Taxonomic Study of the Genus *Brachybacterium*: Proposal of *Brachybacterium conglomeratum* sp. nov., nom. rev., *Brachybacterium paraconglomeratum* sp. nov., and *Brachybacterium rhamnorum* sp. nov.

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On the basis of morphological, physiological, and chemotaxonomic characteristics and DNA-DNA hybridization data, we describe three new *Brachybacterium* species: *Brachybacterium conglomeratum* sp. nov., nom. rev. (type strain, IFO 15472); *Brachybacterium paraconglomeratum* sp. nov. (type strain, IFO 15224); and *Brachybacterium rhamnorum* sp. nov. (type strain, IFO 15203). These new species include some previously misclassified strains and an isolate obtained from corn steep liquor. Although *B. conglomeratum* and *B. paraconglomeratum* are similar in their physiological characteristics, they can be differentiated on the basis of their cellular fatty acid patterns and low levels of DNA-DNA hybridization. *B. rhamnorum* is characterized by the presence of large amounts of rhamnose in its cell wall.

The genus *Brachybacterium* was established by Collins et al. (2). This genus is characterized by the following chemotaxonomic features: the peptidoglycan type is the meso-diaminopimelic acid (DAP)-D-Glu₂ type (variation A4γ) (15); the principal menaquinone is MK-7; mycolic acids are absent; and the G+C content of the DNA is 68 to 72 mol%. Until now, the genus *Brachybacterium* has been represented by two species, *Brachybacterium faecium* (2) and *Brachybacterium nesterenkovi* (5), but it has been suggested that several other bacterial strains belong to this genus (16). Bogdanovsky et al. (1) determined the peptidoglycan structures of "*Micrococcus conglomeratus*" strains and *Micrococcus roseus* (formerly *Staphylococcus lactis*) and found that the peptidoglycan type of these bacteria is the meso-DAP-D-Glu₂ type (variation A4γ), a type which has been found only in the genus *Brachybacterium*. Nogina et al. (11) suggested that these organisms should be assigned to the genus *Brachybacterium*, although their taxonomic positions at the species level were not certain. In addition, one strain isolated in our laboratory had the morphological, biochemical, and chemotaxonomic characteristics peculiar to the genus *Brachybacterium*.

In this paper, we describe the phenotypic and chemotaxonomic characteristics of the strains mentioned above, and on the basis of these characteristics and the results of a DNA-DNA hybridization study, we describe three new *Brachybacterium* species.

MATERIALS AND METHODS

Microorganisms and cultures. The strains which we used are listed in Table 1. *B. faecium* NCIB 9860^T (T = type strain) NCIB 9859, and NCIB 9861 were obtained from the National Collection of Industrial Bacteria, eight strains of "*M. conglomeratus*" and one *Brachybacterium* sp. strain were obtained from the Czech Collection of Microorganisms, and *M. roseus* NCIC 15264 was obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London, England. *Brachybacterium* sp. strain H-6S^T (= IFO 15203^T) was isolated in our laboratory from commercially available corn steep liquor. All strains were cultured at 28°C with aerobic shaking in a peptone-yeast extract

medium supplemented with brain heart infusion (PY-BHI medium), which contained 1% peptone, 0.2% yeast extract, 0.5% brain heart infusion (Difco Laboratories), 0.2% NaCl, and 0.2% glucose (pH 7.0). Cells were harvested by centrifugation, washed with water, and then lyophilized.

Morphological characteristics. Cell morphology was determined by using cells grown on yeast extract-peptone-brain heart infusion agar (1% yeast extract, 0.5% peptone, 0.2% brain heart infusion, 2% agar [pH 7.0]). Each sample used for scanning electron microscopy with a model JSM 5400 scanning electron microscope (JEOL, Ltd., Tokyo, Japan) was prepared by fixing cells with 1% glutaraldehyde and then dehydrating them with a graded acetone series and then in a Hitachi model HCP-2 critical point drying apparatus.

Determination of phenotypic characteristics. Cultures were grown on PY-BHI medium containing 1.5% agar. Motility was determined with a light microscope by the hanging drop method. All tests were performed at 28°C. Catalase activity was determined by monitoring bubble formation in a 3% hydrogen peroxide solution. Oxidase activity was determined by monitoring the oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates was determined in medium containing 1.0% peptone, 0.5% NaCl, 0.003% bromocresol purple, and 0.5% carbohydrate (pH 7.2). Assimilation of carbohydrates and organic acids was determined in medium containing 0.5% carbohydrate or 0.5% organic acid (sodium salt), 0.1% NH₄NO₃, 0.1% KH₂PO₄, 0.05% MgSO₄ · 7H₂O, and 0.02% KCl (pH 7.2).

