyze the indicated reactions: 1, CO dehydrogenase/acetyl-CoA synthetase; 2, formyl-methanofuran dehydrogenase; 3, formyl-methanofuran:H$_4$MPT formyltransferase; 4, combined activities of methenyl-H$_4$MPT cyclohydrolase, methylene-H$_4$MPT dehydrogenase; methylene-H$_4$MPT reductase; 5, phosphotransacetylase; 6, acetate kinase; 7, methyl-H$_4$MPT:CoM methyltransferase; 8, combined activities of methyl-CoM reductase, membranous heterodisulfide reductase, and membranous F$_{420}$H$_2$ dehydrogenase complex (F$_{420}$, coenzyme F$_{420}$). Abbreviations: MF = methanofuran; H$_4$MPT =

tetrahydromethanopterin; HSCoM = coenzyme M. [The figure is based on information in Lessner et al.[52] (further details on how these processes are coupled to the chemiosmotic conservation of energy can be found in this reference).]

Functional Diversity of Acetogenic Prokaryotes

The large phylogenetic diversity of acetogens, as well as the nonmonophyletic nature of many acetogenic genera, make it likely that acetogens possess broad functional diversity. Indeed, in contrast to the narrow substrate range of methanogens, acetogens utilize a wide variety of electron donors and electron acceptors (Fig. 10) and can engage alternative terminal electron-accepting processes when challenged with O_2. Thus, acetogens catalyze a variety of redox reactions by which they create fusion points in the carbon and other biological cycles (referred to below as "intercycle coupling").
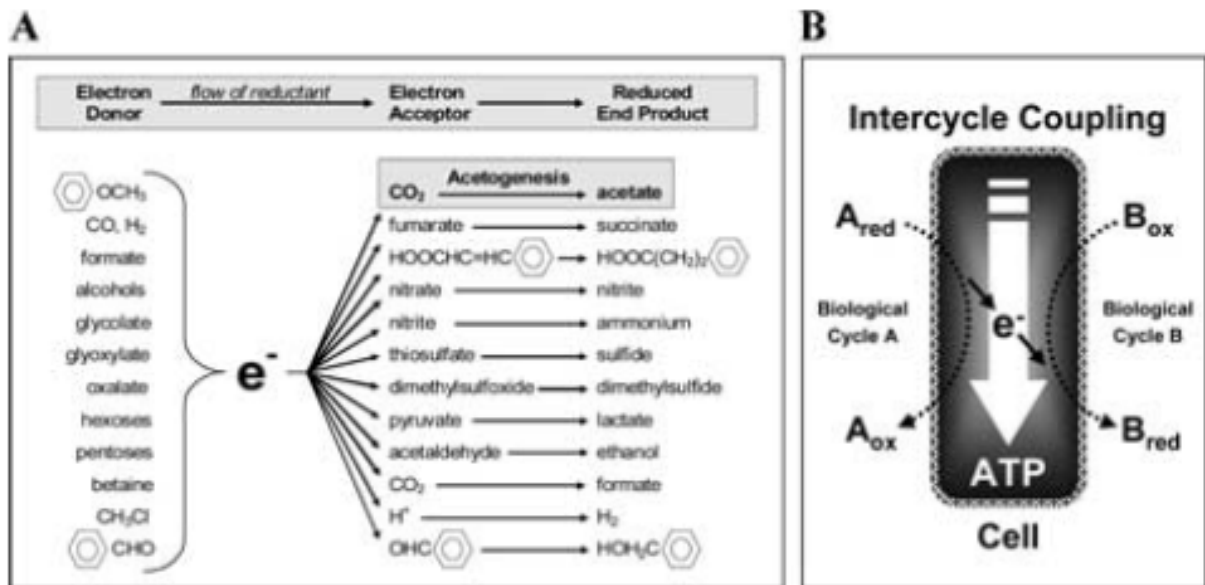
Figure 10. (**A**) Diverse redox couples that can be utilized by acetogens. For a more complete list of electron donors, see Drake et al.[41,42] Abbreviations: e⁻ = reducing equivalent. (**B**) Intercycle coupling (see text). (Modified from Drake et al.[122] and Drake and Küsel[40] and used with the kind permission of IOS Press and CRC Press.)

