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DNA-rRNA Hybridization Studies among *Brochothrix* spp. and Some Other Gram-Positive Bacteria

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As determined by DNA-rRNA hybridization, it is obvious that the recently described species *Brochothrix campestris* belongs to the genus *Brochothrix*. This species is closely related to *Listeria* species and is also related to *Enterococcus faecalis*, *Bacillus cereus*, and *Bacillus subtilis*. *Brochothrix campestris* is not closely related to the other bacteria which we tested.

The genus *Brochothrix* includes the following two species: *Brochothrix thermosphacta*, which was first placed in the genus *Microbacterium* as *Microbacterium thermosphactum* (12), and *Brochothrix campestris*, which was isolated from soil and was identified by DNA-DNA hybridization (22). As determined by this method, strains of *Brochothrix campestris* and *Brochothrix thermosphacta* exhibit low levels of DNA homology (13 to 17%).

It is now well established that *Brochothrix thermosphacta* differs markedly from members of the genus *Microbacterium* (3, 4, 15, 17). In fact, *Brochothrix thermosphacta* is more closely related to *Listeria monocytogenes*, as shown by the results of 16S rRNA cataloging (11). Furthermore, *Brochothrix thermosphacta* was placed in the *Clostridium-Lactobacillus-Bacillus* branch in *Bergey's Manual of Systematic Bacteriology* (9).

The purposes of this work were to determine whether the recently described species *Brochothrix campestris* belongs in the genus *Brochothrix* and to establish the relationship between *Brochothrix campestris* and some other genera in the clostridial branch by using DNA-rRNA hybridization.

The sources of the strains which we used are shown in Table 1. Most of the strains were grown in APT broth (Difco Laboratories); *Streptococcus* strains were grown in Eugon broth (Difco), *Escherichia coli* was grown in lauryl tryptose broth (Difco), and *Arthrobacter globiformis* was grown in *Corynebacterium* agar according to the instructions of the Deutsche Sammlung von Mikroorganismen. Cells were lysed by using the method of Rocourt et al. (14) and Champomier et al. (2). DNAs were extracted and purified as described by Brenner et al. (1).

rRNA from *Brochothrix campestris* ATCC 43754^T (= CIP 102920^T) (T = type strain) was labeled in vivo and extracted by using the method described by Gilman (8) with the modifications described below. Cells cultivated in 200 ml of APT broth (Difco) supplemented with 9.25 MBq of [5,6-^{3}H]uracil (1.48 TBq mmol^{-1}; Amersham Corp.) were harvested at the beginning of the stationary phase, suspensed in 10 mM Tris–10 mM EDTA (pH 8.0) containing 10 mM vanadyl ribonucleoside complex (GIBCO Laboratories or Bethesda Research Laboratories, Inc.) and 8 mg of lysozyme per ml, and incubated for 1 h at 37°C. After centrifugation, the pellet was suspended in lysis buffer (10 mM Tris, 10 mM EDTA, 10 mM NaCl, 1% sodium dodecyl sulfate, pH 7.4) containing proteinase K (final concentration, 0.2 mg/ml) and 1% diethylpyrocarbonate (Tebu) and incubated for 30 min at 37°C. The nucleic acids were extracted with phenol-chloroform and precipitated with 0.5 M NaCl and ethanol. They were suspended in DNase buffer (20 mM Tris, 10 mM $MgCl_2$, pH 8.0) containing 60 U of DNase (RNase free; Appligene) for 1 h at 37°C and extracted as described above. The nucleic acids were purified as described by Champomier et al. (2). The specific activity of ^{3}H-labeled RNA from *Brochothrix campestris* was 10,500 cpm per μg of RNA. Nitrocellulose filters (Sartorius) were loaded with 100-μg portions of DNA as described by Champomier et al. (2). DNA-RNA hybridization and thermal denaturation values were determined by using the method of De Ley and De Smedt (6).

With a difference in the temperatures at which 50% of the bound rRNAs eluted from the filters for the homologous and heterologous hybrids [$\Delta T_{m(e)}$] of 1°C, it is obvious that the two *Brochothrix* species are genuinely related at the genus level (Table 1).

Several authors (7, 10, 21) have suggested that strains for which the $\Delta T_{m(e)}$ is less than 6°C can be included in the same RNA homology group. With $\Delta T_{m(e)}$ values of 4.5 and 5.0°C, the close relationship of *Brochothrix campestris* to *Listeria innocua* and *Listeria monocytogenes* is obvious (Table 1). This relationship is not surprising since the species of these two genera share many common characteristics (Table 1). Furthermore, both taxa possess catalase and cytochromes (5, 13) and contain predominantly methyl-branched-chain fatty acids (16).