Other phenotypic characteristics, production of indole and H₂S, the Voges-Proskauer test reaction, nitrate reduction, and hydrolysis of starch, gelatin, casein, and esculin were determined by the methods described by Cowan and Steel (3).

Analyses of chemotaxonomic characteristics. Cell walls were prepared from ca. 500 mg (dry weight) of cells by mechanical disruption with an ultrasonic oscillator and were purified as described by Schleifer and Kandler (15). The amino acid compositions of complete wall hydrolysates were determined by high-performance liquid chromatography (HPLC) (19), as described by Yokota et al. (20). For the cell wall sugar analysis, cell walls were hydrolyzed with 2 N HCl at 100°C for 2 h, dried in vacuo, and then analyzed by HPLC by the method of Mikami and Ishida (8). Glycolate tests were performed by the method of Uchida and Aida (18). Fatty acids were extracted from dry cells (50 mg) by acid methanolysis, and fatty acid contents were determined by gas-liquid chromatography as described previously (17). Free lipids were extracted from dry cells (100 mg) and purified by the method of Minnikin et al. (10), and free lipid contents were determined by two-dimensional thin-layer chromatography, using Kieselgel 60 F₂₅₄ plates (Merck, Darmstadt, Germany). Lipid spots were revealed by spraying the chromatograms with 10% molybdophosphoric acid in ethanol and then heating them at 150°C for 15 min. Specific spray reagents for lipid phosphate, α-glycol (periodate-Schiff reagent), sugars (α-naphthol), and free amino groups (ninhydrin in water-saturated butanol) were also used. Mycolic acids were analyzed by the method of Minnikin et al. (9). Menaquinones were extracted from 200 mg of dry cells with chloroform-methanol (2:1, vol/vol), purified by thin-layer chromatography, extracted with diethyl ether, dried under a nitrogen stream, and then analyzed by HPLC.

DNA-DNA hybridization. DNA was obtained by the method of Saito and Miura (13). The G+C content of DNA was determined by HPLC by the method of Mesbah et al. (7) after treatment with P₁ nuclease and alkaline phosphatase. DNA-DNA hybridization was carried out fluorometrically in microdilution wells by using biotinylated DNA (4).

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RESULTS

Morphological, physiological, and biochemical characteristics. All of the organisms which we examined had similar morphological properties. In the stationary growth phase, cultures consisted mainly of coccoid cells that were 0.5 to 1 µm in diameter, but 6 to 8 h after the organisms were transferred to fresh medium, the cells were oval or short rods that were up to 2 µm long (Fig. 1). Some cells exhibited a V-shaped arrangement. The cells were gram positive, not acid fast, nonmotile, and nonsporing. Colonies on PY-BHI agar were convex and had regular edges, and their surfaces were smooth or rough. Colonies were yellow, yellow-green, pale brown, or greyish white on PY-BHI agar. As shown in Table 2, all strains gave positive results in the tests for catalase activity, growth in broth containing 0 to 7.5% NaCl, hydrolysis of esculin and starch, nitrate reduction, and formation of acid from L-arabinose and D-glucose, while all strains gave negative results in tests for oxidase activity, hydrolysis of Tween 20 and Tween 40, and formation of acid from mannitol and xylitol.

