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 source of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1. Most of the strains which we used are shown in Table 1.

**Table 1.** Most of the strains were grown in APT broth (Difco Laboratories; 12) and 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 Champonier et al. (2). DNAs were extracted and purified as described by Brenner et al. (1).

rRNA from *Brochothrix campestris* ATCC 43754T (= CIP 102920T) (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 [5.6-3H]uracil (1.48 TBq mmol-1; Amersham Corp.) were harvested at the beginning of the stationary phase, suspended 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 protease 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 MgCl2, 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 [3H]-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-rRNA 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 (ΔTm), 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 ΔTm is less than 6°C can be included in the same RNA homology group. With ΔTm 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* (ΔTm = 5.5°C) and *Bacillus cereus* and *Bacillus subtilis* (ΔTm = 6.3°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 S1,2 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-
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.

We thank J. P. Larpent and P. Muller for helpful advice and M. Cantonnet for typing the manuscript.

LITERATURE CITED


