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# Old Acetogens, New Light

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# Old Acetogens, New Light

Harold L. Drake, Anita S. Gößner, & Steven L. Daniel

**Keywords:** acetogenesis; acetogenic bacteria; acetyl-CoA pathway; autotrophy; bioenergetics; *Clostridium aceticum*; electron transport; intercycle coupling; *Moorella thermoacetica*; nitrate dissimilation

**Abstract:** Acetogens utilize the acetyl-CoA Wood-Ljungdahl pathway as a terminal electron-accepting, energy-conserving, CO<sub>2</sub>-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<sub>2</sub>-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 <sup>14</sup>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<sub>2</sub> to the acetyl moiety of acetyl-coenzyme A (CoA), for the conservation of energy, and for the assimilation of CO<sub>2</sub> into cell carbon. Initially studied primarily for their novel CO<sub>2</sub>-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<sub>2</sub> into biomass or the oxidation of acetate.<sup>1-3</sup>

Table 1. Acetogenic bacteria isolated to date

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

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

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

AOR	Thermophilic digester	+	Rod	Thermophilic	1988; Lee and Zinder <sup>106</sup>
CS1Van	Human feces	+	Rod	Mesophilic	1993; Wolin and Miller <sup>236</sup>
CS3Glu	Human feces	+	Coccoid rod	Mesophilic	1993; Wolin and Miller <sup>236</sup>
CS7H	Human feces	+	Rod	Mesophilic	1993; Wolin and Miller <sup>236</sup>
D	Rumen fluid, deer	-	Rod	Mesophilic	1995; Rieu-Lesme <i>et al.</i> <sup>237</sup>
DMG58	River mud	-	Rod	Mesophilic	1984; Möller <i>et al.</i> <sup>228</sup>
EE121	Granular reactor sludge	+	Rod	NR	1990; Plugge <i>et al.</i> <sup>238</sup>
HA	Horse feces	-	Coccobacillus	NR	1995; Miller and Wolin <sup>181</sup>
I52	Human feces	-	Coccoid rod	Mesophilic	1994; Wolin and Miller <sup>239</sup>
S5a2	Human feces	+	Coccus	NR	1996; Bernalier <i>et al.</i> , <sup>127</sup> Leclerc <i>et al.</i> <sup>212,213</sup>
Ser8	Rumen, newborn lamb	NR	NR	NR	1995; Chaucheyras <i>et al.</i> <sup>240</sup>
SS1	406-m-deep sediment	+	Oval rod	Mesophilic	1993; Liu and Sufita <sup>96</sup>
TH-001	Sewage sludge	-	Rod	Mesophilic	1985; Frazer and Young <sup>241</sup>
VK64	Human feces	+	Coccus	NR	1996; Bernalier <i>et al.</i> <sup>127</sup>
X-8	Vegetable wastewater	-	Rod	Mesophilic	1982; Samain <i>et al.</i> <sup>242</sup>
ZT	Tundra soil	+	Rod	Psychrophilic	1992; Kotsyurbenko <i>et al.</i> , <sup>243</sup> Nozhevnikova <i>et al.</i> <sup>244</sup>
417/2	Oil field	-	Rod	Mesophilic	1992; Davydova-Charakhchyan <i>et al.</i> <sup>185</sup>
417/5	Oil field	-	Rod	Mesophilic	1992; Davydova-Charakhchyan <i>et al.</i> <sup>185</sup>
"New acetogenic bacterium"	Rumen, 15-hour-old lamb	+	Coccoid rod	Mesophilic	1996; Rieu-Lesme <i>et al.</i> <sup>245</sup>

<sup>a</sup>Bacteria listed appear to use the acetyl-CoA pathway for the synthesis of acetate and growth (modified from Drake,<sup>246</sup> Drake,<sup>3</sup> and Drake *et al.*<sup>41,42</sup>). 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.

<sup>b</sup>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.

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

<sup>d</sup>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<sub>2</sub> are reduced to acetate) by F. Fischer and associates.<sup>4</sup> Their work demonstrated that microbial populations in sewage were competent in the formation of acetate from the H<sub>2</sub>-dependent reduction of CO<sub>2</sub>. 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).<sup>5</sup> This spore-forming, mesophilic bacterium was shown to grow at the expense of H<sub>2</sub>-CO<sub>2</sub> according to the following stoichiometry<sup>5-7</sup>:



**Figure 1**

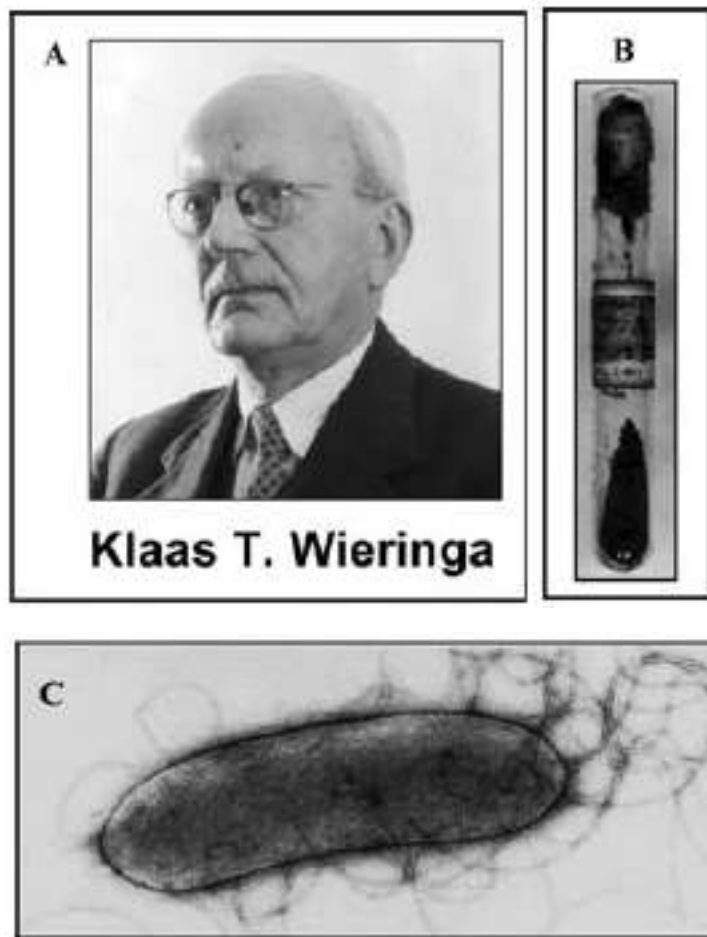


Figure 1. (A) Klaas Tammo Wieringa, who isolated the first acetogen, *Clostridium acetivum*, in 1936.<sup>5</sup> (B) A culture tube, dated 7 May 1947, containing spores of *C. acetivum* in dried soil. The tube was obtained from H. A. Barker and contained spores derived from Wieringa's culture of *C.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

In 1936, this reaction constituted a unique mechanism for the fixation of CO<sub>2</sub>. Unfortunately, the culture of *C. acetivum* was later lost and no further work, except for one study to define its nutritional requirements,<sup>8</sup> was done with this acetogen until it was reisolated in the early 1980s (Fig. 1).<sup>9-11</sup> 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

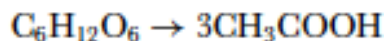
M. W. Beijerinck (Delft; Söhngen's dissertation is dated July 1906)]<sup>12</sup> to join him at the Landbouwhogeschool (Agricultural University). Upon arrival in Wageningen, Wieringa participated in designing and constructing the Laboratory of Microbiology that opened in 1922. Wieringa received his 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.<sup>9-11</sup> 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 collaboration with Söhngen is acknowledged by Wieringa in the introductory comments of his early works on *C. aceticum*<sup>5-7</sup> and is attested to by Wieringa's obituary on Söhngen, who passed away on December 24, 1934.<sup>12</sup> Indeed, it was H<sub>2</sub>-CO<sub>2</sub> methanogenic enrichments that Söhngen and Wieringa had set up before Söhngen's death that yielded subtle hints toward H<sub>2</sub>-dependent acetogenesis. A slight discrepancy in the amount of carbon recovered during the H<sub>2</sub>-dependent formation of methane led Wieringa to conclude that transfers of H<sub>2</sub>-CO<sub>2</sub> methanogenic enrichments must also contain a physiologically new type of organism:

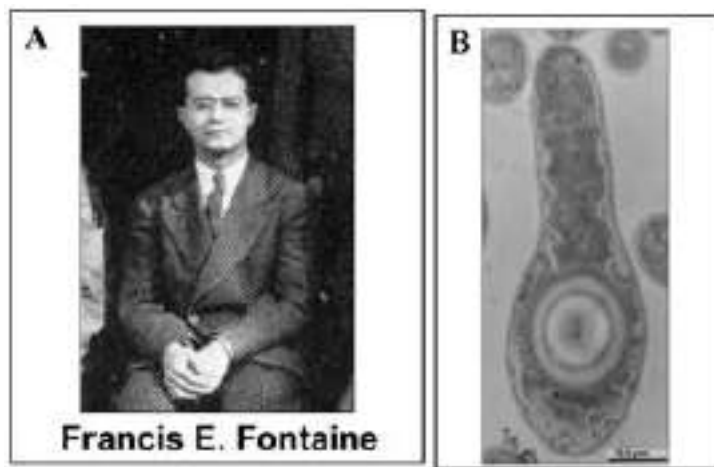
*Now it has been found that subcultures of this type sometimes may absorb large amounts of hydrogen without any formation of CH<sub>4</sub>. Microscopical examination of such cultures shows the presence of club-shaped spore-bearing bacilli.*<sup>6</sup>

With a specially constructed gas-absorption apparatus that encouraged the growth of this novel, nonmethanogenic, H<sub>2</sub>-consuming, spore-forming autotroph, Wieringa was able to enrich and isolate *C. aceticum*.<sup>5</sup> 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)<sup>13</sup>:







**C**

**TABLE 7**  
Summary of glucose fermentations by *C. thermoaceticum*

STRAIN	ORIGINAL GLUCOSE	GLUCOSE FERMENTED	ACETIC ACID	WATER- SOLUBLE GLUCOSE
	mM/100 ml.	mM/100 ml.	mM/100 ml.	
127-1	15.09	3.88	10.12	2.51
14-4-1	14.88	3.96	10.40	2.53
127-2	15.09	8.05	21.00	2.51
70-2a	12.22	8.19	21.00	2.57
56-204	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
180-123	12.24	10.29	26.20	2.54
200-155	12.15	11.26	25.40	2.53
200-154	12.15	11.54	26.05	2.50
Average of 21 fermentations...		8.64	25.06	2.55

Figure 2. (A) Francis Ephraim Fontaine, who isolated the second acetogen, *Clostridium thermoaceticum*, in 1942.<sup>13</sup> 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.*<sup>48</sup>). (From Drake<sup>3</sup> and used with kind permission of Springer.) (C) Original data from Fontaine *et al.*<sup>13</sup> 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),<sup>14-16</sup> and, thus, is widely distributed in soils that experience elevated (thermophilic) temperatures.

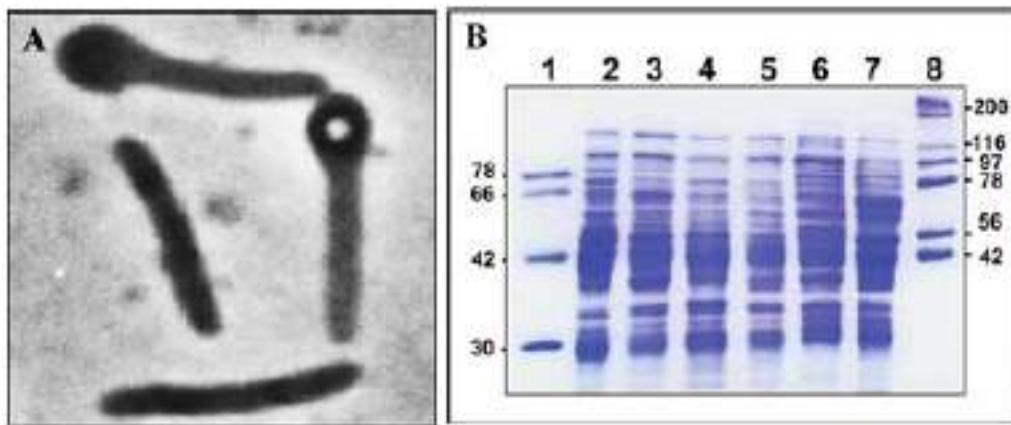


Figure 3. (A) *M. thermoacetica* PT1 (DSM 12993) obtained from Kansas prairie soil. (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis of *M. thermoacetica* isolates obtained from Kansas and Egyptian soils. Cells were cultivated on fructose; Lanes 2–7 are protein profiles 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.*<sup>43</sup>). (Parts (A) and (B) are from Drake and Daniel<sup>94</sup> 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).<sup>17,18</sup> This spore-forming, mesophilic bacterium was isolated from sewage sludge and produced both formate and acetate during glucose-dependent fermentation.<sup>17,18</sup> 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.<sup>19–22</sup> Surprisingly, with all of its metabolic capabilities relative to carbon and reductant flow, *C. formicoaceticum* is unable to grow at the expense of  $H_2-CO_2$ .<sup>18,23</sup>

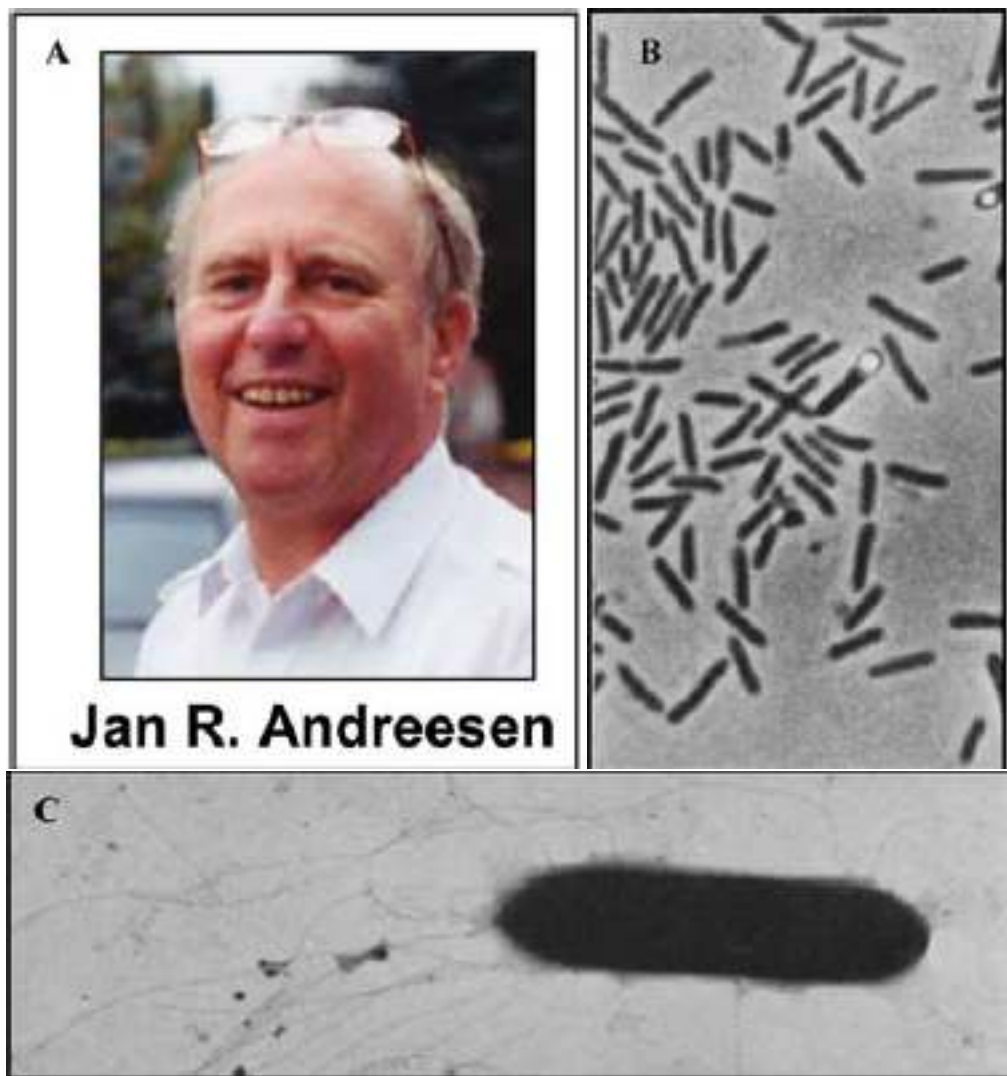


Figure 4. (A) Jan Remmer Andreesen, who in 1970 validated the isolation of the third acetogen, *Clostridium formicoaceticum*.<sup>17,18</sup> (B) *C. formicoaceticum* A1 obtained from an agar slant culture. (C) Electron micrograph showing the peritrichous flagellation of *C. formicoaceticum* A1. (Parts (B) and (C) are from Andreesen *et al.*<sup>18</sup> 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).<sup>3,24,25</sup> 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*<sup>a</sup>**

