Two Coryneform Bacteria Isolated from the Surface of French Gruyère and Beaufort Cheeses Are New Species of the Genus *Brachybacterium: Brachybacterium alimentarium* sp. nov. and *Brachybacterium tyrofermentans* sp. nov.†

KARIN SCHUBERT, 1,† WOLFGANG LUDWIG, 2 NINA SPRINGER, 2 REINER MICHAEL KROPPIENSTEDT, 3 JEAN-PIERRE ACCOLAS, 4 AND FRANZ FIEDLER 1

Institut für Genetik und Mikrobiologie der Universität München, 80638 Munich, 1 Lehrstuhl für Mikrobiologie, Technische Universität München, 80290 Munich, 2 and DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, 38124 Braunschweig, 3 Germany, and Station de Recherches Laitières, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas Cedex, France 4

New species names, *Brachybacterium alimentarium* and *Brachybacterium tyrofermentans*, are proposed for two coryneform bacteria isolated from the surfaces of Gruyère and Beaufort cheeses. These two species are similar in their biochemical and chemotaxonomic characteristics but distinct from previously described bacteria. The most distinctive characteristics are the presence of meso-diaminopimelic acid-containing peptidoglycan with a D-Glu-D-Asp-D-Glu type and resembles the meso-diaminopimelic acid-D-Asp-D-Glu type found in members of the genus *Brachybacterium* (6, 11). The cell walls of two of the isolates were particularly interesting. These cell walls contained a peptidoglycan type not found in any previously described bacteria and also contained esters of uric acid. Deamination of arginine did not occur or occurred only after 1 month (1). The peptidoglycan type of strains CNRZ 925T and CNRZ 926T, made their taxonomic position interesting. The peptidoglycan type of strains CNRZ 925T and CNRZ 926T is the meso-diaminopimelic acid-D-Asp-D-Glu type and resembles the meso-diaminopimelic acid-(D-Glu)2 type found in members of the genus *Brachybacterium* (6, 11).

We studied a number of taxonomic characteristics of strains CNRZ 925T and CNRZ 926T and compared these organisms with strains belonging to the genus *Brachybacterium*.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** Strains CNRZ 925T and CNRZ 926T were obtained from the Centre National de Recherches Zootechniques (CNRZ), Jouy-en-Josas, France. Reference strain *Brachybacterium conglomeratum* NCMB 989 was obtained from the National Collection of Industrial and Marine Bacteria (NCMB), Aberdeen, United Kingdom, and reference strains *B. conglomeratum* CCM 2136 and *B. nesterenkovii* CCM 2432 were obtained from the Czechoslovak Collection of Microorganisms (CCM), Brno, Czech Republic. *Brachybacterium faecium* DSM 4810T, *B. nesterenkovii* DSM 4575T, *Arthrobacter globiformis* DSM 20124T, and *Derma bacter lomisii* DSM 7083T were obtained from the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), Braunschweig, Germany.

The strains were grown aerobically at 30°C in a medium containing (per liter) 10 g of tryptone, 5 g of yeast extract, 5 g of glucose, and 5 g of NaCl (pH 7.2); strains CNRZ 925T and CNRZ 926T were also grown in a medium containing (per liter) 17 g of tryptone, 3 g of soy peptone, 5 g of yeast extract, 2.5 g of glucose, 5 g of NaCl, 2.5 g of KH2PO4, and 1 ml of Tween 80 (pH 7.3). For solid media 12 g of agar per liter was added.

**Utilization of sugars, tyrosine, and xanthine, NO3- and NO2- reduction, and hydrolysis of casein, starch, and gelatin were studied by using the methods of Seiler et al. (25). Utilization of each of the 20 naturally occurring amino acids (100 mg/liter) was determined on MOPS (morpholinepropanesulfonic acid) medium (21) supplemented with (per liter) 40 mg of adenosine, 40 mg of guanosine, 40 mg of cytidine, 40 mg of thymidine, 40 mg of uridine, 0.1 mg of vitamin B12, 0.1 mg of p-aminobenzoic acid, 2 mg of biotin, 2 mg of folic acid, 5 mg of riboflavin, 5 mg of pantothenic acid, 5 mg of lipoic acid, and 10 mg of pyridoxine-HCl. Decreases in the concentrations of single amino acids were determined with an amino acid analyzer (Biotronic, Maintal, Germany).

