NOTE

Reclassification of *Brevibacterium incertum* (Breed 1953) as *Desemzia incerta* gen. nov., comb. nov.

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Phylogenetic analysis of 16S rDNA indicates that *Brevibacterium incertum* is not a member of the genus *Brevibacterium* but related to species of the genus *Carnobacterium*. Hence, *Brevibacterium incertum* is not a member of the class *Actinobacteria* but belongs to the phylogenetically defined broad *Bacillus*–*Lactobacillus* cluster. Based upon properties that taxonomically clearly distinguishes *Brevibacterium incertum* from species of the phylogenetic sister genus *Carnobacterium*, *Brevibacterium incertum* is reclassified as *Desemzia incerta* gen. nov., comb. nov.

Keywords: *Desemzia incerta*, 16S rDNA analysis

In the course of a study on insect-associated bacteria, a Gram-positive bacterium was isolated by Steinhaus (1941) from the ovaries of the lyreman cicada, *Tibicen linnei*. The physiological and cultural characteristics resembled those of the genus *Listeria*, but because of the taxonomic uncertainty which surrounded the *Listeria* group (Steinhaus, 1941) at that time, the isolate was tentatively classified as *Bacterium incertum*. Breed (1953) transferred this species to the genus *Brevibacterium* as *Brevibacterium incertum*. Over the years, the genus became a dumping ground for misclassified strains with superficial morphological and physiological similarities. Several *Brevibacterium* species have been transferred to other genera: *Brevibacterium protophormiae* to *Arthrobacter* (Stackebrandt *et al.*, 1983); *Brevibacterium fermentans* and *Brevibacterium lyticum* to *Cellulomonas* (Stackebrandt *et al.*, 1982); *Brevibacterium albidum*, *Brevibacterium citreum*, *Brevibacterium luteum* and *Brevibacterium pusillum* to *Curtobacterium* (Yamada & Komagata, 1972; Farrow *et al.*, 1994); *Brevibacterium ammoniagenes* (Collins, 1987), *Brevibacterium divaricatum* and *Brevibacterium vitarumen* (Lanéelle *et al.*, 1980) to *Corynebacterium*; *Brevibacterium acetylicum* to *Exiguobacterium* (Farrow *et al.*, 1994); *Brevibacterium imperiale* (Collins *et al.*, 1983b) and *Brevibacterium oxydans* to *Microbacterium* (Schumann *et al.*, 1998); and *Brevibacterium saperdae*

and *Brevibacterium testaceum* to *Aureobacterium* (Collins *et al.*, 1983a). The unification of *Aureobacterium* and *Microbacterium* in a redefined genus *Microbacterium* has recently been proposed by Takeuchi & Hatano (1998).

Analysis of the amino acid composition of the peptidoglycan of *B. incertum* (Schleifer & Kandler, 1972) revealed the presence of L-lysine, while strains of the type species *Brevibacterium linens* contained meso-diaminopimelic acid. Subsequent studies on the composition of isoprenoid quinones and fatty acids (Collins & Kroppenstedt, 1983) confirmed the misclassification of *B. incertum* as, in contrast to *B. linens*, menaquinones were absent and fatty acids contained substantial amounts of monounsaturated fatty acids of the *cis*-vacenic acid series. Collins & Kroppenstedt (1983) pointed out that the lack of isoprenoid quinones suggests a relatedness of *B. incertum* to certain other Gram-positive taxa, such as *Erysipelothrix*, *Gemella*, *Lactobacillus* or *Streptococcus*, which lack respiratory quinones (Collins & Jones, 1981), while the type of fatty acids indicated a relationship to the lactic acid bacteria (see Kroppenstedt & Kutzner, 1978; Collins & Kroppenstedt, 1983). Subsequently, the species *Brevibacterium incertum* was listed as species *incertae sedis* in *Bergey's Manual of Systematic Bacteriology* (Jones & Kiedd, 1986).