Year	Event
Events prior to the isolation of <i>Clostridium thermoaceticum</i>	
1927	Isolation of <i>Clostridium scatologenes</i> (Weinberg and Ginsbourg <sup>46</sup> ); shown to be an acetogen in 2000 (Küsel <i>et al.</i> <sup>47</sup> )
1932	H <sub>2</sub> -dependent conversion of CO <sub>2</sub> to acetate in sewage sludge (Fischer <i>et al.</i> <sup>4</sup> )
1936	Discovery of first acetogen, <i>Clostridium acetium</i> ; total synthesis of acetate from H <sub>2</sub> -CO <sub>2</sub> (Wieringa <sup>5–7</sup> ) (NOTE: Culture was lost.)
1942	Discovery of second acetogen, <i>Clostridium thermoaceticum</i> ; conversion of glucose to 3 acetate (Fontaine <i>et al.</i> <sup>13</sup> )
1944	Acetogenic conversion of pyruvate to acetate (Barker <sup>247</sup> )
1945–1952	Synthesis of acetate from <sup>14</sup> CO <sub>2</sub> (Barker and Kamen <sup>33</sup> ) or <sup>13</sup> CO <sub>2</sub> (Wood <sup>34,132</sup> )
1955	Formate as a methyl-group precursor (Lentz and Wood <sup>248</sup> )
1964	Methylcobalamin as methyl-group precursor (Poston <i>et al.</i> <sup>249</sup> )
1965	Autotrophic synthesis of cell-carbon precursors from CO <sub>2</sub> (Ljungdahl and Wood <sup>250</sup> )
1966–1969	Proposal of one-carbon pathway for the tetrahydrofolate/corrinoid-mediated synthesis of acetate from CO <sub>2</sub> (Ljungdahl <i>et al.</i> <sup>251</sup> Ljungdahl and Wood <sup>252</sup> )
1973–1976	Discovery that tungsten is a biologically active metal in formate dehydrogenase (Andreesen and Ljungdahl, <sup>35</sup> Ljungdahl and Andreesen, <sup>37</sup> Ljungdahl <sup>36</sup> )
1973–1986	Resolution of the tetrahydrofolate pathway (reviewed in Ljungdahl <sup>25</sup> )
1978–1980	Discovery of CO dehydrogenase as a nickel-containing enzyme (Diekert and Thauer, <sup>60</sup> Drake <i>et al.</i> <sup>62</sup> )
1981	Resolution of enzymes required for synthesis of acetyl-CoA from pyruvate and methyltetrahydrofolate (Drake <i>et al.</i> <sup>253</sup> )
1981–1982	Demonstration that CO replaces the carboxyl-group of pyruvate and undergoes an exchange reaction with acetyl-CoA (Drake <i>et al.</i> <sup>254</sup> Hu <i>et al.</i> <sup>61</sup> )
1982	Discovery of hydrogenase (Drake <sup>137</sup> )
1983	Purification of CO dehydrogenase (Diekert and Ritter, <sup>59</sup> Ragsdale <i>et al.</i> <sup>255</sup> )
1983	Use of H <sub>2</sub> and CO under organotrophic conditions (Kerby and Zeikus <sup>256</sup> )
1984	Resolution of nutritional requirements (Lundie and Drake <sup>257</sup> )
1984	Enzyme system for H <sub>2</sub> -dependent synthesis of acetyl-CoA (Pezacka and Wood <sup>258</sup> )
1984–1986	CO dehydrogenase is acetyl-CoA synthase (Pezacka and Wood, <sup>258,259</sup> Ragsdale and Wood <sup>260</sup> ), and CO is the carbonyl precursor in the acetyl-CoA pathway under growth conditions (Diekert <i>et al.</i> <sup>58</sup> Martin <i>et al.</i> <sup>113</sup> )
1985–1991	Catalytic mechanism of acetyl-CoA synthase (reviewed in Ragsdale <sup>84</sup> )
1986–1990	H <sub>2</sub> - and CO-dependent electron-transport system coupled to the synthesis of ATP (Ivey and Ljungdahl, <sup>261</sup> Hugenholtz and Ljungdahl, <sup>262,263</sup> Das <i>et al.</i> <sup>264</sup> )
1990	Chemolithoautotrophic growth on H <sub>2</sub> -CO <sub>2</sub> and CO-CO <sub>2</sub> (Daniel <i>et al.</i> <sup>43</sup> )
1991	Integrated model for catabolic, anabolic, and bioenergetic features of the acetyl-CoA Wood–Ljungdahl pathway (Wood and Ljungdahl <sup>24</sup> )

SOURCE: Modified from Drake *et al.*<sup>41,42</sup>

<sup>a</sup>*Clostridium thermoaceticum* was reclassified to *Moorella thermoacetica* in 1994 (Collins *et al.*<sup>48</sup>).

SOURCE: Modified from Drake *et al.*<sup>41</sup>

<sup>a</sup>*Clostridium thermoaceticum* was reclassified to *Moorella thermoacetica* in 1994 (Collins *et al.*<sup>48</sup>).

*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.*<sup>26</sup>

The phrase “recent work on carbon dioxide uptake” was referring to various studies that assessed the uptake of carbon dioxide into organic carbon.<sup>4,27–31</sup> 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<sub>2</sub> proposed by Fontaine was demonstrated experimentally a few years later in 1945 with the landmark <sup>14</sup>C-studies of Barker and Kamen.<sup>32,33</sup> These studies were the first in biology to make use of carbon-14 and demonstrated that *C. thermoaceticum* incorporated <sup>14</sup>CO<sub>2</sub> equally into both carbons of acetate.<sup>32,33</sup> That *C. thermoaceticum* was capable of synthesizing acetate from two molecules of CO<sub>2</sub> was later confirmed in 1952 by H. G. Wood using <sup>13</sup>CO<sub>2</sub> and mass spectrometry.<sup>34</sup> 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<sub>2</sub>. 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<sub>2</sub> 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.<sup>35–37</sup>

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).<sup>3,24,25,38–42</sup> 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<sub>2</sub>-CO<sub>2</sub> and CO-CO<sub>2</sub> was demonstrated.<sup>43</sup> 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.<sup>3,24,25,38,41,42,44,45</sup> 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.<sup>46</sup> In 2000, K. Küsel and co-workers<sup>47</sup> 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. thermoacetikum* was reclassified as *Moorella thermoacetica* when the taxonomy of the genus *Clostridium* was reorganized.<sup>48</sup> The organism will be referred to as *Moorella thermoacetica* throughout the remainder of this article.

## **Acetyl-CoA Wood–Ljungdahl 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<sub>2</sub>-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 biphosphate, oxaloacetate, and acetyl-CoA, respectively) for the initial fixation of CO<sub>2</sub>.<sup>24,49</sup> 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).<sup>50–53</sup> These concepts were well conceived many years ago.<sup>49</sup> 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<sub>2</sub> to form acetate may have been used by the earliest life forms rather than the more complicated cyclic mechanisms of autotrophism.*<sup>49</sup>

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.<sup>1,2,54–57</sup>

As illustrated in [Figure 5](#), much of Ljungdahl's work focused on understanding how CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> to CO and the synthesis of acetyl-CoA as shown in [Figure 5](#), but can also oxidizes CO to CO<sub>2</sub>; this latter reaction is the historical basis for referring to acetyl-CoA synthase as CO dehydrogenase.<sup>58–62</sup> 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.<sup>24,38–42,44,45,63–75</sup>

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 ( $\text{ATP}_{\text{SLP}}$ ) for each hexose (e.g., glucose) that is converted to three acetates (Fig. 6), a number that is higher than the amount of  $\text{ATP}_{\text{SLP}}$  formed by normal fermentations (e.g., homolactate fermentation yields two  $\text{ATP}_{\text{SLP}}$  per glucose consumed). However, when the acetyl-CoA pathway operates under autotrophic conditions (e.g., during growth at the expense of  $\text{H}_2$  and  $\text{CO}_2$ ), there is no net gain in  $\text{ATP}_{\text{SLP}}$  (1  $\text{ATP}_{\text{SLP}}$  is required for the activation of formate, and 1  $\text{ATP}_{\text{SLP}}$  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.<sup>44,76,77</sup> 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 that 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.<sup>39,44,75</sup> 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 dual function and translocates sodium ions out of the cell; the subsequent gradient of sodium ions drives the formation of ATP by sodium-dependent ATPase.<sup>76,77</sup> 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.<sup>78-80</sup> Motility can likewise be dependent on sodium.<sup>81</sup> Some acetogens might also employ sodium-proton antiporters for generating electrochemical gradients.<sup>82</sup>

Lars G. Ljungdahl

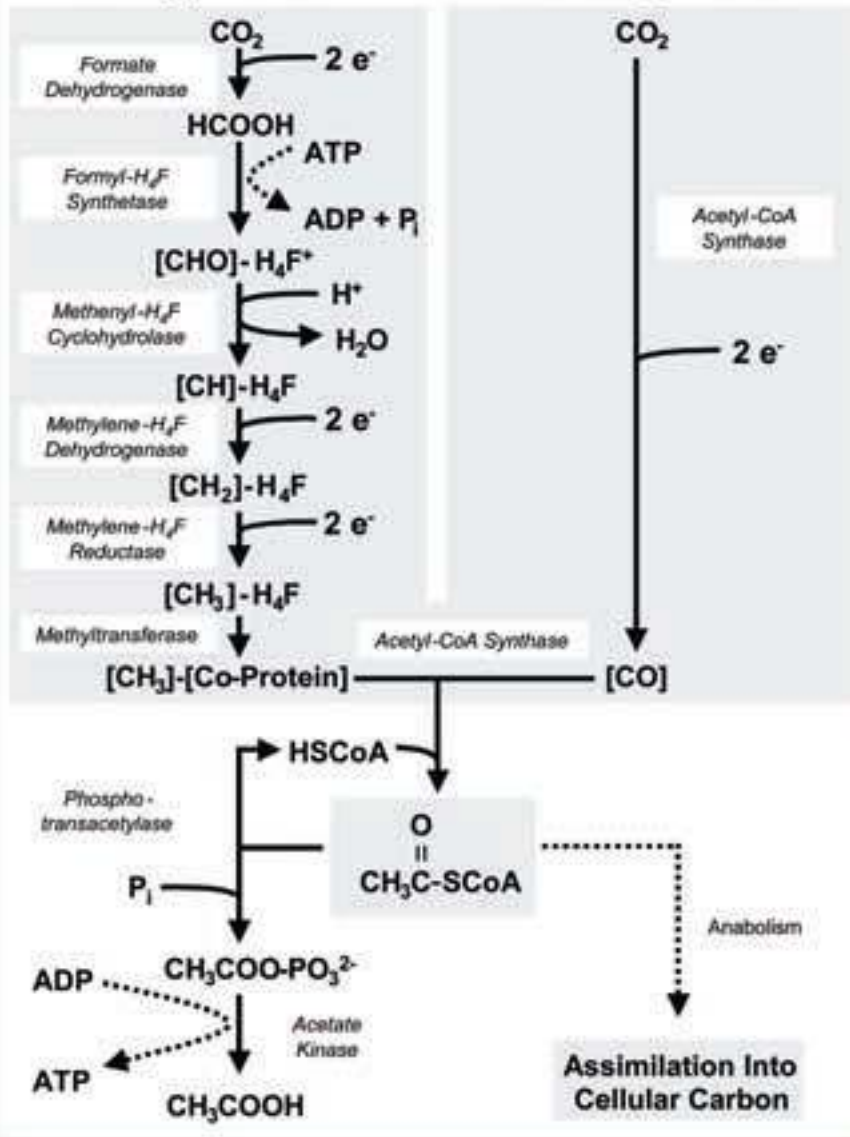


Harland G. Wood



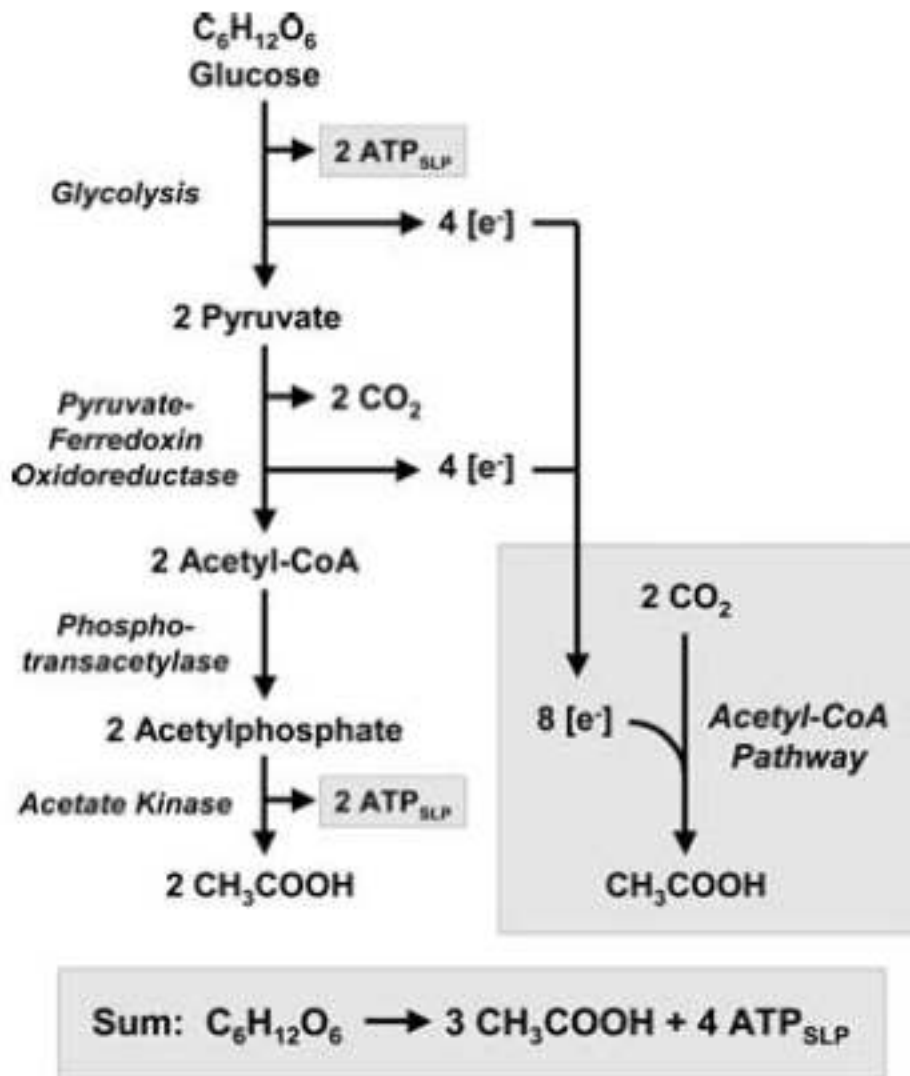
Methyl Branch

Carbonyl Branch





**Figure 5.** The acetyl-CoA Wood–Ljungdahl pathway. *Brackets* indicate that a particular C<sub>1</sub> unit is bound to a cofactor or structurally associated with an enzyme. Abbreviations: H<sub>4</sub>F = tetrahydrofolate; HSCoA = coenzyme A; P<sub>i</sub>= inorganic phosphate; e<sup>-</sup>= electron; Co-Protein = corrinoid enzyme; ATP = adenosine 5'-triphosphate. (The pathway is from Müller *et al.*<sup>77</sup> 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<sup>94</sup> and are used with the kind permission of Elsevier.)



