***Brevibacterium paucivorans* sp. nov., from human clinical specimens**

University of Louvain,
Faculty of Medicine,
Microbiology Unit,
UCL/5490, Av. Hippocrate
54, B-1200 Brussels, Belgium

Georges Wauters, Jacqueline Charlier, Michèle Janssens
and Michel Delmée

Author for correspondence: Georges Wauters. Tel: +32 2 7645490. Fax: +32 2 7649440.
e-mail: wauters@mblg.ucl.ac.be

Seven isolates from various human body sites displayed general chemotaxonomic and phenotypic characteristics of the genus *Brevibacterium*. This was corroborated by the 16S rRNA gene sequence analysis of strain CF62^T, showing a sequence similarity of 99% to *Brevibacterium mcbrellneri*. However, DNA–DNA hybridization, a peculiar amino acid content of the cell wall and some phenotypic properties clearly suggested that these strains belong to a new species, for which the name *Brevibacterium paucivorans* sp. nov. is proposed. The type strain of *B. paucivorans* is CF62^T (= DSM 13657^T = LMG 19814^T). The DNA G+C content of the type strain is 55.8 mol%.

Keywords: *Brevibacterium paucivorans*, taxonomy

INTRODUCTION

Brevibacterium species have been encountered increasingly in humans, either as commensals or as opportunistic pathogens (Funke *et al.*, 1997). Four species have been recognized in humans: *Brevibacterium casei* (Collins *et al.*, 1983), *Brevibacterium epidermidis* (Collins *et al.*, 1983), *Brevibacterium mcbrellneri* (McBride *et al.*, 1993) and *Brevibacterium otitidis* (Pascual *et al.*, 1996). *Brevibacterium avium* has been isolated from poultry (Pascual & Collins, 1999). *Brevibacterium iodinum* (Collins *et al.*, 1980) and *Brevibacterium linens* (Fiedler *et al.*, 1981) are found mainly in food and in the environment. *Brevibacterium liquefaciens* has proved to be a synonym of *Arthrobacter nicotianae* (Stackebrandt *et al.*, 1983) and three species, *Brevibacterium halotolerans*, *Brevibacterium frigoritolerans* and *Brevibacterium stationis*, have not been subjected to genetic studies and their taxonomic position is still uncertain.

Members of the genus *Brevibacterium* are characterized by an oxidative metabolism, a high proportion of branched cellular fatty acids, mainly anteiso 15:0 and anteiso 17:0, and a peptidoglycan type A1 γ with meso-diaminopimelic acid as the diamino acid (Funke *et al.*, 1997). We describe seven strains, displaying a peculiar cell wall composition, isolated from various

human body sites. Their phenotypic, chemotaxonomic and genetic characteristics suggest that they all belong to a single species in the genus *Brevibacterium*, for which the name *Brevibacterium paucivorans* sp. nov. is proposed.

METHODS

Bacterial strains. The seven strains were isolated from various clinical specimens: CF41 from cerebrospinal fluid, CF45 from a groin abscess, CF62^T from blood, CF67 from an ear discharge, CF69 from an intravascular catheter, CF73 from a wound and CF89 from a groin swab. They were stored in glycerol at –20 °C and cultured on blood agar at 37 °C in air for the purposes of the study. The following reference strains were included in the study for phenotypic comparison: *B. casei* ATCC 35513^T, DSM 9657 and DSM 9658, *B. epidermidis* ATCC 49089, DSM 9585 and DSM 9586, *B. mcbrellneri* DSM 9583^T and DSM 9584, *B. otitidis* DSM 10718^T, *B. iodinum* LMG 2201 and *B. linens* LMG 3915.