Chemical characteristics. The chemical characteristics of the strains which we studied are summarized in Tables 3 and 4. All strains contained menaquinone MK-7, and the DNA base compositions of the strains ranged from 70.2 to 72.6 mol%. All strains contained high levels of glycolate in the glycan moieties of their cell walls, which suggests that muramic acid occurred in the N-glycolyl form rather than the more common N-acetyl form. Mycolic acids were absent. Galactose and glucose were common cell wall sugars in all strains, while the cell wall preparations of *B. nesterenkovi* IMV Ac-752^T and "*M. conglomeratus*" CCM 2432 also contained small amounts of rhamnose. *Brachybacterium* sp. strain H-6S^T contained rhamnose as a major component of its cell wall. 6-Deoxytalose was detected in the cell wall preparation of the type strain of *B. faecium*, strain NCIB 9860. The amino acids in the cell wall hydrolysates of all of the strains except *M. nesterenkovi* IMV Ac-752^T and "*M. conglomeratus*" CCM 2432 were *meso*-DAP, alanine, glutamic acid, and glycine (molar ratio, 1:2:2~3:1), whereas the amino acids in the cell wall hydrolysates of strains IMV Ac-752^T and CCM 2432 were *meso*-DAP, alanine, and glutamic acid (molar ratio, 1:2:2~3); glycine was not present in the cell wall hydrolysates of strains IMV Ac-752^T and CCM 2432. On the basis of the molar ratios of the amino acids we suggest that the peptidoglycan structure type of all strains is type A4γ. Figure 2 shows the results of our two-dimensional thin-layer chromatography analysis of the polar lipids of representative strains. The diagnostic phospholipid patterns indicated that diphosphatidylglycerol, phosphatidylglycerol, glycolipids, and unidentified phosphoglycolipids (ninhydrin-positive unknown phosphoglycolipid or ninhydrin-negative unknown phosphoglycolipid) were present in the strains which we studied. In this analysis we identified six different phospholipid profiles (profiles A through F). As shown in Fig. 2, profile B, which was found in *B. nesterenkovi* IMV Ac-752^T, was composed of three lipids, diphosphatidylglycerol, glycolipid, and phosphatidylglycerol. Profile A, which was found in *B. faecium* NCIB 9860^T, contained a ninhydrin-negative unknown phosphoglycolipid in addition to the lipids found in profile B. Profile C, which was found in *B. faecium* NCIB 9861, contained a ninhydrin-positive unknown phosphoglycolipid in addition to the lipids found in profile A. Like profile A, profile D was composed of four lipids, diphosphatidylglycerol, a ninhydrin-negative unknown phosphoglycolipid, glycolipid, and phosphatidylglycerol, but two kinds of glycolipids were present in profile D and the positions of these components on thin-layer chromatography plates were different. In *Brachybacterium con-*

TABLE 1. Bacterial strains studied

Species	Strain ^a	IFO ^a no.	Other designation(s) ^a	Source	Reference	Species after reclassification
<i>Brachybacterium faecium</i>	NCIB 9859	15223		Poultry deep litter	14	<i>Brachybacterium conglomeratum</i>
<i>Brachybacterium faecium</i>	NCIB 9860 ^T	14762 ^T		Poultry deep litter	14	<i>Brachybacterium puraconglomeratum</i>
<i>Brachybacterium faecium</i>	NCIB 9861 ^T	15224 ^T		Poultry deep litter	14	<i>Brachybacterium puraconglomeratum</i>
<i>Brachybacterium nesterenkovi</i>	IMV Ac-752 ^T	15384 ^T		Cheese	This study	<i>Brachybacterium rhamnosum</i>
<i>Brachybacterium</i> sp.	H-6S ^T	15203 ^T		Corn steep liquor		<i>Brachybacterium conglomeratum</i>
<i>Brachybacterium</i> sp.	CCM 4385	15464	IAM 1470, Komagata 5-7, ATCC 19012	Oil brine	6	<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2135	15262	IAM 1452, Komagata 5-3, ATCC 19101	Oil brine	6	<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2134	15263		Oil brine		<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2136	15266	IAM 1448			<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2137	15267	IAM 1480			<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2511	15471				<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2589 ^T	15472 ^T	AJ 1015 ^T , Komagata 5-2 ^T	Oil brine	6	<i>Brachybacterium conglomeratum</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2590	15473	AJ 1018, Komagata 6-3	Oil brine	6	<i>Brachybacterium conglomeratum</i>
<i>Micrococcus roseus</i>	NCTC 7281	15264				<i>Brachybacterium nesterenkovi</i>
" <i>Micrococcus conglomeratus</i> "	CCM 2432	15265	NCTC 7567			<i>Brachybacterium nesterenkovi</i>

^a NCIB, National Collection of Industrial Bacteria, Ltd., Aberdeen, Scotland; IMV, Institute of Microbiology and Virology of the Academy of Sciences of the Ukraine, Kiev, Ukraine; CCM, Czech Collection of Microorganisms, J. E. Purkyně University, Brno, Czech Republic; ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; IFO, Institute for Fermentation, Osaka, Japan.

FIG. 1. Scanning electron micrographs of *Brachybacterium* strains grown on PY-BHI agar for 1 day. (A) *B. faecium* NCIB 9860^T. (B) *B. paraconglomeratum* NCIB 9861^T. (C) *B. nesterenkovi* IMA Ac-752^T. (D) *B. rhamnosum* H-6S^T. (E) *B. conglomeratum* CCM 2589^T.

not be clearly differentiated on the basis of their phospholipid patterns. All of the strains produced fatty acid profiles which consisted primarily of anteiso methyl-branched acids, including 12-methyltetradecanoic (anteiso-C_{15:0}) and 14-methylhexadecanoic (anteiso-C_{17:0}) (Table 4). The results of a cluster analysis based on fatty acid compositions are shown in Fig. 3; this analysis revealed that *B. nesterenkovi* and *Brachybacterium rhamnosum* strains belong to a complex together with *B. conglomeratum* strains, but that *B. faecium* and *B. paraconglomeratum* strains belong on different branches.