**Figure 6.** Homoacetogenic conversion of glucose to acetate. The two molecules of CO<sub>2</sub> that are reduced to acetate in the acetyl-CoA pathway can be derived from exogenous CO<sub>2</sub> rather than the CO<sub>2</sub> that is produced via the decarboxylation of pyruvate (see text). Abbreviations: ATP<sub>SLP</sub>= ATP that is produced by substrate-level phosphorylation; [e<sup>-</sup>], reducing equivalent. (From Müller *et al.*<sup>77</sup> and used with the kind permission of Horizon Bioscience.)

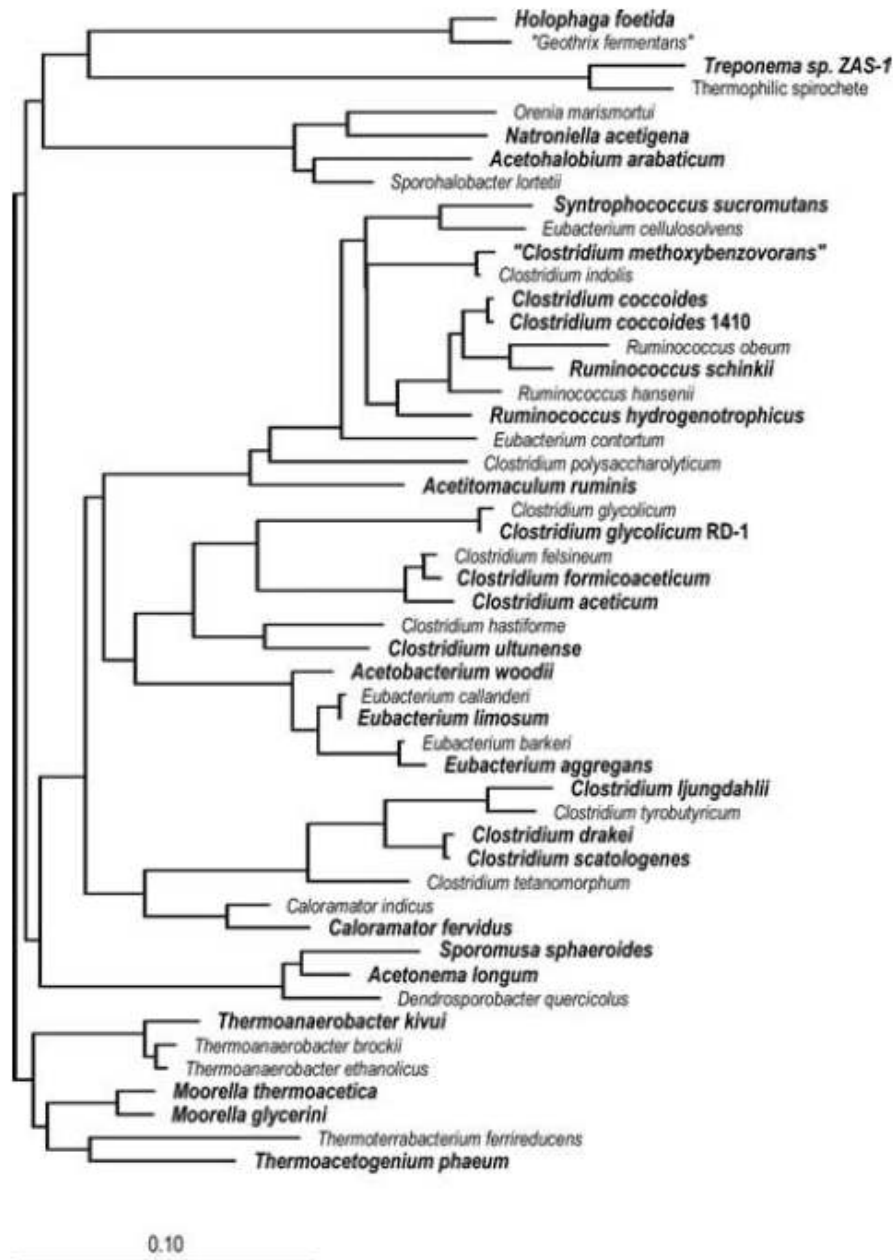
### Organismal Diversity of Acetogenic Prokaryotes

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<sub>2</sub>, (2) conservation of energy, and (3) assimilation of CO<sub>2</sub> into biomass.<sup>3,41,42</sup> 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,<sup>3,41,42</sup> referring to microorganisms as acetogens when they form acetate by processes that do not involve the reductive synthesis of acetate from CO<sub>2</sub> 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*<sup>83</sup>) to 62 mol% (*Holophaga foetida*<sup>84</sup>). Acetogens have been assigned to 22 different bacterial genera<sup>41,42</sup>: *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 pyruvate, and cell extracts have hydrogenase and CO dehydrogenase activities.<sup>85</sup> 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 coccooides*<sup>86</sup> and *C. scatologenes*<sup>47</sup>). 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

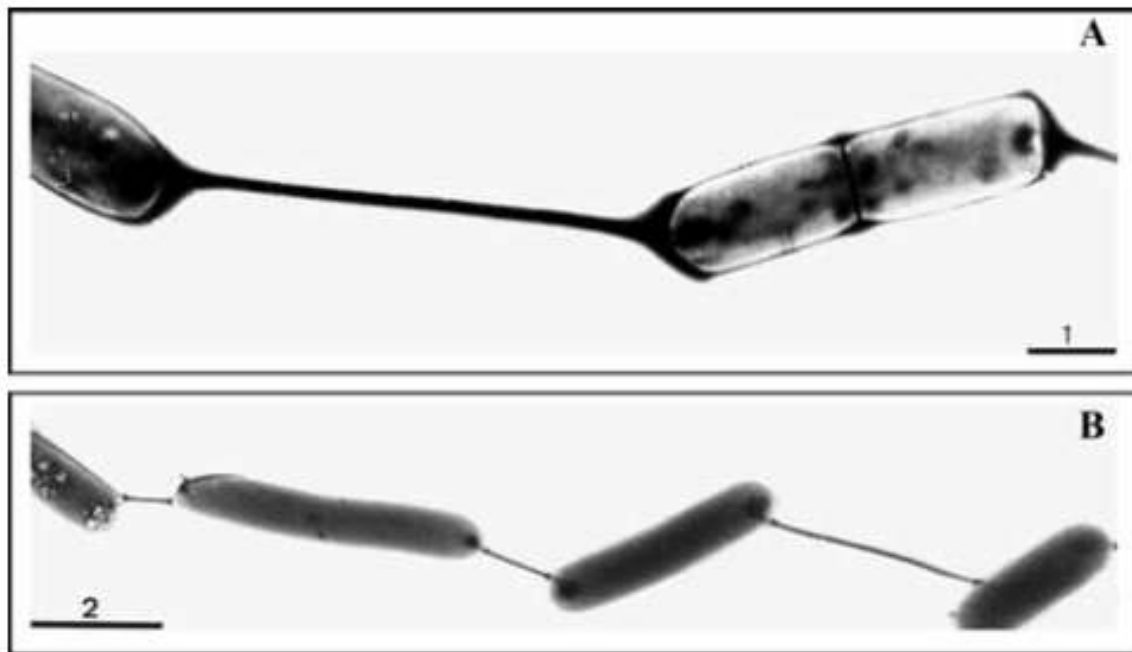
strain of *C. glycolicum* does not display acetogenic capabilities, but *C. glycolicum* strain RD-1<sup>87</sup> and *C. glycolicum* strain 22<sup>88</sup> are acetogens. Prolonged cultivation under certain conditions in the laboratory might cause certain acetogens to lose the capacity to engage the acetyl-CoA pathway and grow acetogenically.<sup>41,42</sup> DNA-DNA hybridization 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<sup>47</sup> and later shown to be a unique species by DNA-DNA hybridization and reclassified as *Clostridium drakei*.<sup>89</sup>



**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.*<sup>41,42</sup> 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<sup>90</sup> and *H. foetida* from freshwater sediment<sup>84</sup>), soils (e.g., psychrotrophic *Acetobacterium tundrae* from tundra soil and Antarctic surficial material,<sup>91,92</sup> the mesophile *Sporomusa silvacetica* from forest soil,<sup>93</sup> and the thermophile *M. thermoacetica* from Egyptian and Kansas soils that experience thermophilic temperatures<sup>14–16,94</sup>), the subsurface (e.g., strain SS1 and “*Acetobacterium psammolithicum*” from subsurface sediment and sandstone, respectively<sup>95,96</sup>), acidic coal mine ponds (e.g., *C. drakei*<sup>47,89</sup>), salt-lake soda deposits (e.g., *Natroniella acetigena* from the soda deposits at Lake Magadi, Kenya<sup>97</sup>), fecal material (e.g., *C. coccoides* and *Bryantella formatexigens* from human feces,<sup>86,98</sup> *Treponema primitia* from the termite hindgut,<sup>99,100</sup> and *C. ljungdahlii* from chicken manure/waste<sup>83,101</sup>), and estuarine and salt-marsh plants (e.g., *C. glycolicum* RD-1 from the roots of the sea grass *Halodule wrightii*<sup>87</sup> and *Sporomusa rhizae* from the roots of the needlerush *Juncus roemerianus*<sup>102</sup>).

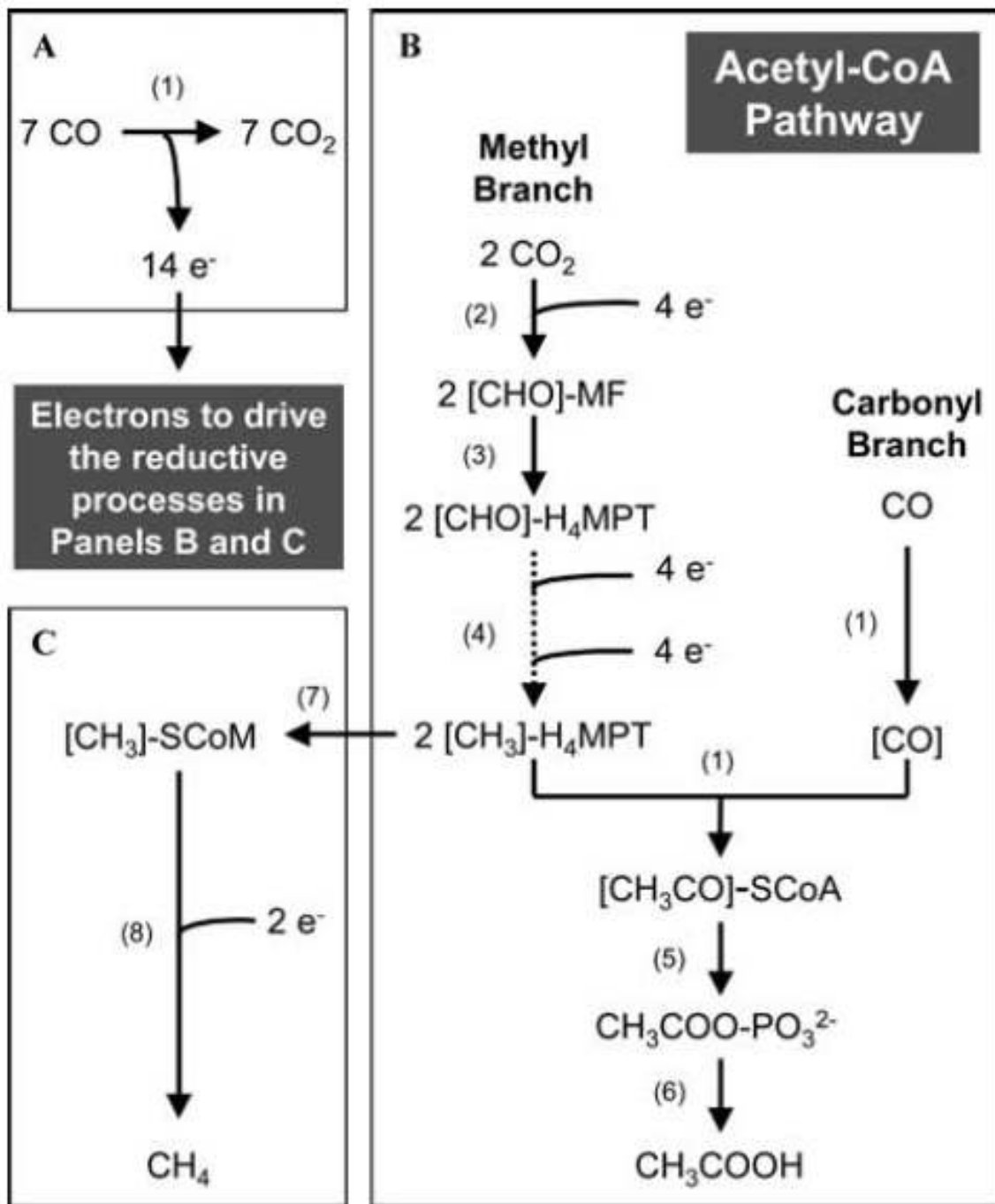
The diverse habitat range of acetogens demonstrates that organisms that can utilize the acetyl-CoA pathway are adapted to a broad range of *in situ* conditions. Many acetogens are spore-formers, a feature that likely aids in their *in situ* survival. Indeed, spores of *M. thermoacetica* have a decimal reduction time (i.e., the time required to decrease the population of viable spores by 90%) of nearly 2 hours at 121°C.<sup>103</sup> 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.*<sup>87</sup> and used with the kind permission of the American Society for Microbiology.) (B) The nitrogen-fixing bacterium *Clostridium akagii*. (From Drake *et al.*<sup>41,42</sup> 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.<sup>87,181,182</sup> Bars are in micrometers.

The usage of the acetyl-CoA pathway for autotrophic assimilation of carbon and acetate utilization by methanogens,<sup>1,2,104,105</sup> and the apparent reversibility of the pathway in some organisms (e.g., strain AOR<sup>106</sup> and *T. phaeum*<sup>107</sup>) 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).<sup>52,108</sup> Similar observations have been made with the archaeon *Archaeoglobus fulgidus* VC16, that is, this archaea can grow via CO-dependent acetogenesis.<sup>109</sup> The capacity of anaerobic archaea to consume and oxidize CO has been known for decades,<sup>110</sup> 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.

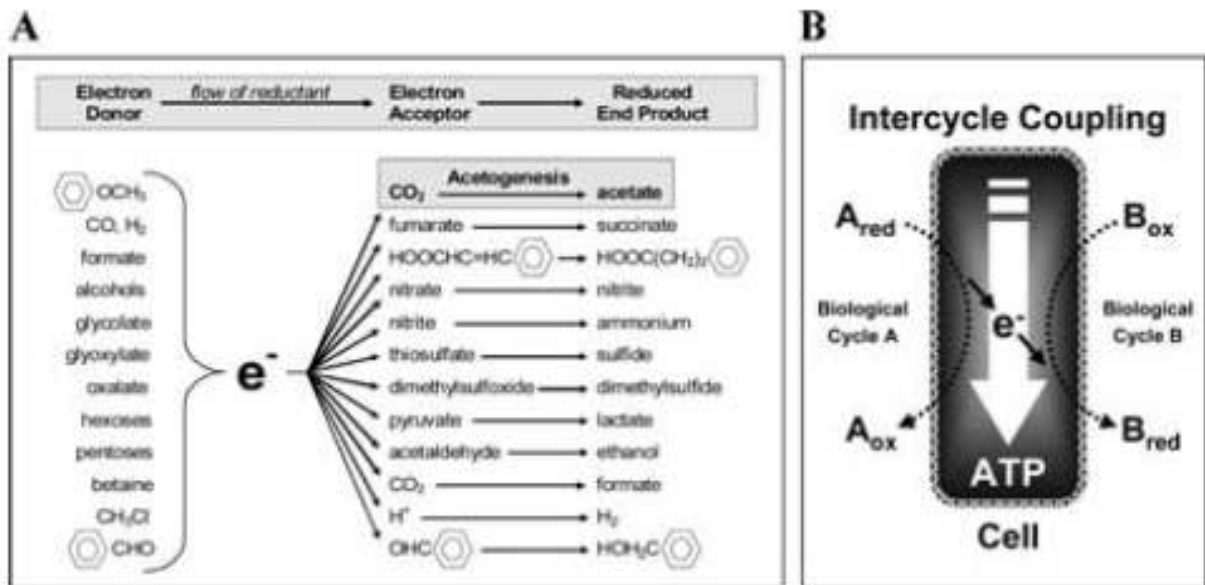


**Figure 9.** Metabolic processes by which carbon monoxide is utilized by *Methanosarcina acetivorans*. **(A)** Oxidation of carbon monoxide; **(B)** reductive synthesis of acetate via the acetyl-CoA pathway; **(C)** production of methane. *Parenthetical numbers* identify enzymes that catalyze the indicated reactions: 1, CO dehydrogenase/acetyl-CoA synthetase; 2, formyl-methanofuran dehydrogenase; 3, formyl-methanofuran:H<sub>4</sub>MPT formyltransferase; 4, combined activities of methenyl-H<sub>4</sub>MPT cyclohydrolase, methylene-H<sub>4</sub>MPT dehydrogenase; methylene-H<sub>4</sub>MPT reductase; 5, phosphotransacetylase; 6, acetate kinase; 7, methyl-H<sub>4</sub>MPT:CoM methyltransferase; 8, combined activities of methyl-CoM reductase, membranous heterodisulfide reductase, and membranous F<sub>420</sub>H<sub>2</sub> dehydrogenase complex (F<sub>420</sub>, coenzyme F<sub>420</sub>). Abbreviations: MF = methanofuran; H<sub>4</sub>MPT =

tetrahydromethanopterin; HSCoM = coenzyme M. [The figure is based on information in Lessner *et al.*<sup>52</sup> (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<sub>2</sub>. 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.*<sup>41,42</sup> Abbreviations: e<sup>-</sup> = reducing equivalent. (B) Intercycle coupling (see text). (Modified from Drake *et al.*<sup>122</sup> and Drake and Küsel<sup>40</sup> and used with the kind permission of IOS Press and CRC Press.)

