NOTE

***Brevibacterium avium* sp. nov., isolated from poultry**Cristina Pascual¹ and Matthew D. Collins²

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Two strains of a *Brevibacterium*-like bacterium originating from bumble-foot lesions of domestic fowls were subjected to a polyphasic taxonomic study. The phenotypic characteristics of the bacterium were consistent with its assignment to the genus *Brevibacterium* although comparative 16S rRNA gene sequencing showed that the organism represents a distinct subline within the genus. Chromosomal DNA–DNA pairing studies confirmed that the unidentified bacterium was genomically distinct and worthy of separate species status. Based on the phenotypic and genotypic distinctiveness of the bacterium from poultry, a new species, *Brevibacterium avium*, is proposed. The type strain of *Brevibacterium avium* is NCIMB 703055^T.

Keywords: *Brevibacterium avium* sp. nov., taxonomy, phylogeny, coryneform bacteria

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 mcbrellneri* (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 & Keddle, 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 703055^T 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 703055^T was labelled with ³H-labelled nucleotides by using a Megaprime kit (Amersham). DNA–DNA hybridization experiments were performed by using an

Abbreviations: RDP, Ribosomal Database Project; ΔT_m , 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 703055^T is Y17962.

S1 nuclease trichloroacetic acid procedure (Grimont *et al.*, 1980); the reaction mixtures were incubated at 70 °C for 16 h. The ΔT_m 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 (using the Kimura-two correction parameter) (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 casein, gelatin and xanthine. They also displayed extracellular DNase activity. Neither of the strains 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, glycerol, 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, glycogen, 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-xylose, L-xylose or methyl β -xyloside. Using the API ZYM system, enzymic activities for acid and alkaline phosphatase, esterase C_4 , ester lipase C_8 , leucine arylamidase, cystine arylamidase and phosphoramidase were detected. Enzymic activities for chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, lipase C_{14} , 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*.

Fig. 1. Dendrogram showing the phylogenetic position of *Brevibacterium avium* sp. nov. within the genus *Brevibacterium*. The tree, constructed using the neighbour-joining method, was based on a comparison of approximately 1351 nt. Bootstrap values (>90%), expressed as a percentage of 500 replications, are given at branching points. Bar, 1%.

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 101125^T and *B. epidermidis* NCDO 2286^T were the closest relatives of the poultry bacterium with sequence divergence values of 2.2 and 2.3%, respectively. *B. casei* NCDO 2048^T and *B. iodinum* NCDO 613^T were the next nearest relatives of the unknown organism (displaying 2.6% sequence divergence), with *B. mcbrellneri* CIP 104342^T and *B. otitidis* NCFB 3053^T more distantly related (5.6 and 4.4% sequence divergence, respectively). The 16S rDNA 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 703055^T) 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 703055^T, this organism displayed 91% reassociation with NCIMB 703056 at 70 °C with insignificant divergence (ΔT_m , 0.1 °C). In contrast, strain NCIMB 703055^T exhibited much lower levels of reassociation with *B. linens* CIP 101125^T (30%), *B. casei* NCDO 2048^T (20%), *B. epidermidis* NCFB 2286^T (39%) and *B. otitidis* NCFB 3053^T (8%). Taking 70% reassociation as the cut-off point for species delimitation using the S1 procedure, these findings confirm

Table 1. Tests useful for differentiating species of the genus *Brevibacterium*

Strains: 1, *B. linens*; 2, *B. iodinum*; 3, *B. casei*; 4, *B. epidermidis*; 5, *B. mcbrellneri*; 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 & Keddle (1986), McBride *et al.* (1993), Funke & Carlotti (1994) and Pascual *et al.* (1996).

Characteristic	1	2	3	4	5	6	7
Colony morphology and consistency	Smooth, creamy	Smooth, creamy	Smooth, creamy	Smooth, creamy	Contoured friable, dry	Smooth, creamy	Smooth, creamy
Pigment	Yellow-orange	Whitish-grey plus iodine crystals	Whitish-grey	Whitish-yellow	Whitish-beige	Whitish-yellow	Whitish-grey
Growth at 37 °C	w	+	+	+	+	+	+
Nitrate reduction	+	+	v(–)	v(+)	–	–	+
α -Glucosidase activity	–	–	v	–	–	–	–
Xanthine hydrolysis	+	+	+	+	v	–	+
Utilization of:							
D-Arabinose	–	–	+	–	–	–	–
L-Arabinose	–	–	–	–	–	–	+
D-Arabitol	v(–)	–	–	+	–	–	+
Gluconate	–	–	+	+	–	–	–
Mannitol	v(–)	–	–	+	–	–	+

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 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 entire margin, round, smooth, greyish-white in colour and of butyrous consistency. Good growth occurs at 22, 30 and 37 °C but is optimal at 37 °C. The organism is catalase- and oxidase-positive and has a respiratory mode of metabolism. Tyrosine is decomposed and extracellular DNase is produced. Casein, gelatin and xanthine are hydrolysed but aesculin, starch and urea are not. Nitrate is reduced to nitrite. No acid is produced from glucose, maltose, mannitol, sucrose or xylose. L-Arabinose, D-arabitol, galactose, D-glucose, glycerol, D-fructose, D-mannose and mannitol are utilized but adonitol, amygdalin, D-arabinose, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, β -gentiobiose, gluconate, 2-keto-gluconate, 5-ketogluconate, N-acetylglucosamine, methyl α -D-glucoside, glycogen, inositol, inulin, lactose, D-lyxose, maltose, methyl α -D-mannoside, melibiose, melibiose, D-raffinose, rhamnose, ribose, salicin, sorbitol, L-sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose, L-xylose and methyl β -xyloside are not. Alkaline and acid phos-

phatase, esterase C₄, ester lipase C₈, leucine arylamidase, cystine arylamidase and phosphoamidase are detected but lipase C₁₄, valine arylamidase, trypsin, chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase and α -mannosidase activities are not. The peptidoglycan contains meso-diaminopimelic acid. Galactose is present in the cell wall but not arabinose. Mycolic acids are not present. Isolated from bumble-foot-like manifestations of poultry, though their pathogenic role is unclear. Isolates have been found to be non-pathogenic for experimentally injected animals and are thought to be secondary invaders (Mohan, 1981). The type strain is NCIMB 703055^T.

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