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Proposal of Neotype for *Clostridium thermohydrosulfuricum* and the Merging of *Clostridium tartarivorum* with *Clostridium thermosaccharolyticum*

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The deoxyribonucleic acid (DNA) relationships among strains of the thermophilic species *Clostridium thermohydrosulfuricum, C. thermosaccharolyticum,* and *C. tartarivorum,* other saccharolytic, proteolytic, and acetate-producing clostridia, and some sulfate-reducing bacteria were studied by DNA-DNA hybridization experiments. Strains of the species *C. thermohydrosulfuricum* were found to constitute a genotypically homogeneous group clearly unrelated to the strains of the species *C. thermosaccharolyticum* and other competitor strains used. Strains of the species *C. tartarivorum* showed a high level of homology with the reference strain *C. thermosaccharolyticum* ATCC 7956.The guanine-plus-cytosine contents of the acetate-forming clostridia *C. formicoaceticum* and *C. thermoaceticum* were 34.0 and 54.0 mol%, respectively.

Since the first description of *Clostridium thermosaccharolyticum* (8), some other thermophilic saccharolytic clostridia have been described. Mercer and Vaughn (12) found tartrate-fermenting clostridia in different sources and defined the species *C. tartarivorum.* From extraction juices of beet sugar factories Klaushofer and Parkkinen (7) isolated a clostridium which they called *C. thermohydrosulfuricum* because of its high temperature limits for growth (up to 75°C) and the striking capacity to produce large amounts of H_2S by reduction of sulfite. A comparison of strains of the three above-mentioned *Clostridium* species, which in addition to the study of biochemical and cultural features also included the fine structure of the cell wall surface, led to an expanded description of the species *C. thermohydrosulfuricum* (4, 13). The only difference that was found between *C. thermosaccharolyticum* and *C. tartarivorum* was the utilization of tartrate by the latter. On the contrary, *C. thermohydrosulfuricum* differed from the two other species not only by a higher maximum temperature growth limit of almost 10°C, but also by having hexagonal cell wall surface patterns, in contrast to the rectangular surface patterns of *C. thermosaccharolyticum* and *C. tartarivorum.*

The present study was designed to investigate the taxonomic relationship between strains of *C. thermohydrosulfuricum, C. tartarivorum,* and *C. thermosaccharolyticum* by comparing the guanine-plus-cytosine (G+C) contents of their deoxyribonucleic acids (DNA) and the nucleotide sequence similarities of the DNA preparations by DNA-DNA homology experiments. Reference strains of other saccharolytic, proteolytic, and acetate-forming clostridia and some sulfate-reducing bacteria were also compared with these organisms.

MATERIALS AND METHODS

Bacterial strains and culture. The bacterial strains used were as follows. Thirteen strains of *C. thermohydrosulfuricum* isolated from extraction juices of different beet sugar factories during the campaigns of 1966, 1969, 1970, and 1971 and previously identified as *C. thermohydrosulfuricum* (3) were examined; they were E100-69, E101-69, H100-69, H101-69, L77-66, L91-71, L92-71, L110-69, L111-69, S100-69, S101-69, S102-70, and T15-66.

The strains of *C. thermosaccharolyticum* used were ATCC 7956 (NCIB 9385), D120-70, D90-71, ZU51, ZU53, ZU54, ZU56, ZU57, and ZU58 (from extraction juice); and ZU68, ZU69, S201-71, S202-71, S203-71, D204-71, E205-71, E207-71, and S208-71 (from white sugar). All the strains were derived from different Austrian or Italian beet sugar factories or white sugar samples.

The strains of *C. tartarivorum* used included T9-1 and T9-3R, obtained from R. H. Vaughn, University of California, Davis, Calif.

Desulfotomaculum nigrificans strains used were NCIB 8395, and NCIB 8706; and B200 and T206-71 from white sugar.

Other strains used included *Desulfovibrio vulgaris* NCIB 8303, *C. thermoaceticum* DSM 521, *C. formicoaceticum* ATCC 27076, *C. sporogenes* ATCC 319, *C. bifermentans* ATCC 19299, *C. perfringens* ATCC 13124, *C. acetobutylicum* ATCC 824, *C. pasteurianum* ATCC 6013, *C. felsineum* McClung 538, *C. butyricum* ATCC 860, *C. bejierinckii* ATCC 17795, and *C. tyrobutyricum* ATCC 25755.

TABLE 1. *G+C content and DNA homology values among the strains of clostridia studied*