Electron Acceptors and Intercycle Coupling

CO₂ and Acetogenesis

The growth of acetogens can be impaired when exogenous CO_2 is not available.[18,22,111,112] Given the fact that CO_2 is the terminal electron acceptor during acetogenesis (i.e., the reductive synthesis of acetate from CO_2), the importance of the availability of CO_2 might seem obvious. However, the stoichiometry of glucose-dependent acetogenesis (i.e., three acetate produced per glucose consumed) does not require CO_2 (i.e., CO_2 is not a substrate in the reaction). However, sugars are often not utilized optimally (indeed, might not be used at all) in the absence of supplemental CO_2. During glycolysis, oxidative reactions precede the decarboxylation of pyruvate (which yields CO_2). Thus, the recycling of electron

carriers often necessitates an adequate supply of supplemental CO_2. Consistent with the flow of carbon predicted in Figure 6, growth of *M. thermoacetica* with uniformly labeled [^{14}C]glucose yields the following percent relative distribution of recovered ^{14}C: 31%[^{14}C]carbonates/CO_2, 62%[^{14}C]acetate, and 6%[^{14}C]biomass (Martin and Drake, unpublished data). Furthermore, large amounts of exogenous CO_2 are reduced to acetate during glucose-dependent acetogenesis.[113] The decarboxylation of carboxylated aromatic compounds by certain acetogens can augment the availability of CO_2 for acetogenesis.[112,114] Likewise, carbonic anhydrase might optimize the availability of intracellular CO_2 in acetogens.[115]

Alternative Electron Acceptors and Intercycle Coupling

Many acetogens can utilize one or more terminal electron-accepting processes in addition to acetogenesis (Fig. 10A). For example, nitrate is the preferred electron acceptor for *M. thermoacetica* and is dissimilated to nitrite and ammonium.[116,117] Closely related acetogens differ in their ability to utilize alternative electron acceptors. For example, the thermophilc acetogen *Moorella glycerini* is a close relative of *M. thermoacetica* but does not dissimilate nitrate.[118]