## Electron Acceptors and Intercycle Coupling

### CO<sub>2</sub> and Acetogenesis

The growth of acetogens can be impaired when exogenous CO<sub>2</sub> is not available.<sup>18,22,111,112</sup> Given the fact that CO<sub>2</sub> is the terminal electron acceptor during acetogenesis (i.e., the reductive synthesis of acetate from CO<sub>2</sub>), the importance of the availability of CO<sub>2</sub> might seem obvious. However, the stoichiometry of glucose-dependent acetogenesis (i.e., three acetate produced per glucose consumed) does not require CO<sub>2</sub> (i.e., CO<sub>2</sub> 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<sub>2</sub>. During glycolysis, oxidative reactions precede the decarboxylation of pyruvate (which yields CO<sub>2</sub>). Thus, the recycling of electron

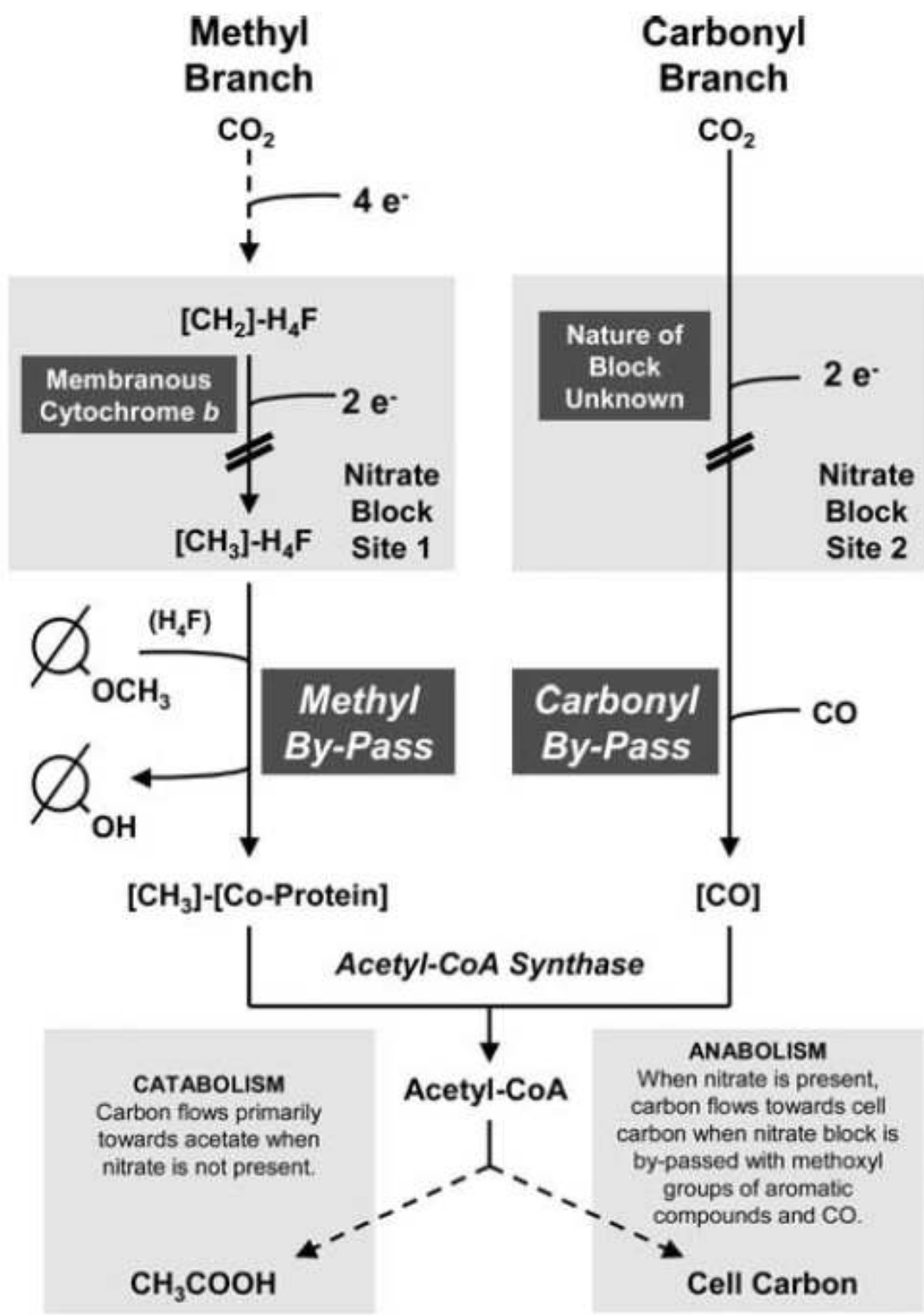
carriers often necessitates an adequate supply of supplemental CO<sub>2</sub>. Consistent with the flow of carbon predicted in [Figure 6](#), growth of *M. thermoacetica* with uniformly labeled [<sup>14</sup>C]glucose yields the following percent relative distribution of recovered <sup>14</sup>C: 31% [<sup>14</sup>C]carbonates/CO<sub>2</sub>, 62% [<sup>14</sup>C]acetate, and 6% [<sup>14</sup>C]biomass (Martin and Drake, unpublished data). Furthermore, large amounts of exogenous CO<sub>2</sub> are reduced to acetate during glucose-dependent acetogenesis.<sup>113</sup> The decarboxylation of carboxylated aromatic compounds by certain acetogens can augment the availability of CO<sub>2</sub> for acetogenesis.<sup>112,114</sup> Likewise, carbonic anhydrase might optimize the availability of intracellular CO<sub>2</sub> in acetogens.<sup>115</sup>

## 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.<sup>116,117</sup> Closely related acetogens differ in their ability to utilize alternative electron acceptors. For example, the thermophilic acetogen *Moorella glycerini* is a close relative of *M. thermoacetica* but does not dissimilate nitrate.<sup>118</sup>

Nitrate dissimilation of *M. thermoacetica* is noteworthy, since the standard redox potential of the CO<sub>2</sub>/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.<sup>116,117</sup> The dissimilation of nitrate significantly increases the growth efficiency of *M. thermoacetica*. H<sub>2</sub>-dependent growth yields are enhanced eightfold when nitrate, rather than CO<sub>2</sub>, is utilized as a terminal electron acceptor.<sup>116</sup> 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<sub>2</sub>-dependent dissimilation of CO<sub>2</sub> to acetate via the acetyl-CoA pathway is -95 kJ per mol reaction, while that of the H<sub>2</sub>-dependent dissimilation of nitrate to ammonium is -600 kJ per mol reaction.<sup>119</sup> *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.<sup>116</sup> Nitrite can also be used by *M. thermoacetica* as an energy-conserving terminal electron acceptor.<sup>120</sup> 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.<sup>116</sup> 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.<sup>116,121</sup> This matter has recently been addressed<sup>94</sup>:





**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.<sup>120</sup> Abbreviations: H<sub>4</sub>F = tetrahydrofolate; CoA = coenzyme-A; Co-Protein = corrinoid enzyme. (Modified from Drake and Küsel<sup>40</sup> and Drake and Daniel<sup>94</sup> 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<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.<sup>116</sup> 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.<sup>40,41,122</sup>*

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).<sup>40</sup> For example, the H<sub>2</sub>-dependent reduction of CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>. However, an acetogen might also use multiple terminal electron acceptors simultaneously. For example, the acetogen *Ruminococcus productus* simultaneously reduces phenylacrylates to phenylpropionates and CO<sub>2</sub> to acetate,<sup>123</sup> and also reduces CO<sub>2</sub> to acetate concomitant to lactate fermentation during growth at the expense of fructose.<sup>124</sup> *A. woodii* likewise has the potential to simultaneously utilize phenylacrylates (e.g., caffeate) and the acetyl-CoA pathway as terminal electron-accepting processes.<sup>125</sup> 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<sub>2</sub> Transfer

Although energy might not be conserved by the reduction of protons when pure cultures of acetogens vent H<sub>2</sub> as a trace byproduct of acetogenesis, H<sub>2</sub>-producing acetogens (e.g., *A. woodii*) can form close trophic associations with H<sub>2</sub>-consuming partners.<sup>126</sup> Such participation in interspecies H<sub>2</sub> transfer suggests that the production of H<sub>2</sub> 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<sub>2</sub>-consuming partner in the interspecies transfer of H<sub>2</sub>.<sup>127-129</sup>

## Electron Donors

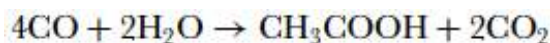
It is not possible that Fotaine and his co-workers could have known that the organism they would isolate, *M. thermoacetica*, would become not only the organism from which the acetyl-CoA pathway would be resolved (Table 2), but would also be the most metabolically robust acetogen characterized to the 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 substrates.

Most of the acetogens isolated to date can utilize hexoses or pentoses for growth and acetogenesis.<sup>41,42,130,131</sup> Tracer studies demonstrated that the glycolytic Embden–Meyerhof–Parnas (EMP) pathway is operative in *M. thermoacetica*.<sup>132</sup> 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,<sup>133</sup> 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.<sup>98</sup> 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<sup>84,134,135</sup> 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<sub>2</sub> is chemically the simplest source of reductant in nature, and H<sub>2</sub> is the only noncarbonaceous source of reductant that is currently known to be growth supportive for acetogens. Although H<sub>2</sub>-dependent autotrophic acetogenesis is a distinguishing feature of acetogens, H<sub>2</sub>-dependent growth (i.e., cell yields) is usually poor, and the ability of an acetogen to grow autotrophically at the expense of H<sub>2</sub> might therefore go undetected when the organism is first isolated (as was the case with *M. thermoacetica*<sup>136,137</sup>). Acetogens can contain multiple hydrogenases, and activity levels can vary with growth conditions.<sup>136,137</sup> 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,<sup>116</sup> 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<sub>2</sub>, as has been proposed for the hydrogenase activity detected during the heterotrophic growth of *M. thermoacetica*.<sup>94</sup>

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).<sup>94</sup> One-carbon substrates can be either oxidized or be directly assimilated into the acetyl-CoA pathway. For example, as can be visualized in [Figure 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.

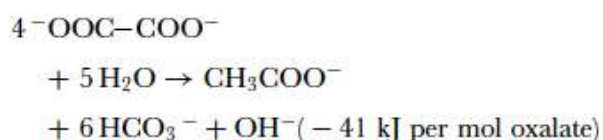
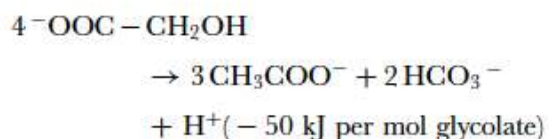
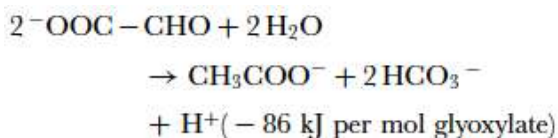
CO-dependent acetogenesis yields the following stoichiometry:



During this specialized process, three molecules of CO are oxidized to CO<sub>2</sub>, yielding six electrons that are then used to reduce CO<sub>2</sub> 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<sub>2</sub>, while the carboxyl group of acetate is derived from CO.<sup>113</sup> Traces of H<sub>2</sub> and methane can be produced during CO-dependent growth.<sup>111</sup>

Acetogens can oxidize a variety of alcohols and organic acids. For example, *M. thermoacetica* can utilize methanol, ethanol, *n*-propanol, *n*-butanol, formate, oxalate, glyoxylate, glycolate, pyruvate, and lactate.<sup>94</sup> As noted earlier, the potential to oxidize a particular substrate may be dependent upon the electron acceptor utilized (e.g., short-chain alcohols are not growth supportive for *M. thermoacetica* when CO<sub>2</sub> is used as a terminal electron acceptor, but are growth supportive when nitrate is used as a terminal electron acceptor).

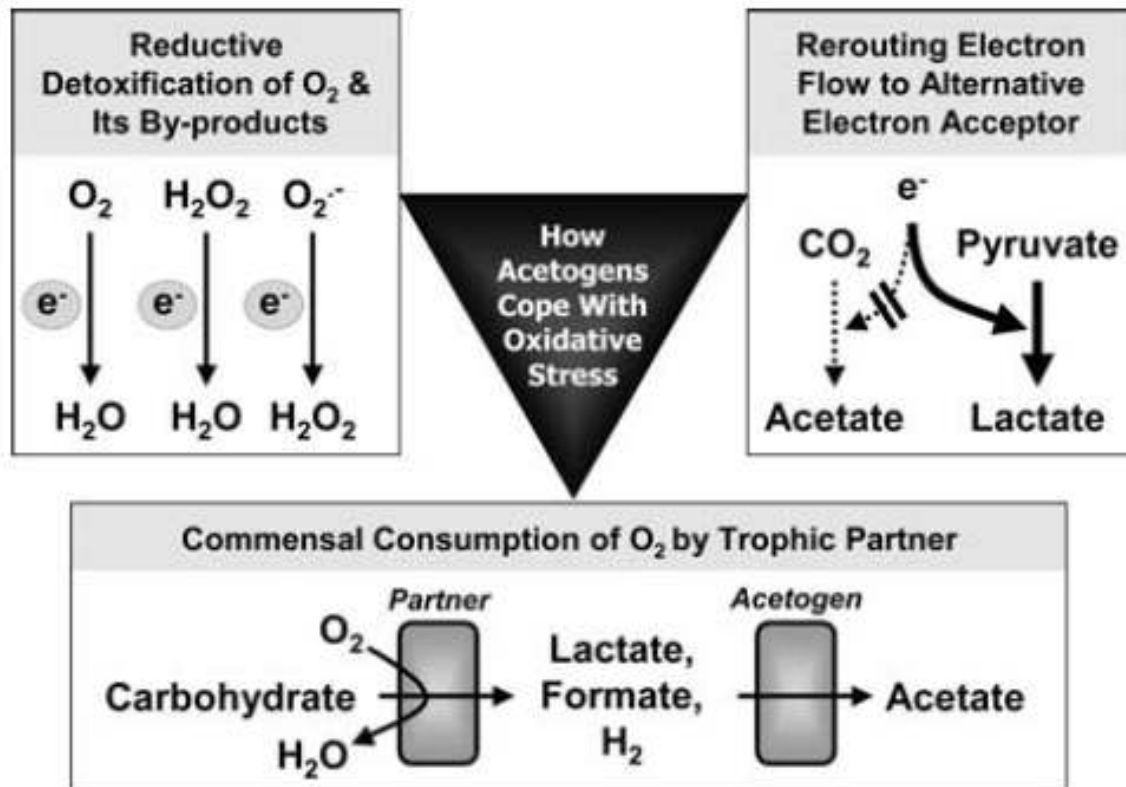
Although the use of glyoxylate, glycolate, and oxalate has not been widely demonstrated in acetogens or other anaerobes, these two-carbon compounds are readily used by *M. thermoacetica* according to the following reactions (kJ per mol calculated from Thauer *et al.*<sup>119</sup>)<sup>138,139</sup>:



The enzyme system by which *M. thermoacetica* catabolizes oxalate requires a utilizable electron acceptor.<sup>140</sup> 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.<sup>120,139,141</sup> Reducing equivalents that are theoretically derived from these two-carbon substrates appear to be managed differently and yield dissimilar growth efficiencies.<sup>94</sup>

### Response to O<sub>2</sub> 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<sub>2</sub> (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<sub>2</sub> enrichment harbor high numbers of culturable acetogens, including classic and novel acetogenic species.<sup>14,93,102,142,143</sup> Acetogens in redox-unstable habitats are likely challenged with O<sub>2</sub> 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),<sup>5,6</sup> 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<sub>2</sub>, 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<sup>-</sup>= electron. (Modified from Müller *et al.*<sup>77</sup> and used with the kind permission of Horizon Bioscience.)

