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A Phylogenetic Analysis of Anaerobic Eubacteria Capable of Synthesizing Acetate from Carbon Dioxide

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Abstract. *Acetobacterium woodii, Acetogenium kivui, Clostridium aceticum, C. acidiurici, C. cylindrosporum, C. formicoaceticum, C. thermoaceticum, Eubacterium limosum*, and *Peptococcus glycinophilus* were characterized by oligonucleotide cataloging of their 16S ribosomal RNA to determine whether the ability to synthesize acetate from CO_2 is a phylogenetic trait. The ability to synthesize acetate from CO_2 apparently is not a valid phylogenetic marker. The *Eubacterium* and *Peptococcus* species examined here are less related to other species in their genera than they are to different species of *Clostridium*. The *Eubacterium* species examined here show little relatedness to the genus *Propionibacterium*. The acetogenic eubacteria belong to the phylogenetic group defined basically by the Gram-positive sporeforming anaerobes.

A group of bacteria capable of reducing CO_2 to acetate was listed in a 1969 review on the production of acetate from CO_2 by heterotrophs [9]: *Clostridium acidiurici, C. cylindrosporum, Peptococcus glycinophilus, Eubacterium limosum (Butyribacterium rettgeri), C. aceticum, C. formicoaceticum* (*Clostridium* strain A_1), and *C. thermoaceticum. Clostridium aceticum*, which was recently recovered [3], can also use H_2 to reduce CO_2 to acetate. Two nonsporeforming bacteria able to form acetate from H_2 and CO_2, *Acetobacterium woodii* [2] and *Acetogenium kivui* [7], have been isolated since the aforementioned review [9] appeared. Recently, *Eubacterium limosum* has been shown capable of using H_2 to reduce CO_2 to acetate [12]. In this study, we decided to use oligonucleotide cataloging of ribosomal RNA (rRNA) to determine whether the ability to synthesize acetate from CO_2 can be considered a phylogenetic trait. The methanogens, which synthesize CH_4 from CO_2, have been shown to form a phylogenetically related group by comparative cataloging of 16S rRNA [1].

In a previous publication, *Acetobacterium woodii* and *Eubacterium limosum* were shown to be related to each other, and both are related to *Clostridium barkeri* [14]. The association between anaerobic eubacteria capable of producing acetate from CO_2 and other clostridia will be discussed here. Johnson and Francis defined several groups in the genus *Clostridium* by 23S rRNA homology [6]. *Clostridium butyricum, C. scatologenes*, and *C. pasteurianum* are members of homology group I, a low mol% G+C cluster. *Clostridium lituseburense* is the type species of homology group II, another low mol% G+C cluster. *Clostridium aminovalericum* is a species with a low mol% G+C in its DNA and is not a member of either of these [6] homology groups. *Clostridium barkeri*, whose DNA has a higher mol% G+C, is not a member of either 23S rRNA homology groups. These species were selected to represent the clostridia in our study. The relationship between the genera *Eubacterium* and *Propionibacterium* also will be discussed.

Materials and Methods

Clostridium acidiurici ATCC 7906, *C. aminovalericum* ATCC 13725, *C. cylindrosporum* ATCC 7905, *C. formicoaceticum* ATCC

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Table 1. Oligonucleotide catalogs for 16S rRNAs of: 1, *Acetogenium kivui*; 2, *Clostridium aceticum*; 3, *C. acidiurici*; 4, *C. aminovalericum*; 5, *C. formicoaceticum*; 6, *C. pasteurianum*; 7, *C. scatologenes*; 8, *C. thermoaceticum*; 9, *Peptococcus aerogenes*; 10, *P. glycinophilus*.