Nitrate dissimilation of *M. thermoacetica* is noteworthy, since the standard redox potential of the CO_2/acetate half-cell reaction is –290 mV (classically thought of as essential to the growth of this acetogen), while that of the nitrate/nitrite half-cell reaction is 430 mV. Indeed, not only is nitrate dissimilation preferred to acetogenesis, growth of *M. thermoacetica* is significantly enhanced during nitrate dissimilation.[116,117] The dissimilation of nitrate significantly increases the growth efficiency of *M. thermoacetica*. H_2-dependent growth yields are enhanced eightfold when nitrate, rather than CO_2, is utilized as a terminal electron acceptor.[116] This enhancement of growth is consistent with the thermodynamics of these alternative terminal electron-accepting processes. The standard change in Gibbs free energy for H_2-dependent dissimilation of CO_2 to acetate via the acetyl-CoA pathway is –95 kJ per mol reaction, while that of the H_2-dependent dissimilation of nitrate to ammonium is –600 kJ per mol reaction.[119] *M. thermoacetica* cannot use ethanol or *n*-propanol as substrates for acetogenesis. However, both ethanol and *n*-propanol are readily utilized as electron donors when nitrate is available for dissimilation.[116] Nitrite can also be used by *M. thermoacetica* as an energy-conserving terminal electron acceptor.[120] These observations (1) demonstrate that *M. thermoacetica* is a facultative nitrate dissimilator rather than a so-called homoacetogen, (2) suggest that the acetogenic nature of this classic, model acetogen might have been overlooked if it had been isolated with a nitrate-rich cultivation medium, and (3) demonstrate that the acetyl-CoA pathway in *M. thermoacetica* is not constitutive. A membranous *b*-type cytochrome that is required on the methyl branch of the acetyl-CoA pathway is not present in the membrane when *M. thermoacetica* is grown in the presence of nitrate.[116] This cytochrome deficiency in the membrane appears to disable the acetyl-CoA pathway (Fig. 11). There are conflicting reports on the occurrence of active forms of the enzymes of the acetyl-CoA pathway in nitrate-dissimilating cells.[116,121] This matter has recently been addressed[94]:
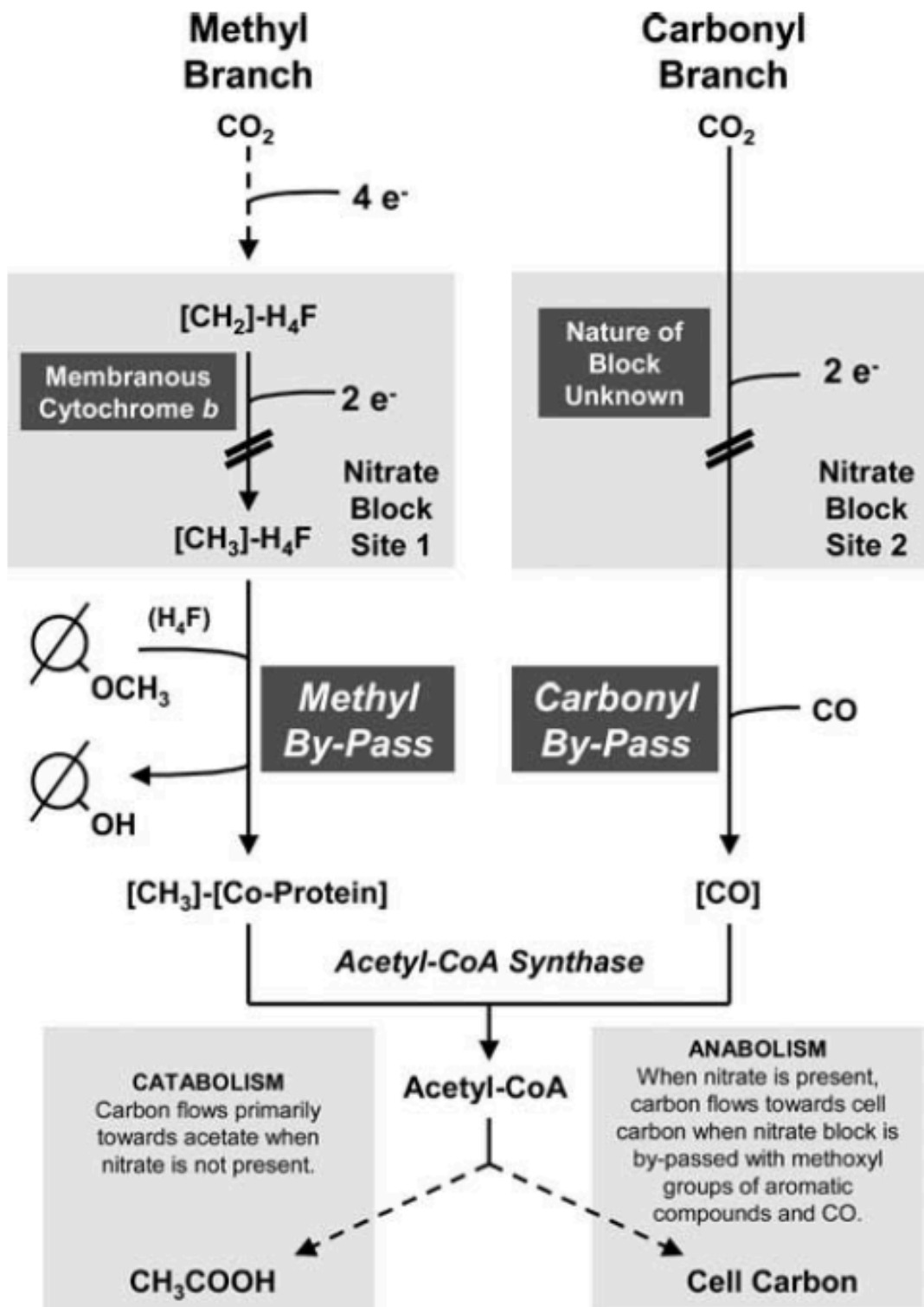
Figure 11. Scheme illustrating the proposed two major sites where the acetyl-CoA pathway is blocked when nitrate is dissimilated to ammonium by *M. thermoacetica*. The dissimilation of nitrite to ammonium appears to have the same

affect.[120] Abbreviations: H₄F = tetrahydrofolate; CoA = coenzyme-A; Co-Protein = corrinoid enzyme. (Modified from Drake and Küsel[40] and Drake and Daniel[94] and used with the kind permission of Springer and Elsevier.)

The acetyl-CoA pathway is linked to anabolism, and the cell's inability to form acetyl-CoA from CO_2 (required for the assimilation of carbon when CO_2 is the sole source of carbon) during the dissimilation of nitrate is overcome with preformed methyl and carbonyl groups.[116] 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_2 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.[40,41,122]*

The engagement of diverse redox couples enables acetogens to catalyze intercycle coupling, that is, to form junction points within and between biological cycles (Fig. 10B).[40] For example, the H_2-dependent reduction of CO_2 to acetate links the hydrogen and carbon cycles, and the oxidation of organic substrates via the dissimilation of nitrate fuses the carbon and nitrogen cycles. Intercycle coupling not only provides the cell with a means of conserving energy but forms a basis for linking biological cycles at the ecosystem level.