On the basis of morphological, physiological, and chemotaxonomic characteristics, all of the strains were identified as members of the genus *Brachybacterium*.

DNA-DNA hybridization. On the basis of DNA-DNA hybridization values (Table 5) we separated the strains which we studied into five distinct DNA relatedness groups. The *B. faecium* strains (NCIB 9860^T, NCIB 9859, and NCIB 9861) exhibited low levels of DNA-DNA homology with each other (13 to 49%). The type strain of *B. faecium*, NCIB 9860, and strain NCIB 9861 did not exhibit a high level of homology with any other strain tested, but *B. faecium* NCIB 9859 exhibited high levels of homology with nine strains of "*M. conglomeratus*" (CCM 2135, CCM 2134, CCM 2136, CCM 2137, CCM

glomeratum, two profiles (profiles E and F) were found. Both profile E and profile F were composed of five lipids, but two kinds of ninhydrin-positive unknown phosphoglycolipids and two types of glycolipids were found in profiles E and F, respectively. Therefore, the *B. conglomeratum* (profiles E and F) and *Brachybacterium paraconglomeratum* (profile C) could

TABLE 2. Phenotypic characteristics of *Brachybacterium* strains

Characteristic	<i>B. faecium</i> NCIB 9860 ^T	<i>B. nesterenkovi</i> strains		<i>B. paracon-</i> <i>glomeratum</i> NCIB 9861 ^T	<i>B. rham-</i> <i>nosum</i> H-6S ^T	<i>B. conglomeratum</i> strains									
		IMV Ac-752 ^T	CCM 2432			NCIB 9859	CCM 4385	CCM 2135	CCM 2134	CCM 2136	CCM 2137	CCM 2511	CCM 2589 ^T	CCM 2590	NCTC 7281
Growth rate ^a	S	R	R	S	R	R	R	R	R	S	R	R	S	S	R
Color of colonies ^b	P	L-Y	Y	P	W-Y	P	Y-G	P	P	P	P	P	P	P	L-Y
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in the presence of:															
2.0% NaCl	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
5.0% NaCl	+	+	+	+	+	++	+	+	+	++	++	+	+	+	+
7.5% NaCl	+	+w	+w	+	+	++	+	+	+	+w	+	+	+	+	+w
10.0% NaCl	+w	-	-	+w	+w	++	+w	+w	+	+w	+	+w	+w	+w	-
15.0% NaCl	+w	-	-	+w	-	+	+w	+w	+w	+w	+w	+w	+w	+w	-
Growth at pH 5.0	-	+	+	-	+	+	+	+	+	-	+	+	-	+	-
Hydrolysis of:															
Gelatin	-	-	-	-	+	+w	-	+w	-	-	-	-	-	-	-
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	w	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+w	+	+	+
Tween 20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tween 40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tween 60	-	-	-	-	-	-	-	-	-	+w	+	+w	+w	-	+w
Tween 80	-	-	-	-	-	-	-	-	-	+	+	+w	+w	-	+w
Urease production	-	-	-	+	+	+w	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H ₂ S production	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+
Voges-Proskauer test	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+
Indole formation	+w	-	-	+	-	+	+	+	+	+w	+	+	+w	+	-
Acid production from:															
D-Arabinose	+w	-	-	-	-	-	-	-	-	-	-	+w	-	-	-
L-Arabinose	+w	+	+	+	+	+	+	+	+	+	+	+w	+w	+	+
Cellobiose	-	-	+	+w	+	-	+w	-	+w	-	+w	-	-	-	-
D-Fructose	-	-	-	+	+	+	+	+	+	-	+	-	-	-	+
D-Galactose	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	+	+	+w	-	-	+w	+w	+w	-	+w	-	-	-	+
Maltose	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
D-Mannose	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-
Melezitose	+w	-	-	+w	+	+	-	-	-	-	+w	-	-	-	-
Raffinose	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
L-Rhamnose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
D-Ribose	+w	+	+w	-	+w	-	-	-	-	-	+	+w	-	+	+
Salicin	-	+	+	+	+	-	+	+	+	-	+	-	-	+	+
Sucrose	-	-	+	+	+	+	+	+	+	-	+	-	-	-	-
Trehalose	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
D-Xylose	-	-	+	-	+	+	-	+	-	+	-	-	-	-	-
Adonitol	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+
Inositol	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a R, rapid; S, slow.^b P, pale brown; L-Y, light yellow; W-Y, whitish yellow; Y-G, yellow-green; Y, yellow.^c ++, strongly positive; +, positive; +w, weakly positive; -, negative.

