**NOTE**

*Brevibacterium avium* sp. nov., isolated from poultry

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The genus *Brevibacterium* was created by Breed (1953), with *Brevibacterium linens* as the type species, for a number of Gram-positive, non-spore-forming, short, non-branching rods formerly classified in the genus *Bacterium*. Due to the poor delimitation of the genus, it soon became a repository for a broad range of species with very diverse morphological, biochemical and physiological features. The description of the genus *Brevibacterium* was subsequently emended to embrace only those species which closely resemble the type species, *B. linens* (Collins et al., 1980). In addition to *B. linens*, five other species of the genus are currently recognized, viz. *Brevibacterium casei* (Collins et al., 1983), *Brevibacterium epidermidis* (Collins et al., 1983), *Brevibacterium iodinum* (Collins et al., 1980), *Brevibacterium mbrellneri* (McBride et al., 1993) and *Brevibacterium otitidis* (Pascual et al., 1996). Dairy milk products have long been recognized as a habitat of brevibacteria, where the bacteria contribute to the aroma and the colour (from *B. linens*) of surface-ripened cheeses (Jones & Keddie, 1986). Some *Brevibacterium* species are residents of human skin and, in recent years, have been implicated in human disease (e.g. Gruner et al., 1993, 1994; Funke & Carlotti, 1994; Funke et al., 1997; Neumeister et al., 1993; Reinert et al., 1995). Mohan (1981) reported the isolation of some *Brevibacterium*-like organisms from diseased poultry. The organisms from diseased poultry were recovered from aspirates at necropsy from bumble-foot (granuloma) lesions and although they phenotypically resembled brevibacteria, identification at the species level was not performed (Mohan, 1981). In this article, we report the detailed phenotypic characteristics of the bacterium from poultry and the results of molecular genetic taxonomic analyses. Based on the findings of this polyphasic taxonomic study, a new species, *Brevibacterium avium*, is described.

Strains NCIMB 703055ᵀ and 703056 were originally isolated from bumble-foot lesions of poultry by Mohan (1981). Assimilation reactions (Funke & Carlotti, 1994) were tested in the API 50CH system (API bioMérieux) using the AUX medium (API bioMérieux); the results were read after 48 h incubation. Enzymic activities were determined by means of the API ZYM system (API bioMérieux) as described by the manufacturer. Hydrolysis of casein, tyrosine and xanthine was detected by clearing of the medium around the colonies after incubation for up to 10 d (Nash & Krenz, 1991). The presence of mycolic acids was investigated by the procedure of Minnikin et al. (1975). Cell wall composition was determined as described by Pitcher (1983). DNA for DNA–DNA relatedness studies was isolated and purified by the method of Marmur (1961). DNA from strain NCIMB 703055ᵀ was labelled with ³²P-labelled nucleotides by using a Megaprime kit (Amersham). DNA–DNA hybridization experiments were performed by using an

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Abbreviations: RDP, Ribosomal Database Project; ΔTᵦ, the difference between the denaturation temperature of a DNA homoduplex and heteroduplex.

The GenBank accession number for the 16S rRNA gene sequence of strain NCIMB 703055ᵀ is Y17962.
S1 nuclease and a trichloroacetic acid procedure (Grimont et al., 1980); the reaction mixtures were incubated at 70 °C for 16 h. The ΔTm value (i.e. the difference between the denaturation temperature of a DNA homoduplex and heteroduplex) was determined as described by Grimont et al. (1980). The 16S rRNA genes of the two poultry isolates were amplified by PCR and directly sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the isolates were determined by performing database searches. These sequences were retrieved from the GenBank or Ribosomal Database Project (RDP) databases and aligned with the newly determined sequences using the program PILEUP (Devereux et al., 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The two poultry isolates consisted of Gram-positive rods which displayed a coryneform morphology. On blood agar, they formed smooth, convex colonies which were greyish-white in colour. The isolates were obligately aerobic and catalase- and oxidase-positive. Both strains decomposed tyrosine and hydrolysed aesculin, starch or urea. They showed nitrate reductase activity. Using the API 50CH system, both poultry isolates utilized L-arabinose, D-arabitol, galactose, D-glucose, glyceral, D-fructose, D-mannose and mannitol. Neither of the isolates utilized adonitol, amygdalin, D-arabinose, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, β-gentiobiose, gluconate, 2-ketogluconate, 5-ketogluconate, methyl α-D-glucoside, N-acetylglucosamine, glycerogen, inositol, inulin, lactose, D-lyxose, maltose, methyl α-D-mannoside, melezitose, melibiose, D-raffinose, rhamnose, ribose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylene, L-xylene or methyl β-xylidine. Using the API ZYM system, enzymic activities for acid and alkaline phosphatase, esterase C4, ester lipase C3, leucine arylamidase, cysteine arylamidase and phosphoamidase were detected. Enzymic activities for chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, lipase C14, α-mannosidase, trypsin and valine arylamidase were negative. The above cellular morphology and biochemical characteristics of the poultry isolates are consistent with their assignment to the genus *Brevibacterium*.