Not all of the redox couples illustrated in Figure 10A conserve energy. For example, the reduction of aldehyde groups of aromatic compounds appears to merely vent excess reductant under certain conditions and is not coupled to the conservation of energy. Nonetheless, their ability to facilitate a diverse number of redox reactions means that acetogens form a large variety of reduced end-products and not just acetate. As noted earlier, (1) exogenous CO_2 might determine how efficiently a particular electron donor is metabolized by an acetogen, and (2) an acetogen may utilize alternative terminal electron acceptors in preference to CO_2. However, an acetogen might also use multiple terminal electron acceptors simultaneously. For example, the acetogen *Ruminococcus productus* simultaneously reduces phenylacrylates to phenylpropionates and CO_2 to acetate,[123] and also reduces CO_2 to acetate concomitant to lactate fermentation during growth at the expense of fructose.[124] *A. woodii* likewise has the potential to simultaneously utilize phenylacrylates (e.g., caffeate) and the acetyl-CoA pathway as terminal electron-accepting processes.[125] However, these two terminal processes might be selectively engaged by certain electron donors (e.g., *A. woodii* preferentially uses acetogenesis when reductant is derived from methanol).

Interspecies H_2 Transfer

Although energy might not be conserved by the reduction of protons when pure cultures of acetogens vent H_2 as a trace byproduct of acetogenesis, H_2-producing acetogens (e.g., *A. woodii*) can form close trophic associations with H_2-consuming partners.[126] Such participation in interspecies H_2 transfer suggests that the production of H_2 by acetogens can be coupled to the conservation of energy under certain conditions. In contrast, acetogens from certain gastrointestinal tract systems

(e.g., human and termite) can be the H_2-consuming partner in the interspecies transfer of H_2.[127–129]

Electron Donors

It is not possible that Fotaine and his co-workers could have known that the organism they would isolate, *M. thermoacetica*, would become not only the organism from which the acetyl-CoA pathway would be resolved (Table 2), but would also be the most metabolically robust acetogen characterized to the present date. Because the constraints of this presentation will not provide for an elaborate assessment of the diverse catabolic potentials of all of the acetogens known to date, this section will concentrate mostly on the ability of *M. thermoacetica* to activate reductant from diverse substates.

Most of the acetogens isolated to date can utilize hexoses or pentoses for growth and acetogenesis.[41,42,130,131] Tracer studies demonstrated that the glycolytic Embden–Meyerhof–Parnas (EMP) pathway is operative in *M. thermoacetica*.[132] However, there is limited information on the ability of acetogens to metabolize polymers (e.g., cellulose and lignin). Most acetogens isolated to date do not appear to be able to degrade high-molecular-weight polymers. However, a cellulose-degrading strain of *M. thermoacetica* was recently reported,[133] suggesting that the genus *Moorella* might contain cellulolytic strains. The acetogen *B. formatexigens* initially had the ability to use amorphous cellulose, but this ability was lost after prolonged growth under laboratory conditions.[98] It thus seems likely that future studies will resolve additional strains of acetogens that are able to use polymers of monosaccharides. Although most of the acetogens isolated to date are unable to degrade aromatic rings, the ability of the acetogen *H. foetida* to degrade aromatic rings[84,134,135] indicates that some acetogens have this metabolic potential. The degradation of lignin by an acetogen has not been reported.

In contrast to the chemical complexity of polymers, H_2 is chemically the simplest source of reductant in nature, and H_2 is the only noncarbonaceous source of reductant that is currently known to be growth supportive for acetogens. Although H_2-dependent autotrophic acetogenesis is a distinguishing feature of acetogens, H_2-dependent growth (i.e., cell yields) is usually poor, and the ability of an acetogen to grow autotrophically at the expense of H_2 might therefore go undetected when the organism is first isolated (as was the case with *M. thermoacetica*[136,137]). Acetogens can contain multiple hydrogenases, and activity levels can vary with growth conditions.[136,137] Hydrogenase can be expressed during heterotrophic growth, but its physiological role under such conditions is unclear. During the heterotrophic dissimilation of nitrate by *M. thermoacetica*, the specific activity of hydrogenase is 14-fold lower than it is when growth is coupled to heterotrophic acetogenesis,[116] suggesting that hydrogenase is indeed important to the heterotrophic growth of this acetogen. Oxidoreductases that are detected by standard hydrogenase assays might be involved in intracellular reductant flow rather than the consumption or production of extracellular H_2, as has been proposed for the hydrogenase activity detected during the heterotrophic growth of *M. thermoacetica*.[94]