Phenotypic characterization. Most phenotypic characteristics were studied as outlined previously (Funke *et al.*, 1997; Wauters *et al.*, 1998, 2000). Acid production from 2,3-butylene glycol was tested on medium containing the following (l⁻¹): 1 g proteose peptone, 1 g yeast extract, 50 ml 2,3-butylene glycol, 0.025 g phenol red and 17 g agar. The medium was dispensed in 2 ml amounts in small tubes and distributed to slants after sterilization. Slants were inoculated heavily with bacteria from a 24 h blood agar plate and incubated for 4 d. The medium turned yellow when acid was produced from the butylene glycol. Pyrazinamidase was detected on medium containing 10 g peptone and 2.5 g 2-pyrazine carboxamide l⁻¹ distilled water. One ml of the broth

The EMBL accession number for the 16S rRNA gene sequence of strain CF62^T is AJ251463.

was inoculated heavily and incubated for 24 h. Pyrazinoic acid was detected by adding one drop of a 1.0% (w/v) solution of ferrous sulfate. Pyrrolidonyl arylamidase and α -glucosidase were tested for by using diagnostic tablets (Rosco). Carbohydrate assimilation was performed on 50 CH strips using AUX medium (bioMérieux). API ZYM and API CORYNE (bioMérieux) strips were used according to the manufacturer's instructions, except for gelatin, which was read after up to 5 d.

Antibiotic susceptibility. Susceptibility was evaluated by MIC determination using E-test strips (PDM, Solna, Sweden), on Mueller–Hinton blood agar incubated at 37 °C for 24 h. Penicillin, ampicillin, cefotaxime, cephalothin, erythromycin, ciprofloxacin, gentamicin and vancomycin were tested and the results were interpreted according to the criteria established for staphylococci by the National Committee for Clinical Laboratory Standards (1997).

Chemotaxonomic properties. Cellular fatty acids were assayed by GLC using a Delsi Chromatograph as described previously (Wauters *et al.*, 1996). The amino acid composition of the peptidoglycan was studied by N. Weiss at the DSMZ (Braunschweig, Germany) using a TLC method as outlined by Schleifer & Kandler (1972).

16S rRNA gene sequence determination. Sequence analysis was performed at the DSMZ by C. Sproer. Approximately 95% of the 16S rRNA gene sequence of the strains was determined by direct sequencing of PCR-amplified 16S rDNA. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of the PCR products were carried out as described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using the ABI PRISM Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) according to the manufacturer's protocol. Sequence reactions were electrophoresed using an Applied Biosystems 373A DNA Sequencer. The resulting sequence data were put into the alignment editor ae2 (Maidak *et al.*, 1996), aligned manually and compared with representative 16S rRNA gene sequences of organisms belonging to the *Corynebacterium* subgroup of the Gram-positive bacteria (Maidak *et al.*, 1996). For comparison, 16S rRNA sequences were obtained from the EMBL database or RDP (Maidak *et al.*, 1996). 16S rRNA gene similarity values were calculated by pairwise comparison of the sequences within the alignment. For construction of the phylogenetic dendrogram, operations of the PHYLIP package (version 3.5.1; J. Felsenstein, Department of Genetics, University of Washington, Seattle, WA, USA) were used. Pairwise evolutionary distances were computed from percentage similarities by the correction of Jukes & Cantor (1969) and, based on the evolutionary distance values, the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). The root of the tree was determined by including the 16S rRNA gene sequence of *Dermatophilus congolensis* in the analysis.

DNA–DNA hybridization. DNA–DNA hybridization determinations were carried out at the DSMZ. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was performed as described by De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980), using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermo-programmer and plotter. Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992).

RESULTS AND DISCUSSION

Gram staining of the seven strains showed short and plump coryneform rods. Colonies on blood agar reached 1–2 mm diameter after 48 h of incubation at 37 °C. They were opaque, glistening and smooth, but sometimes sticky. Aerobic growth was observed at 30 and 37 °C but not at 20 °C. There was no sugar acidification, either fermentatively or oxidatively. Urease, nitrate reduction, indole production, decarboxylation of lysine and ornithine and arginine dihydrolase were negative. Gelatin was hydrolysed weakly and slowly but casein hydrolysis was negative. Tyrosine and xanthine hydrolysis were also negative. Ethylene glycol was acidified but 2,3-butylene glycol was not. Methanethiol production was positive. Pyrrolidone arylamidase and pyrazinamidase were negative. Using 50 CH strips, no carbohydrates were assimilated. With the API ZYM system, only esterase lipase (C8), leucine arylamidase and phosphoamidase were positive in all strains; trypsin was positive in some strains. The API CORYNE code obtained with the seven strains was uniformly 0000004.