Production of indole and ammonification were determined by using a medium containing 40 g of peptone per liter. The presence of liberated NH3 was determined with Nessler’s reagent, and the presence of indole was determined with Kovács’s reagent. Leucinease activity and tellurite reduction were examined by using a medium containing (per liter) 3 g of meat extract, 10 g of casein peptone, 1 g of yeast extract, 10 g of sodium pyruvate, 12 g of glucose, 5 g of lithium chloride, and 15 g of agar (pH 6.8) to which 50 ml of egg yolk emulsion per liter was added.

© 1996, International Union of Microbiological Societies

† We dedicate this publication to Prof. Dr. Otto Kandler on the occasion of his 75th birthday.

* Corresponding author. Mailing address: Institut für Genetik und Mikrobiologie der Universität München, Maria-Ward-Strasse 1a, 80638 Munich, Germany. Phone: (089) 17 91 98 77. Fax: (089) 17 91 98 49.
and 3 ml of a 3.5% potassium tellurite solution per liter were added after the
medium was cooled to 50°C.

Lipase activity was determined on tributyrin agar containing (per liter) 2.5 g of
meat extract, 2.5 g of casein peptone, 3 g of yeast extract, 10 ml of glycolyltrbuty-
tyrate, and 12 g of agar (pH 7.5). Gas production was determined in culture
medium by using a Durham tube. Urease production was examined on the urea
agar base of Christensen (Merck) to which 5 ml of a 40% urea solution was
added after the medium was cooled to 50°C. Denitrification was examined in
nitrate broth (Merck). A control medium without KNO₃ was included. Tolerance
to NaCl was tested in culture medium containing 0 to 20% NaCl. Susceptibility
to antibiotics was tested in culture medium containing penicillin G, cycloserine,
chloramphenicol, streptomycin, rifampin, kanamycin, or bacitracin (each at a
concentration of 0.5 mg/ml). The presence of catalase was tested by applying 3%
H₂O₂ to colonies grown on solid medium. Oxidase activity was determined by
using the modified oxidase test of Fuller and Schleifer (8).

Chemotaxonomic characteristics. Fatty acids were isolated by the method of
Minnikin et al. (19), and fatty acid contents were determined by gas-liquid
chromatography as described by Kroppenstedt and Kützer (15). Menaquino-
es were isolated by the method of Collins et al. (7) and were identified by high-
performance liquid chromatography (HPLC) (14). Cell walls were prepared and
total amino acids were analyzed as described previously (24). Phosphorus con-
tents were determined by the method of Ames (3). The erythritol, sugars, and
glucosamine in teichoic acids or polysaccharides in the cell walls were analyzed
by gas-liquid chromatography as described previously (24). Diaminoglucuronic
acid was detected with an amino acid analyzer (Biotronic), and diaminoglucu-
ronic acid contents were determined by using trifluoroacetic acid hydrolysates (2
M trifluoroacetic acid, 3 h, 100°C) or hydrofluoric acid hydrolysates (60% HF, 16
h, 0°C [4]; followed by treatment with 2 M trifluoroacetic acid). The glycolic acid
contents of cell wall hydrolysates (4 M HCl, 1 h, 100°C) were determined by
cation-exchange HPLC by using an Aminex IPX-87H column and the method
described by Guerrant et al. (10).

Sequence determination. DNA purification, in vitro amplification of 16S rRNA-
encoding DNA, and direct sequencing of amplified DNA were performed as de-
scribed previously (26).

