Isolation of *Corynebacterium falsenii* and description of *Corynebacterium aquilae* sp. nov., from eagles

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Biochemical, molecular chemical and molecular genetic studies were performed on seven unidentified Gram-positive, rod-shaped organisms recovered from eagles. The strains were provisionally identified as *Corynebacterium jeikeium* with the commercial API Coryne system, but they were able to grow under anaerobic conditions and were non-lipophilic. Comparative 16S rRNA gene sequencing studies demonstrated that the isolates belonged phylogenetically to the genus *Corynebacterium*. Three strains were identified genotypically as *Corynebacterium falsenii*; the remaining four strains corresponded to a hitherto unknown lineage within the genus *Corynebacterium*, associated with a small subcluster of species that included *Corynebacterium diphtheriae* and its close relatives. The unknown bacterial strains were readily distinguished from these and other species of the genus by biochemical tests. Based on both phenotypic and phylogenetic evidence, it is proposed that the unknown bacterial strains from eagles should be classified as *Corynebacterium aquilae* sp. nov. (type strain is S-613⁷ = CECT 5993⁷ = CCUG 46511⁷).

Corynebacteria represent a major group of diphtheroid organisms within the Gram-positive, high-G+C content *Actinobacteria*. The genus *Corynebacterium* has undergone considerable expansion in the past decade and, at the time of writing, over 50 species are recognized. Corynebacteria have been isolated from a wide range of environments (e.g. dairy products, soil, sewage, sediments, aquatic sources and animals) but the vast majority of novel species described in recent years have originated from human clinical sources (e.g. Funke *et al.*, 1997, 1998; Sjödén *et al.*, 1998; Collins *et al.*, 1999a; Renaud *et al.*, 2001), due to increasing concern about their potential pathogenic significance (Lagrou *et al.*, 1998; Riegel, 1998). Corynebacteria also occur as part of the indigenous flora of animals other than man, but presently there is little information on the nature of these corynebacterial species and their host distribution. However, there are indications from the implementation of improved molecular taxonomic methodologies that much new corynebacterial species diversity remains to be discovered from animal sources (e.g. Fernández-Garayzábal *et al.*, 1997, 1998; Pascual *et al.*, 1998; Collins *et al.*, 1999b, 2001a, b). In the course of a study of the normal flora of the respiratory tract of eagles, we have characterized seven *Corynebacterium*-like organisms by using phenotypic, molecular chemical and molecular genetic methods. Based on the results of this study, we report the isolation of *Corynebacterium falsenii* from birds for the first time and describe a hitherto unknown *Corynebacterium* species, *Corynebacterium aquilae* sp. nov.