The strains were susceptible to all antibiotics tested with the exception of strain CF73, which was resistant to gentamicin and ciprofloxacin.

The cellular fatty acids were predominantly of the branched type, predominantly anteiso C15:0 and anteiso C17:0, although the sum of the two components did not always reach 75% of the total cellular fatty acids, as is the rule in other *Brevibacterium* (Funke *et al.*, 1997).

The peptidoglycan analysis revealed the presence of *meso*-diaminopimelic acid as the diamino acid. A unique feature was the presence in the cell wall of an excess of glutamic acid and glycine, which could be removed by hydrofluoric acid treatment in the cold. This suggested that there was a non-peptidoglycan component containing glutamic acid and glycine that could be split off by hydrofluoric acid.

16S rRNA gene sequencing of CF62^T showed the highest similarity, 99%, to *B. mcbrellneri* (Table 1), and the overall phenotypic and chemotaxonomic properties of strain CF62^T were consistent with its membership of the genus *Brevibacterium* (Funke *et al.*, 1997). DNA–DNA hybridization between strain CF62^T and the type strain of *B. mcbrellneri*, DSM 9583^T, showed a relationship of 65.4%. DNA of strains CF41, CF45, CF67 and CF69 displayed homology levels of 86.3, 89.1, 90.2 and 78.5%, respectively, with labelled DNA of CF62^T, suggesting that they belong to a single species. The DNA G + C content of strain CF62^T was 55.8 mol%, which is rather low in comparison with other *Brevibacterium* (Gruner *et al.*, 1994).

Although the 65.4% DNA–DNA hybridization of CF62^T to *B. mcbrellneri* was rather close to the 70% limit required to conclude that they constitute different

Table 1. 16S rRNA gene sequence similarity between *B. paucivorans* CF62^T and other *Brevibacterium* species and related taxa

Species	Accession number	16S rRNA similarity to CF62 ^T (%)
<i>Brevibacterium liquefaciens</i>	AJ251417	92.1
<i>Brevibacterium casei</i>	AJ251418	93.4
<i>Brevibacterium avium</i>	Y17962	94.0
<i>Brevibacterium epidermidis</i>	X76565	93.8
<i>Brevibacterium iodinum</i>	X83813	93.9
<i>Brevibacterium linens</i>	X77451	93.8
<i>Brevibacterium mcbrellneri</i>	X93594	99.0
<i>Brevibacterium otitidis</i>	X93593	94.9
<i>Kocuria rosea</i>	X87756	92.5
<i>Nesterenkonia halobia</i>	X80747	92.0
<i>Arthrobacter atrocyaneus</i>	X80746	90.9
<i>Dermabacter hominis</i>	X91034	91.6
<i>Dermatophilus congolensis</i>	M59067	91.8

Table 2. Characteristics that differentiate *B. paucivorans* from other *Brevibacterium* species

Taxa are identified as: 1, *B. paucivorans*; 2, *B. mcbrellneri*; 3, *B. casei*; 4, *B. epidermidis*; 5, *B. otitidis*; 6, *B. iodinum*; 7, *B. linens*. Characteristics are scored as: +, positive; –, negative; (+), delayed positive; +/–, most strains positive; –/+, most strains negative; w, weak reaction; v, variable.

Characteristic	1	2	3	4	5	6	7
Colonies	Greyish, smooth or sticky	Greyish, dry, friable	White-greyish, smooth	White-yellow, smooth	Yellowish, smooth	Greyish, smooth	Yellow, smooth
Growth at 20 °C	–	–	+	+	–	+	+
Hydrolysis of							
Casein	–	+	+	+	+	+	+
Gelatin	(+) ^w	+/(+)	+	+	+	+	+
Nitrate reduction	–	–	–/+	+/-	–	+	+
Acid production from	–	+	–	–	–	–	–
2,3-butylene glycol							
Utilization of:							
D-Arabinose	–	–	+	–	–	–	–
Mannitol	–	–	–	+	–	–	–
Gluconate	–	–	+	+	–	–	–
α-Glucosidase	–	–	+/-	–	–	–	–
Pyrrolidone peptidase	–	–	v	–	+	–	–
Pyrazinamidase	–	–	+	+	+	+	+