Phylogenetic analysis. 16S rRNA sequences were compared with about 1,800
homologous primary structures from bacteria (16, 20) by using the alignment tool
developed by the ARB program package (28). Phylogenetic analyses were performed by
using distance matrix, maximum-parminony, and maximum-likelihood methods
and the corresponding tools of the ARB (28) and PHYLIP (9) program pack-
geiges, as well as the fastDNAml program (16). The compositions of the data sets
varied with respect to the reference sequences and the alignment positions
included. Positional variations at the individual alignment positions were deter-
mined by using the tools of the ARB package and were used as criteria to remove
or include variable positions.

Isolation of DNA and DNA-DNA hybridization. Chromosomal DNA was iso-
lated from bacterial cells grown to the early stationary phase by using the method
of Marmur (17). The DNA purity and concentrations of DNA were determined
spectrophotometrically. DNA-DNA hybridization experiments were performed by
using DNA labelled with α-³²P-dCTP (Amersham, Little Chalfont, United
Kingdom) by nick translation (12). The hybridization experiments were per-
formed on nitrocellulose filters in 2X SSC (1X SSC is 0.15 M NaCl plus 0.015 M
sodium citrate, pH 7.0) at 68°C for 20 h. Nonspecifically bound DNA was
removed by stringent washing in 0.1X SSC containing 0.1% sodium dodecyl
sulfate for 3 h at 68°C. The DNA reassociation rates were determined at the
beginning of saturation (45 μg of DNA) by measuring ³²P contents with a
scintillation counter (model MR 300; Kontron). DNA from A. globiformis DSM
20124T was used as the negative control.

DNA base composition. The average guanine-plus-cytosine (G+C) molar ratio
dNA was determined by the thermal denaturation method (18). Reference
DNA was obtained from Micrococcus luteus DSM 20030T.

Nucleotide sequence accession numbers. The nucleotide sequences of strain
CNRZ 925T, strain CNRZ 926T, B. faecium DSM 4810T, B. nesterenkovii DSM
9533T, B. conglobatum NCIMB 9859, and D. hominis DSM 7083T have been
deposited in the EMBL Data Library under accession numbers X91031, X91657,
X91032, X91033, X91030, and X91034, respectively.

RESULTS

Cultural and morphological properties. In the exponential phase of growth strains CNRZ 925T and CNRZ 926T were
nonmotile, club-shaped rods that occurred in pairs. After 48 h the cells reached the stationary phase and became irregular
cocci that occurred in small (CNRZ 925T) or relatively large (CNRZ 926T) agglomerations (Fig. 1).
After 2 days on agar media both strains formed medium-sized, smooth, circular, low-convex colonies with entire margins (diameter, 3 to 5 mm). The colors of the colonies of strains CNRZ 925T and CNRZ 926T were vivid lemon and pale flaxen, respectively.

Growth occurred at temperatures above 20°C, and optimal growth occurred at 30°C. No growth occurred at ≥37°C. The doubling times at 30°C in the culture medium used were 1.25 h (CNRZ 925T) and 2.75 h (CNRZ 926T).

**Biochemical properties.** We determined biochemical characteristics of strains CNRZ 925T and CNRZ 926T, B. faecium DSM 4810T, B. nesterenkovi DSM 9573T and CCM 2432, and B. conglomeratum CCM 2136 and NCIMB 9859. All of these strains exhibited catalase and oxidase activities but not lecithinase activity. None hydrolyzed casein or tributyrin. None utilized β-galactosidase. The pH optimum for growth occurred at temperatures above 20°C, and optimal growth occurred at 30°C. The doubling times at 30°C in the culture medium used were 1.25 h (CNRZ 925T) and 2.75 h (CNRZ 926T).

**Chemotaxonomic characteristics.** All of the strains contained menaquinones in their respiratory chains. The major menaquinone was an unsaturated menaquinone with seven isoprenoid units (MK-7). In addition, minor amounts of MK-8 were present in most strains; the only exception was strain CNRZ 925T, which contained substantial amounts of MK-8 (Table 2).