Competitor strains	Source[a]	Homology (%) of reference strains				G+C (mol %)
		C. thermosaccharolyticum		*C. thermohydrosulfuricum* E100-69	*C. formicoaceticum* ATCC 27076	
		ATCC 7956	ZU 51			
Clostridium thermohydrosulfuricum						
E100-69	Extraction juice	0	4	100	0	32.0
E101-69	Extraction juice			80		29.5
H100-69	Extraction juice			95		
H101-69	Extraction juice	8	6	87		
L77-66	Extraction juice			100		
L91-71	Extraction juice	30	0	81		32.0
L92-71	Extraction juice	35		102		32.0
L110-69	Extraction juice			95		30.5
L111-69	Extraction juice			100		31.0
S100-69	Extraction juice			102		
S101-69	Extraction juice			98		31.0
S102-70	Extraction juice			100		31.0
T15-66	Extraction juice			87		32.0
C. thermosaccharolyticum						
7956	ATCC	100	91	19	0	29.0
D120-70	Extraction juice	100	95	49	0	30.0
D90-71	Extraction juice	84	99			
ZU51	Extraction juice	88	100	10		
ZU53	Extraction juice	85	92	40		31.0
ZU54	Extraction juice	81	78	36		32.0
ZU56	Extraction juice	84	90	16		32.0
ZU57	Extraction juice	77	87	37	6	30.0
ZU58	Extraction juice	82	100	43		31.0
ZU68	White sugar	98	102	26		
ZU69	White sugar	97	88	15		
S201-71	White sugar	95	100		5	
S202-7	White sugar	102	100			
S203-71	White sugar	86	99			
D204-71	White sugar	91	102	16		
E205-71	White sugar	85	100	30		
E207-71	White sugar	86	98			
S208-71	White sugar	98	102			
C. tartarivorum						
T9-3R	Vaughn	87	92	38		30.0
T9-1	Vaughn	96	88	29		30.5
Desulfotomaculum nigrificans						
8395	NCIB	0	0	0	2	45.0
8706	NCIB	10	17	0	0	45.0
B200-71	White sugar	0	0	0	7	46.5
T206-71	White sugar	2	0	0	3	45.5
Desulfovibrio vulgaris 8303	NCIB	0	0	0	0	61.0
C. thermoaceticum 521	DSM	0	0	0	3	54.0
C. formicoaceticum 27076	ATCC	16	4	7	100	34.0
C. sporogenes 319	ATCC	7		0	10	26.0
C. bifermentans 19299	ATCC	21		0	2	29.0
C. perfringens 13124	ATCC	0		4	2	25.0
C. acetobutylicum 824	ATCC	0		0		29.0
C. pasteurianum 6013	ATCC	15		0	8	29.0
C. felsineum 538	McClung	0		0	2	25.0
C. butyricum 860	ATCC	14		0	8	27.5
C. bejierinckii 17795	ATCC	10		0	0	28.0
C. tyrobutyricum 25755	ATCC	14		0	0	28.5

[a] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; NCIB, The National Collection of Industrial Bacteria, Torry Research Station, London, England; Vaughn, R. H. Vaughn, University of California, Davis, Calif.; McClung, Indiana University, Bloomington, Ind.; DSM, Deutsche Sammlun für Mikroorganismen, München, German.

The medium employed and culture conditions for DNA isolation from saccharolytic and proteolytic clostridia were described previously (11). *D. vulgaris* and the strains of *D. nigrificans* were grown in Starkey Sporovibrio medium without iron salts and by replacing glucose with 0.35% sodium pyruvate. *C. formicoaceticum* and *C. thermoaceticum* were grown in the media indicated by Gottwald et al. (2). The incubation temperature was 55°C for thermophilic strains and 37°C for mesophilic species. GasPak system (BBL Microbiology Systems) was used to obtain anaerobic conditions.

DNA extraction, DNA base composition, and DNA hybridization. DNA extraction and purification procedures were performed by the method of Marmur (9); the G+C content of the DNA preparations was determined by the thermal denaturation method (10) by using a thermal denaturation analyzer (Beckman Instruments, Inc.). DNA homology experiments were conducted with the membrane filter competition procedure under the conditions suggested by Johnson and Ordal (6). A detailed description of the procedure as used in this Institute was described previously (11). The strains used as reference organisms for DNA-DNA hybridization were as follows: *C. thermohydrosulfuricum* Klaushofer and Parkkinen E100-69; *C. thermosaccharolyticum* McClung ATCC 7956 (NCIB 9385) and ZU51 (from extraction juice); *C. formicoaceticum* Andreesen, Gottschalk and Schlegel ATCC 27076.

RESULTS AND DISCUSSION

The G+C content values and the results of the DNA-DNA hybridization experiments are presented in Table 1. Strains of *C. tartarivorum, C. thermohydrosulfuricum,* and *C. thermosaccharolyticum* had similar G+C content values, ranging from 29 to 32 mol%. The data for the G+C contents of *C. formicoaceticum* and *C. thermoaceticum* were the first reported in the literature for these species, whereas the values obtained for the other species examined are in close agreement with those of other authors (1, 5). Strains of the "sulfide spoilage" bacterium, *D. nigrificans,* were also included in this study. This bacterium shares several characteristics with *C. thermohydrosulfuricum,* namely, the presence of spores, thermophily, and H_2S production from sulfur compounds (3). The G+C content values for *D. nigrificans,* ranging from 45.0 to 46.5 mol%, were similar to those obtained in other laboratories. These significantly different values from those of the species *C. thermohydrosulfuricum* and *C. thermosaccharolyticum* indicate that *D. nigrificans* cannot be closely related to the other species.

The strains of *C. thermohydrosulfuricum* formed a very homogeneous group of organisms as measured by DNA homology (Table 1). Four of the strains had from 80 to 87% homology to the reference strain (E100-69), and the remaining eight ranged from 95 to 102%. The *C. thermosaccharolyticum* strains had similar levels of homology to the two reference strains ATCC 7956 and ZU51. The question as to whether, on account of their biochemical reactions, it would be justifiable to consider *C. thermohydrosulfuricum* as a highly thermophilic representative of the species *C. thermosaccharolyticum* (3, 4) must be answered in the negative. There is no doubt that *C. thermohydrosulfuricum,* on the basis of the data obtained here, is a well-defined species. As the original type strain was lost, we propose, according to Hollaus and Sleytr (4), E100-69 as the neotype strain.

The homology results for the *C. tartarivorum* strains show that these organisms belong to the *C. thermosaccharolyticum* group. Since *C. tartarivorum* is the most recently assigned name, it should be considered as a synonym of *C. thermosaccharolyticum.* Therefore, as suggested by Hollaus and Sleytr (4), these strains must be regarded as representing a tartrate-fermenting biotype.

As predicted from the G+C content values, the *D. nigrificans* strains had negligible homology with the reference strains for the other species. The *C. formicoaceticum* reference strain was unrelated to all of the organisms tested.

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