species, several chemotaxonomic and phenotypic properties indicate clearly that our strains belong to a species distinct from *B. mcbrellneri*. Indeed, the peculiar amino acid content of the cell wall is not a feature of *B. mcbrellneri*. The cell morphology and culture characteristics are also different: cells of the new strains are stained evenly and they are short and plump, whereas those of *B. mcbrellneri* are somewhat irregular and thin. The glistening smooth or sticky colonies of the strains are in sharp contrast to the dry and crumbly colonies of *B. mcbrellneri* (McBride *et al.*, 1993). Biochemically, the study strains can be further differentiated from *B. mcbrellneri* by the lack of casein hydrolysis. Furthermore, acidification of 2,3-butylene

glycol is absent in the new strains and in other *brevibacteria*, while it is positive in *B. mcbrellneri*, a feature unique to this species. In addition, gelatin hydrolysis is delayed and weaker than in other *Brevibacterium* species and depends on the technique used. The seven strains differ from *B. otitidis* by their lack of tyrosine hydrolysis and by the absence of pyrrolidonyl peptidase and pyrazinamidase. They are distinct from *B. casei* and *B. epidermidis* by their inability to grow at 20 °C and to assimilate any carbohydrate in the 50CH system and by the lack of decomposition of casein, tyrosine and xanthine. The main characteristics that differentiate the new species from other *brevibacteria* are summarized in Table 2.

Fig. 1. Unrooted tree showing the phylogenetic position of *B. paucivorans* CF62^T within the genus *Brevibacterium* and related taxa. Bar, 10 nucleotide substitutions per 100 nucleotides.

There is genetic, chemotaxonomic and phenotypic evidence that strains CF62^T, CF41, CF45, CF67 and CF69 belong to a single species in the genus *Brevibacterium*, distinct from the other species described in this genus and related most closely to *B. mcbrellneri* (Fig. 1). The name *Brevibacterium paucivorans* sp. nov. is proposed for this new species. Although strains CF73 and CF89 have not been subjected to genetic analysis, their chemotaxonomic and phenotypic properties suggest strongly that they belong to the new species.

The seven strains of *B. paucivorans* were isolated from different body sites, but no pathological role in disease was proved. The ecological niche of these organisms is not known and their clinical significance remains to be assessed.

Description of *Brevibacterium paucivorans* sp. nov.

Brevibacterium paucivorans (pau.ci.vo'rans. L. adj. *paucus* little; L. v. *vorare* to eat; N.L. adj. *paucivorans* eating little, because few substrates are assimilated).

Cells are Gram-positive, non-motile, non-sporulating, short and plump rods with a diphtheroid arrangement.

Colonies are greyish, glistening and smooth or sticky and approximately 2 mm in diameter after 48 h of incubation at 37 °C on blood agar. Growth occurs aerobically at 37 °C but not at 20 °C. Catalase is positive. No carbohydrates are acidified or assimilated. Urease and pyrazinamidase are negative and nitrate is not reduced. Methanethiol is produced. Casein, tyrosine, xanthine and aesculin are not hydrolysed. Gelatin is liquefied weakly and slowly. Ethylene glycol is acidified but 2,3-butylene glycol is not. Pyrrolidonyl peptidase is negative. Using the API ZYM system, leucine arylamidase and phosphoamidase are positive, esterase lipase (C8) is weakly positive and trypsin is positive in some strains. Alkaline phosphatase, esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, α -chymotrypsin, acid phosphatase, α - and β -galactosidase, β -glucuronidase, α - and β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are negative. Cellular fatty acids are of the branched type, with anteiso 15:0 and anteiso 17:0 being the major components. The diamino acid of the peptidoglycan is *meso*-diaminopimelic acid and the cell wall contains an excess of glutamic acid and glycine that are not bound to the peptidoglycan. The organisms are isolated from various human body sites.