The amino acids present in all cell wall hydrolysates were alanine, glutamic acid, and meso-diaminopimelic acid (molar ratio, 2:2:3:1). In addition, glycine and amide groups were found in strains CNRZ 925T, CNRZ 926T, DSM 4810T, and NCIMB 9859. In strains CNRZ 925T and CNRZ 926T one of the three glutamate residues was replaced by aspartate. Glycolic acid was present in all strains. The detailed compositions of the peptidoglycan constituents are shown in Table 2.

**Phylogenetic considerations.** We determined almost complete 16S rRNA gene sequences for strains CNRZ 925T and CNRZ 926T, B. faecium DSM 4810T, B. nesterenkovi DSM 9573T and CCM 2432, and B. conglomeratum CCM 2136 and NCIMB 9859. A matrix of the overall levels of 16S rRNA primary structure similarity for the bacteria which we investigated is shown in Table 4. The 16S rRNA sequence of D. hominis was nearly identical to the sequence described by Cai and Collins (5). The closest relatives of strains CNRZ 925T and CNRZ 926T were B. faecium and B. conglomeratum, as indicated by similarity values ranging from 97.1 to 97.5%, whereas B. nesterenkovi was less closely related (similarity values, 97.2 and 97.5%).
the brachybacteria were considerably lower (94.1 to 94.8%). All other currently available 16s rRNA sequences of gram-positive bacteria with high DNA G+C contents are less than 94.2% similar to the sequences of the previously described brachybacteria and strains CCM 925T and CRR 926T. The phylogenetic relationships of the organisms which we studied and a selection of related reference organisms are shown in Fig. 2.

DNA-DNA hybridization. The results of DNA-DNA hybridization experiments are shown in Table 5. The DNA-DNA complementarity values for strains CCR 925T and CRR 926T were 10.8 and 17.7% in a reverse experiment. The complementarity values for strain CCM 925T and the previously described brachybacteria ranged from 11.4 to 38.1%, and the complementarity values for strain CCM 926T and the previously described brachybacteria which we investigated ranged from 11.8 to 35.8%. The levels of DNA relatedness between A. globiformis DSM 20124T and the brachybacteria were not significant (0 to 2.4%).

**DISCUSSION**

The taxonomic position of a number of strains that exhibit peptidoglycan variation A4γ of Schleifer and Kandler (22) has been uncertain. These bacteria have been placed in the genera Micrococcus and Arthrobacter (11, 22), and on the basis of DNA-DNA hybridization data, Schleifer and Lang (23) placed them in a separate genus, which they did not define further. Later, Collins et al. (6) described the genus Brachybacterium for some of these strains. Gvozdyak et al. (11) found that several strains isolated from dairy products belonged to a new species of this genus, and recently, Takeuchi et al. (29) described three additional species of the genus Brachybacterium, bringing the number of species in this genus to five (B. faecium Collins, Brown, and Jones 1988, B. nesterenkovii Gvozdyak, Nogina, and Schumann 1992, B. congoloneurum Takeuchi, Fang, and Yokota 1995, Brachybacterium paradoxum Takenouchi, Fang, and Yokota 1995, and Brachybacterium rhamnosum Takeuchi, Fang, and Yokota 1995). The main chemotaxonomic characteristic of these species is the occurrence of peptidoglycan variation A4γ [meso-diaminopimelic acid-(D-Glu)] type (22). A glycine amide substituent at the α-carboxyl group of D-Glu in the peptidoglycan does not occur in B. nesterenkovii. Muramic acid occurs mostly in the glycolylated form instead of the acetylated form (29). In all species, the principal menaquinone is MK-7; the principal fatty acids are anteiso-C15:0 and anteiso-C17:0 acids. The G+C contents of the DNAs of these species range from 68 to 72 mol%. The main sugars in cell walls are galactose and glucose; in B. rhamnosum and B. nesterenkovii rhamnose is also found (29).

