International Journal of Systematic and Evolutionary Microbiology (2000), **50**, 661–663 Printed in Great Britain

NOTE

Reclassification of *Actinomyces humiferus* (Gledhill and Casida) as *Cellulomonas humilata* nom. corrig., comb. nov.

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The placement of *Actinomyces humiferus* within the genus *Actinomyces* has always been controversial. *A. humiferus* differs from typical members of the genus both phenotypically and in possessing a relatively high DNA G+C content. Comparative 16S rRNA gene sequencing has shown that *A. humiferus* is related only distantly to other species of the genus *Actinomyces* and is, in fact, a member of the genus *Cellulomonas*. On the basis of phylogenetic evidence, it is proposed that *A. humiferus* be reclassified in the genus *Cellulomonas* as *Cellulomonas humilata* nom. corrig., comb. nov.

Keywords: *Actinomyces humiferus*, *Cellulomonas humilata*, taxonomy, phylogeny

In 1969, Gledhill and Casida described the characteristics of a Gram-positive, non-spore-forming, rod-shaped bacterium, which they named *Actinomyces humiferus* (Gledhill & Casida, 1969). Morphologically, the organism was reported to produce hyphal-like structures with true branching that fragmented into diphtheroid and coccoid elements, and was considered to resemble *Actinomyces* species in producing branched filamentous 'spider-like' microcolonies (Gledhill & Casida, 1969). Based primarily upon these morphological features, together with its catalase-negative reaction and nutritional characteristics, the genus *Actinomyces* was considered to be the most appropriate taxonomic niche for the species. The placement of *A. humiferus* in the genus *Actinomyces* has, however, always been controversial (Schaal, 1986, 1992). Unlike most other *Actinomyces* species, which are found in association with humans and other warm-blooded animals, *A. humiferus* has been isolated exclusively from organically rich soils, where it is reported to be a numerically predominant inhabitant (Gledhill & Casida, 1969). *A. humiferus* differs from other *Actinomyces* species in being aerobic to micro-aerophilic, inhibited or at least not stimulated by increased CO_2 and sensitive to lysozyme. In addition, *A. humiferus* has an optimum growth temperature of 30 °C and grows poorly or not at all at 37 °C (Gledhill & Casida, 1969). It also has a higher DNA G+C content (73 mol%) than other *Actinomyces* species (Schaal, 1986).

During the past few years, great progress has been made in elucidating the phylogenetic interrelationships of species of the genus *Actinomyces*. Comparative 16S rRNA gene sequencing studies (e.g. Pascual Ramos *et al.*, 1997; Lawson *et al.*, 1997) have shown that *Actinomyces* species are phylogenetically very diverse and that the genus *Actinomyces* should be restricted to the type species *Actinomyces bovis* and its close relatives. Although the precise phylogenetic position of *A. humiferus* was not established in the aforementioned studies, it was apparent that the species was related only remotely to authentic *Actinomyces* species and displayed a closer affinity to *Rothia dentocariosa* (Pascual Ramos *et al.*, 1997; Lawson *et al.*, 1997). This latter species is a member of the family *Micrococcaceae* (Stackebrandt *et al.*, 1997) but, unfortunately, other representatives of this family and related taxa were not included in the comparative phylogenetic analyses of Pascual Ramos *et al.* (1997) and Lawson *et al.* (1997). To rectify this situation, we conducted 16S rRNA sequence database searches to ascertain the nearest relatives of *A. humiferus*. The searches indicated that the nearest relatives of *A. humiferus* were certain members of the *Micrococcineae* (Stackebrandt *et al.*, 1997) with cellulomonads, *Cellulomonas turbata* (= *Oerskovia turbata*) and *Promicromonospora enterophila* displaying highest sequence relatedness (approx. 95–97% 16S rRNA similarity). In contrast, *A. humiferus* displayed far lower 16S rRNA sequence relatedness to *A. bovis* and its near relatives (<90% similarity; data not shown).

A tree, constructed by the neighbour-joining method, depicting the phylogenetic interrelationships of *A. humiferus* (Fig. 1) demonstrates its high affinity with

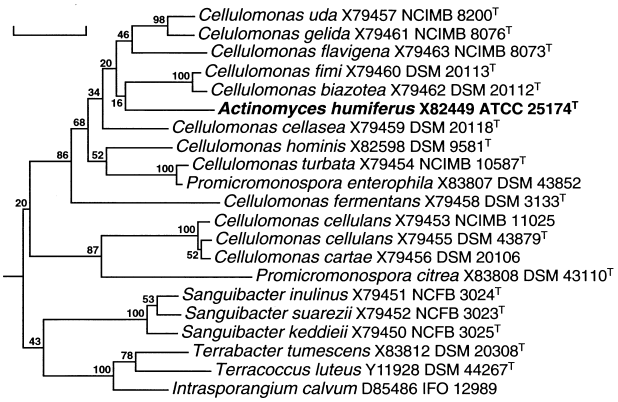

Fig. 1. Unrooted tree constructed by the neighbour-joining method showing the phylogenetic interrelationships of *A. humiferus*, based on 16S rRNA (excluding variable region V1). Bootstrap values (from 1000 tree replicates generated using the programs SEQBOOT, DNADIST and CONSENSE of the PHYLIP package; Felsenstein, 1989) are given at the branching points. Bar, 2% sequence divergence.

the genus *Cellulomonas*. Although *A. humiferus* does not exhibit a particularly close or significant association with any individual *Cellulomonas* species, it is consistently placed within the robust *Cellulomonas* clade, which includes *Cellulomonas flavigena*, the type species of the genus, and *C. turbata*, formerly the type species of the genus *Oerskovia*. The genealogical intermixing of *Cellulomonas* species and former *Oerskovia* species (*C. turbata* and *Cellulomonas cellulans* NCIMB 11025 = *Oerskovia xanthineolytica*) shown in Fig. 1 confirms earlier phylogenetic studies (e.g. Fernandez-Garayzabal *et al.*, 1995; Rainey *et al.*, 1995) and strongly supports the unification of the cellulomonads and oerskoviae as a single genus, *Cellulomonas* (Stackebrandt *et al.*, 1980, 1982; Rainey *et al.*, 1995).