2511, CCM 2589, CCM 2590, and CCM 2432), *M. roseus* NCTC 7281, and *Brachybacterium* sp. strain CCM 4385. "*M. conglomeratus*" CCM 2432 exhibited a high level of homology with the type strain of *B. nesterenkovi*, IMV Ac-752.

DISCUSSION

On the basis of biochemical and chemical criteria, the 15 strains which we studied were found to belong to the genus *Brachybacterium*.

Schleifer and Lang (16) reported that the cell morphology of "*M. conglomeratus*" strains is uniform and that only coccoid

cells occur throughout the various phases of growth. In our study, we found that stationary-phase cultures of the 15 strains which we studied contained only coccoid cells and that early-log-phase cultures contained rod-shaped cells.

Collins et al. (2) placed strains CCM 9860^T, CCM 9859, and CCM 9861 in the species *B. faecium* on the basis of their physiological and chemotaxonomic characteristics. On the other hand, Schleifer and Lang (16) reported that the levels of DNA-DNA homology between *B. faecium* CCM 9860^T (= IFO 14762^T) and the other two *B. faecium* strains, CCM 9861 and CCM 9859, revealed moderate levels of relatedness (60 to 70 and 40 to 60%, respectively), and they suggested that a level of

TABLE 3. Chemotaxonomic characteristics

Species	Strain	Mena-quinone	Acyl type	Mycolic acid	Phospho-lipid profile ^a	G+C content (mol%)	Cell wall composition ^b							
							Sugars				Amino acids			
							Gal	Glc	Rha	6Tal	meso-DAP	Glu	Gly	Ala
<i>B. faecium</i>	NCIB 9860 ^T	MK-7	Glycolyl	–	A	72.1	1.0	0.32		0.46	1.0	2.46	1.28	2.00
<i>B. nesterenkovii</i>	IMV Ac-752 ^T	MK-7	Glycolyl	–	B	72.0	1.0	0.45	0.53		1.0	1.61		1.61
	CCM 2432	MK-7	Glycolyl	–	B	71.4	1.0	0.28	0.66		1.0	2.35		2.32
<i>B. paraconglomeratum</i>	NCIB 9861 ^T	MK-7	Glycolyl	–	C	68.6	1.0	0.49			1.0	3.22	1.31	2.22
<i>B. rhamnosum</i>	H-6S ^T	MK-7	Glycolyl	–	D	71.5	1.0	0.76	4.52		1.0	2.57	0.88	1.96
<i>B. conglomeratum</i>	NCIB 9859	MK-7	Glycolyl	–	E	70.5	1.0	0.69			1.0	3.34	1.21	2.12
	CCM 4385	MK-7	Glycolyl	–	E	71.2	1.0	0.47			1.0	2.56	1.53	3.12
	CCM 2135	MK-7	Glycolyl	–	F	70.8	1.0	0.50			1.0	2.07	1.35	2.70
	CCM 2134	MK-7	Glycolyl	–	F	71.4	1.0	0.53			1.0	1.22	0.78	1.49
	CCM 2136	MK-7	Glycolyl	–	E	71.6	1.0	0.42			1.0	1.82	1.00	1.75
	CCM 2137	MK-7	Glycolyl	–	E	72.6	1.0	0.22			1.0	2.32	1.23	2.32
	CCM 2511	MK-7	Glycolyl	–	E	70.2	1.0	0.44			1.0	1.83	1.47	2.78
	CCM 2589 ^T	MK-7	Glycolyl	–	E	70.6	1.0	0.42			1.0	2.69	1.63	2.86
	CCM 2590	MK-7	Glycolyl	–	E	70.9	1.0	0.21			1.0	2.08	1.12	2.00
	NCTC 7281	MK-7	Glycolyl	–	E	71.5	1.0	0.20			1.0	2.51	1.40	2.86

^a See Fig. 2.^b Gal, galactose; Glc, glucose; Rha, rhamnose; 6Tal, 6-deoxytalose; Glu, glutamic acid; Gly, glycine; Ala, alanine.

DNA-DNA homology of about 65% represents the borderline value for subspecies differentiation. In our study, the levels of DNA-DNA homology among these strains were less than 50%, and therefore, we concluded that they should be classified as members of separate species. Our dendrogram based on the quantitative fatty acid data (Fig. 3) also showed that *B. faecium* CCM 9860^T, CCM 9861, and CCM 9859 should be separated from each other.