Oligonucleotide sequence	Present in organism number[a]
5-mers	
CCCCG	1–6, 8–10
CCCAG	1, 8
CCACG	6–7
ACCCG	2–6, 9–10; 4
CAACG	1–10; 7–8, 10
ACACG	1–10
AACCG	5, 8
CAAAG	4
AACAG	2–3, 5
AAACG	2, 4–5, 9
AAAAG	4
UCCCG	1–6, 8–10
CCUCG	3
CCCUG	8
UCCAG	3, 5–7
UACCG	5, 8
CUCAG	1–10; 2
CUACG	1–2, 5
CCUAG	2
CACUG	8
AUCCG	8
ACUCG	9
ACCUG	2, 5–7, 10
UACAG	1–5, 7, 10
UAACG	1–7, 9–10
CUAAG	9
CAAUG	1, 8
AUCAG	1–5, 8–10; 4, 9–10
AUACG	6, 8
ACUAG	4
ACAUG	1
AAUCG	1, 4, 8
AACUG	2–3, 5–9; 3, 6–7
UAAAG	1–10; 1–5, 7, 9–10
AUAAG	5, 3, 10; 3
AAUAG	2, 5–6, 8; 6
AAAUG	1–10
UCCUG	7, 9
CUCUG	2, 5–10
CCUUG	1, 5–6, 8
UUCAG	2, 5
UUACG	3
UCUAG	6–7
UAUCG	1–10; 8
AUCUG	4
ACUUG	2, 5–7, 10
UUAAG	1–2, 4–10; 8, 10
UAAUG	1, 4, 7; 4
AUUAG	1–2, 4–10; 6–7
AUAUG	4
AAUUG	1–2, 4–10; 4
UUUCG	2, 5
UCUUG	2–5, 9–10; 4
CUUUG	6–7
UUUAG	9–10
6-mers	
CCACCG	9
CCAAAG	2, 5
CACAAG	1–10
CAAACG	4
ACAACG	10
AAACCG	3, 9
AAAAAG	4
UCCACG	1–10
CUACCG	8
ACCCUG	1
UCCAAG	1
UAACCG	7
CUAACG	2–3, 5, 7
CCAAUG	4
ACACUG	10
AACCUG	8
UCAAAG	9
UACAAG	2, 5
UAAACG	1–10
CAUAAG	4
ACUAAG	4
AAUCAG	2, 5–7
AAUACG	1, 3–4, 6–8
AACAUG	9
AAAUCG	9
AAACUG	1, 7–8, 10
UUCCCG	1–10
CCUUCG	1–2, 5, 7–8
UCUCAG	2, 5, 8
UCUACG	8
UACCUG	4, 8
CUCUAG	9
CCUUAG	2
CAUCUG	10
CACUUG	1, 3–4
AUCCUG	1–10
ACUUCG	4
UUAACG	1
UCUAAG	10
UACUAG	1, 6, 8, 10
UAAUCG	1–10
UAACUG	2, 4–8, 10
CAUUAG	6–7
AUAUCG	1
AUACUG	3, 8
UAUAAG	3–4
AUUAAG	4
AUAAUG	2–4, 6–7, 9–10
AAUUAG	9
UCUUCG	4, 6, 10
CUUUCG	9
CCUUUG	3, 9–10
CAUUUG	7–9
UUUAAG	7, 10
UUAAUG	9
UAAUUG	7
AUAAUG	2, 5
UCUUUG	7
7-mers	
CCCAAAG	10
CAAACAG	1, 4, 6–10
AACAACG	6
CCUCCCG	6
UACCCCG	1, 8
CACUCCG	2–3, 5, 9
CUAACCG	3, 9
CCAUCAG	10
CAACUCG	1, 3–4, 9–10
CAACCUG	7
UACAACG	3
UAACACG	6–7, 9
CAUACAG	4
AUCACAG	3
ACACAUG	9
AAUACCG	9–10
AAACUCG	2, 5, 7
UACAAAG	8
UAACAAG	9
CAUAAAG	2, 5
CACAAUG	2–7, 9–10
CAAUAAG	1, 4, 7
AUCAAAG	9–10
AUAACAG	2, 4–5
CCUUCCG	7
CCCUUCG	6–8
UACUCCG	6, 10
UACCUCG	2, 5
CAUUCCG	1
UUAACCG	6–7
UCCUAAG	3
UCAUCAG	8
UAAUCCG	6–7
UAACCUG	4, 6, 9
AUCUCAG	9
AACUUCG	10
AACUCUG	4
UCAUAAG	1
UAAUACG	2–7, 9–10
CAUUAAG	2–3, 5
AAAUUCG	6
AUUAAAG	8
UUAUCCG	2–5, 10
UCCUAUG	9
UAUUCCG	7
CUUCUAG	1
CUUACUG	4
[U,CCU]AUG	10
CAUUUCG	2, 5
UAUCUAG	6
UAAUCUG	2, 5, 10
AAUUUCG	9
AAUCUUG	1, 8
AUAUUAG	2–5, 9–10
AAUAUUG	3–4, 6–7, 9–10
UUCUCUG	9
CUUUCUG	6
UUUCUAG	6
UCUUUAG	2, 5, 10
UAUUUAG	4
CUUUUUG	6
8-mers	
ACAACACG	1
