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Reclassification of *Micrococcus agilis* (Ali-Cohen 1889) to the Genus *Arthrobacter* as *Arthrobacter agilis* comb. nov. and Emendation of the Genus *Arthrobacter*

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Phylogenetic evidence derived from a 16S ribosomal DNA analysis indicated that the type strain of *Micrococcus agilis*, DSM 20550 (= ATCC 966 = CCM 2390), is less closely related to the type species of the genus *Micrococcus*, *Micrococcus luteus*, than to the type species of the genus *Arthrobacter*, *Arthrobacter globiformis*, and related *Arthrobacter* species. The phylogenetic position of *M. agilis* is supported by the presence of peptidoglycan variation A3α and by the presence of MK-9(H_2) as the major isoprenolog, a characteristic also found in strains of *A. globiformis*, *Arthrobacter crystallopoietes*, *Arthrobacter atrocyaneus*, *Arthrobacter citreus*, *Arthrobacter aurescens*, *Arthrobacter ilicis*, *Arthrobacter ureafaciens*, *Arthrobacter oxydans*, *Arthrobacter histidinolovorans*, and *Arthrobacter nicotinovorans*. The last six species and *M. agilis* are characterized by the presence of threonine in the interpeptide bridge of the peptidoglycan. Threonine has not been found in the peptidoglycans of other *Arthrobacter* species or in members of the genus *Micrococcus*. Despite the fact that a morphological life cycle is not known, these data support the proposal that *M. agilis* should be transferred to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov.

A recent phylogenetic analysis of the 16S ribosomal DNAs (rDNAs) of the type strains of 15 species belonging to the genera *Arthrobacter* and *Micrococcus* indicated that three *Micrococcus* species (*Micrococcus luteus*, *Micrococcus lylae*, and *Micrococcus agilis*) should be placed within the radiation that contains members of the genus *Arthrobacter*, while the other *Micrococcus* species are more distantly related (7). The degrees of relatedness between *M. agilis* and certain members of the genus *Arthrobacter* were significantly higher than the degrees of relatedness between *M. agilis* and the other two *Micrococcus* species and even between many *Arthrobacter* species. This result suggested that *M. agilis* is a member of the genus *Arthrobacter* sensu stricto (6), but that its development is blocked so that only one stage of the life cycle typical of *Arthrobacter* species is observed (18).

In order to place *M. agilis* more precisely within the radiation of the 16S rDNA tree, we extended the analysis to include all known type strains belonging to the genera *Arthrobacter* and *Micrococcus*. The 16S rDNAs were analyzed as described previously (14). The inclusion of six *Arthrobacter* sequences which we determined, the sequences of *Arthrobacter aurescens* DSM 20116^T (T = type strain), *Arthrobacter histidinolovorans* DSM 20115^T, *Arthrobacter ilicis* DSM 20138^T, *Arthrobacter oxydans* DSM 20119^T, *Arthrobacter sulfureus* DSM 20167^T, and *Arthrobacter uratoxydans* DSM 20647^T, and the sequences of other *Micrococcus* species (data not shown) did not significantly change the topology of the phylogenetic tree (17). The position of *M. agilis* (Fig. 1) indicates that this organism is a member of the *Arthrobacter* species group, as defined by peptidoglycan variation A3α, which contains *Arthrobacter globiformis*, the type species of the genus. This group is referred to as group I. The second recognized group of *Arthrobacter* species (group II) (i.e., species with peptidoglycan type A4α) appears to be more closely related to *M. luteus* and *M. lylae*. An analysis

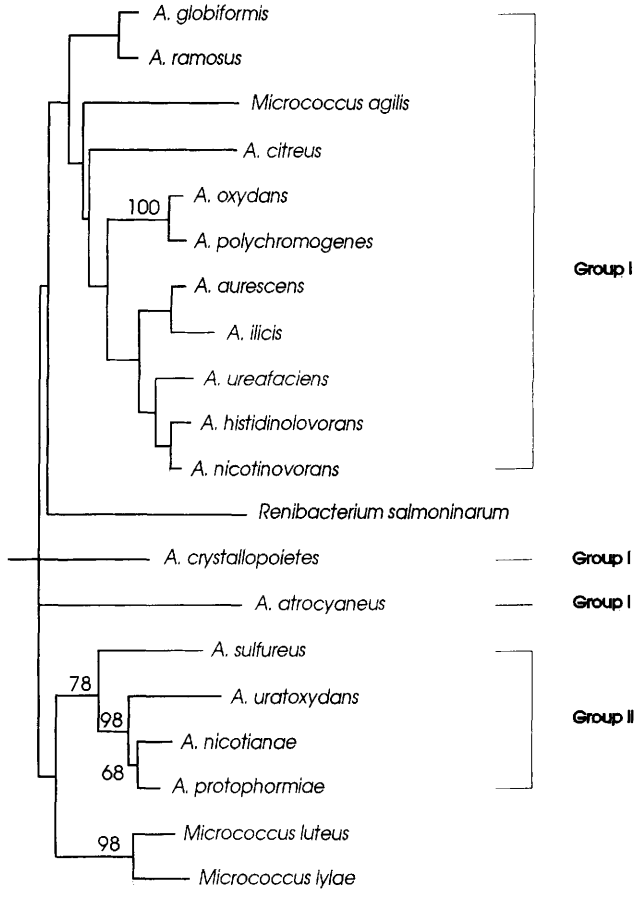

FIG. 1. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of *M. agilis* and related organisms belonging to the genera *Micrococcus*, *Arthrobacter*, and *Renibacterium*. The branching pattern was generated as described previously (14). Numbers refer to bootstrap values. Bar = 1.5 substitutions per 100 nucleotides.