On the basis of morphological, physiological, and chemotaxonomic characteristics and DNA-DNA hybridization data, we describe three new *Brachybacterium* species: *Brachybacterium conglomeratum* sp. nov., nom. rev. (type strain, CCM 9860 [= IFO 15472]) for strains CCM 9860, NCIB 9859, CCM 2134, CCM 2135, CCM 2136, CCM 2137, CCM 2511, CCM 2589, CCM 2590, CCM 4385, and NCTC 7281; *Brachybacterium paraconglomeratum* sp. nov. (type strain, NCIB 9861 [= IFO 15224]) for strain NCIB 9861 (formerly a *B. faecium* strain); and *Brachybacterium rhamnosum* sp. nov. (type strain, H-6S [= IFO 15203]) for strain H-6S, which was isolated from corn steep liquor. Strain NCTC 7281, which was received as *M.*

roseus, is not a type strain and is not an *M. roseus* strain; this strain was identified as a member of *B. conglomeratum* in this study.

Differential characteristics of these new *Brachybacterium* species are shown in Table 6.

The physiological characteristics of *B. paraconglomeratum* and *B. conglomeratum* are similar, but these taxa can be distinguished on the basis of their cellular fatty acid types (Fig. 3) and low DNA-DNA hybridization values. *B. rhamnosum* is characterized by the presence of large amounts of rhamnose in its cell wall, and *B. nesterenkovi* is characterized by the absence of glycine in its cell wall. *B. nesterenkovi* IMV Ac-752^T and CCM 2432 were included in the same cluster as *B. conglomeratum* and *B. rhamnosum* on the dendrogram based on quantitative fatty acid data (Fig. 3), but these two strains are quite different from the other strains in the cluster in their chemotaxonomic characteristics (e.g., cell wall sugars and phospholipid type) and DNA-DNA homology values. *B. faecium* NCIB 9860^T is characterized by the presence of 6-deoxytalose in its cell wall.

TABLE 4. Cellular fatty acid compositions

Species	Strain	% of total fatty acids											
		iso- 14:0	14:0	iso- 15:0	anteiso- 15:0	15:0	15:1	iso- 16:0	16:0	iso- 17:0	anteiso- 17:0	18:0	18:1
<i>B. faecium</i>	NCIB 9860 ^T	0.8		18.8	58.1		2.7	3.2		2.2	10.1	4.0	
<i>B. nesterenkovi</i>	IMV Ac-752 ^T	3.1	2.9	3.7	42.3		2.9	11.1			12.4	16.9	4.5
	CCM 2432	1.6	2.7	4.0	49.6		3.1	12.3			17.2	7.1	2.4
<i>B. paraconglomeratum</i>	NCIB 9861 ^T	0.9	2.1	2.8	57.1	5.8	4.1	6.9	6.4	0.9	5.1	4.6	3.1
<i>B. rhamnosum</i>	H-6S ^T	1.9	1.0		48.2	13.0	9.2	3.0	17.0		6.1	0.5	
<i>B. conglomeratum</i>	NCIB 9859	2.5	1.9	4.6	61.6	4.6	12.2	1.9		1.8	9.0		
	CCM 4385	1.1	0.9	10.6	41.7	6.3	14.2	2.6	2.9	3.5	15.5	0.7	
	CCM 2135	0.8	0.7	12.7	39.9		11.4	5.0			26.9	2.5	
	CCM 2134	1.0	0.9	4.1	28.5		18.6	10.5		2.9	25.3	8.3	
	CCM 2136	0.7	0.6	5.0	30.1		12.4	7.1		6.2	36.7	1.3	
	CCM 2137	1.1	0.9	2.9	29.2		21.8	9.1		2.6	28.6	3.7	
	CCM 2511	1.7	0.9	4.3	57.9		8.1	5.4		1.4	14.2	6.2	
	CCM 2589 ^T	2.2	1.4	10.6	48.9		11.2	7.3		1.2	8.5	8.5	
	CCM 2590	1.7	0.9	2.7	28.8		22.6	9.0		2.2	27.5	4.6	
	NCTC 7281	1.4	0.7	7.9	47.1		13.4	5.5		1.4	18.5	4.0

FIG. 2. Two-dimensional thin-layer chromatograms of polar lipids from *B. faecium* NCIB 9860^T (A), *B. nesterenkovi* IMV Ac-752^T (B), *B. paraconglomeratum* NCIB 9861^T (C), *B. rhamnosum* H-6S^T (D), *B. conglomeratum* CCM 2589^T (E), and *B. conglomeratum* CCM 2134 (F). Chloroform-methanol-water (65:25:4) was used in the first dimension, and chloroform-methanol-acetate-water (80:12:15:4) was used in the second dimension. Lipids were revealed by spraying the chromatograms with 10% molybdophosphoric acid in ethanol. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, glycolipid; PGL, ninhydrin-negative unknown phosphoglycolipid; PNL, ninhydrin-positive unknown phosphoglycolipid.