ACAAACCG	8
AACACCAG	2–5, 7–8
CUCAACCG	2–3, 5
CCCACAUG	2, 5
CCACACUG	1–3, 5, 8
ACAUCCCG	10
UACCCAAG	7
CCUCAAAG	6–7
CAAUACCG	6
ACCAUCAG	6
ACAACUCG	5
AACCUCAG	3
AAACCCUG	2–5, 9
UACCAAAG	6
AAUACCAG	1
AAACACUG	8
AUACAAAG	2, 5
CUUAACCG	8
CCUACAUG	3, 7, 10
CCACAUUG	4, 6–7, 9–10
CACUCUAG	1–7, 10
AUCCUCAG	10
AUACCCUG	1–10
ACAUCCUG	8
AAUUCCCG	1, 8
UCCAUAAG	4
UCAUAACG	9
UACCAUAG	9–10
CUACAAUG	1–10
AUAACUCG	2, 10
AUAACCUG	4
ACUACAUG	4
AAUCACUG	2, 5, 9
AACUAUCG	10
AAACUCUG	10
AUAAAUCG	3, 9
AAUACUAG	4, 7
AUCCUUCG	3
ACUCUCUG	1–2, 5, 8
UACCUUAG	3
CCUUUAAG	8
CAUCUUAG	2, 5
ACUUACUG	9
UCUUAAAG	8
UAUUACAG	9
AUCUUAAG	10
AUAUCUAG	7
AAUUACUG	1, 8, 10
UUAAUAAG	4
CUUUACUG	10
AUUCUCUG	7
ACUUUCUG	3
AUUUAUCG	5
AUUUACUG	4, 6–7
AAUUAUUG	3
AUUUAUUG	2, 5
9-mers	
CAACACCCG	1, 7–8
ACCCAACCG	4
CAACCCCUG	1, 10
UACCACACG	1
UACACACCG	2–10
UAACACCCG	3, 6
CUCACCAAG	3–4, 6–9
CUACACACG	1–10
AUCCAACCG	10
CCUACCAAG	1–2, 5, 10
CAAUACCCG	9
[CCA,CA,CA]UG	3
UAAAACCCG	1
AUAACACCG	8
AAAAUCCCG	7
ACUACAAAG	4
CCAAUAAAG	2, 5
UCCCAUCAG	1
CUCAACCUG	1, 6
ACUCCUACG	1–3, 5–8
CAACCCUUG	2, 5
AACCCUUCG	9–10
CUAAUACCG	1–2, 4–5, 8, 10
CUAACUACG	1–10
AAACCUUCG	6
CCCCUUAUG	2, 5, 10
UCCCAUUAG	3, 8
UCAUCUCAG	4
CUCUAUCAG	4
CCCUAUAUG	1
UCACUUAAG	1
CUAAACUUG	6
CAUCUUAAG	2
AAUUCCUAG	2–7, 9–10
AAUUACCUG	7
AAAUCUUCG	8
AUACUUAAG	6
CAUCUUCUG	8
CCCUUUAUG	9
UUUCACAUG	6
CUUAACUUG	7
CAUCUUAUG	2
UUAACUUAG	4
AUUAAUUAG	9
UUUAAUUCG	1–10
10-mers	
ACCCAACCCG	1
AAAAACCCCG	8
CUCAACCCCG	4, 10
ACCUAACCCG	8
AAACUACCCG	4
AAACUCAAAG	1–5, 8–10
ACAUCCCCUG	7
AACCUUACCG	8
UCACAAACUG	9
ACAACUCUAG	3
CACUCUAAAG	8
AACUCUAAAG	9
ACCCCUUCUG	8
ACAUCUCCUG	6
CCUUAUACAG	4
UAAAACUCUG	3
AUUAAUACCG	6–7
UCUCCCUUCG	3
ACUCCUUCUG	6–7
UUCAACUUCG	4
CUAUUCUAAG	2
CAAUUUUAAG	5
AAACUUAUUG	2, 5
AAACUAUUUG	3
UUUCCUUUCG	10
UCUUUAAUUG	8
11-mers	
AACCCAAAAAG	1
[CAA,CUA]CAAAG	10
CAACUCACCUG	8
UCAAACCUCAG	2, 5
UCAAAACUCCG	3
CCUAACACAUG	1–3, 5, 8–9
AUAACUCACCG	10
AUAACACCUCG	1
AACCUUACCAG	1, 4, 9
UAAAAUACCCG	1
CUCAACCCUUG	9
ACAUCCCUUCG	7
ACAUCCCUCUG	3–4
AACCUUACCUG	2, 5
CUUAACACAUG	4, 6–7, 10
CUACCAUUAAG	6–7
CCAUCAUUAAG	3
AUUAAUACCCG	9
CAUAACAUUAG	7
AAACUUAAUCG	10
UAUUCCACCUG	4
CCCCUUAUAUG	3
ACAUAAUAUUG	9
[CUUU,CCCUU]CG	9
ACAUCCUUUUG	2, 5
UAUUUCACAUG	7
UAAC(C,U)UUAUG	6
UUUUCCCUUCG	2
12-mers	
AAUCCCAAAAAG	8
AAAAUCAAACAG	9
CCCUUAUCACCG	10
AACCUUACCUAG	6–7
AAUCUCAUAAAG	2