The type strain is CF62^T (= DSM 13657^T = LMG 19814^T). Three other strains have been deposited in the DSMZ: CF45 (= DSM 13658), CF67 (= DSM 13659) and CF69 (= DSM 13660). The G + C content of the DNA of the type strain is 55.8 mol%. It was isolated from human blood.

REFERENCES

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Collins, M. D., Jones, D., Keddle, R. M. & Sneath, P. H. A. (1980). Reclassification of *Chromobacterium iodinum* (Davis) in a redefined genus *Brevibacterium* (Breed) as *Brevibacterium iodinum* nom. rev.; comb. nov. *J Gen Microbiol* **120**, 1–10.
- Collins, M. D., Farrow, J. A. E., Goodfellow, M. & Minnikin, D. E. (1983). *Brevibacterium casei* sp. nov. and *Brevibacterium epidermidis* sp. nov. *Syst Appl Microbiol* **4**, 388–395.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.
- Fiedler, F., Schäffler, M. J. & Stackebrandt, E. (1981). Biochemical and nucleic acid hybridisation studies on *Brevibacterium linens* and related strains. *Arch Microbiol* **129**, 85–93.
- Funke, G., von Graevenitz, A., Clarridge, J. E., III & Bernard, K. A. (1997). Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* **10**, 125–159.
- Gruner, E., Steigerwalt, A. G., Hollis, D. G., Weyant, R. S., Weaver, R. E., Moss, C. W., Daneshvar, M., Brown, J. M. & Brenner, D. J. (1994). Human infections caused by *Brevibacterium casei*, formerly CDC groups B-1 and B-3. *J Clin Microbiol* **32**, 1511–1518.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- McBride, M. E., Ellner, K. M., Black, H. S., Clarridge, J. E. & Wolf, J. E. (1993). A new *Brevibacterium* sp. isolated from infected genital hair of patients with white piedra. *J Med Microbiol* **39**, 255–261.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **24**, 82–85.
- National Committee for Clinical Laboratory Standards (1997). *Minimum inhibitory concentration (MIC) interpretative standards (µg ml⁻¹) for organisms other than Haemophilus spp., Neisseria gonorrhoeae, and Streptococcus spp.* NCCLS document M7-A4. Wayne, PA: National Committee for Clinical Laboratory Standards.
- Pascual, C. & Collins, M. D. (1999). *Brevibacterium avium* sp. nov., isolated from poultry. *Int J Syst Bacteriol* **49**, 1527–1530.
- Pascual, C., Collins, M. D., Funke, G. & Pitcher, D. G. (1996). Phenotypic and genotypic characterization of two *Brevibacterium* strains from the human ear: description of *Brevibacterium otitidis* sp. nov. *Med Microbiol Lett* **5**, 113–123.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Stackebrandt, E., Fowler, V. J., Fiedler, F. & Seiler, H. (1983). Taxonomic studies on *Arthrobacter nicotianae* and related taxa: description of *Arthrobacter uratoxydans* sp. nov. and *Arthrobacter sulfureus* sp. nov. and reclassification of *Brevibacterium protophormiae* as *Arthrobacter protophormiae* comb. nov. *Syst Appl Microbiol* **4**, 470–486.
- Wauters, G., Driessen, A., Ageron, E., Janssens, M. & Grimont, P. A. D. (1996). Propionic acid-producing strains previously designated as *Corynebacterium xerosis*, *C. minutissimum*, *C. striatum*, and CDC group I₂ and group F₂ coryneforms belong to the species *Corynebacterium amycolatum*. *Int J Syst Bacteriol* **46**, 653–657.
- Wauters, G., Van Bosterhaut, B., Janssens, M. & Verhaegen, J. (1998). Identification of *Corynebacterium amycolatum* and other nonlipophilic fermentative corynebacteria of human origin. *J Clin Microbiol* **36**, 1430–1432.
- Wauters, G., Charlier, J., Janssens, M. & Delmée, M. (2000). Identification of *Arthrobacter oxydans*, *Arthrobacter luteolus* sp. nov., and *Arthrobacter albus* sp. nov., isolated from human clinical specimens. *J Clin Microbiol* **38**, 2412–2415.