The one-carbon nature of the acetyl-CoA pathway provides for the efficient use of both one-carbon substrates and one-carbon side chains of aromatic compounds. For example, *M. thermoacetica* can use CO, formate, and methanol as sources of reductant, and is also able to utilize the methoxyl groups of a wide variety of aromatic compounds (e.g., 1,2,3-trimethoxybenzene, 4-hydroxy-3-methoxybenzyl alcohol, 2,3-dimethoxybenzoate, and 2,6-dimethoxyphenol).[94] One-carbon substrates can be either oxidized or be directly assimilated into the acetyl-CoA pathway. For example, as can be visualized in <u>Figure</u> 5, formate and CO can enter the methyl or carbonyl branches of the pathway, respectively, or be oxidized as sources of reductant. Methanol and methyl-level groups are disproportionated when utilized for acetogenesis.
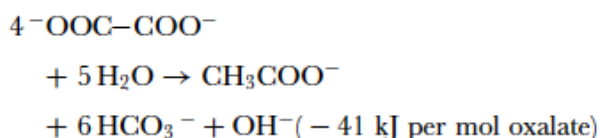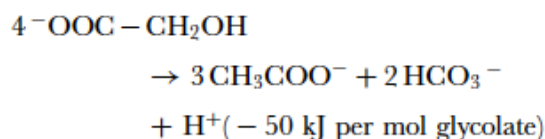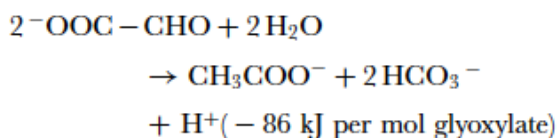
CO-dependent acetogenesis yields the following stoichiometry:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$$
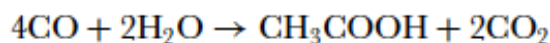
During this specialized process, three molecules of CO are oxidized to CO_2, yielding six electrons that are then used to reduce CO_2 on the methyl branch of the acetyl-CoA pathway. CO enters the carbonyl branch directly as a preformed carbonyl-level molecule. Thus, during CO-dependent acetogenesis, the methyl group of acetate is derived from CO_2, while the carboxyl group of acetate is derived from CO.[113] Traces of H_2 and methane can be produced during CO-dependent growth.[111]

Acetogens can oxidize a variety of alchohols and organic acids. For example, *M. thermoaetica* can utilize methanol, ethanol, *n*-propanol, *n*-butanol, formate, oxalate, glyoxylate, glycolate, pyruvate, and lactate.[94] As noted earlier, the potential to oxidize a particular substate may be dependent upon the electron acceptor utilized (e.g., short-chain alcohols are not growth supportive for *M. thermoacetica* when CO_2 is used as a terminal electron acceptor, but are growth supportive when nitrate is used as an terminal electron acceptor).

Although the use of glyoxylate, glycolate, and oxalate has not been widely demonstrated in acetogens or other anaerobes, these two-carbon compounds are readily used by *M. thermoacetica* according to the following reactions (kJ per mol calculated from Thauer *et al.*[119])[138,139]:

$$2\,{}^-OOC - CHO + 2\,H_2O$$
$$\rightarrow CH_3COO^- + 2\,HCO_3{}^-$$
$$+ H^+(-86 \text{ kJ per mol glyoxylate})$$

$$4\,{}^-OOC - CH_2OH$$
$$\rightarrow 3\,CH_3COO^- + 2\,HCO_3{}^-$$
$$+ H^+(-50 \text{ kJ per mol glycolate})$$

$$4\,{}^-OOC{-}COO^-$$
$$+ 5\,H_2O \rightarrow CH_3COO^-$$
$$+ 6\,HCO_3{}^- + OH^-(-41 \text{ kJ per mol oxalate})$$

The enzyme system by which *M. thermoacetica* catabolizes oxalate requires a utilizable electron acceptor.[140] Glyoxylate, glycolate, and oxalate appear to be metabolized by different mechanisms in *M. thermoacetica*. For example, glyoxylate and oxalate are utilized when nitrate is dissimilated, whereas glycolate is not utilized under this condition.[120,139,141] Reducing equivalents that are theoretically derived from these two-carbon substrates appear to be managed differently and yield dissimilar growth efficiencies.[94]

