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 CO₂ 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 Micrococccinae (Stackebrandt et al., 1997) with cellulomonads, Cellulomonas turbata (= Oerskovia turbata) and Promicromonospora enterophilae 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
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 cellomonads 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 coccoïd 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 cellomonads. *A. humiferus* is aerobic to micro-aerophilic, catalase-negative and ferments carbohydrates (Gledhill & Casida, 1969). Most cellomonads 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(H₄) is the major lipoquinone (M. D. Collins, unpublished data). These data are not inconsistent with the close affinity of the bacterium to the cellomonad 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. corr. 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. corr., 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 coccoïd 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 CO₂ 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-ribose, D-xylene, 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(H4) 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 25174T, 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.

References


