Actinomyces vaccimaxillae sp. nov., from the jaw of a cow

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A previously undescribed Actinomyces-like bacterium was isolated from a lesion in the jaw of a cow. Based on its cellular morphology and the results of biochemical testing, the organism was tentatively identified as a member of the genus Actinomyces. Comparative 16S rRNA gene sequencing studies showed that the bacterium represents a hitherto unknown species within the genus Actinomyces, and is related to a group of species that includes Actinomyces turicensis and its close relatives. It is proposed that the unknown organism be classified as Actinomyces vaccimaxillae sp. nov. (the type strain is CCUG 46091T = CIP 107423T).

In the last decade the number of described Actinomyces species has increased dramatically, and currently the genus encompasses 25 species. Most of the newly described species have originated from human clinical specimens and to a lesser extent, from animals, where they occur as mucosal contaminants, or some possibly represent unknown opportunistic pathogens (e.g. Collins et al., 2000; Funke et al., 1994, 1997; Pascual et al., 1997, 1999; Wüst et al., 1995). Despite the rapid increase in the number of recognized Actinomyces species, it is clear that much new diversity remains to be discovered from human and animal sources (Hall et al., 1999, 2001). In the course of an ongoing study of taxonomically problematic Actinomyces-like organisms, using a polyphasic taxonomic approach, we describe Actinomyces vaccimaxillae sp. nov., from a cow.

Bacterial strain R10176 was isolated at the Veterinary Investigation Centre, Shrewsbury, and was referred to the Anaerobe Reference Unit, PHLS, University Hospital of Wales, for identification. It was cultured from pus from a jaw lesion in an adult cow. Strain R10176 has been deposited at CCUG as CCUG 46091T, and at CIP as CIP 107423T. It was characterized biochemically by using both conventional tests (Phillips, 1976) and the API Rapid ID32Strep, API Rapid 32A, API Coryne and API ZYM systems according to the manufacturer’s instructions (bioMérieux). Volatile and non-volatile end products of glucose metabolism were detected by GLC (Holdeman & Moore, 1974). PAGE analysis of whole-cell proteins was performed as described by Pot et al. (1994). For densitometric analysis, normalization and interpretation of protein patterns, the GCW 3.0 software package (Applied Maths) was used. The similarity between all pairs of traces was expressed by the Pearson product–moment correlation, converted for convenience to a percentage similarity. The 16S rRNA gene of the isolate was amplified by PCR and sequenced directly by using a Taq DyeDexoxy Terminator Cycle Sequencing kit and a model 373A automatic DNA sequencer (both Applied Biosystems). The closest known relatives of the new isolate were determined by performing GenBank/EMBL database searches, and their sequences and those of other known related strains were retrieved and aligned with the newly determined sequence using the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually using the program GeneDoc (Nicholas et al., 1997) and a distance matrix was calculated using the program DNADIST (using the Kimura two-parameter correction) (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 unidentified organism consisted of Gram-positive, coccobacillary to short diphtheroid-shaped rods, which were non-acid-fast, non-spore-forming and non-motile. After 48 h anaerobic incubation on Fastidious Anaerobe Agar with 5% horse blood, colonies were <1 mm diameter, convex, smooth, entire-edged, opaque white and non-haemolytic. The organism was catalase-negative and grew...
poorly in air and in air plus 10% CO₂. Lactic, succinic and acetic acids were the end products of glucose fermentation. As determined by traditional methods, the organism produced acid from L-arabinose, fructose, glucose, D-ribose, salicin, sucrose, trehalose and D-xylose. It failed to produce acid from cellobiose, lactose, mannitol or D-raffinose. The organism hydrolysed aesculin but not gelatin or starch; it did not produce lecinthinase, lipase or urease, was indole-negative and did not reduce nitrate. The strain could not be identified using the API biochemical kits. Using the API Rapid ID 32Strep, API Rapid ID32A and API Coryne systems, the strain produced profiles 2(6)/0102000300, 0410073725 and 2150752 respectively. Using the API ZYM kit, the only observed positive tests were for acid phosphatase (weak), α-fucosidase and leucine arylamidase; all other enzyme tests were negative. Although most of the results obtained from the API test kits were consistent with each other, nevertheless some differences were observed. Acid production from sucrose but not from lactose was obtained using the API Rapid ID 32Strep system. However, using the API Coryne system, the opposite results were obtained. Using the API Coryne kit, alkaline phosphatase was detected but not with the API Rapid ID 32Strep, API Rapid ID32A or API ZYM test systems. Using the API Rapid ID32A kit, α-glucosidase and β-glucosidase were detected. However, negative reactions were obtained for these enzymes using the API ZYM system. It is pertinent to note that method-dependent reactions have been observed previously for some other *Actinomyces* species in commercial API systems (Hoyles *et al.*, 2001; Lawson *et al.*, 2001) and in conventional biochemical tests (Slack & Gerencser, 1975). The cellular morphology and biochemical reactions of the organism were consistent with its tentative assignment to the genus *Actinomyces*, but it did not appear to correspond to any recognized species of this genus. PAGE analysis of whole-cell proteins further demonstrated the phenotypic distinctiveness of CCUG 46091T: it formed a separate lineage and did not display a particularly close affinity with any described *Actinomyces* species. A dendrogram based on protein profiles, illustrating the distinctiveness of the unknown isolate amongst its nearest relatives, is available as supplementary data in IJSEM Online (http://ijis.sgmjournals.org).