In the present analysis, the *Cellulomonas* clade was recovered in 86% of bootstrapped trees (1000 replicates) and the placement of *A. humiferus* within this grouping was also observed by using the Fitch and maximum-parsimony treeing methods (data not shown). Thus, comparative 16S rRNA sequencing provides unequivocal evidence for the removal of *A. humiferus* from the genus *Actinomyces* and its reclassification in the genus *Cellulomonas*.

In our opinion, the phenotypic characteristics of *A. humiferus* should not prevent its assignment to the genus *Cellulomonas*. *A. humiferus* forms a mycelium with true branching that fragments into diphtheroid and coccoid elements (Gledhill & Casida, 1969). Cellulomonads are generally regarded as not producing mycelia, although some primary branching may occur. However, *C. turbata*, which is recovered within the realm of the *Cellulomonas* clade (Fig. 1), produces a well-developed mycelium compared with the morphologically simpler forms exhibited by cellulomonads. *A. humiferus* is aerobic to microaerophilic, catalase-negative and ferments carbohydrates (Gledhill & Casida, 1969). Most cellulomonads are catalase-positive and display both respiratory and fermentative metabolism. However, *Cellulomonas fermentans* is catalase-negative and exhibits solely carbohydrate fermentation (Bagnara *et al.*, 1985). Therefore, we consider the cellular morphology, dissimilar metabolism (fermentative) and negative catalase reaction of *A. humiferus* to be insufficient grounds to preclude its classification in the genus *Cellulomonas*. The cell wall of *A. humiferus* has been shown to contain lysine and ornithine (Gledhill & Casida, 1969). Rhamnose is also reported to be present in the cell wall. Similarly, MK-9(H_4) is the major lipoquinone (M. D. Collins, unpublished data). These data are not inconsistent with the close affinity of the bacterium to the cellulomonad clade. On the basis of the overwhelming phylogenetic evidence, we propose formally that *A. humiferus* be reclassified in the genus *Cellulomonas* as *Cellulomonas humilata* nom. corrig., comb. nov. *C. humilata* may be distinguished easily from all other members of the *Cellulomonas* cluster by its ability to hydrolyse casein and in its failure to reduce nitrate. Other species fail to hydrolyse casein and are able to reduce nitrate. With the exception of *Cellulomonas fermentans*, *C. humilata* also differs from other species in the above-mentioned cluster in being catalase-negative (Funke *et al.*, 1995; Gledhill & Casida, 1969).

Description of *Cellulomonas humilata* (Gledhill and Casida 1969) nom. corrig., comb. nov.

Cellulomonas humilata (hu.mi.la′ta. L. masc. n. *humus* soil; L. adj. part. *latus*, *-a*, *-um* borne; M.L. fem. adj. *humilata* soil-borne).

Cells are predominantly filamentous and branched and often have swollen ends. They stain Gram-positive and are non-acid-fast. After prolonged incubation, they usually fragment into diphtheroid or coccoid elements of varied size and shape. In liquid media, growth is granular or flocculent, forming a white sediment without turbidity. Mature colonies are small, opaque, smooth, entire and convex with a dark central region. Rough colony variants occur occasionally. Pigmentation is not evident. Microaerophilic to aerobic; there is poor or no growth in anaerobic conditions. Growth is not stimulated by increased CO_2 tension. Catalase- and oxidase- negative.

The optimum temperature for growth is approximately 30 °C; poor or no growth is observed at 37 °C. Does not grow on media lacking organic nitrogen. In addition, little if any growth is obtained in certain chemically defined media or those that contain simple peptones. Cells are sensitive to lysis by lysozyme. Fermentation of sugars produces lactic acid as a major end-product and no gas formation. Casein, aesculin and starch are hydrolysed, whereas xanthine, tyrosine and urea are not. Gelatin is weakly decomposed but not liquefied. Litmus milk is acidified and reduced.

Nitrates are not reduced to nitrites. Production of indole from tryptophan and of ammonia from peptone and arginine are negative. Methyl-red test is positive, whereas Voges–Proskauer reaction is negative. Hydrogen sulfide is produced. No growth is obtained in the presence of 4% NaCl. Pyruvate, fumarate, 2-oxoglutarate and gluconate are utilized. Acid is produced from cellobiose, dextrin, D-fructose, D-glucose, D-mannose, D-raffinose, D-xylose, galactose, L-arabinose, maltose, mannitol, melezitose, melibiose, rhamnose, salicin, starch, sucrose, turanose and β-gentiobiose, whereas acid is not produced from adonitol, dulcitol, inositol, inulin, ribose or sorbitol. Acid production from glycerol, lactose and trehalose is variable. The cell wall is reported to contain lysine and ornithine. Rhamnose is the predominant cell-wall sugar, but glucose and fucose may be present in trace amounts. MK-9(H_4) is the major lipoquinone. The natural habitat of *Cellulomonas humilata* is reported to be organically rich soil, from which the organism may be recovered in large numbers. The G+C content of the DNA is 73 mol%. The type strain, ATCC 25174^T, exhibits the characteristics of the species and produces acid from glycerol, lactose and trehalose.

Acknowledgements

We are grateful to Professor Hans Trüper (University of Bonn, Germany) for correcting the species epithet.

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