Bacterial strains S-102 and S-107 were isolated from the mouth of an adult and a juvenile Spanish Imperial eagle (*Aquila adalberti*), respectively. Five strains were isolated from Golden eagles (*Aquila chrysaetos*), three (S-613⁷, S-622 and S-623) from choanae and two (S-672 and S-676) from trachea. Eagles were housed in a rehabilitation centre located in the region of Castilla-La Mancha, Spain. All strains were isolated on Columbia sheep-blood agar plates (bioMérieux) and incubated for 48 h at 37 °C under aerobic and anaerobic (AnaeroGen; Oxoid) conditions. The strains were characterized biochemically by using the API Coryne (version 2.0), API 50 CH and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. The API 50 CH strips were read after up to 7 days incubation at 37 °C. The CAMP (Christie–Atkins–Munch-Petersen) test with *Staphylococcus aureus* ATCC 25923 was performed according to standard procedures (Funke *et al.*, 1997). Lipophilic requirements were determined by growing...
the isolates in brain-heart infusion agar supplemented with 1 % Tween 80, in comparison with brain-heart infusion agar that lacked lipid supplementation. Cell-wall murein was prepared by mechanical disruption of cells and analysis of complete acid hydrolysates, as described by Schleifer & Kandler (1972). Fatty acid methyl esters were prepared and analysed as described by Kämpfer & Kroppenstedt (1996). The presence of mycolic acids was investigated by GLC analysis of trimethylsilylated derivatives (TMS-MAME) (Klatte et al., 1994). For 16S rRNA gene sequence analysis, a large fragment (approx. 1450 bases) of the 16S rRNA gene of the isolates was amplified by PCR and sequenced directly by using a Taq DyeDeoxy Terminator Cycle Sequencing kit and a model 373A automatic DNA sequencer (both from Applied Biosystems). The closest known relatives of the new isolates were determined by performing a database search. 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) by using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The seven strains consisted of Gram-positive, non-motile, non-spore-forming, catalase-positive rods. Colonies were non-haemolytic and non-lipophilic and the CAMP test result was negative. All strains produced acid from glucose and ribose but not from xylose, mannotil, lactose or sucrose. None of the strains reduced nitrate and they did not hydrolyse aesculin, gelatin or urea. Reactions for pyrazinamide and alkaline phosphatase were positive, but no activity was detected for pyrrolidonyl arylamidase, β-glucuronidase, β-glucosidase, α-glucosidase or N-acetyl-β-glucosaminidase. Three strains (S-107, S-672 and S-676) produced acid from maltose, whereas the other four strains did not. The former three strains grew on Columbia sheep-blood agar as whitish, circular, smooth colonies that developed a yellowish pigment after 48–72 h incubation. This pigmentation is produced by only a few non-lipophilic Corynebacterium species (Funke et al., 1997; Sjödén et al., 1998). Two different numerical profiles were obtained with the commercial API Coryne system: 2100304 (strains S-107, S-613T, S-622 and S-623) and 2100324 (S-102, S-672 and S-676), which correspond to a good identification of Corynebacterium jeikeium and an excellent identification within the genus Corynebacterium, respectively. However, C. jeikeium is lipophilic and does not grow anaerobically (Funke et al., 1997), whereas the seven eagle isolates were able to grow under anaerobic conditions and were non-lipophilic.

The eagle isolates were further characterized biochemically by using the API 50 CH and API ZYM systems. All strains produced acid from D-fructose, D-mannose, glycerol, ribose, N-acetylglucosamine and galactose. None of the strains produced acid from maltose, trehalose, D-xylene, L-xylene, mannotil, lactose, sucrose, erythritol, D-arabinose, L-arabinose, adonitol, methyl β-D-glucoside, amygdalin, arbutin, salicin, cellobiose, melibiose, inulin, melezitose, D-raffinose, xylitol, β-gentibiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate, 5-ketogluconate or glycogen. All strains gave positive reactions for alkaline phosphatase, ester lipase C8, esterase C4, naphthol-AS-BI-phosphohydrolase, acid phosphatase and leucine arylamidase. No activity was detected for trypsin, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, α-galactosidase, β-galactosidase, N-acetyl-β-glucosaminidase, valine arylamidase or cystine arylamidase. Strains S-102, S-672 and S-676 gave a positive reaction for lipase C14 and were negative for chymotrypsin. On the other hand, strains S-107, S-613T, S-622 and S-623 gave a positive reaction for chymotrypsin and were negative for lipase C14. Morphological and biochemical characteristics of both groups of strains were consistent with their provisional assignment to the genus Corynebacterium.

In order to investigate the phylogenetic relationships of the two groups of strains, their 16S rRNA gene sequences were determined by direct sequencing of in vitro-amplified rRNA gene products. The almost-complete 16S rRNA gene sequences (>1400 nt) of two representative strains of each group (S-676, S-672, S-622 and S-613T) and approximately 1000 nt of those of the other three strains were determined and subjected to comparative analysis. The isolates from eagles formed two separate phylogenetic groups that were consistent with their phenotypic division, with >99.5 % 16S rRNA sequence similarity within groups. Sequence searches of GenBank revealed that the unknown isolates belonged phylogenetically to the genus Corynebacterium (data not shown). Treeing analysis clearly demonstrated that the two groups of unidentified strains were phylogenetically separate from each other. The 16S rRNA gene sequences of strains S-672 and S-676 displayed 99.9 % similarity with C. falsenii CCUG 33651T, whereas the second group of strains was phylogenetically distinct from all previously described Corynebacterium species. The genotypic identification of isolates S-102, S-672 and S-676 as C. falsenii is consistent with their phenotypic characteristics, although the three eagle strains failed to produce acid from glycogen and did not hydrolyse urea with the API Coryne system. However, the aforementioned strains did hydrolyse urea using Christensen’s urea medium, which also agrees with previous results of studies on C. falsenii (Sjödén et al., 1998). C. falsenii has been isolated from different, normally sterile body sites in humans (Sjödén et al., 1998), but to our knowledge this is the first isolation of this species from birds.