## Reductive Removal of O<sub>2</sub>

Acetogens contain numerous enzymes that can reductively remove O<sub>2</sub> 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.<sup>144-147</sup> These enzymes are effective in protecting acetogens from oxidative stress when the concentration of O<sub>2</sub> is relatively low.

## Use of Alternative Electron Acceptors in Response to O<sub>2</sub>

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<sub>2</sub> and operate at higher redox potentials than does the acetyl-CoA pathway (as noted earlier, the standard redox potential of the CO<sub>2</sub>/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<sub>2</sub>.<sup>87</sup> This acetogen tolerates up to 4% O<sub>2</sub> 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<sub>2</sub> than acetogenesis.

## Trophic Interaction with O<sub>2</sub>-consuming Partner

Acetogens can form symbiotic relationships with O<sub>2</sub>-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<sup>14</sup>), 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*<sup>102</sup>). In both cases, a fermentative nonacetogen that has the capacity to consume O<sub>2</sub> (thus protecting the acetogen from O<sub>2</sub>) while simultaneously forming fermentation products (e.g., lactate, formate, and H<sub>2</sub>) that can be used by the acetogen for acetogenesis. Although such partnerships between acetogens and O<sub>2</sub>-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.<sup>41,148-155</sup> The acetogenic conversion of synthesis gas (i.e., H<sub>2</sub>, CO, and CO<sub>2</sub>) to acetic acid, ethanol, and butanol has also been investigated.<sup>83,156-158</sup> 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.<sup>159</sup> These limitations have hampered the commercialization of acetogens. Nonetheless, the existence of acetogens in acidic habitats (e.g., *C. drakei*<sup>47,89</sup>) 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*<sup>98</sup> and *M. thermoacetica* strain F21,<sup>133</sup> 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.<sup>160</sup> 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<sub>2</sub> transfer.<sup>161</sup> *Clostridium lentocellum* strain SG6 forms high amounts of acetate from cellulose, and product stoichiometries suggest that this organism might utilize CO<sub>2</sub> as a terminal electron acceptor via the acetyl-CoA pathway.<sup>162</sup> 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 due to its inhibitory effects on growth. New strategies to efficiently recover acetate from cultivation broths might circumvent this problem.<sup>163,164</sup>

Acetogens and the enzymes that they produce might be useful in the bioremediation of certain anthropogenic compounds (e.g., trinitrotoluene)<sup>165-167</sup> or the production of fine chemicals (e.g., corrinoids) and enzymes (e.g., acetate kinase).<sup>41,168-171</sup> 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.<sup>24</sup> 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  $\text{CO}_2$ .<sup>172</sup> 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).<sup>173</sup> 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.*<sup>143</sup>). 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.<sup>174,175</sup> Although these functional gene approaches have great promise and utility (e.g., Salmassi and Leadbetter,<sup>176</sup> Pester and Brune<sup>177</sup>), they, too, are compromised by problems of specificity.<sup>178</sup>

## ***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).<sup>52,108</sup> Likewise, the archaeon *A. fulgidus* VC16 can grow via CO-dependent acetogenesis.<sup>109</sup> 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<sub>2</sub> in both domains of the Prokaryotes, the pathway might be important to primary production in certain habitats. As reviewed elsewhere,<sup>41,42,179,180</sup> 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 80<sup>th</sup> birthday, we extend a special thanks to you, Lars.

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## **Conflict of Interest**

The authors declare no conflicts of interest.

## **References**

- 1 Fuchs, G. 1994. Variations of the acetyl-CoA pathway in diversely related microorganisms that are not acetogens. In *Acetogenesis*. H.L.Drake, Ed.: 507–520. New York : Chapman & Hall.
- 2 Fuchs, G. 1986. CO<sub>2</sub> fixation in acetogenic bacteria: variations on a theme. *FEMS Microbiol. Rev.* 39: 181–213.
- 3 Drake, H.L. 1994. Acetogenesis, acetogenic bacteria, and the acetyl-CoA “Wood/Ljungdahl” pathway: past and current perspectives. In *Acetogenesis*. H.L.Drake, Ed.: 3–60. New York : Chapman & Hall.

- 4 Fischer, F., R. Lieske & K. Winzer. 1932. Biologische Gasreaktionen. II. Über die Bildung von Essigsäure bei der biologischen Umsetzung von Kohlenoxyd und Kohlensäure mit Wasserstoff zu Methan. *Biochem. Z.* 245: 2–12.
- 5 Wieringa, K.T. 1936. Over het verdwijnen van waterstof en koolzuur onder anaerobe voorwaarden. *Antonie Leeuwenhoek* 3: 263–273.
- 6 Wieringa, K.T. 1939/1940. The formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. *Antonie Leeuwenhoek J. Microbiol. Serol.* 6: 251–262.
- 7 Wieringa, K.T. 1941. Über die Bildung von Essigsäure aus Kohlensäure und Wasserstoff durch anaerobe Bazillen. *Brennstoff-Chemie* 22: 161–164.
- 8 Karlsson, J.L., B.E. Volcani & H.A. Barker. 1948. The nutritional requirements of *Clostridium aceticum*. *J. Bacteriol.* 56: 781–782.
- 9 Adamse, A.D. 1980. New isolation of *Clostridium aceticum* (Wieringa). *Antonie Leeuwenhoek* 46: 523–531.
- 10 Braun, M., F. Mayer & G. Gottschalk. 1981. *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Arch. Microbiol.* 128: 288–293.
- 11 Gottschalk, G. & M. Braun. 1981. Revival of the name *Clostridium aceticum*. *Int. J. Syst. Bacteriol.* 31: 476.
- 12 Wieringa, K.T. 1935. In memoriam Prof. Dr. Ir. N.L. Söhngen. *Antonie Leeuwenhoek* 2: 1–7.
- 13 Fontaine, F.E., W.H. Peterson, E. McCoy & M.J. Johnson. 1942. A new type of glucose fermentation by *Clostridium thermoaceticum* n. sp. *J. Bacteriol.* 43: 701–715.
- 14 Gößner, A.S., R. Devereux, N. Ohnemüller, et al. 1999. *Thermicanus aegyptius* gen. nov., sp. nov., isolated from oxic soil, a fermentative microaerophile that grows commensally with the thermophilic acetogen *Moorella thermoacetica*. *Appl. Environ. Microbiol.* 65: 5124–5133.
- 15 Gößner, A. & H.L. Drake. 1997. Characterization of a new thermophilic acetogen (PT-1) isolated from aggregated Kansas prairie soil. *Abstr. Ann. Meet. Am. Soc. Microbiol.*, p. 401, Abstr. N-122.
- 16 Goessner, A.S., K. Kuesel, R. Devereux & H.L. Drake. 1998. Occurrence of thermophilic acetogens in Egyptian soils. *Abstr. Ann. Meet. Am. Soc. Microbiol.*, p. 366, Abstr. N-1.
- 17 El Ghazzawi, E. 1967. Neuisolierung von *Clostridium aceticum* Wieringa und stoffwechselphysiologische Untersuchungen. *Arch. Mikrobiol.* 57: 1–19.

- 18 Andreesen, J.R., G. Gottschalk & H.G. Schlegel. 1970. *Clostridium formicoaceticum* nov. spec. isolation, description and distinction from *C. aceticum* and *C. thermoaceticum*. Arch. Microbiol. 72: 154–174.
- 19 Bogdahn, M., J.R. Andreesen & D. Kleiner. 1983. Pathways and regulation of N<sub>2</sub>, ammonium and glutamate assimilation by *Clostridium formicoaceticum*. Arch. Microbiol. 134: 167–169.
- 20 Dorn, M., J.R. Andreesen & G. Gottschalk. 1978. Fermentation of fumarate and L-malate by *Clostridium formicoaceticum*. J. Bacteriol. 133: 26–32.
- 21 Gößner, A., S.L. Daniel & H.L. Drake. 1994. Acetogenesis coupled to the oxidation of aromatic aldehyde groups. Arch. Microbiol. 161: 126–131.
- 22 Matthies, C., A. Freiberger & H.L. Drake. 1993. Fumarate dissimilation and differential reductant flow by *Clostridium formicoaceticum* and *Clostridium aceticum*. Arch. Microbiol. 160: 273–278.
- 23 Lux, M.F. & H.L. Drake. 1992. Re-examination of the metabolic potentials of the acetogens *Clostridium aceticum* and *Clostridium formicoaceticum*: chemolithoautotrophic and aromatic-dependent growth. FEMS Microbiol. Lett. 95: 49–56.
- 24 Wood, H.G. & L.G. Ljungdahl. 1991. Autotrophic character of acetogenic bacteria. In Variations in Autotrophic Life. J.M.Shively & L.L.Barton, Eds.: 201–250. San Diego, CA : Academic Press.
- 25 Ljungdahl, L.G. 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. Annu. Rev. Microbiol. 40: 415–450.
- 26 Fontaine, F.E. 1941. The fermentation of cellulose and glucose by thermophilic bacteria. Ph.D. dissertation. University of Wisconsin-Madison .
- 27 Barker, H.A., S. Ruben & J.V. Beck. 1940. Radioactive carbon as an indicator of carbon dioxide reduction. IV. The synthesis of acetic acid from carbon dioxide by *Clostridium acidi-urici*. Proc. Natl. Acad. Sci. USA 26: 477–482.
- 28 Phelps, A.S., M.J. Johnson & W.H. Peterson. 1939. Carbon dioxide utilization during the dissimilation of glycerol by the propionic acid bacteria. Biochem. J. 33: 726–728.
- 29 Stephenson, M. & L.H. Stickland. 1933. Hydrogenase. III. The bacterial formation of methane by the reduction of one-carbon compounds by molecular hydrogen. Biochem. J. 27: 1517–1527.
- 30 Wood, H.G. & C.H. Werkman. 1936. The utilization of carbon dioxide in the dissimilation of glycerol by the propionic acid bacteria. Biochem. J. 30: 48–53.

- 31 Wood, H.G. & C.H. Werkman. 1940. Heavy carbon as a tracer in bacterial fixation of carbon dioxide. *J. Biol. Chem.* 135: 789–790.
- 32 Kamen, M.D. 1963. The early history of carbon-14. *J. Chem. Educ.* 40: 234–242.
- 33 Barker, H.A., Kamen, M.D. 1945. Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. *Proc. Natl. Acad. Sci. USA* 31: 219–225.
- 34 Wood, H.G. 1952. A study of carbon dioxide fixation by mass determination on the types of C<sup>13</sup>-acetate. *J. Biol. Chem.* 194: 905–931.
- 35 Andreesen, J.R. & L.G. Ljungdahl. 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. *J. Bacteriol.* 116: 867–873.
- 36 Ljungdahl, L.G. 1976. Tungsten, a biologically active metal. *Trends Biochem. Sci.* 1: 63–65.
- 37 Ljungdahl, L.G. & J.R. Andreesen. 1975. Tungsten, a component of active formate dehydrogenase from *Clostridium thermoaceticum*. *FEBS Lett.* 54: 279–282.
- 38 Ragsdale, S.W. 1997. The Eastern and Western branches of the Wood/Ljungdahl pathway: how the East and West were won. *Biofactors* 6: 3–11.
- 39 Das, A. & L.G. Ljungdahl. 2003. Electron-transport system in acetogens. In *Biochemistry and Physiology of Anaerobic Bacteria*. L.G.Ljungdahl, M.W.Adams, L.L.Barton, et al. , Eds.: 191–204. New York : Springer-Verlag.
- 40 Drake, H.L. & K. Küsel. 2005. Acetogenic clostridia. In *Handbook on Clostridia*. P.Dürre, Ed.: 719–746. Boca Raton , FL .: CRC Press.
- 41 Drake, H.L., K. Küsel & C. Matthies. 2004. Acetogenic prokaryotes. In *The Prokaryotes An Evolving Electronic Resource for the Microbiological Community*, 3rd ed. M.Dworkin, S.Falkow, E.Rosenberg, et al. , Eds.: Release 3.17, August 2004 <http://springeronline.com>. New York : Springer-Verlag.
- 42 Drake, H.L., K. Küsel & C. Matthies. 2006. Acetogenic prokaryotes. In *The Prokaryotes, Vol. 2*. M.Dworkin, S.Falkow, E.Rosenberg, et al. , Eds.: 354–420. New York : Springer-Verlag.
- 43 Daniel, S.L., T. Hsu, S.I. Dean & H.L. Drake. 1990. Characterization of the H<sub>2</sub>- and CO-dependent chemolithotrophic potentials of the acetogens *Clostridium thermoaceticum* and *Acetogenium kivui*. *J. Bacteriol.* 172: 4464–4471.
- 44 Ljungdahl, L.G. 1994. The acetyl-CoA pathway and the chemiosmotic generation of ATP during acetogenesis. In *Acetogenesis*. H.L.Drake, Ed.: 63–87. New York : Chapman & Hall.

- 45 Ragsdale, S.W. 2004. Life with carbon monoxide. *Crit. Rev. Biochem. Mol. Biol.* 39: 165–195.
- 46 Weinberg, M. & B. Ginsbourg. 1927. Données récentes sur les microbes anaérobies et leur rôle en pathologie, pp. 1–291. Paris : Masson et Cie.
- 47 Küsel, K., T. Dorsch, G. Acker, et al. 2000. *Clostridium scatologenes* strain SL1 isolated as an acetogenic bacterium from acidic sediments. *Int. J. Syst. Evol. Microbiol.* 50: 537–546.
- 48 Collins, M.D., P.A. Lawson, A. Willems, et al. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* 44: 812–826.
- 49 Wood, H.G. 1991. Life with CO or CO<sub>2</sub> and H<sub>2</sub> as a source of carbon and energy. *FASEB J.* 5: 156–63.
- 50 Ferry, J.G. & C.H. House. 2006. The stepwise evolution of early life driven by energy conservation. *Mol. Biol. Evol.* 23: 1286–1292.
- 51 Russell, M.J. & W. Martin. 2004. The rocky roots of the acetyl-CoA pathway. *Trends Biochem. Sci.* 29: 358–363.
- 52 Lessner, D.J., L. Li, Q. Li, et al. 2006. An unconventional pathway for reduction of CO<sub>2</sub> to methane in CO-grown *Methanosarcina acetivorans* revealed by proteomics. *Proc. Natl. Acad. Sci. USA* 103: 17921–17926.
- 53 Miyakawa, S., H. Yamanashi, K. Kobayashi, et al. 2002. Prebiotic synthesis from CO atmospheres: implications for the origins of life. *Proc. Natl. Acad. Sci. USA* 99: 14628–14631.
- 54 Schouten, S., M. Strous, M.M.M. Kuypers, et al. 2004. Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria. *Appl. Environ. Microbiol.* 70: 3785–3788.
- 55 Svetlitchnyi, V., H. Dobbek, T. Meins, et al. 2004. A functional Ni-Ni-[4Fe-4S] cluster in the monomeric acetyl-CoA synthase from *Carboxydothemus hydrogenoformans*. *Proc. Natl. Acad. Sci. USA* 101: 446–451.
- 56 Svetlitchnyi, V., C. Peschel, G. Acker & O. Meyer. 2001. Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium *Carboxydothemus hydrogenoformans*. *J. Bacteriol.* 183: 5134–5144.
- 57 Henstra, A.M. & A.J.M. Stams. 2004. Novel physiological features of *Carboxydothemus hydrogenoformans* and *Thermoterrabacterium ferrireducens*. *Appl. Environ. Microbiol.* 70: 7236–7240.