Like B. nesterenkovii, the strains described in this paper were isolated from dairy products (Beaufort and Gruyère cheeses), and they have similar characteristics. The peptidoglycans of these strains are variation A4γ peptidoglycans but differ from the peptidoglycans of the previously described brachybacteria. They contain d-Asp–D-Glu rather than d-Glu as the interpeptide bridge. The α-carboxyl group of D-glutamic acid in the peptide subunit is replaced by glycine amide, as is the case in most previously described brachybacteria. Muramic acid occurs in the N-glycolylated form. The principal menaquinone is MK-7.

**TABLE 2. Chemotaxonomic characteristics**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Menaquinone composition (%)</th>
<th>Amino acids</th>
<th>Relative amount of cell wall components</th>
<th>Sugars and phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MK-7</td>
<td>MK-8</td>
<td>Asp Mur Glu Gly Ala m-Dpm* GlcN NH3 Glycine acid*</td>
<td>Erythritol P GlcUANAc* Rhamicose Gal Glic GlcN</td>
</tr>
<tr>
<td>CCM 2136</td>
<td>0.7</td>
<td>0.6</td>
<td>6.2</td>
<td>37.6</td>
</tr>
<tr>
<td>CCM 2432</td>
<td>2.1</td>
<td>3.3</td>
<td>4.5</td>
<td>57.2</td>
</tr>
<tr>
<td>CCR 925T</td>
<td>1.0</td>
<td>4.1</td>
<td>64.3</td>
<td>3.4</td>
</tr>
<tr>
<td>CCR 926T</td>
<td>1.5</td>
<td>1.6</td>
<td>69.4</td>
<td>4.3</td>
</tr>
<tr>
<td>CNMB 9859</td>
<td>2.1</td>
<td>3.3</td>
<td>4.5</td>
<td>57.2</td>
</tr>
<tr>
<td>CCR 2432</td>
<td>2.1</td>
<td>3.3</td>
<td>4.5</td>
<td>57.2</td>
</tr>
<tr>
<td>CCR 926T</td>
<td>1.5</td>
<td>1.6</td>
<td>69.4</td>
<td>4.3</td>
</tr>
<tr>
<td>CCR 925T</td>
<td>1.0</td>
<td>4.1</td>
<td>64.3</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* The abbreviations for m-Dpm, meso-diaminopimellic acid.

+ Qualitative estimate.

ND, not detected.

a As no commercial N,N′-diacetyl-2,3-diamino-2,3-dideoxyglucuronic acid was available, the actual amount could not be determined without investigating the teichoic acid fragments; 80% of the erythritol was unsubstituted (amount of erythritol liberated by HF compared with the total amount of erythritol). The amount of acetate as substituent was not determined.

**TABLE 3. Fatty acid compositions of strains CCR 925T and CRR 926T and other Brachybacterium strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fatty acid composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i-C14:0</td>
</tr>
<tr>
<td>CCR 925T</td>
<td>1.0</td>
</tr>
<tr>
<td>CCR 926T</td>
<td>1.5</td>
</tr>
<tr>
<td>CNMB 9859</td>
<td>8.2</td>
</tr>
<tr>
<td>CCM 2136</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* The abbreviations for fatty acids are illustrated by the following examples: C16:0, hexadecanoic acid; C12:0, hexadecenoic acid; i-C15:0, 13-methyltetradecanoic acid; i-C15:1ω9c, 12-methyltetradecenoic acid; C18:1ω7c, C19:0, 10-octadecenoic acid; C18:2ω6c, cyclopropanoic acid.
MK-7, but MK-8 is also present in substantial amounts in strain CNRZ 925T (Table 2). The fatty acids are primarily straight-chain, saturated, iso- and anteiso-methyl, branched fatty acids; 12-methyltetradecanoic (anteiso-C15:0) acid is the predominant fatty acid in strains CNRZ 925T and CNRZ 926T (64.3 and 69.1% of the total fatty acids, respectively) (Table 3). Other fatty acids are present at levels of less than 10%. Thus, strains CNRZ 925T and CNRZ 926T clearly differ from the previously described brachybacteria, which contain 37.6 to 57.2% 12-methyltetradecanoic (anteiso-C15:0) acid and substantial amounts of 14-methylhexadecanoic (anteiso-C17:0) acid (Table 3).