[a] Multiple occurrences of a sequence in a given organism are denoted by repeating the organism's number; e.g., 1–6; 4, 6; 6 signifies a double occurrence in organism 4 and a triple occurrence in organism 6.

Table 1. (Continued)

Oligonucleotide sequence	Present in organism number[a]
AAUCUCAAUAAG	3
UAACCUACCUUG	2, 5
CAUCACCUUAUG	1
CAUUUCAAACUG	6
UACUAAUUAAAG	7
ACCUUUCCUUCG	4
CUUUAAUUAAAG	6
13-mers	
AAUCCCAAAAAAG	9
AAUCCCAUAAAAG	10
UAAACACAAUAAG	8
UUAACACAAUAAG	9
UCAAAUCAUCAUG	1–2, 5–6, 8–10
UUAACACAUUAAG	10
CAU(AU,AAU)UAAAG	6
14-mers	
CCCAAACUCCUACG	4, 10
AAAACUCAUCCCAG	6
CCAAUCUCAAAAAG	4
UCCAAACUCCUACG	9
ACCUCACCUUAAAG	1
AUUAAAACUCAAAG	6–7
ACAACAUUAAUAAG	6
CCCCUUAUAUCCCG	8
15-mers	
CAACCUACCCUUCAG	1
AAACUAAUAAACUUG	9
UAAUCUACCCUUCAG	8
16-mers	
CAACCCUUAUCCUUAG	9
CAACCCUUACCUUUAG	3, 8
CAACCCCUAUCUUUAG	4
CAACCCUUAUCAUUAG	6–7
17-mers	
AACCUUACCUAAACUUG	10
AACCUUACCAACAUUUG	3
19-mers	
AAACCUUCCUUAUACAAAG	3
24-mers	
CAAAACUUUUAAAACUCAUCUCAG	7
termini	
5′end	
pUAUUUUG	3
pUAAUUUG	4
pCUUUAUUG	10
pUUUUAUUAAG	2
pUUUUAAAUUG	7
pUAUAAAUUUG	1
3′end	
$AUCACCUCCUUUCU_{OH}$	1–8, 10
$AUCACCUCCUUUCUA_{OH}$	1, 8
Post-transcriptionally modified	
U̇G	3, 8, 10
AAĠ	3–4, 9–10
ȦȦG	1–5, 8–10
ĊCCG	1–10
CCĠCG	1–10
ĊAACG	1–10
AU̇UAG	2–3, 5–7, 9–10
ȦȦCCUG	6–7
U̇AACAAG	1–8, 10
UACACACĊG	1
CCCC(U̇,U)AUG	4, 6–7
UCAĊACCACG	2–3, 8
UĊAĊACCACG	1
U̇CAĊACCAUG	4–7, 9–10
UĊAAAUCAUCAUG	3–4, 7