- 58 Diekert, G., M. Hansch & R. Conrad. 1984. Acetate synthesis from 2CO<sub>2</sub> in acetogenic bacteria: is carbon monoxide an intermediate? *Arch. Microbiol.* 138: 224–228.
- 59 Diekert, G. & M. Ritter. 1983. Purification of the nickel protein carbon monoxide dehydrogenase of *Clostridium thermoaceticum*. *FEBS Lett.* 151: 41–44.
- 60 Diekert, G.B. & R.K. Thauer. 1978. Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. *J. Bacteriol.* 136: 597–606.
- 61 Hu, S.I., H.L. Drake & H.G. Wood. 1982. Synthesis of acetyl coenzyme A from carbon monoxide, methyltetrahydrofolate, and coenzyme A by enzymes from *Clostridium thermoaceticum*. *J. Bacteriol.* 149: 440–448.
- 62 Drake, H.L., S.I. Hu & H.G. Wood. 1980. Purification of carbon monoxide dehydrogenase, a nickel enzyme from *Clostridium thermocaceticum*. *J. Biol. Chem.* 255: 7174–80.
- 63 Das, A. & L.G. Ljungdahl. 2000. Acetogenesis and acetogenic bacteria. In *Encyclopedia of Microbiology*, 2nd ed., Vol. 1. J.Lederberg, Ed.: 18–27. San Diego , CA : Academic Press.
- 64 Ragsdale, S.W. 1991. Enzymology of the acetyl-CoA pathway of CO<sub>2</sub> fixation. *Crit. Rev. Biochem. Mol. Biol.* 26: 261–300.
- 65 Ragsdale, S.W. 1994. CO dehydrogenase and the central role of this enzyme in the fixation of carbon dioxide by anaerobic bacteria. In *Acetogenesis*. H.L.Drake, Ed.: 88–126. New York : Chapman & Hall.
- 66 Diekert, G. & G. Wohlfarth. 1994. Metabolism of homoacetogens. *Antonie Leeuwenhoek* 66: 209–221.
- 67 Radfar, R., R. Shin, G.M. Sheldrick, et al. 2000. The crystal structure of N(10)-formyltetrahydrofolate synthetase from *Moorella thermoacetica*. *Biochemistry* 39: 3920–3926.
- 68 Ragsdale, S.W. 2000. Nickel containing CO dehydrogenases and hydrogenases. *Subcell. Biochem.* 35: 487–518.
- 69 Lindahl, P.A. & B. Chang. 2001. The evolution of acetyl-CoA synthase. *Origins Life Evol. Biosphere.* 31: 403–434.
- 70 Maynard, E.L. & P.A. Lindahl. 2001. Catalytic coupling of the active sites in Acetyl-CoA synthase, a bifunctional CO-channeling enzyme. *Biochemistry* 40: 13262–13267.
- 71 Leapart, A.B., H.T. Spencer & C.R. Lovell. 2002. Site-directed mutagenesis of a potential catalytic and formyl phosphate binding site and substrate inhibition of N-10-formyltetrahydrofolate synthetase. *Arch. Biochem. Biophys.* 408: 137–143.

- 72 Lindahl, P.A. 2002. The Ni-containing carbon monoxide dehydrogenase family: light at the end of the tunnel? *Biochemistry* 41: 2097–2105.
- 73 Darnault, C., A. Volbeda, E.J. Kim, et al. 2003. Ni-Zn-[Fe<sub>4</sub>-S<sub>4</sub>] and Ni-Ni-[Fe<sub>4</sub>-S<sub>4</sub>] clusters in closed and open subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase. *Nat. Struct. Biol.* 10: 271–279.
- 74 Ragsdale, S.W. 2003. Pyruvate ferredoxin oxidoreductase and its radical intermediate. *Chem. Rev.* 103: 2333–2346.
- 75 Das, A. & L.G. Ljungdahl. 1995. Bioenergetics of acetogenic thermophilic clostridia, the homoacetogens—An overview. *Biochem. (Life Sci. Adv.)* 14: 33–38.
- 76 Müller, V. 2003. Energy conservation in acetogenic bacteria. *Appl. Environ. Microbiol.* 69: 6345–6353.
- 77 Müller, V., F. Inkamp, A. Rauwolf, K. Küsel & H.L. Drake. 2004. Molecular and cellular biology of acetogenic bacteria. In *Strict and Facultative Anaerobes: Medical and Environmental Aspects*. M.Nakano & P.Zuber, Eds.: 251–281. Norfolk: UK. Horizon Scientific Press.
- 78 Geerligs, G., P. Schönheit & G. Diekert. 1989. Sodium dependent acetate formation from CO<sub>2</sub> in *Peptostreptococcus productus* (strain Marburg). *FEMS Microbiol. Lett.* 57: 253–258.
- 79 Heise, R., V. Müller & G. Gottschalk. 1989. Sodium dependence of acetate formation by the acetogenic bacterium *Acetobacterium woodii*. *J. Bacteriol.* 171: 5473–5478.
- 80 Yang, H.C. & H.L. Drake. 1990. Differential effects of sodium on hydrogen- and glucose-dependent growth of the acetogenic bacterium *Acetogenium kivui*. *Appl. Environ. Microbiol.* 56: 81–86.
- 81 Müller, V. & S. Bowien. 1995. Differential effects of sodium ions on motility in the homoacetogenic bacteria *Acetobacterium woodii* and *Sporomusa sphaeroides*. *Arch. Microbiol.* 164: 363–369.
- 82 Terracciano, J.S., W.J.A. Schreurs & E.R. Kashket. 1987. Membrane H<sup>+</sup> conductance of *Clostridium thermoaceticum* and *Clostridium acetobutylicum*: evidence for electrogenic Na<sup>+</sup>/H<sup>+</sup> antiport in *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.* 53: 782–786.
- 83 Tanner, R.S., L.M. Miller & D. Yang. 1993. *Clostridium ljungdahlii* sp. nov., and acetogenic species in clostridial rRNA homology group I. *Int. J. Sys. Bacteriol.* 43: 232–236.
- 84 Liesack, W., F. Bak, J.-U. Kreft & E. Stackebrandt. 1994. *Holophaga foetida* gen. nov., sp. nov., a new, homoacetogenic bacterium degrading methoxylated aromatic compounds. *Arch. Microbiol.* 162: 85–90.

- 85 Pikuta, E.V., R.B. Hoover, A.K. Bej, et al. 2003. *Tindallia californiensis* sp. nov., a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California. *Extremophiles* 7: 327–334.
- 86 Kamlage, B., B. Gruhl & M. Blaut. 1997. Isolation and characterization of two new homoacetogenic hydrogen-utilizing bacteria from the human intestinal tract that are closely related to *Clostridium coccoides*. *Appl. Environ. Microbiol.* 63: 1732–1738.
- 87 Küsel, K., A. Karnholz, T. Trinkwalter, et al. 2001. Physiological ecology of *Clostridium glycolicum* RD-1, an aerotolerant acetogen isolated from sea grass roots. *Appl. Environ. Microbiol.* 67: 4734–4741.
- 88 Ohwaki, K. & R.E. Hungate. 1977. Hydrogen utilization by clostridia in sewage sludge. *Appl. Environ. Microbiol.* 33: 1270–1274.
- 89 Liou, J.S.-C., D.L. Balkwell, G.R. Drake & R.S. Tanner. 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. *Int. J. Syst. Evol. Microbiol.* 55: 2085–2091.
- 90 Balch, W.E., S. Schoberth, R.S. Tanner & R.S. Wolfe. 1977. *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. *Int. J. Syst. Bacteriol.* 27: 355–361.
- 91 Sattley, W.M. & M.T. Madigan. 2007. Cold-active acetogenic bacteria from surficial sediments of perennially ice-covered Lake Fryxell, Antarctica. *FEMS Microbiol. Lett.* 9: 1836–1841.
- 92 Simankova, M.V., O.R. Kotsyurbenko, E. Stackebrandt, et al. 2000. *Acetobacterium tundrae* sp. nov., a new psychrophilic acetogenic bacterium from tundra soil. *Arch. Microbiol.* 174: 440–447.
- 93 Kuhner, C.H., C. Frank, A. Grieshammer, et al. 1997. *Sporomusa silvacetica* sp. nov., an acetogenic bacterium isolated from aggregated forest soil. *Int. J. Syst. Bacteriol.* 47: 352–358.
- 94 Drake, H.L. & S.L. Daniel. 2004. Physiology of the thermophilic acetogen *Moorella thermoacetica*. *Res. Microbiol.* 155: 869–883.
- 95 Krumholz, L.R., S.H. Harris, S.T. Tay & S.M. Suflita. 1999. Characterization of two subsurface H<sub>2</sub>-utilizing bacteria, *Desulfomicrobium hypogeium* sp. nov. and *Acetobacterium psammolithicum* sp. nov., and their ecological roles. *Appl. Environ. Microbiol.* 65: 2300–2306.



- 96 Liu, S. & J.M. Suflita. 1993. H<sub>2</sub>/CO<sub>2</sub>-dependent anaerobic O-demethylation activity in subsurface sediments and by an isolated bacterium. *Appl. Environ. Microbiol.* 59: 1325–1331.
- 97 Zhilina, T.N., G.A. Zavarzin, E.N. Detkova & F.A. Rainey. 1996. *Natroniella acetigena* gen. nov. sp. nov., an extremely halophilic, homoacetogenic bacterium: a new member of Haloanaerobiales. *Curr. Microbiol.* 32: 320–326.
- 98 Wolin, M.J., T.L. Miller, M.D. Collins & P.A. Lawson. 2003. Formate-dependent growth and homoacetogenic fermentation by a bacterium from human feces: description of *Bryantella formatexigens* gen. nov., sp. nov. *Appl. Environ. Microbiol.* 69: 6321–6326.
- 99 Graber, J.R. & J.A. Breznak. 2004. Physiology and nutrition of *Treponema primitia*, an H<sub>2</sub>-CO<sub>2</sub>-acetogenic spirochete from termite hindguts. *Appl. Environ. Microbiol.* 70: 1307–1314.
- 100 Graber, J.R., J.R. Leadbetter & J.A. Breznak. 2004. Description of *Treponema azotonutricium* sp. nov., and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl. Environ. Microbiol.* 70: 1315–1320.
- 101 Barik, S., Prieto, S.B. Harrison, et al. 1988. Biological production of alcohols from coal through indirect liquefaction. *Appl. Biochem. Biotech.* 18: 363–378.
- 102 Gößner, A.S., K. Küsel, G. Acker, et al. 2006. Trophic interaction of the aerotolerant anaerobe *Clostridium intestinale* and the acetogen *Sporomusa rhizae* sp. nov. isolated from roots of the black needlerush *Juncus roemerianus*. *Microbiology* 152: 1209–1219.
- 103 Byrer, D.E., F.A. Rainey & J. Wiegel. 2000. Novel strains of *Moorella thermoacetica* form unusually heat-resistant spores. *Arch. Microbiol.* 174: 334–339.
- 104 Ferry, J.G. 1994. CO dehydrogenase of methanogens. In *Acetogenesis*. H.L. Drake, Ed.: 539–556. New York : Chapman & Hall.
- 105 Whitman, W.B. 1994. Autotrophic acetyl coenzyme A biosynthesis in methanogens. In *Acetogenesis*. H.L. Drake, Ed.: 521–538. New York : Chapman & Hall.
- 106 Lee, M.J. & S.H. Zinder. 1988. Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H<sub>2</sub>-CO<sub>2</sub>. *Appl. Environ. Microbiol.* 54: 124–129.
- 107 Hattori, S., A.S. Galushko, Y. Kamagata & B. Schink. 2005. Operation of the CO dehydrogenase/acetyl coenzyme A pathway in both acetate oxidation and acetate formation by the syntrophically acetate-oxidizing bacterium *Thermoacetogenium phaeum*. *J. Bacteriol.* 187: 3471–3476.

- 108 Rother, M. & W.W. Metcalf. 2004. Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. *Proc. Natl. Acad. Sci. USA* 101: 16929–16934.
- 109 Henstra, A.M., C. Dijkema & A.J.M. Stams. 2007. *Archaeoglobus fulgidus* couples CO oxidation to sulfate reduction and acetogenesis with transient formate accumulation. *Environ. Microbiol.* 9: 1836–1841.
- 110 Daniels, L., G. Fuchs, R.K. Thauer & J.G. Zeikus. 1977. Carbon monoxide oxidation by methanogenic bacteria. *J. Bacteriol.* 132: 118–126.
- 111 Savage, M.D., Z.G. Wu, S.L. Daniel, et al. 1987. Carbon monoxide-dependent chemolithotrophic growth of *Clostridium thermoautotrophicum*. *Appl. Environ. Microbiol.* 53: 1902–1906.
- 112 Hsu, T., S.L. Daniel, M.F. Lux & H.L. Drake. 1990. Biotransformations of carboxylated aromatic compounds by the acetogen *Clostridium thermoaceticum*: generation of growth-supportive CO<sub>2</sub> equivalents under CO<sub>2</sub>-limited conditions. *J. Bacteriol.* 172: 212–217.
- 113 Martin, D.R., A. Misra & H.L. Drake. 1985. Dissimilation of carbon monoxide to acetic acid by glucose-limited cultures of *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.* 49: 1412–1417.
- 114 Hsu, T.D., M.F. Lux & H.L. Drake. 1990. Expression of an aromatic-dependent decarboxylase which provides growth-essential CO<sub>2</sub> equivalents for the acetogenic (Wood) pathway of *Clostridium thermoaceticum*. *J. Bacteriol.* 172: 5901–5907.
- 115 Braus-Stromeyer, S.A., G. Schnappauf, G.H. Braus, et al. 1997. Carbonic anhydrase in *Acetobacterium woodii* and other acetogenic bacteria. *J. Bacteriol.* 179: 7197–7200.
- 116 Fröstl, J.M., C. Seifritz & H.L. Drake. 1996. Effect of nitrate on the autotrophic metabolism of the acetogens *Clostridium thermoautotrophicum* and *Clostridium thermoaceticum*. *J. Bacteriol.* 178: 4597–4603.
- 117 Seifritz, C., S.L. Daniel, A. Gößner & H.L. Drake. 1993. Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. *J. Bacteriol.* 175: 8008–8013.
- 118 Slobodkin, A., A.L. Reysenbach, F. Mayer & J. Wiegel. 1997. Isolation and characterization of the homoacetogenic thermophilic bacterium *Moorella glycerini* sp. nov. *Int. J. Syst. Bacteriol.* 47: 969–974.
- 119 Thauer, R.K., K. Jungermann & K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* 41: 100–180.