Our results also demonstrated that teichoic acids with the unusual compound erythritol as the polyol and diaminoglucuronic acid as a substituent occur in strains CNRZ 925T and CNRZ 926T (24). In addition to the teichoic acids, galactose (CNRZ 925T) or galactose and glucose (CNRZ 926T) were found to constitute separate polysaccharides in the cell walls. An investigation of the cell walls of B. faecium DSM 4810T revealed a similar cell wall structure; erythritol, phosphorus, diaminoglucuronic acid, glucosamine, galactose, and glucose occurred in the cell walls of this organism. On the basis of the results of the cell wall analysis alone we could not determine which constituents belonged to the teichoic acid. Assuming that the cell wall compositions of strains DSM 4810T, CNRZ 925T, and CNRZ 926T are similar, glucose and galactose probably form a separate polysaccharide. Thus, B. faecium, the type species of the genus Brachybacterium (6), is obviously similar to strains CNRZ 925T and CNRZ 926T in cell wall structure.

Gvozdyak et al. (11) described the occurrence of rhamnose in B. nesterenkovi, whereas no rhamnose was present in B. faecium. Recently, Takeuchi et al. (29) showed that rhamnose occurs in the cell walls of B. nesterenkovi. Our investigation of cell walls, which included extraction of accessory polymers, confirmed this finding and corroborated the hypothesis that galactose occurs in the cell walls of Brachybacterium strains. However, our experiments revealed that glucose is not a cell wall constituent in B. nesterenkovi and that 6-deoxytose is not a cell wall constituent in B. faecium (29). Instead (B. nesterenkovi) or in addition (B. faecium), glucosamine was found. The reason for these differences in the compositions of the carbohydrate moieties of the cell walls is not clear, but the differences might be due to nutritional differences (i.e., addition of brain heat infusion to the culture medium) (29). In this context it seems important to emphasize that when accessory carbohydrate polymers in bacterial cell walls are examined, taxonomically relevant data can be obtained only when polysaccharides and/or teichoic acids are extracted, fractionated, and structurally investigated.

We believe that the chemotaxonomic data described above indicate that strains CNRZ 925T and CNRZ 926T belong to the genus Brachybacterium. This view was clearly validated by 16S rRNA sequence data. For each of these isolates the levels of sequence similarity to B. faecium and B. conglomeratum, the closest relatives, were more than 97%, indicating that the organisms are related at the genus level or possibly at the species level. DNA-DNA hybridization experiments performed with B. faecium DSM 4810T, B. nesterenkovi DSM 9573T, B. conglomeratum NCIMB 9859, strain CNRZ 925T, and strain CNRZ 926T resulted in values well below 70%, the level of relatedness which separates organisms at the species level (27). The levels of DNA relatedness to B. faecium DSM 4810T, B. nesterenkovi DSM 9573T, and B. conglomeratum NCIMB 9859 were between 11.4 and 38.1% for both CNRZ 925T and CNRZ 926T; these values were similar to the values obtained by Gvozdyak et al. (11) for the relationships between B. nesterenkovi and B. faecium (22%) and by Takeuchi et al. (29) for the relationships among the five Brachybacterium species which they investigated (2 to 49%). The 16S rRNA sequence data and the DNA-DNA hybridization data clearly showed that