27076, *C. pasteurianum* ATCC 6013, *C. scatologenes* ATCC 25775, *Peptococcus aerogenes* ATCC 14963, and *P. glycinophilus* ATCC 23195 were obtained from the American Type Culture Collection, Rockville, Maryland. *Acetogenium kivui* ATCC 33488 was obtained from J. A. Leigh, *Clostridium aceticum* strain Wieringa from G. Gottschalk, and *C. thermoaceticum* DSM 521 from L. G. Ljungdahl. Organisms were grown under anaerobic conditions under a N_2:CO_2 (80:20) gas phase at 37°C in medium containing (g/liter): yeast extract, 2; tryptone, 2; peptone, 2; piperazine-*N,N′*-bis(2-ethanesulfonic acid) (PIPES), 10; fructose, 5; $NaHCO_3$, 2.5; cysteine · HCl, 0.1; $Na_2S \cdot 9H_2O$, 0.1; trace mineral solution and vitamin solution [19], 10 ml each. Initial medium pH was 6.8 to 7.2. Yeast extract, tryptone, and peptone were dephosphorylated by precipitating the phosphate as magnesium-ammonium-phosphate [11]. The medium was supplemented with 20 mg $FeSO_4 \cdot 7H_2O$ and incubation was at 60°C for *Acetogenium kivui.* For *Clostridium aceticum,* tris(hydroxymethyl)methylaminopropanesulfonic acid (TAPS) replaced PIPES, the medium was supplemented with 0.4 mg cyanocobalamin, and the initial pH was 8.3. For *C. acidiurici* and *C. cylindrosporum,* 1 g nondephosphorylated yeast extract replaced the yeast extract, tryptone, and peptone; 2 g uric acid replaced the fructose; and *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES) replaced PIPES in the medium. Neutralized δ-amino-*n*-valeric acid · HCl replaced fructose for *C. aminovalericum.* For *C. thermoaceticum,* 0.5 g sodium thioglycollate replaced the cysteine · HCl and the $Na_2S \cdot 9H_2O$, and incubation was at 55°C. Medium was supplemented with 10 g L-glutamic acid, monosodium salt, for *Peptococcus aerogenes.* For *P. glycinophilus,* glycine replaced fructose. Source and culture conditions for *Acetobacterium woodii, Clostridium barkeri, C. butyricum, C. lituseburense, Eubacterium limosum, E. tenue, Propionibacterium acnes,* and *Pr. freudenreichii* have been previously published [13,14].

For labeling strains, carrier-free $^{32}PO_4$ (0.4 to 0.6 mCi/ml) was added to growing cultures. Cells were harvested after 3 to 4 generations and burst with a French pressure cell at 20,000 lb/in^2. RNA was extracted and purified as described elsewhere [18,20].

Determination of the oligonucleotide catalogs of RNase T_1 digests of 16S rRNA and the analysis of the data have been described in detail [1,4,16].

Results and Discussion

Table 1 lists the oligonucleotide catalogs for those eubacteria whose catalogs have not appeared previously [13,14]. Table 2 gives S_{AB} values for the binary comparisons of the catalogs, and the resulting dendrogram is shown in Fig. 1. *Escherichia coli* appears in Fig. 1 as a representative of a Gram-negative eubacterium.

Seven clusters of organisms emerge from Fig. 1: One containing *Clostridium formicoaceticum, C. aceticum, Eubacterium tenue, C. lituseburense,* and *C. acidiurici;* a second composed of *Peptococcus glycinophilus* and *C. aminovalericum*; a third including *C. pasteurianum, C. scatologenes,* and *C. butyricum;* a fourth containing *Eubacterium limosum, C. barkeri,* and *Acetobacterium woodii;* a fifth defined by *Peptococcus aerogenes;* a sixth composed of *Acetogenium kivui* and *C. thermoaceticum;* and the seventh comprising the *Propionibacterium* species. The extremely close relationship

Table 2. Binary comparisons among the 16S rRNA catalogs of some anaerobic eubacteria. Top triangle: number of bases in sequences common to each pair of catalogs (for hexamers and larger). Bottom triangle: S_{AB} values for each pair of catalogs [4].