FIG. 3. Dendrogram showing the relationships among the 15 strains studied. The dendrogram was based on fatty acid composition data. Data were analyzed by using the NTSYS-pc program package (12), and clustering was performed by using the unweighted pair group arithmetic average method.

The new *Brachybacterium* species are described below.

Description of *Brachybacterium conglomeratum* sp. nov., nom. rev. *Brachybacterium conglomeratum* (con.glo.me.ra'tum. L. part. adj. *conglomeratum*, rolled together) is gram positive, nonmotile, and nonsporing. In the stationary growth phase, cells are coccoid; however, when a culture is transferred to fresh medium, the cells become oval or rod shaped. Colonies are circular, entire, low convex, smooth, opaque, and whitish yellow or pale brown. Aerobic or facultatively anaerobic. Catalase positive and oxidase negative. Grows well in the presence of 7.5% NaCl and weakly in the presence of 10 to 15% NaCl. Some strains grow at pH 5.0. Starch, esculin, and

urea are hydrolyzed, but Tween 20 and Tween 40 are not hydrolyzed. Some strains hydrolyze Tween 60 and Tween 80. Nitrate is reduced to nitrite. Acid is produced from L-arabinose, D-galactose, and D-glucose. Acid is not produced from trehalose, mannitol, sorbitol, and xylitol.

The cell wall peptidoglycan is based on *meso*-DAP. The cell wall sugars are galactose and glucose. The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; anteiso-C_{15:0} and anteiso-C_{17:0} are the predominant fatty acids. The major polar phospholip-

TABLE 5. DNA-DNA homology values

Species	Strain	% DNA-DNA reassociation with:									
		<i>B. faecium</i> NCIB 9860 ^T	<i>B. nesterenkovi</i> strains		<i>B. paracon-</i> <i>glomeratum</i> NCIB 9861 ^T	<i>B. rhamnosum</i> H-6S ^T	<i>B. conglomeratum</i> strains				
			IMV Ac-752 ^T	CCM 2432			NCIB 9859	CCM 4385	CCM 2135	CCM 2136	CCM 2589 ^T
<i>B. faecium</i>	NCIB 9860 ^T	100	5	18	19	28	17	26	27	28	19
<i>B. nesterenkovi</i>	IMV Ac-752 ^T	5	100	98	21	7	5	5	3	20	13
	CCM 2432	27	92	100	32	6	24	31	35	38	17
<i>B. paraconglomeratum</i>	NCIB 9861 ^T	19	12	19	100	11	13	14	14	14	10
<i>B. rhamnosum</i>	H-6S ^T	11	3	13	5	100	9	9	8	31	22
<i>B. conglomeratum</i>	NCIB 9859	49	19	23	31	32	100	125	103	104	76
	CCM 4385	12	7	31	17	11	78	100	73	66	NT ^a
	CCM 2135	14	10	36	10	9	71	61	100	71	NT
	CCM 2134	9	8	2	16	7	61	57	76	69	NT
	CCM 2136	30	26	5	20	34	76	59	83	100	95
	CCM 2137	32	20	8	26	23	87	95	105	98	79
	CCM 2511	12	15	30	17	38	66	95	83	70	69
	CCM 2589 ^T	12	10	14	19	17	70	87	76	82	100
	CCM 2590	22	14	18	17	12	93	81	99	79	NT
	NCTC 7281	25	16	34	26	17	83	96	94	80	64

^a NT, not tested.