Response to O_2 and Oxidative Stress

Acetogens have been classically referred to as obligate, if not strict, anaerobes. Indeed, many enzymes central to acetogenesis are extremely sensitive to O_2 (i.e., oxidation), and the decades of work on resolving the acetyl-CoA pathway were very much impaired by this sensitivity. During those years, it was certainly logical to assume that acetogens are strict anaerobes. However, acetogens have been isolated from redox-unstable environments. Indeed, aerated soils and the rooting zones of estuarine and salt-marsh macrophytes that experience periods of O_2 enrichment harbor high numbers of culturable acetogens, including classic and novel acetogenic species.[14,93,102,142,143] Acetogens in redox-unstable habitats are likely challenged with O_2 and must cope with periods of oxidative stress. Thus, and in retrospect of the fact that the first acetogen, *C. aceticum*, was isolated from soil that was likely subject to periodic wetting and drying (i.e., aeration),[5,6] it is not surprising that recent studies have demonstrated that acetogens have several adaptation strategies by which they might deal with oxidative stress under *in situ* conditions (Fig. 12).
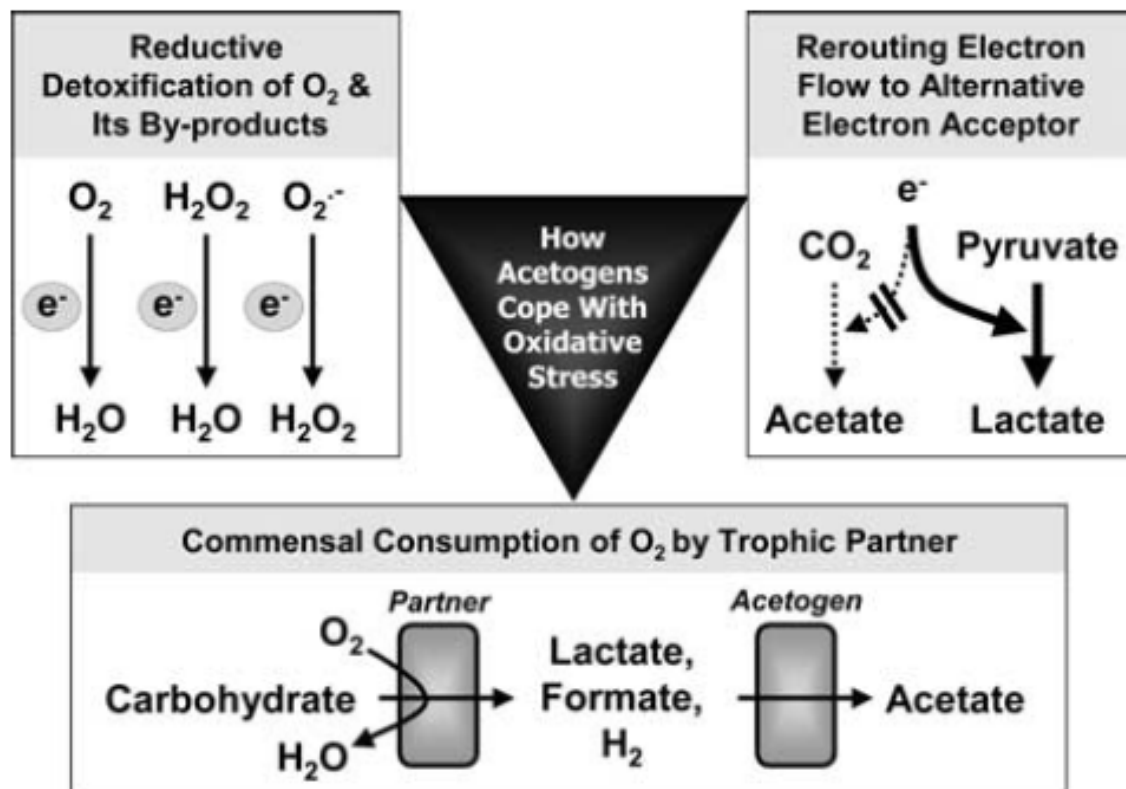
Figure 12. Mechanisms by which acetogens cope with oxidative stress. Abbreviations: X = products (e.g., H_2, formate, lactate) that are derived from the

partial oxidation of carbohydrates [in some cases, short-chain polymers (e.g., stachyose) that are not substrates for the acetogen]; e⁻ = electron. (Modified from Müller et al.[77] and used with the kind permission of Horizon Bioscience.)

Reductive Removal of O$_2$

Acetogens contain numerous enzymes that can reductively remove O$_2$ and its toxic byproducts (e.g., superoxide and peroxide). These enzymes include peroxidase, reduced nicotinamide adenine dinucleotide (NADH) -oxidase, rubredoxin oxidoreductase (a superoxide reductase), rubrerythrin (a peroxidase), superoxide dismutase, catalase, and cytochrome *bd* oxidase.[144–147] These enzymes are effective in protecting acetogens from oxidative stress when the concentration of O$_2$ is relatively low.