	Organism																	
Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *Acetobacterium woodii*		210	203	227	219	334	199	193	197	193	239	198	380	196	208	206	170	156
2. *Acetogenium kivui*	0.36		227	214	210	194	174	217	213	181	194	289	225	201	171	218	150	163
3. *Clostridium aceticum*	0.33	0.39		287	247	244	243	532	284	208	235	237	236	296	210	260	182	172
4. *C. acidiurici*	0.37	0.37	0.47		271	244	245	271	265	218	231	250	249	269	255	238	153	168
5. *C. aminovalericum*	0.34	0.35	0.39	0.42		227	289	251	255	267	277	207	253	257	248	307	179	176
6. *C. barkeri*	0.54	0.34	0.40	0.40	0.35		207	234	244	208	226	221	394	243	205	226	152	143
7. *C. butyricum*	0.31	0.29	0.38	0.38	0.44	0.32		247	264	366	387	180	216	261	226	263	174	175
8. *C. formicoaceticum*	0.33	0.40	0.91	0.46	0.41	0.40	0.41		268	212	239	227	226	292	214	256	182	172
9. *C. lituseburense*	0.31	0.36	0.46	0.42	0.39	0.39	0.41	0.45		235	242	210	233	445	233	280	171	157
10. *C. pasteurianum*	0.31	0.31	0.33	0.35	0.41	0.33	0.57	0.36	0.37		395	193	216	246	211	255	177	177
11. *C. scatologenes*	0.37	0.33	0.37	0.36	0.42	0.36	0.59	0.40	0.38	0.61		222	242	261	210	256	181	188
12. *C. thermoaceticum*	0.32	0.51	0.39	0.41	0.33	0.36	0.29	0.39	0.34	0.31	0.35		213	214	175	200	157	162
13. *Eubacterium limosum*	0.63	0.40	0.39	0.41	0.41	0.66	0.35	0.40	0.38	0.35	0.39	0.36		240	220	234	164	151
14. *E. tenue*	0.31	0.35	0.48	0.44	0.40	0.39	0.41	0.50	0.71	0.39	0.41	0.35	0.40		210	284	183	166
15. *Peptococcus aerogenes*	0.33	0.29	0.34	0.41	0.38	0.33	0.35	0.36	0.37	0.33	0.33	0.28	0.36	0.34		262	150	150
16. *P. glycinophilus*	0.32	0.37	0.41	0.38	0.47	0.36	0.40	0.43	0.44	0.40	0.39	0.32	0.38	0.45	0.41		170	171
17. *Propionibacterium acnes*	0.29	0.27	0.31	0.26	0.29	0.26	0.28	0.32	0.29	0.30	0.30	0.27	0.29	0.31	0.25	0.28		317
18. *Pr. freudenreichii*	0.26	0.29	0.29	0.28	0.28	0.24	0.28	0.30	0.26	0.29	0.31	0.27	0.26	0.28	0.25	0.28	0.55	

found here between *C. aceticum* and *C. formicoaceticum* is supported by their nutritional characteristics and by DNA-DNA homology [3]. Both are homoacetate fermenters and the main difference between these two clostridia is that *C. aceticum* can use H_2 as a substrate. *Eubacterium tenue* is a specific relative of *C. lituseburense*. *Eubacterium limosum, C. barkeri,* and *Acetobacterium woodii* share an uncommon murein structure [14]. *Eubacterium limosum* and *A. woodii* can use H_2 to reduce CO_2 to acetate [2,12]. *Peptococcus aerogenes,* which, like *C. acidiurici* and *C. cylindrosporum,* can ferment purines, was not specifically related to any other species studied here. *Acetogenium kivui* and *C. thermoaceticum* are both thermophilic homoacetate fermenters; *Ac. kivui* can also use H_2 as a substrate [7]. *Clostridium thermosaccharolyticum* is also a member of this cluster (C. R. Woese, personal communication). We have been unsuccessful in obtaining a complete catalog of *C. cylindrosporum* and are continuing our efforts with this organism. However, the partial catalog of *C. cylindrosporum* suggests that it is not a specific relative of *C. acidiurici* or *C. lituseburense* (C. R. Woese, personal communication). The 1980 American Type Culture Collection Catalogue of Strains lists *C. cylindrosporum,* a purine fermenter, as a strain of *C. lituseburense,* a moderately saccharolytic and moderately peptolytic species; *Peptococcus aerogenes* is listed as a strain of *Peptococcus asaccharolyticus*.