- 120 Seifritz, C., H.L. Drake & S.L. Daniel. 2003. Nitrite as an energy-conserving electron sink for the acetogenic bacterium *Moorella thermoacetica*. *Curr. Microbiol.* 46: 329–333.
- 121 Arendsen, A.F., M.Q. Soliman & S.W. Ragsdale. 1999. Nitrate-dependent regulation of acetate biosynthesis and nitrate respiration by *Clostridium thermoaceticum*. *J. Bacteriol.* 181: 1489–1495.
- 122 Drake, H.L., S.L. Daniel, K. Küsel, et al. 1997. Acetogenic bacteria: What are the in situ consequences of their diverse metabolic versatilities? *Biofactors* 6: 13–24.
- 123 Misoph, M., S.L. Daniel & H.L. Drake. 1996. Bidirectional usage of ferulate by the acetogen *Peptostreptococcus productus* U-1: CO<sub>2</sub> and aromatic acrylate groups as competing electron acceptors. *Microbiology* 142: 1983–1988.
- 124 Misoph, M. & H.L. Drake. 1996. Effect of CO<sub>2</sub> on the fermentation capacities of the acetogen *Peptostreptococcus productus* U-1. *J. Bacteriol.* 178: 3140–3145.
- 125 Dilling, S., F. Inkamp, S. Schmidt & V. Müller. 2007. Regulation of caffeate respiration in the acetogenic bacterium *Acetobacterium woodii*. *Appl. Environ. Microbiol.* 73: 3630–3636.
- 126 Winter, J.U. & R.S. Wolfe. 1980. Methane formation from fructose by syntrophic associations of *Acetobacterium woodii* and different strains of methanogens. *Arch. Microbiol.* 124: 73–39.
- 127 Bernalier, A., M. Lelait, V. Rochet, et al. 1996. Acetogenesis from H<sub>2</sub> and CO<sub>2</sub> by methane- and non-methane-producing human colonic bacterial communities. *FEMS Microbiol. Immunol.* 19: 193–202.
- 128 Doré, J., P. Pochart, A. Bernalier, et al. 1995. Enumeration of H<sub>2</sub>-utilizing methanogenic archaea, acetogenic and sulfate-reducing bacteria from human feces. *FEMS Microbiol. Ecol.* 17: 279–284.
- 129 Leadbetter, J.R., T.M. Schmidt, J.R. Graber & J.A. Breznak. 1999. Acetogenesis from H<sub>2</sub> plus CO<sub>2</sub> by spirochetes from termite guts. *Science* 283: 686–689.
- 130 Schink, B. 1994. Diversity, ecology, and isolation of acetogenic bacteria. In *Acetogenesis*. H.L. Drake, Ed.: 197–235. New York : Chapman & Hall.
- 131 Ljungdahl, L.G. 1983. Formation of acetate using homoacetate fermenting anaerobic bacteria. In *Organic Chemicals from Biomass*. D.L. Wise, Ed.: 219–248. Menlo Park , CA : Benjamin/Cummings Publishing Company.
- 132 Wood, H.G. 1952. Fermentation of 3,4-C<sup>14</sup>- and 1-C<sup>14</sup>-labeled glucose by *Clostridium thermoaceticum*. *J. Biol. Chem.* 199: 579–583.

- 133 Karita, S., K. Nakayama, M. Goto, et al. 2003. A novel cellulolytic, anaerobic, and thermophilic bacterium, *Moorella* sp. strain F21. *Biosci. Biotechnol. Biochem.* 67: 183–185.
- 134 Bak, F., K. Finster, F. Rothfuß. 1992. Formation of dimethylsulfide and methanethiol from methoxylated aromatic compounds and inorganic sulfide by newly isolated anaerobic bacteria. *Arch. Microbiol.* 157: 529–534.
- 135 Kreft, J.U. & B. Schink. 1993. Demethylation and degradation of phenylmethylethers by the sulfide-methylating homoacetogenic bacterium strain TMBS 4. *Arch. Microbiol.* 159: 308–315.
- 136 Kellum, R. & H.L. Drake. 1984. Effects of cultivation gas phase on hydrogenase of the acetogen *Clostridium thermoaceticum*. *J. Bacteriol.* 160: 466–469.
- 137 Drake, H.L. 1982. Demonstration of hydrogenase in extracts of the homoacetate-fermenting bacterium *Clostridium thermoaceticum*. *J. Bacteriol.* 150: 702–709.
- 138 Daniel, S.L. & H.L. Drake. 1993. Oxalate- and glyoxylate-dependent growth and acetogenesis by *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.* 59: 3062–3069.
- 139 Seifritz, C., J.M. Frostl, H.L. Drake & S.L. Daniel. 1999. Glycolate as a metabolic substrate for the acetogen *Moorella thermoacetica*. *FEMS Microbiol. Lett.* 170: 399–405.
- 140 Daniel, S.L., C. Pilsel & H.L. Drake. 2004. Oxalate metabolism by the acetogenic bacterium *Moorella thermoacetica*. *FEMS Microbiol. Lett.* 231: 39–43.
- 141 Seifritz, C., J.M. Fröstl, H.L. Drake & S.L. Daniel. 2002. Influence of nitrate on oxalate- and glyoxylate-dependent growth and acetogenesis by *Moorella thermoacetica*. *Arch. Microbiol.* 178: 457–464.
- 142 Küsel, K., C. Wagner & H.L. Drake. 1999. Enumeration and metabolic product profiles of the anaerobic microflora in the mineral soil and litter of a beech forest. *FEMS Microbiol. Ecology* 29: 91–103.
- 143 Küsel, K., H.C. Pinkart, H.L. Drake & R. Devereux. 1999. Acetogenic and sulfate-reducing bacteria inhabiting the rhizoplane and deep cortex cells of the sea grass *Halodule wrightii*. *Appl. Environ. Microbiol.* 65: 5117–5123.
- 144 Das, A., E.D. Coulter, D.M. Kurtz & L.G. Ljungdahl. 2001. Five-gene cluster in *Clostridium thermoaceticum* consisting of two divergent operons encoding rubredoxin oxidoreductase-rubredoxin and rubrerythrin-type A flavoprotein-high-molecular-weight rubredoxin. *J. Bacteriol.* 183: 1560–1567.
- 145 Karnholz, A., K. Küsel, A. Gößner, et al. 2002. Tolerance and metabolic response of acetogenic bacteria toward oxygen. *Appl. Environ. Microbiol.* 68: 1005–1009.

- 146 Silaghi-Dumitrescu, R., E.D. Coulter, A. Das, et al. 2003. A flavodiiron protein and high molecular weight rubredoxin from *Moorella thermoacetica* with nitric oxide reductase activity. *Biochemistry* 42: 2806–2815.
- 147 Das, A., R. Silaghi-Dumitrescu, L.G. Ljungdahl & D.M. Kurtz, Jr. 2005. Cytochrome bd oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *J. Bacteriol.* 187: 2020–2029.
- 148 Busche, R.M. 1991. Extractive fermentation of acetic acid: economic tradeoff between yield of *Clostridium* and concentration of *Acetobacter*. *Appl. Biochem. Biotechnol.* 28–29: 605–621.
- 149 Cheryan, M., S. Parekh, M. Shah & K. Witjitra. 1997. Production of acetic acid by *Clostridium thermoaceticum*. *Adv. Appl. Microbiol.* 43: 1–33.
- 150 Ljungdahl, L.G., J. Hugenholtz & J. Wiegel. 1989. Acetogenic and acid-producing clostridia. In *Clostridia*. N.P.Minton & D.J.Clarke, Eds.: 145–191. New York : Plenum Press.
- 151 Ljungdahl, L.G., L.H. Carreira, R.J. Garrison, et al. 1985. Comparison of three thermophilic acetogenic bacteria for production of calcium magnesium acetate. *Biotechnol. Bioeng. Symp.* 15: 207–223.
- 152 Schwartz, R.D. & F.A. Keller, Jr. 1982. Isolation of a strain of *Clostridium thermoaceticum* capable of growth and acetic acid production at pH 4.5. *Appl. Environ. Microbiol.* 43: 117–123.
- 153 Shah, M.M. & M. Cheryan. 1995. Improvement of productivity in acetic acid fermentation with *Clostridium thermoaceticum*. *Appl. Biochem. Biotechnol.* 51–52: 413–422.
- 154 Wiegel, J., L.H. Carreira, R.J. Garrison, et al. 1990. Calcium magnesium acetate (CMA) manufacture from glucose by fermentation with thermophilic homoacetogenic bacteria. In *Calcium Magnesium Acetate*. D.L.Wise, Y.Levendis & M.Metghalchi, Eds.: 359–416. Amsterdam , The Netherlands : Elsevier.
- 155 Wiegel, J. 1994. Acetate and the potential of homoacetogenic bacteria for industrial applications. In *Acetogenesis*. H.L.Drake, Ed.: 484–504. New York : Chapman & Hall.
- 156 Grethlein, A.J. & M.K. Jain. 1992. Bioprocessing of coal-derived synthesis gases by anaerobic bacteria. *Trends Biotechnol.* 10: 418–423.
- 157 Phillips, J.R., E.C. Clausen & J.L. Gaddy. 1994. Synthesis gas as substrate for the biological production of fuels and chemicals. *Appl. Biochem. Biotechnol.* 45/46: 145–157.

- 158 Abrini, J., H. Naveau & E.-J. Nyns. 1994. *Clostridium autoethanogenum*, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. *Arch. Microbiol.* 161: 345–351.
- 159 Baronofsky, J.J., W.J.A. Schreurs & E.R. Kashket. 1984. Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.* 48: 1134–1139.
- 160 Le Ruyet, P., H.C. Dubourguier & G. Albagnac. 1984. Homoacetogenic fermentation of cellulose by a coculture of *Clostridium thermocellum* and *Acetogenium kivui*. *Appl. Environ. Microbiol.* 48: 893–894.
- 161 Miller, T.L. & M.J. Wolin. 1995. Bioconversion of cellulose to acetate with pure cultures of *Ruminococcus albus* and a hydrogen-using acetogen. *Appl. Environ. Microbiol.* 61: 3832–3835.
- 162 Ravinder, T., M.V. Swamy, G. Seenayya & G. Reddy. 2001. *Clostridium lentocellum* SG6—A potential organism for fermentation of cellulose to acetic acid. *Bioresour. Technol.* 80: 171–177.
- 163 Eggeman, T. & D. Verser. 2005. Recovery of organic acids from fermentation broths. *Appl. Biochem. Biotechnol.* 121–124: 605–618.
- 164 Eggeman, T. & D. Verser. 2006. The importance of utility systems in today's biorefineries and a vision for tomorrow. *Appl. Biochem. Biotechnol.* 129–132: 361–381.
- 165 Egli, C., T. Tschan, R. Scholtz, et al. 1988. Transformation of tetrachloromethane to dichloromethane and carbon dioxide by *Acetobacterium woodii*. *Appl. Environ. Microbiol.* 54: 2819–2824.
- 166 Traunecker, J., A. Preuß & G. Diekert. 1991. Isolation and characterization of a methyl chloride utilizing, strictly anaerobic bacterium. *Arch. Microbiol.* 156: 416–421.
- 167 Duhamel, M. & E.A. Edwards. 2007. Growth and yields of dechlorinators, acetogens, and methanogens during reductive dechlorination of chlorinated ethenes and dihaloelimination of 1,2-dichloroethane. *Environ. Sci. Technol.* 41: 2303–2310.
- 168 Lebloas, P., P. Loubiere & N.D. Lindley. 1994. Use of unicarbon substrate mixtures to modify carbon flux improves vitamin B12 production with the acetogenic methylotroph *Eubacterium limosum*. *Biotechnol. Lett.* 16: 129–132
- 169 Schulz, M., H. Leichmann, H. Günther & H. Simon. 1995. Electromicrobial regeneration of pyridine nucleotides and other preparative redox transformations with *Clostridium thermoaceticum*. *Appl. Microbiol. Biotechnol.* 42: 916–922.

- 170 Koesnandar, S. Ago, N. Nishio & S. Nagai. 1989. Production of extracellular 5-aminoleuvulinic acid by *Clostridium thermoaceticum* grown in minimal medium. *Biotechnol. Lett.* 11: 567–572.
- 171 Koesnandar, N. Nishio, A. Yamamoto & S. Nagai. 1991. Enzymatic reduction of cystine into cysteine by cell-free extract of *Clostridium thermoaceticum*. *J. Ferment. Bioeng.* 72: 11–14.
- 172 Breznak, J.A. & M.D. Kane. 1990. Microbial H<sub>2</sub>/CO<sub>2</sub> acetogenesis in animal guts: nature and nutritional significance. *FEMS Microbiol. Rev.* 7: 309–313.
- 173 Graber, J.R. & J.A. Breznak. 2005. Folate cross-feeding supports symbiotic homoacetogenic spirochetes. *Appl. Environ. Microbiol.* 71: 1883–1889.
- 174 Lovell, C.R. 1994. Development of DNA probes for the detection and identification of acetogenic bacteria. In *Acetogenesis*. H.L. Drake, Ed.: 236–253. New York : Chapman & Hall.
- 175 Lovell, C.R. & A.B. Leaphart. 2005. Community-level analysis: key genes of CO<sub>2</sub>-reductive acetogenesis. *Methods Enzymol.* 397: 454–469.
- 176 Salmassi, T.M. & J.R. Leadbetter. 2003. Analysis of genes of tetrahydrofolate-dependent metabolism from cultivated spirochaetes and the gut community of the termite *Zootermopsis angusticollis*. *Microbiology* 149: 2529–2537.
- 177 Pester, M. & A. Brune. 2006. Expression profiles of *fhs* (FTHFS) genes support the hypothesis that spirochaetes dominate reductive acetogenesis in the hindgut of lower termites. *Appl. Environ. Microbiol.* 8: 1261–1270.
- 178 Leaphart, A.B., M.J. Friez & C.R. Lovell. 2003. Formyltetrahydrofolate synthetase sequences from salt marsh plant roots reveal a diversity of acetogenic bacteria and other bacterial functional groups. *Appl. Environ. Microbiol.* 69: 693–696.
- 179 Kotelnikova, S. 2002. Microbial production and oxidation of methane in deep subsurface. *Earth Sci. Rev.* 58: 367–395.
- 180 Krumholz, L.R. 2000. Microbial communities in the deep subsurface. *Hydrogeol. J.* 8: 4–10.
- 181 Kuhner, C.H., C. Matthies, G. Acker, et al. 2000. *Clostridium akagii* sp. nov. and *Clostridium acidisoli* sp. nov.: acid-tolerant, N<sub>2</sub>-fixing clostridia isolated from acidic forest soil and litter. *Int. J. Syst. Evol. Microbiol.* 50: 873–881.
- 182 Matthies, C., C.H. Kuhner, G. Acker & H.L. Drake. 2001. *Clostridium uliginosum* sp. nov., a novel acid-tolerant anaerobic bacterium with connecting filaments. *Int. J. Syst. Evol. Microbiol.* 51: 1119–1125.

- 183 Greening, R.C. & J.A.Z. Leedle. 1989. Enrichment and isolation of *Acetitomaculum ruminis*, gen. nov., sp. nov.: acetogenic bacteria from the bovine rumen. *Arch. Microbiol.* 151: 399–406.
- 184 Sleat, R., R.A. Mah & R. Robinson. 1985. *Acetoanaerobium noterae* gen. nov., sp. nov.: an anaerobic bacterium that forms acetate from H<sub>2</sub> and CO<sub>2</sub>. *Int. J. Syst. Bacteriol.* 35: 10–15.
- 185 Davydova-Charakhchyan, I.A., A.N. Mileeva, L.L. Mityushina & S.S. Belyaev. 1992. Acetogenic bacteria from oil fields of Tataria and western Siberia. *Mikrobiologiya* 61: 306–315.
- 186 Kotsyurbenko, O.R., M.V. Simankova, A.N. Nozhevnikova, et al. 1995. New species of psychrophilic acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov. *Arch. Microbiol.* 163: 29–34.
- 187 Eichler, B. & B. Schink. 1984. Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. *Arch. Microbiol.* 140: 147–152.
- 188 Tanaka, K. & N. Pfennig. 1988. Fermentation of 2-methoxyethanol by *Acetobacterium malicum* sp. nov. and *Pelobacter venetianus*. *Arch. Microbiol.* 149: 181–187.
- 189 Braun, M. & G. Gottschalk. 1982. *Acetobacterium wieringae* sp. nov., a new species producing acetic acid from molecular hydrogen and carbon dioxide. *Zentralbl. Bakteriolog. Hyg., I. Abt. Originale C* 3368–376.
- 190 Dörner, C. & B. Schink. 1991. Fermentation of mandelate to benzoate and acetate by a homoacetogenic bacterium. *Arch. Microbiol.* 156: 302–306.
- 191 Sembiring, T. & J. Winter. 1989. Anaerobic degradation of *o*-phenylphenol by mixed and pure cultures. *Appl. Microbiol. Biotechnol.* 31: 89–92.
- 192 Sembiring, T. & J. Winter. 1990. Demethylation of aromatic compounds by strain B10 and complete degradation of 3-methoxybenzoate in co-culture with *Desulfosarcina* strains. *Appl. Microbiol. Biotechnol.* 33: 233–238.
- 193 Schramm, E. & B. Schink. 1991. Ether-cleaving enzyme and diol dehydratase involved in anaerobic polyethylene glycol degradation by a new *Acetobacterium* sp. *Biodegradation* 2: 71–79.
- 194 Conrad, R., F. Bak, H.J. Seitz, B. Thebrath, et al. 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol. Ecol.* 62: 285–294.
- 195 Wagener, S. & B. Schink. 1988. Fermentative degradation of nonionic surfactants and polyethylene glycol by enrichment cultures and by pure cultures of



homoacetogenic and propionate-forming bacteria. *Appl. Environ. Microbiol.* 54: 561–565.