### TABLE 4. Similarity matrix based on 16S rRNA sequences

<table>
<thead>
<tr>
<th>Organism</th>
<th>Brachybacterium tyroferrans CNRZ 926T</th>
<th>Brachybacterium conglomeratum NCIMB 9859</th>
<th>Brachybacterium faecium DSM 4810T</th>
<th>Brachybacterium nesterenkovi DSM 9573T</th>
<th>Dermabacter hominis</th>
<th>Brevibacterium casei</th>
<th>Brevibacterium limosum</th>
<th>Brevibacterium linens</th>
<th>Brevibacterium subtilis</th>
<th>Brevibacterium gallicum</th>
<th>Arthrobacter globiformis</th>
<th>Micrococcus luteus</th>
<th>Rothia dentocariosa</th>
<th>Dermatophilus congolensis</th>
<th>Dermabacter corninis</th>
<th>Brevibacterium fynfermentans</th>
<th>Curtobacterium citreum</th>
<th>Terrabacter turnescens</th>
<th>Carboxybacillus citreus</th>
<th>Carboxybacillus callosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>% 16S rRNA sequence similarity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
maximum-parsimony analyses of more comprehensive data sets containing all contents that were the 40 closest relatives of strains CNRZ 925T and CNRZ 926T. The topology of the tree was evaluated by performing distance matrix and maximum-parsimony analyses of more comprehensive data sets containing all available 16S rRNA sequences from gram-positive bacteria with high DNA G+C contents. Only alignment positions which were occupied by identical residues in at least 50% of all available homologous sequences from gram-positive bacteria with high DNA G+C contents were included in the calculations. The inclusion of more variable positions did not alter the topology of the subtree containing strains CNRZ 925T and CNRZ 926T and Brachybacterium and Demamabacter strains. Multifurcations in the tree branches for which a relative order could not be determined unambiguously or a common branching order was not significantly supported when different treeing methods were used. Bar = 10% estimated sequence divergence.

TABLE 5. DNA-DNA hybridization data for strains CNRZ 925T and CNRZ 926T and other Brachybacterium strains

<table>
<thead>
<tr>
<th>Source of unlabelled DNA</th>
<th>% DNA relatedness with 35S-labelled DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNRZ 925T</td>
</tr>
<tr>
<td>CNRZ 925T</td>
<td>100</td>
</tr>
<tr>
<td>CNRZ 926T</td>
<td>10.8</td>
</tr>
<tr>
<td>DSM 4810T</td>
<td>19.4</td>
</tr>
<tr>
<td>DSM 9573T</td>
<td>16.4</td>
</tr>
<tr>
<td>CCM 2432</td>
<td>11.4</td>
</tr>
<tr>
<td>NCIMB 9859</td>
<td>38.1</td>
</tr>
<tr>
<td>CCM 2136</td>
<td>14.3</td>
</tr>
<tr>
<td>DSM 20124T</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* A. globiformis DSM 20124T was used as a negative control.

Our findings indicate that CNRZ 925T and CNRZ 926T are not members of previously described Brachybacterium species. The levels of DNA relatedness between CNRZ 925T and CNRZ 926T, corresponding to hybridization values of 10.8 and 17.7% (Table 5), also indicate that these organisms belong to separate species.

The cell wall peptidoglycan is based on meso-diaminopimelic acid with a D-Asp-D-Glu interpeptide bridge (variation A4\v). Two teichoic acids are present; one contains erythritol and N,N'-diacetyl-2,3-diamino-2,3-dideoxyglyceruronic acid, and the other contains N-acetylgalactosamine and galactose as sugar constituents. The principal menaquinone is MK-7. MK-8 is present in substantial amounts. The long-chain fatty acids are primarily straight-chain, saturated, iso- and anteiso-methyl-branched fatty acids, with 12-methyltetradecanoic (anteiso-C16:0) acid predominating. The G+C content of the DNA is 73 mol% (as determined by the thermal denaturation method). Isolated from the surfaces of Beaufort and Gruyère cheeses. The type strain is CNRZ 925.

The peptidoglycan is based on meso-diaminopimelic acid with a D-Asp-D-Glu interpeptide bridge (vari-ation A4\v). Two teichoic acids are present; one contains erythritol, N-acetylgalactosamine, and N,N'-diacetyl-2,3-diamino-2,3-dideoxyglyceruronic acid. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso- and anteiso-methyl-branched fatty acids, with 12-methyltetradecanoic (anteiso-C16:0) acid predominating. The G+C content of the DNA is 73 mol% (as determined by the thermal denaturation method). Isolated from the surfaces of Beaufort and Gruyère cheeses. The type strain is CNRZ 926.

REFERENCES