To clarify the taxonomic position of *B. incertum*, the type strain DSM 20581^T was analysed in this study with respect to 16S rDNA (Rainey *et al.*, 1996), the base composition of DNA (Marmur, 1961; Mesbah *et*

Fig. 1. 16S rDNA sequence based phylogenetic dendrogram showing the phylogenetic position of *Brevibacterium incertum* and related taxa. Bootstrap values (expressed as percentages of 500 replications) of 55% and higher are indicated at the branch points. Scale bar, 5 inferred nucleotide substitutions per 100 nucleotides.

al., 1989) and isoprenoid quinone content (Groth *et al.*, 1996; Tindall, 1990). The 16S rDNA sequences of *Trichococcus flocculiformis* DSM 2094^T and *Enterococcus hirae* DSM 20160^T were included as references. Comparison of almost the complete sequence of 16S rDNA of strain DSM 20581^T [1510 bp; 98% of the *Escherichia coli* sequence (Brosius *et al.*, 1978)] to the homologous sequences of species of Gram-positive bacteria indicated that *B. incertum* showed the highest similarity values to members of the genus *Carnobacterium* (95.1–96.2%) (EMBL accession numbers X54268–X54271, S86170, L08623, Z73313), while those determined with *Abiotrophia defectiva* (D50541), *Enterococcus hirae* (Y17302), *Facklamia hominis* (Y10772), *Globicatella sanguinis* (S50214), *Lactobacillus casei* (X61135), *Lactosphaera pasteurii* (X87150), *Trichococcus flocculiformis* (Y17301), *Vagococcus* sp. (X54258, X54272) and *Weissella kandleri* (X52570) were lower (91–94.3%). The phylogenetic dendrogram (Fig. 1), constructed from evolutionary distance values (Jukes & Cantor, 1969), using the algorithm of DeSoete (1983), shows *B. incertum* to branch close to the branching point of the most

unrelated *Carnobacterium* species. High bootstrap values for the branching point of *B. incertum* support this phylogenetic affiliation. Dendrograms generated on the basis of maximum-likelihood analysis and neighbour-joining analysis (Felsenstein, 1993) show that *B. incertum* branches deeply within the radiation of *Carnobacterium* species (not shown). None of these branching points were supported by statistical analysis.

B. incertum resembles *Carnobacterium* species morphologically, in a fermentative metabolism, base composition of DNA (40 mol %) and in the composition of fatty acids (comprising predominantly straight-chain saturated and monounsaturated acids) (Collins & Kroppenstedt, 1983; Collins *et al.*, 1987). However, while *B. incertum* contains large amounts of vaccenic acid ($C_{18:1}$, $\Delta 11$), *Carnobacterium* species contain oleic acid ($C_{18:1}$, $\Delta 9$) as octadecenoic acid. The lack of isoprenoid quinones in *B. incertum*, originally reported by Collins & Kroppenstedt (1983), was confirmed in this study by HPLC (Groth *et al.*, 1996) and electron-impact mass spectrometry (Collins, 1994). None of the substances extracted by a method developed for lipoquinone analysis (Tindall, 1990) gave rise to chromatographic patterns of menaquinones or ubiquinones, mass spectral nuclear fragments or molecular ions characteristic for isoprenoid quinones (Collins, 1994). No data on isoprenoid quinones are available for species of *Carnobacterium*. *B. incertum* and *Carnobacterium* species differ significantly in the amino acid composition of peptidoglycan. While *B. incertum* possesses peptidoglycan type L-lysine-D-glutamic acid, variation A4 α (Schleifer & Kandler, 1972), carnobacteria exhibit the *meso*-diaminopimelic acid direct cross-linked type, variation A1 γ (Collins *et al.*, 1987).

Strain DSM 20581^T grows well on Columbia blood agar (Benson Dickenson; no. 4354005) within 24 h, while good growth is observed in Caso medium (Oxoid; no. CM 129) and in PYG medium (DSMZ catalogue of strains, medium no. 104) after 48 h. L-Lactate, but no D-lactate, is produced from glucose in the latter two media (Hohorst, 1966), lowering the pH to pH 6.3. The growth temperature range on Columbia blood agar confirmed the data given by Jones & Keddie (1986). Physiological reactions towards the substrate panel provided by the API ID32 Strep kit (bioMérieux) gave the following results: acids are produced from ribose, lactose, trehalose, raffinose, maltose, melibiose and saccharose; acids are not produced from mannitol, sorbitol, L-arabinose or melizitose. The reaction for fructose is different to that reported by Steinhaus (1941). Tests for β -glucosidase, β -glucuronidase, α -galactosidase, glycyl-tryptophan arylamidase and β -mannosidase are positive; arginine dehydrolase, β -glucosidase and β -galactosidase are negative. Acetoin production is negative. Most of these tests were not performed by Steinhaus (1941) thus we have extended the physiological characterization of strain 20581^T.