A tree constructed by using the neighbour-joining method, depicting the phylogenetic position of the second group of eagle isolates within the genus Corynebacterium, is shown in Fig. 1. The unknown group (as exemplified by strain S-613T) formed a distinct lineage within the genus Corynebacterium, loosely associated with a small subcluster...
Corynebacterium aquilae sp. nov., from eagles

Corynebacterium aquilae (a’qui.lae. N.L. gen. n. aquilae pertaining to the eagle, Aquila).

Cells are Gram-positive, non-motile, non-spore-forming rods. Colonies are whitish, low convex, dry, rough and 1–2 mm in diameter after 48 h incubation at 37 °C on sheep-blood agar. Facultatively anaerobic, catalase-positive and oxidase-negative. Non-haemolytic, CAMP-negative and non-lipophilic. Nitrate is not reduced. Acid is produced from D-glucose, D-fructose, D-mannose, glycerol, ribose, N-acetylglucosamine and galactose, but not from maltose, trehalose, D-xylene, L-xylene, mannitol, lactose, sucrose, erythritol, D-arabinose, L-arabinose, adonitol, methyl β-xyllose, L-sorbose, rhamnose, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, cellobiase, melibiose, inulin, melezitose, D-raffinose.

Fig. 1. Unrooted tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of Corynebacterium aquilae sp. nov. Bootstrap values (percentage of 500 replications) are given at branching points. Bar, 1 % sequence divergence.

Table 1. Characteristics that differentiate C. aquilae sp. nov. from its nearest phylogenetic relatives

<table>
<thead>
<tr>
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<th>4‡</th>
<th>5</th>
<th>6</th>
<th>7§</th>
<th>8§</th>
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<td>Nitrate reduction</td>
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<td>Pyrazinamidase</td>
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<td>Pyrrolidonyl arylamidase</td>
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<td>Alkaline phosphatase</td>
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<td>-</td>
<td>V</td>
<td>+D</td>
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<td>ND</td>
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<td>V</td>
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*C. argentoratense differs also from C. aquilae by its inability to produce acid from galactose and by exhibiting cystine arylamidase activity.
†C. testudinis is z-chymotrypsin-negative and β-glucosidase-positive.
‡C. felinum is z-chymotrypsin-negative.
§C. ulcerans and C. pseudotuberculosis display reverse CAMP reactions.
‖Variable, according to Funke et al. (1997).
xylitol, β-gentibiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate, 5-ketogluconate or glycogen. Gelatin, urea and aesculin are not hydrolysed. Alkaline and acid phosphatases, ester lipase C8, esterase C4, naphthol-AS-BI-phosphohydrolase, leucine arylamidase and chymotrypsin activities are detected. No activity is detected for lipase C14, z-glucosidase, β-glucuronidase, β-mannosidase, β-galactosidase, β-galactosidase, α-fucosidase, N-acetyl-β-glucosaminidase, valine arylamidase, trypsin or cysteine arylamidase. Cell wall contains meso-diaminopimelic acid. Mycolic acids (C_{30}–C_{36}) are present. Fatty acids (C_{14:0}, C_{16:0} and C_{18:0}) and monounsaturated (C_{16:1}ω9c and C_{18:1}ω9c) types.

The type strain, S-613\textsuperscript{T} (=CECT 5993\textsuperscript{T} = CCUG 46511\textsuperscript{T}), was isolated from the choanae of the Golden eagle (A. chrysaetos).

Acknowledgements

The authors thank N. Montero for her technical assistance.

References