196 Frings, J. & B. Schink. 1994. Fermentation of phenoxyethanol to phenol and acetate by a homoacetogenic bacterium. *Arch. Microbiol.* 162: 199–204.

197 Frings, J., C. Wondrak & B. Schink. 1994. Fermentative degradation of triethanolamine by a homoacetogenic bacterium. *Arch. Microbiol.* 162: 103–107.

198 Emde, R. & B. Schink. 1987. Fermentation of triacetin and glycerol by *Acetobacterium* sp. No energy is conserved by acetate excretion. *Arch. Microbiol.* 149: 142–148.

199 Schuppert, B. & B. Schink. 1990. Fermentation of methoxyacetate to glycolate and acetate by newly isolated strains of *Acetobacterium* sp. *Arch. Microbiol.* 153: 200–204.

200 Inoue, K., S. Kageyama, K. Miki, et al. 1992. Vitamin B12 production by *Acetobacterium* sp. and its tetrachloromethane-resistant mutants. *J. Ferment. Bioeng.* 73: 76–78.

201 Kotsyurbenko, O.R., A.N. Nozhevnikova, T.I. Soloviova & G.A. Zavarin. 1996. Methanogenesis at low temperatures by microflora of tundra wetland soil. *Antonie Leeuwenhoek* 69: 75–86.

202 Zhilina, T.N. & G.A. Zavarzin. 1990. Extremely halophilic, methylotrophic, anaerobic bacteria. *FEMS Microbiol. Rev.* 87: 315–322.

203 Kane, M.D. & J.A. Breznak. 1991. *Acetonema longum* gen. nov. sp. nov., an H<sub>2</sub>/CO<sub>2</sub> acetogenic bacterium from the termite, *Pterotermes occidentis*. *Arch. Microbiol.* 156: 91–98.

204 Zeikus, J.G., L.H. Lynd, T.E. Thompson, et al. 1980. Isolation and characterization of a new, methylotrophic, acidogenic anaerobe, the Marburg strain. *Curr. Microbiol.* 3: 381–386.

205 Patel, B.K.C., C. Monk, H. Littleworth, et al. 1987. *Clostridium fervidus* sp. nov., a new chemoorganotrophic acetogenic thermophile. *Int. J. Syst. Bacteriol.* 37: 123–126.

206 Kaneuchi, C., Y. Benno & T. Mitsuoka. 1976. *Clostridium coccoides*, a new species from the feces of mice. *Int. J. Syst. Bacteriol.* 26: 482–486.

207 Rieu-Lesme, F., C. Dauga, G. Fonty & J. Doré. 1998. Isolation from the rumen of a new acetogenic bacterium phylogenetically closely related to *Clostridium difficile*. *Anaerobe* 4: 89–94.

208 Schink, B. 1984. *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.* 137: 250–255.

- 209 Mechichi, T., M. Labat, T.H.S. Woo, et al. 1998. *Eubacterium aggregans* sp nov., a new homoacetogenic bacterium from olive mill wastewater treatment digester. *Anaerobe* 4: 283–291.
- 210 Schnürer, A., B. Schink & B.H. Svensson. 1996. *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. *Int. J. Syst. Bacteriol.* 46: 1145–1152.
- 211 Adamse, A.D. & C.T.M. Velzeboer. 1982. Features of a *Clostridium*, strain CV-AA1, an obligatory anaerobic bacterium producing acetic acid from methanol. *Antonie Leeuwenhoek* 48: 305–313.
- 212 Leclerc, M., A. Bernalier, G. Donadille & M. Lelait. 1997. H<sub>2</sub>/CO<sub>2</sub> metabolism in acetogenic bacteria isolated from the human colon. *Anaerobe* 3: 307–315.
- 213 Leclerc, M., A. Bernalier, M. Lelait & J.-P. Grivet 1997. <sup>13</sup>C-NMR study of glucose and pyruvate catabolism in four acetogenic species isolated from the human colon. *FEMS Microbiol. Lett.* 146: 199–204.
- 214 Sharak Genthner, B.R., C.L. Davies & M.P. Bryant. 1981. Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H<sub>2</sub>-CO<sub>2</sub>-utilizing species. *Appl. Environ. Microbiol.* 42: 12–19.
- 215 Balk, M., J. Weijma, M.W. Friedrich & A.J. Stams. 2003. Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. *Arch. Microbiol.* 179: 315–320.
- 216 Wiegel, J., M. Braun & G. Gottschalk. 1981. *Clostridium thermoautotrophicum* species novum, a thermophile producing acetate from molecular hydrogen and carbon dioxide. *Curr. Microbiol.* 5: 255–260.
- 217 Sakai, S., Y. Nakashimada, H. Yoshimoto, et al. 2004. Ethanol production from H<sub>2</sub> and CO<sub>2</sub> by a newly isolated thermophilic bacterium, *Moorella* sp. HUC22-1. *Biotechnol. Lett.* 26: 1607–1612.
- 218 Zhilina, T.N., E.N. Detkova, F.A. Rainey, et al. 1998. *Natronoincola histidinovorans* gen. nov., sp. nov., a new alkaliphilic acetogenic anaerobe. *Curr. Microbiol.* 37: 177–185.
- 219 Krumholz, L.R. & M.P. Bryant. 1985. *Clostridium pfennigii* sp. nov. uses methoxyl groups of monobenzenoids and produces butyrate. *Int. J. Syst. Bacteriol.* 35: 454–456.
- 220 Bernalier, A., V. Rochet, M. Leclerc, et al. 1996. Diversity of H<sub>2</sub>/CO<sub>2</sub>-utilizing acetogenic bacteria from feces of non-methane-producing humans. *Curr. Microbiol.* 33: 94–99.

- 221 Bernalier, A., A. Willems, M. Leclerc, et al. 1996. *Ruminococcus hydrogenotrophicus* sp. nov., a new H<sub>2</sub>/CO<sub>2</sub>-utilizing acetogenic bacterium isolated from human feces. *Arch. Microbiol.* 166: 176–183.
- 222 Lorowitz, W.H. & M.P. Bryant. 1984. *Peptostreptococcus productus* strain that grows rapidly with CO as the energy source. *Appl. Environ. Microbiol.* 47: 961–964.
- 223 Geerligs, G., H.C. Aldrich, W. Harder & G. Diekert. 1987. Isolation and characterization of a carbon monoxide utilizing strain of the acetogen *Peptostreptococcus productus*. *Arch. Microbiol.* 148: 305–313.
- 224 Rieu-Lesme, F., B. Morvan, M.D. Collins, et al. 1996. A new H<sub>2</sub>/CO<sub>2</sub>-using acetogenic bacterium from the rumen: description of *Ruminococcus schinkii* sp. nov. *FEMS Microbiol. Lett.* 140: 281–286.
- 225 Ollivier, B., R. Cordruwisch, A. Lombardo & J.-L. Garcia. 1985. Isolation and characterization of *Sporomusa acidovorans* sp. nov., a methylotrophic homoacetogenic bacterium. *Arch. Microbiol.* 142: 307–310.
- 226 Boga, H.I., W. Ludwig & A. Brune. 2003. *Sporomusa aerivorans* sp. nov., an oxygen-reducing homoacetogenic bacterium from the gut of a soil-feeding termite. *Int. J. Syst. Evol. Microbiol.* 53: 1397–1404.
- 227 Dehning, I., M. Stieb & B. Schink. 1989. *Sporomusa malonica* sp. nov., a homoacetogenic bacterium growing by decarboxylation of malonate or succinate. *Arch. Microbiol.* 151: 421–426.
- 228 Möller, B., R. Oßmer, B.H. Howard, et al. 1984. *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. *Arch. Microbiol.* 139: 388–396.
- 229 Hermann, M., M.-R. Popoff & M. Sebald. 1987. *Sporomusa paucivorans* sp. nov., a methylotrophic bacterium that forms acetic acid from hydrogen and carbon dioxide. *Int. J. Syst. Bacteriol.* 37: 93–101.
- 230 Breznak, J.A., J.M. Switzer & H.J. Seitz. 1988. *Sporomusa termitida* sp. nov., an H<sub>2</sub>/CO<sub>2</sub>-utilizing acetogen isolated from termites. *Arch. Microbiol.* 150: 282–288.
- 231 Rosencrantz, D., F.A. Rainey & P.H. Janssen. 1999. Culturable populations of *Sporomusa* spp. and *Desulfovibrio* spp. in the anoxic bulk soil of flooded rice microcosms. *Appl. Environ. Microbiol.* 65: 3526–3533.
- 232 Krumholz, L.R. & M.P. Bryant. 1986. *Syntrophococcus sucromutans* sp. nov. gen. nov. uses carbohydrates as electron donors and formate, methoxymonobenzenoids or *Methanobrevibacter* as electron acceptor systems. *Arch. Microbiol.* 143: 313–318.
- 233 Hattori, S., Y. Kamagata, S. Hanada & H. Shoun. 2000. *Thermoacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int. J. Syst. Evol. Microbiol.* 50: 1601–1609.

- 234 Leigh, J.A., F. Mayer & R.S. Wolfe. 1981. *Acetogenium kivui*, a new thermophilic hydrogen-oxidizing, acetogenic bacterium. *Arch. Microbiol.* 129: 275–280.
- 235 Davidova, I.A. & A.J.M. Stams. 1996. Sulfate reduction with methanol by a thermophilic consortium obtained from a methanogenic reactor. *Appl. Microbiol. Biotechnol.* 46: 297–302.
- 236 Wolin, M.J. & T.L. Miller. 1993. Bacterial strains from human feces that reduce CO<sub>2</sub> to acetic acid. *Appl. Environ. Microbiol.* 59: 3551–3556.
- 237 Rieu-Lesme, F., G. Fonty & J. Doré. 1995. Isolation and characterization of a new hydrogen-utilizing bacterium from the rumen. *FEMS Microbiol. Lett.* 125: 77–82.
- 238 Plugge, C.M., J.T.C. Grotenhuis & A.J.M. Stams. 1990. Isolation and characterization of an ethanol-degrading anaerobe from methanogenic granular sludge. In *Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer*. J.-P. Belaich, M. Bruschiand & J.-L. Garcia, Eds.: 439–442. FEMS Symposium No. 54. New York : Plenum Press.
- 239 Wolin, M.J. & T.L. Miller. 1994. Acetogenesis from CO<sub>2</sub> in the human colonic ecosystem. In *Acetogenesis*. H.L. Drake, Ed.: 365–385. New York : Chapman & Hall.
- 240 Chaucheyras, F., G. Fonty, G. Bertin & P. Gouet. 1995. In vitro H<sub>2</sub> utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archaea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 61: 3466–3467.
- 241 Frazer, A.C. & L.Y. Young. 1985. A gram-negative anaerobic bacterium that utilizes O-methyl substituents of aromatic acids. *Appl. Environ. Microbiol.* 49: 1345–1347.
- 242 Samain, E., G. Albangnac, H.C. Dubourguier & J.-P. Touzel. 1982. Characterization of a new propionic acid bacterium that ferments ethanol and displays a growth factor-dependent association with a gram-negative homoacetogen. *FEMS Microbiol. Lett.* 15: 69–74.
- 243 Kotsyurbenko, O.R., M.V. Simankova, N.P. Bolotina, et al. 1992. Psychrotrophic homoacetogenic bacteria from several environments. 7th Int. Symp. C1 Compounds, Abstr. C136.
- 244 Nozhevnikova, A., O.R. Kotsyurbenko & M.V. Simankova. 1994. Acetogenesis at low temperature. In *Acetogenesis*. H.L. Drake, Ed.: 416–431. New York : Chapman & Hall.
- 245 Rieu-Lesme, F., C. Dauga, B. Morvan, et al. 1996. Acetogenic coccoid spore-forming bacteria isolated from the rumen. *Res. Microbiol.* 147: 753–764.
- 246 Drake, H.L. 1992. Acetogenesis and acetogenic bacteria. In *Encyclopedia of Microbiology*, Vol. 1. J. Lederberg, Ed.: 1–15. San Diego , CA : Academic Press.

- 247 Barker, H.A. 1944. On the role of carbon dioxide in the metabolism of *Clostridium thermoaceticum*. Proc. Natl. Acad. Sci. USA 30: 88–90.
- 248 Lentz, K. & H.G. Wood. 1955. Synthesis of acetate from formate and carbon dioxide by *Clostridium thermoaceticum*. J. Biol. Chem. 215: 645–654.
- 249 Poston, J.M., E.R. Stadtman & K. Kuratomi. 1964. Methyl-vitamin B12 as source of methyl groups for synthesis of acetate by cell-free extracts of *Clostridium thermoaceticum*. Ann. N.Y. Acad. Sci. 112: 804–806.
- 250 Ljungdahl, L. & H.G. Wood. 1965. Incorporation of C14 from carbon dioxide into sugar phosphates, carboxylic acids, and amino acids by *Clostridium thermoaceticum*. J. Bacteriol. 89: 1055–1064.
- 251 Ljungdahl, L., E. Irion & H.G. Wood. 1966. Role of corrinoids in the total synthesis of acetate from CO<sub>2</sub> by *Clostridium thermoaceticum*. Fed. Proc. 25: 1642–1648.
- 252 Ljungdahl, L.G. & H.G. Wood. 1969. Total synthesis of acetate from CO<sub>2</sub> by heterotrophic bacteria. Annu. Rev. Microbiol. 23: 515–538.
- 253 Drake, H.L., S.I. Hu & H.G. Wood. 1981. Purification of five components from *Clostridium thermoaceticum* which catalyze synthesis of acetate from pyruvate and methyltetrahydrofolate. Properties of phosphotransacetylase. J. Biol. Chem. 256: 11137–11144.
- 254 Drake, H.L., S.-I. Hu & H.G. Wood. 1981. The synthesis of acetate from carbon monoxide plus methyltetrahydrofolate and the involvement of the nickel enzyme, CO dehydrogenase. Abstr. Ann. Meet. Am. Soc. Microbiol., p. 144, Abstr. K-42.
- 255 Ragsdale, S.W., J.E. Clark, L.G. Ljungdahl, et al. 1983. Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron-sulfur protein. J. Biol. Chem. 258: 2364–2369.
- 256 Kerby, R. & J.G. Zeikus. 1983. Growth of *Clostridium thermoaceticum* on H<sub>2</sub>/CO<sub>2</sub> or CO as energy source. Curr. Microbiol. 8: 27–30.
- 257 Lundie, L.L., Jr. & H.L. Drake. 1984. Development of a minimally defined medium for the acetogen *Clostridium thermoaceticum*. J. Bacteriol. 159: 700–7003.
- 258 Pezacka, E. & H.G. Wood. 1984. The synthesis of acetyl-CoA by *Clostridium thermoaceticum* from carbon dioxide, hydrogen, coenzyme A and methyltetrahydrofolate. Arch. Microbiol. 137: 63–69.
- 259 Pezacka, E. & H.G. Wood. 1984. Role of carbon monoxide dehydrogenase in the autotrophic pathway used by acetogenic bacteria. Proc. Natl. Acad. Sci. USA 81: 6261–6265.

260 Ragsdale, S.W. & H.G. Wood. 1985. Acetate biosynthesis by acetogenic bacteria. Evidence that carbon monoxide dehydrogenase is the condensing enzyme that catalyzes the final steps of the synthesis. *J. Biol. Chem.* 260: 3970–3977.

261 Ivey, D.M. & L.G. Ljungdahl. 1986. Purification and characterization of the F1-ATPase from *Clostridium thermoaceticum*. *J. Bacteriol.* 165: 252–257.

262 Hugenholtz, J. & L.G. Ljungdahl. 1989. Electron transport and electrochemical proton gradient in membrane vesicles of *Clostridium thermoautotrophicum*. *J. Bacteriol.* 171: 2873–2875.

263 Hugenholtz, J. & L.G. Ljungdahl. 1990. Metabolism and energy generation in homoacetogenic clostridia. *FEMS Microbiol. Rev.* 7: 383–389.

264 Das, A., J. Hugenholtz, H. Van Halbeek & L.G. Ljungdahl. 1989. Structure and function of a menaquinone involved in electron transport in membranes of *Clostridium thermoautotrophicum* and *Clostridium thermoaceticum*. *J. Bacteriol.* 171: 5823–5829.