DNA–DNA reassociation values obtained for four *Carnobacterium* species that showed higher than 96.5% 16S rDNA sequence similarity were lower than 45% (Collins *et al.*, 1987). These low values clearly supported the validity of these species and we conclude that no DNA–DNA reassociation experiments are necessary between *B. incertum* and *Carnobacterium* species to confirm the species status of *B. incertum*. While the phylogenetic analysis did not *per se* exclude the reclassification of *B. incertum* as a member of *Carnobacterium* (Fig. 1), we consider the marked differences in the composition of the peptidoglycan and in the isomeric type of octadecenoic acid as the predominant fatty acid to be of such high taxonomic significance that we propose the reclassification of *B. incertum* in a new genus for which the name *Desemzia* gen. nov. is proposed.

Description of *Desemzia* gen. nov.

Desemzia [De.sem'zi.a. M.L. fem., arbitrary name, derived from the abbreviation DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH)].

Morphological, cultural and physiological properties are those of Jones & Keddle (1986), who combined the original data of Steinhaus (1941) and Breed (1953). The chemotaxonomic data have been published by Collins & Kroppenstedt (1983). Short rods, 0.5–0.8 × 1.0–1.5 µm, occurring singly and occasionally in pairs. Gram-positive. Cells from older cultures tend to lose the ability to retain the Gram stain. Not acid-fast. Endospores not formed. Microaerophilic. Metabolism fermentative. L-Lactic acid but no D-lactic acid is produced from glucose. Catalase-negative, oxidase-negative. The peptidoglycan contains lysine as the diagnostic amino acid (peptidoglycan type L-lysine-D-glutamic acid; variation A4α). Mycolic acids are not present. Isoprenoid quinones not present. The fatty acids are composed of predominantly straight chain saturated and monounsaturated acids. The major fatty acids are hexadecanoic (C_{16:0}), hexadecenoic (C_{16:1}) and *cis*-vaccenic (C_{18:1}, ω7). The G+C content of the DNA is 40 mol%. Phylogenetically, a member of the *Bacillus*–*Lactobacillus* group of the subphylum *Clostridium*–*Bacillus*, phylum of Gram-positive bacteria, domain *Bacteria*. Isolated from the ovaries of the lyreman cicada, *Tibicen linnei*. Habitat is not known. The type species is *Desemzia incerta* comb. nov.

Description of *Desemzia incerta* comb. nov.

[Basonym: *Brevibacterium incertum* (Steinhaus 1941) Breed 1953, 13^{A1}]

The description includes data compiled by Jones & Keddle (1986) and data generated in this study.

Short rods about 0.5–0.8 × 1.0–1.5 µm, with rounded ends and cocco–bacillary forms. Occur singly, in pairs or in chains. Gram-positive; cells from older cultures tend to lose the ability to retain the Gram-stain. Not

acid-fast. Endospores are not formed. Motile with one or two flagella. On PYE media colonies are tiny with no distinctive pigmentation. Microaerophilic. Metabolism fermentative. L-Lactic but no D-lactic acid is produced from glucose. Acid is produced from glucose, fructose, lactose, maltose, mannose, saccharose, raffinose, melibiose, ribose and trehalose. No acid is produced from galactose, rhamnose, mannitol, dulcitol, inositol, sorbitol, L-arabinose or melizitose. Catalase-negative, oxidase-negative. Sodium hippurate is hydrolysed. Gelatin, casein and starch are not hydrolysed. Cellulose is not attacked. Urease is not produced. DNase, arginine dehydrolase, β-glucosidase and β-galactosidase are not produced. Reactions for β-glucosidase, β-glucuronidase, α-galactosidase, glycyl-tryptophane arylamidase and β-mannosidase are positive. Acetyl methylcarbinol, indole and H₂S are not produced. Nitrates are not reduced to nitrites. Acetoin production negative. The chemotaxonomic properties are as given for the genus. The accession number for 16S rDNA is Y17300. The type strain is DSM 20581^T (= ATCC 8363^T, = NCIB 9892^T).

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