The ability to form acetate from CO_2 does not appear to be a valid phylogenetic trait. All species known to be able to synthesize acetate from CO_2 fall into a large grouping that is basically defined by the anaerobic sporeforming rods: the clostridia. The capability of spore formation seems to be a valid phylogenetic marker, but the inability to form spores does not exclude other eubacteria from the clostridial grouping [5]. Members of the nonsporeforming genera *Acetobacterium, Acetogenium, Eubacterium,* and *Peptococcus* are related specifically

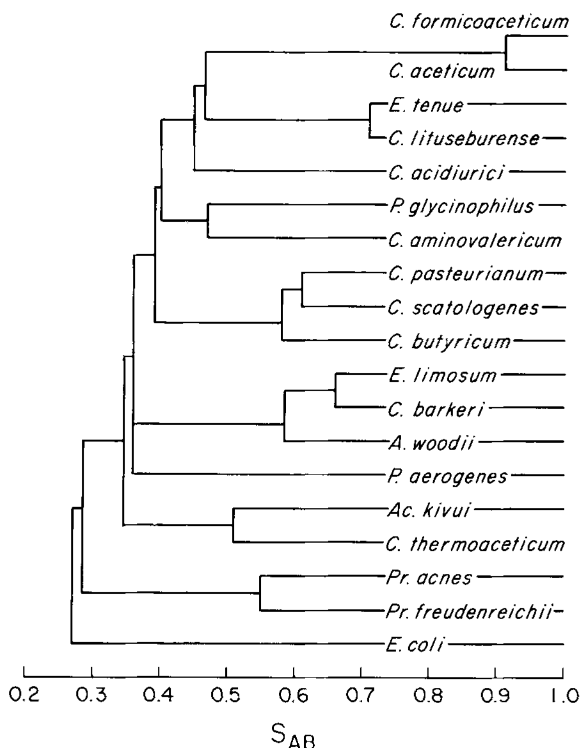

Fig. 1. Dendrogram of relationships among the anaerobic eubacteria able to synthesize acetate from CO_2 and related clostridia, *Propionibacterium* species, and *Escherichia coli*. The figure was constructed by average linkage clustering (between the merged groups) from the S_{AB} values in Table 2. See first column of Table 2 for the full spelling of generic names.

to the clostridia. For the genera *Eubacterium* and *Peptococcus*, different species within the genus are less related to each other than they are to different species of *Clostridium*.

Evidence has been presented suggesting a common biochemistry for the synthesis of acetate from CO_2 in *Acetobacterium woodii, C. formicoaceticum*, and *C. thermoaceticum* [15]. This similar biochemistry was not useful in predicting a high degree of relatedness among these organisms. In addition to *A. woodii* having a rare murein type, the mol% G+C of DNA for *A. woodii, C. formicoaceticum*, and *C. thermoaceticum* is, respectively, 39, 34, and 54 [2,8]. This supports our finding that *C. thermoaceticum* is not a close relative of either *A. woodii* or *C. formicoaceticum*. It has been shown that *C. acidiurici* synthesizes acetate from CO_2 by a different mechanism than *C. thermoaceticum* [17]. It was suggested that *C. cylindrosporum* and *P. glycinophilus* use the same pathway as *C. acidiurici*, while *E. limosum* probably uses the same mechanism as *C. thermoaceticum* [17]. This establishment of two pathways for acetate synthesis is a further indication that acetate production from CO_2 is not a good predictor of phylogenetic relatedness.

Analysis of metabolic end products does not immediately indicate where phylogenetic groupings of anaerobes might fall. For example, *Acetobacterium woodii* is a homoacetate fermenter, the primary end products of *E. limosum* metabolism are acetate and butyrate, and the major end products of metabolism for *C. barkeri* are butyrate and lactate, yet these organisms are closely related, as indicated by murein analysis and 16S rRNA analysis [14].

The *Eubacterium* species examined here are not related to the *Propionibacterium* species. The clostridial grouping is only slightly more related to the genus *Propionibacterium* than it is to *Escherichia coli*. This result questions the current placement of *Eubacterium* in the family Propionibacteriaceae [10], as already pointed out by Stackebrandt and Woese [13].

We have not yet surveyed a sufficient number of clostridia to formally propose a comprehensive phylogenetic scheme. In general, this work is consistent with the 23S rRNA homology groups of clostridia found by Johnson and Francis [6], and expands on their work by the inclusion of further genera. We intend to extend this study—by examining other species of *Clostridium, Eubacterium*, and *Peptococcus*, and members of the genera *Butyrivibrio, Desulfotomaculum, Peptostreptococcus*, and *Ruminococcus*—to provide a framework for the phylogeny of the Gram-positive anaerobes relative to the clostridia.

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