To investigate the phylogenetic relationships of the unknown organism, its almost-complete 16S rRNA gene sequence (>1400 nucleotides) was determined. Sequence database searches confirmed that the unknown isolate was most closely related to species of the genus *Actinomyces*. Highest sequence similarity values were found with *Actinomyces meyeri* (94·9%), *Actinomyces funkei* (93·5%), *Actinomyces turicensis* (94·4%), *Actinomyces georgiae* (94·4%), *Actinomyces odontolyticus* (94·4%), *Actinomyces suimastitidis* (94·4%) and phylogenetically related organisms. Treeing analysis further demonstrated the placement of the unidentified bacterium within the genus *Actinomyces*, where it formed a distinct subline within a small subcluster of species which included *A. funkei*, *A. georgiae*, *Actinomyces hyovaginalis*, *A. meyeri*, *A. odontolyticus*, *A. radinage*, *A. suimastitidis* and *A. turicensis* (Fig. 1).

Phylogenetically, the unknown bacterium forms a distinct subline within the *Actinomyces* genus and displays an affinity with a group of species that includes *A. turicensis* and related species. Bootstrap resampling, however, showed that the unknown bacterium did not share a statistically significant association with any individual member of this rRNA subcluster. Sequence divergence values of approximately 5–8% reinforced the separateness of CCUG 46091T from all currently recognized members of this subcluster. Although there is no precise correlation between 16S rRNA sequence divergence values and species delineation, it is now generally accepted that organisms displaying values of 3% or more do not belong to the same species (Stackebrandt & Goebel, 1994). The observed >5% sequence divergence between CCUG 46091T and all currently defined *Actinomyces* species is therefore consistent with separate species status. Therefore, based on the polyphasic taxonomic approach described here, we propose the name *Actinomyces vaccimaxillae* sp. nov. for the novel isolate. Tests that are useful for distinguishing *Actinomyces vaccimaxillae* from its closest relatives are shown in Table 1.

**Description of Actinomyces vaccimaxillae** sp. nov.

*Actinomyces vaccimaxillae* (vac.ci.ma’xill.lae. L. n. vacca cow; L. n. maxilla jaw; N.L. gen. n. vaccimaxillae of a cow’s jaw).

Cells are non-spore-forming, non-motile, Gram-positive, cocco-bacillary to short diphtheroid-shaped rods. Non-acid-fast. After 48 h anaerobic incubation on Fastidious
Table 1. Tests which are useful in distinguishing Actinomyces vaccimaxillae sp. nov. from its nearest phylogenetic relatives

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Anaerobe Agar with 5% horse blood, colonies are < 1 mm diameter, convex, smooth, entire-edged, opaque white and non-haemolytic. Facultatively anaerobic and catalase-negative. Lactic and succinic acids, together with minor amounts of acetic acid, are the end products of glucose fermentation. Using conventional tests, aesculin is hydrolysed but gelatin and starch are not. Leucithinase, lipase and urease are not produced. Indole is negative and nitrate is not reduced. With the API Rapid ID 32Strep system, acid is formed from L-arabinose, ribose, sucrose and trehalose, but not from D-arabitol, cycloextrin, glycojen, lactose, maltose, mannitol, melibiose, melezitose, methyl β-D-glucopyranoside, pullulan, D-raffinose, sorbitol, or tagatose. Hippurate is not hydrolysed and acetoain is not produced. Alanine-phenylalanine-proline arylamidase and β-glucosidase are detected. No activity is detected for arginine dihydrolase, alkaline phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, glucyl-cysteinylpropanoyl arylamidase, N-acetyl-β-glucosaminidase, pyrogallol arylamidase, β-mannosidase or urease. With the API Rapid ID32A system, acid is not produced from mannose or raffinose, indole is not formed and nitrate is not reduced. Activity is detected for alanine arylamidase, arginine arylamidase, α-fucosidase, α-glucosidase, β-glucosidase, glycine arylamidase, histidine arylamidase, proline arylamidase, leucyl-glycine arylamidase, leucine arylamidase, phenylalanine arylamidase, serine arylamidase and tyrosine arylamidase. No activity is detected for α-arabinosidase, α-galactosidase, β-galactosidase, pyrogallol arylamidase, alkaline phosphatase, arginine dihydrolase, β-galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl-glutamic acid arylamidase, β-glucuronidase, N-acetyl-β-glucosaminidase or urease. With the API Coryne kit, acid is formed from glucose, glycojen, lactose, mannitol, ribose and D-xylose, but not from maltose or sucrose; aesculin is hydrolysed but gelatin is not, and nitrate is not reduced. Alkaline phosphatase, α-glucosidase and pyrazinamidase are detected, whereas β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, pyrrolidonyl arylamidase and urease are not. Using the API ZYM system, acid phosphatase (weak reaction), α-fucosidase and leucine arylamidase are detected, but alkaline phosphatase, α-glucosidase, β-glucosidase, β-glucuronidase, β-glucosaminidase, esterase C4, ester lipase C8, lipase C14, α-mannosidase, phosphoamidase, chymotrypsin, trypsin, cysteine arylamidase and valine arylamidase are not detected.

Isolated from pus from a lesion in the jaw of a cow. Habitat is not known. The type strain is CCUG 46091T = CIP 107423T.

Acknowledgements

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References