To establish the phylogenetic position of the poultry isolates, their almost complete 16S rRNA gene sequences were determined by PCR direct DNA sequencing. Pairwise analysis revealed that the 16S rRNA gene sequences of the two isolates were identical. Sequence searches of GenBank and RDP databases showed that species of the genus *Brevibacterium* were the closest phylogenetic relatives of the unidentified bacterium (16S rRNA sequence similarities of >94%). Other taxa were more distantly related (data not shown). Comparative sequence analysis showed that *B. linens* CIP 101125T and *B. epidermidis* NCDO 2286T were the closest relatives of the poultry bacterium with sequence divergence values of 2.2 and 2.3%, respectively. *B. casei* NCDO 2048T and *B. iodinum* NCDO 613T were the next nearest relatives of the unknown organism (displaying 2.6% sequence divergence), with *B. mcbrellneri* CIP 104342T and *B. oitidis* NCFB 3053T more distantly related (5.6 and 4.4% sequence divergence, respectively). The 16S rRNA nucleotide signature of the poultry bacterium was found to be consistent with signature data for *Brevibacterium* published by Stackebrandt et al. (1997). A tree depicting the phylogenetic position of the poultry bacterium (as exemplified by strain NCIMB 703055T) within the genus *Brevibacterium* is shown in Fig. 1 and shows that the organism forms a new subline, close to but distinct from *B. linens* and near relatives (viz. *B. casei*, *B. epidermidis* and *B. iodinum*).

In view of the high levels of 16S rRNA sequence relatedness of the unknown bacterium with some *Brevibacterium* species, chromosomal DNA–DNA hybridization experiments were performed to establish whether the two poultry strains represent a distinct species. Using 3H-labelled DNA from strain NCIMB 703055T, this organism displayed 91% reassociation with NCIMB 703056 at 70 °C with insignificant divergence (ΔTn, 0.1 °C). In contrast, strain NCIMB 703055T exhibited much lower levels of reassociation with *B. linens* CIP 101125T (30%), *B. casei* NCDO 2048T (20%), *B. epidermidis* NCFB 2286T (39%) and *B. oitidis* NCFB 3053T (8%). Taking 70% reassociation as the cut-off point for species delimitation using the S1 procedure, these findings confirm
that the two strains isolated from bumble-foot lesions of domestic fowl by Mohan (1981) are genomically homogeneous and represent a hitherto unknown species of the genus Brevibacterium. Thus, on the basis of both phenotypic and molecular genetic findings, we propose the bacterium from poultry to be classified as a new species, Brevibacterium avium. Tests which serve to distinguish B. avium from currently described Brevibacterium species are listed in Table 1.

Description of Brevibacterium avium sp. nov.

Brevibacterium avium (a’vi.um. L. n. avis a bird; L. gen. pl. n. avium of birds).

This description is based on the results of the present study and the findings of Mohan (1981). Cells are Gram-positive, non-motile, non-acid-fast, non-spore-forming rods which exhibit a coryneform morphology. Branching is not observed. Colonies are convex, with forming rods which exhibit a coryneform morphology.

Strains: 1, B. linens; 2, B. iodinum; 3, B. casei; 4, B. epidermidis; 5, B. mesodermidis; 6, B. otitidis; 7, B. avium sp. nov. +, Positive; —, negative; v, variable; v(—), variable with most negative; v(+), variable with most positive; w, very weak growth. Data from the present study, and Jones & Keddie (1986), McBride et al. (1993), Funke & Carlotti (1994) and Pascual et al. (1996).

References