Use of Alternative Electron Acceptors in Response to O$_2$

As noted earlier, acetogens can utilize a variety of terminal electron acceptors. Thus, some acetogens can shift reductant flow away from the acetyl-CoA pathway to alternative terminal electron-accepting processes that are less sensitive to O$_2$ and operate at higher redox potentials than does the acetyl-CoA pathway (as noted earlier, the standard redox potential of the CO$_2$/acetate half-cell reaction is −290 mV). For example, *C. glycolicum* RD-1 (isolated from sea-grass roots) is an aerotolerant acetogen that switches from acetogenesis to classic fermentation in response to O$_2$.[87] This acetogen tolerates up to 4% O$_2$ in the headspace of agitated cultures, during which sugars are metabolized via combined lactate–ethanol fermentation. The high standard redox potential of the nitrate/nitrite half-cell reaction (430 mV) suggests that nitrate dissimilation by *M. thermoacetica* would be less sensitive to O$_2$ than acetogenesis.

Trophic Interaction with O$_2$-consuming Partner

Acetogens can form symbiotic relationships with O$_2$-consuming microaerophiles and aerotolerant fermenters. Such relationships have been observed between the acetogen *M. thermoacetica* and the fermentative microaerophilic bacterium *Thermicanus aegyptius* (two thermophiles initially isolated as a co-culture from Egyptian soil[14]), and the acetogen *S. rhizae* and the aerotolerant fermenter *Clostridium intestinale* (two mesophiles initially isolated as a coculture from the roots of the needlerush *J. roemerianus*[102]). In both cases, a fermentative nonacetogen that has the capacity to consume O$_2$ (thus protecting the acetogen from O$_2$) while simultaneously forming fermentation products (e.g., lactate, formate, and H$_2$) that can be used by the acetogen for acetogenesis. Although such partnerships between acetogens and O$_2$-consuming microorganisms have only been documented with laboratory cultures, such interactions might constitute a basis by which certain acetogens are protected from oxidative stress and form trophic linkages to other microorganisms under *in situ* conditions.

Harnessing the Functional Talents of Acetogens

Acetic acid is an important chemical. Its commercial production at the global level in 2001 approximated 10^{10} kg, and numerous studies have evaluated the potential use of acetogens to produce acetic acid or a salt thereof.[41,148–155] The acetogenic conversion of synthesis gas (i.e., H_2, CO, and CO_2) to acetic acid, ethanol, and butanol has also been investigated.[83,156–158] Unfortunately, acetogens are sensitive to acetate and acidic conditions. High concentrations of acetate and protons inhibit the growth of acetogens, mainly because a proton motive force and transmembrane electrical potential cannot be maintained under such conditions.[159] These limitations have hampered the commercialization of acetogens. Nonetheless, the existence of acetogens in acidic habitats (e.g., *C. drakei*[47,89]) suggests that new acetogens might be found that have higher tolerance to acidic conditions.

Despite the broad physiological activities of acetogens, their potential to degrade high-molecular-weight polymers (e.g., cellulose and lignin) appears to be limited. This limitation constitutes another problem for commercializing acetogenesis. However, normal strategies for isolating acetogens do not take such growth potentials into consideration. It is therefore noteworthy that two acetogens, *B. formatexigens*[98] and *M. thermoacetica* strain F21,[133] have recently been shown to degrade cellulose. Co-cultures of anaerobes might also offer promise for application. For example, cocultures of the cellulolytic thermophile *Clostridium thermocellum* and the thermophilic acetogen *Thermoanaerobacter kivui* can produce acetate from cellulose.[160] Cocultures of the cellulolytic mesophile *Ruminococcus albus* and the mesophilic acetogen HA have similar potentials; in coculture, the reducing equivalents derived from cellulose are utilized by HA via interspecies H_2 transfer.[161]*Clostridium lentocellum* strain SG6 forms high amounts of acetate from cellulose, and product stoichiometries suggest that this organism might utilize CO_2 as a terminal electron acceptor via the acetyl-CoA pathway.[162] However, it appears that significant amounts of acetyl-CoA are reduced to ethanol. The metabolism of such organisms might offer new strategies for conversion of cellulose to commercially useful chemicals.