The other organisms which are most closely related to *Brochothrix campestris* are *Enterococcus faecalis* [$\Delta T_{m(e)}$, 5.5°C] and *Bacillus cereus* and *Bacillus subtilis* [$\Delta T_{m(e)}$, 6.5°C]. Indeed, in addition to the common features shown in Table 1, *Bacillus subtilis* and its relatives (19) and *Brochothrix* species are aerobic or facultatively anaerobic and catalase positive. Despite the differences in peptidoglycan type and menaquinone type (Table 1), *Brochothrix campestris* exhibits a high level of relatedness to *Enterococcus faecalis*. Stackebrandt and Teuber (20) have stated previously that the *Brochothrix* line branches off from the main stem at about the same S_{AB} value as the lines leading to the genera *Enterococcus* and *Bacillus*.

Except for *Enterococcus faecalis*, all of the lactic acid bacteria and *Staphylococcus* spp. are distantly related to *Brochothrix campestris* (Table 1). This finding is in accordance with data obtained from comparative cataloging of 16S rRNA oligonucleotides (20).

There is no specific relationship between *Brochothrix* spp. and *A. globiformis* (Table 1), which is a gram-positive bacterium linked to the coryneform taxa, including *Micro-*

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TABLE 1. Levels of rRNA-DNA relatedness and chemotaxonomic properties for *Brochothrix campestris* and other gram-positive bacteria

Strain[a]	Peptidoglycan type[b]	Guanine-plus-cytosine content (mol%)[b]	Menaquinone type[b]	$\Delta T_{m(e)}$ (°C)[c]
Brochothrix campestris ATCC 43754^T	mDAP	38	ND	0
Brochothrix thermosphacta ATCC 11509^T	mDAP	36	MK-7	1.0
Listeria innocua ATCC 33090^T	mDAP	38	MK-7	4.5
Listeria monocytogenes ATCC 19115	mDAP	38	MK-7	5.0
Enterococcus faecalis DSM 20478	L-Lys-L-Ala_{2-3}	38	DMK-9, DMK-8	5.5
Bacillus subtilis DSM 402	mDAP	42.9	MK-7	6.5
Bacillus cereus DSM 626	mDAP	36.2	MK-7	6.5
Carnobacterium piscicola NCDO 2762^T	mDAP	34		7.5
Staphylococcus carnosus DSM 20501^T	L-Lys-Gly_{5-6}	35	MK-7, MK-8	7.8
Staphylococcus epidermidis ATCC 14990^T	L-Lys-Gly_4-L-Ser	34.8	MK-7	8.0
Lactobacillus plantarum ATCC 14917^T	mDAP	45		8.0
Lactobacillus acidophilus ATCC 4356^T	L-Lys-D-Asp	36		8.0
Sporolactobacillus inulinus DSM 20348	mDAP	38	MK-7	8.0
Lactococcus lactis DSM 20481	L-Lys-D-Asp	38.6	MK-9, MK-8	8.5
Streptococcus oralis CIP 102922	L-Lys	39		8.5
Lactobacillus viridescens ATCC 12706^T	L-Lys-L-Ala-L-Ser	42		8.7
Pediococcus damnosus DSM 20331^T	L-Lys-L-Ala-D-Asp	37.1		9.0
Pediococcus pentosaceus DSM 20336^T	L-Lys-L-Ala-D-Asp	38		9.5
Steptococcus mutans CIP 103220	L-Lys-L-Ala_{2-3}	37		9.5
Leuconostoc mesenteroides DSM 20343	L-Lys-L-Ser-L-Ala_2	37		10.0
Arthrobacter globiformis DSM 20124	L-Lys-L-Ala_3	62.0	MK-9(H_2)	13.0
Escherichia coli DSM 30083	mDAP	51.7		13.2

[a] Source of filter-bound DNA. ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of Germany; NCDO, National Collection of Dairy Organisms, Reading, United Kingdom; CIP, Collection de l'Institut Pasteur, Paris, France.
[b] Most of the data are from *Bergey's Manual of Systematic Bacteriology* (18); the data for *Brochothrix campestris* are from reference 22. The chemotaxonomic properties are characteristics of the species. mDAP, *meso*-Diaminopimelic acid; ND, not determined.
[c] Hybridization with 16S and 23S rRNAs from *Brochothrix campestris*.

bacterium, the genus in which *Brochothrix thermosphacta* was first placed.

In conclusion, the recently described species *Brochothrix campestris* belongs in the genus *Brochothrix* and is closely related to the genera *Listeria*, *Enterococcus*, and *Bacillus*.

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