TABLE 6. Differential characteristics of five *Brachybacterium* species

Characteristic	<i>B. faecium</i> (n = 1) ^a	<i>B. nesterenkovi</i> (n = 2)	<i>B. paraconglomeratum</i> (n = 1)	<i>B. rhamnosum</i> (n = 1)	<i>B. conglomeratum</i> (n = 10)
Morphological, physiological, and biochemical characteristics					
Growth rate ^b	S	R	S	R	R,S
Color of colonies ^c	P	L-Y,Y	P	W-Y	P,Y-G,L-Y
Growth in the presence of:					
7.5% NaCl	+ ^d	+w	+	+	100
10.0% NaCl	+w	—	+w	+w	90
15.0% NaCl	+w	—	+w	—	90
Growth at pH 5.0	—	+	—	+	70
Hydrolysis of gelatin	—	—	—	+	20
Hydrolysis of Tween 60	—	—	—	—	50
Hydrolysis of Tween 80	—	—	—	—	50
Urease production	—	—	+	+	100
H ₂ S production	—	+	+	+	80
Voges-Proskauer test	—	—	—	—	40
Indole formation	+w	—	+	—	90
Acid production from:					
D-Arabinose	+w	—	—	—	10
Cellobiose	—	d	+w	+	30
D-Fructose	—	—	+	+	60
D-Galactose	—	+	+	+	90
Lactose	—	+	+w	—	50
D-Mannose	—	—	+	+	50
Melezitose	+w	—	+w	+	20
Raffinose	—	d	—	+	10
D-Ribose	+w	+	—	+w	40
Salicin	—	+	+	+	60
Sucrose	—	—	+	+	50
Trehalose	—	—	—	+	0
D-Xylose	—	d	—	+	30
Adonitol	—	—	+	+	20
Inositol	—	—	+	+	10
Sorbitol	—	—	—	+	0
Chemotaxonomic characteristics					
Cell wall contains rhamnose	—	+	—	++	0
Cell wall contains 6-deoxy-L-talose	+	—	—	—	0
Cell wall contains glycine	+	—	+	+	100
Phospholipid type(s) ^e	A	B	C	D	E,F

^a n is the number of strains examined.^b R, rapid; S, slow.^c P, pale brown; L-Y, light yellow; Y, yellow; W-Y, whitish yellow; Y-G, yellow-green.^d +, positive; ++, strongly positive; +w, weakly positive; —, negative; d, different reactions in different strains. The values are the percentages of strains that are positive.^e See Fig. 2.

ids are diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids, and unidentified, ninhydrin-positive phosphoglycolipids. The G+C content of the DNA is 70.2 to 71.6 mol%; the G+C content of the type strain is 70.6 mol%.

The type strain is CCM 2589 (= IFO 15472).

Description of *Brachybacterium paraconglomeratum* sp. nov.

Brachybacterium paraconglomeratum (pa.ra.con.glo.me.ra'tum. L. part. adj. *para*, alongside of, resembling; M.L. adj. *conglomeratum*, specific epithet of *B. conglomeratum*; M.L. gen. n. *paraconglomeratum*, resembling *B. conglomeratum*) is gram positive, nonmotile, and nonsporing. In the stationary growth phase, cells are coccoid; however, when a culture is transferred to fresh medium, the cells become oval or rod shaped. Colonies are circular, entire, low convex, smooth, and opaque, and the colony color is pale brown. Aerobic or facultatively anaerobic. Catalase positive and oxidase negative. Grows well in the presence of 7.5% NaCl. Does not grow at pH 5.0. Starch, esculin, and urea are hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are not hydrolyzed. H₂S and indole

are produced. Nitrate is reduced to nitrite. Acid is produced from many sugars.

The cell wall peptidoglycan is based on *meso*-DAP. The cell wall sugars are galactose and glucose.

The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; anteiso-C_{15:0} and anteiso-C_{17:0} are the predominant fatty acids. The major polar phospholipids are diphosphatidylglycerol, phosphatidylglycerol, unidentified glycolipids, and unidentified, ninhydrin-positive phosphoglycolipids. The G+C content of the DNA is 68.6 mol%.

The type strain is NCIB 9861 (= IFO 15224).

Description of *Brachybacterium rhamnosum* sp. nov. *Brachybacterium rhamnosum* (rham.nos'um. L. n. *rhamnosum*, rhamnose, referring to the abundance of rhamnose in the cell wall) is gram positive, nonmotile, and nonsporing. In the stationary growth phase, cells are coccoid; however, when a culture is

transferred to fresh medium, the cells become oval or rod shaped. Colonies are circular, entire, low convex, smooth, opaque, and whitish yellow. Aerobic or facultatively anaerobic. Catalase positive and oxidase negative. Grows well in the presence of 7.5% NaCl. Grows at pH 5.0. Gelatin, starch, esculin, and urea are hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are not hydrolyzed. Nitrate is reduced to nitrite. H₂S is produced. Acid is produced from many sugars.

The cell wall peptidoglycan is based on *meso*-DAP. The cell wall sugars are galactose, glucose, and rhamnose. The cell wall muramic acid occurs in the *N*-glycolyl form. Mycolic acids are absent. The principal menaquinone is MK-7. The long-chain fatty acids are primarily straight-chain, saturated, iso and anteiso methyl-branched fatty acids; anteiso-C_{15:0} and anteiso-C_{17:0} are the predominant fatty acids. The major polar phospholipids are diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids. The G+C content of the DNA is 71.5 mol%. Isolated from commercially available corn steep liquor.

The type strain is H-6S (= IFO 15203).

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