The recovery of acetate from cultivation broths is another problem relative to the commercialization of acetogenesis. The concentration of acetate formed by acetogens is relatively low do to its inhibitory effects on growth. New strategies to efficiently recover acetate from cultivation broths might circumvent this problem.[163,164]

Acetogens and the enzymes that they produce might be useful in the bioremediation of certain anthropogenic compounds (e.g., trinitrotoluene)[165–167] or the production of fine chemicals (e.g., corrinoids) and enzymes (e.g., acetate kinase).[41,168–171] However, a commercial application of these potentials has not been reported.

Ecological Impact of Acetogens and the Acetyl-CoA Pathway

It is beyond the scope of this chapter to evaluate in detail the ecology of acetogens and acetogenesis. However, the following generalizations highlight both the ecological importance of acetogens and acetyl-CoA pathway, and also identify some of the challenges that future studies will be confronted by in this area.

In Situ Information on Acetogens

It has been estimated that 10^{12} kg of acetate are synthesized per year in sediments via acetogenesis.[24] Likewise, it has been estimated that 10^{12} kg of acetate are produced annually via acetogenesis in the hindgut of termites, a number that is fivefold greater than the annual amount of methane produced via the methanogenic reduction of CO_2.[172] Despite such estimations that make the ecological importance of acetogens seem obvious, assessing the *in situ* activity of acetogens is extremely problematic. Measuring the turnover of acetate is complicated, and, as noted earlier, acetogens catalyze a large number of redox reactions (i.e., the production of acetate is very likely not their only *in situ* activity). Plus, their interactions with other microbes is not restricted to carbon flow, but is also coupled to interspecies transfer of nutrients (e.g., folates).[173] Thus, although acetate is quantitatively an important trophic link in a wide variety of ecosystems, and although acetogens are important players in the carbon flow of many ecosystems, *in situ* information on acetogens is often conceptual.

Molecular Analysis

As noted earlier, acetogens are not monophyletic, in that many genera that contain acetogens also contain nonacetogens. Thus, broad-based analysis of acetogens as a distinct functional group by 16S rRNA-based approaches is problematic. Nonetheless, highly specific 16S rRNA-based probes and primers designed to target subsets of acetogenic taxa (e.g., a genus that only contains acetogens) have been developed (e.g., Küsel *et al.*[143]). Molecular approaches that are based on the analysis of functional genes central to the acetyl-CoA pathway (e.g., a gene for formyltetrahydrofolate synthetase) have also been developed for analyzing acetogenic bacteria.[174,175] Although these functional gene approaches have great promise and utility (e.g., Salmassi and Leadbetter,[176] Pester and Brune[177]), they, too, are compromised by problems of specificity.[178]

Phylogenetic and Global Distributions of the Acetyl-CoA Pathway

The *in situ* importance of the acetyl-CoA pathway is reflected in its wide distribution. As noted earlier, various forms of the acetyl-CoA pathway are used by both Bacteria and Archaea. However, an understanding of the distribution of the acetyl-CoA pathway is incomplete, as is an understanding of how the acetyl-CoA pathway is utilized in Prokaryotes. Recent evidence suggests that there are yet to be discovered processes that link the acetyl-CoA to the carbon flow of certain habitats. As noted earlier, the methanogen *M. acetivorans* uses the acetyl-CoA pathway to convert CO to both acetate and methane, that is, methane is not the sole reduced end-product of this methanogen (Fig. 9).[52,108] Likewise, the archaeon *A. fulgidus* VC16 can grow via CO-dependent acetogenesis.[109] Such fascinating observations point toward new ways in which the acetyl-CoA pathway might be metabolically linked to the carbon cycle via prokaryotic microbes.

Primary Production

Because the acetyl-CoA pathway and variants thereof facilitates the fixation of CO_2 in both domains of the Prokayotes, the pathway might be important to primary production in certain habitats. As reviewed elsewhere,[41,42,179,180] there is compelling evidence for that in the deep subsurface. Given the likelihood that chemical variations of the acetyl-CoA pathway were important to the evolution of life, that is, to early processes that were coupled to the fixation of carbon and the synthesis of organic molecules (see the second section of this chapter), future studies in this area would likely be very rewarding.

Conclusions

Acetogens and acetogenesis are microbiological discoveries. Those who made these early discoveries could not have known that their observations would foster thousands of studies on the biochemical, cellular, ecological, and evolutionary features of acetogens and the acetyl-CoA pathway. As one travels forward, the voyage made and those who were a part of it are well worth remembering. And on the occasion of your 80th birthday, we extend a special thanks to you, Lars.

Acknowledgments

Conflict of Interest

The authors declare no conflicts of interest.

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