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TABLE 1. Cell wall types, menaquinone compositions, and DNA G+C contents of *M. agilis* and phylogenetically related organisms belonging to the genera *Arthrobacter*, *Micrococcus*, and *Renibacterium*

Organism	Group	Peptidoglycan variant[a]	Peptidoglycan type[b]	Major isoprenolog(s)[c]	G+C content (mol%)[d]
M. agilis		A3α	Lys-Thr-Ala_3	MK-9(H_2)	67.0–70.0
A. citreus	I	A3α	Lys-Thr-Ala_2	MK-9(H_2)	62.9–65.1
A. oxydans	I	A3α	Lys-Ser-Thr-Ala	MK-9(H_2)	62.7–65.9
A. polychromogenes	I	A3α	Lys-Ser-Thr-Ala	MK-9(H_2)	62.9
A. aurescens	I	A3α	Lys-Ala-Thr-Ala	MK-9(H_2)	59.1–61.9
A. ilicis	I	A3α	Lys-Ala-Thr-Ala	MK-9(H_2)	60.0–61.5
A. ureafaciens	I	A3α	Lys-Ala-Thr-Ala	MK-9(H_2)	61.7–63.6
A. histidinolovorans	I	A3α	Lys-Ala-Thr-Ala	MK-9(H_2)	61.3–62.2
A. nicotinovorans[e]	I	A3α	Lys-Ala-Thr-Ala	MK-9(H_2)	62.4
A. globiformis	I	A3α	Lys-Ala_3	MK-9(H_2)	62.0–65.5
A. ramosus	I	A3α	Lys-Ala_4	MK-9(H_2)	62.2–63.1
A. crystallopoietes	I	A3α	Lys-Ala	MK-9(H_2)	63.0–63.8
A. atrocyaneus	I	A3α	Lys-Ser-Ala_{2-3}	MK-9(H_2)	69.5–70.3
R. salmoninarum[f]		A3α	Lys-Gly-Ala	MK-9, MK-10	53.0
A. sulfureus	II	A4α	Lys-Glu	MK-9, MK-10	64.5–66.0
A. uratoxydans	II	A4α	Lys-Ala-Glu	ND[g]	61.2–61.5
A. nicotianae	II	A4α	Lys-Ala-Glu	MK-8	60.2–63.0
A. protophormiae	II	A4α	Lys-Ala-Glu	MK-9, MK-8	63.2–65.9
M. luteus		A2	Lys-peptide subunit	MK-8[h]	70.0–75.5
M. lylae		A3α	Lys-Asp	MK-8(H_2)[h]	67.7–69.5

[a] As defined by Schleifer and Kandler (16).
[b] Data from references 8 and 16.
[c] Data from references 6, 8, and 19.
[d] Data from references 2, 6, and 8.
[e] Data from reference 10.
[f] Data from references 11 and 15.
[g] ND, not determined.
[h] Data from reference 17.

of bootstrap values, however, indicated that most branching points could not be considered statistically significant, as most values were less than 50%. The levels of sequence difference between most pairs of organisms shown in Fig. 1 are too small (between 0.5 and 6.0%) to reliably determine the order in which these highly related species evolved.

Despite the low statistical significance of the tree topology, the clustering is by and large supported by the phenotypic data (Table 1). Not only is the separation of *Arthrobacter* species into groups I and II confirmed (5), but all species that contain threonine in the interpeptide bridge of peptidoglycan form a phylogenically coherent cluster within group I. *M. agilis*, like all other group I species, contains major amounts of isoprenoid quinones of the MK-9(H_2) type (>80% of the total quinones), while *Arthrobacter* group II species, *Renibacterium salmoninarum*, *M. luteus*, and *M. lylae* contain unsaturated menaquinones (4, 15). Group I and II organisms cannot be clearly distinguished on the basis of fatty acid spectra, because all members of the genus *Arthrobacter* produce similar patterns, consisting of major amounts of iso and anteiso methyl-branched acids and with smaller amounts of straight-chain fatty acids (5, 8, 10). A similar pattern has been reported for the type strain of *M. agilis* (2; this study). Comparative studies performed with members of the genera *Arthrobacter* and *Micrococcus* are necessary to determine more precisely the levels of similarity among the fatty acid profiles of individual species. The polar lipid compositions of members of *Arthrobacter* groups I and II are similar (2, 3, 5), but group I species and *M. agilis* contain phosphatidylinositol (5), which is not found in the other *Arthrobacter* species. The previously reported G+C content of *M. agilis* DNA ranges from 67.0 to 70 mol% (2, 8), values which are in the same range as the values obtained for other *Micrococcus* species. Since the values for *Arthrobacter* species are generally about 2 to 3% lower than the values for micrococci, the G+C content of *M. agilis* was redetermined by high-performance liquid chromatography (13). The value obtained was 69.5 mol%, which confirms the previously reported values.

M. agilis (1) and several *Arthrobacter* group I species, including *Arthrobacter ramosus*, *A. ilicis*, *Arthrobacter citreus*, and *Arthrobacter atrocyaneus*, are motile (5). It should be noted in this context that early descriptions of *M. agilis* indicated that motility is frequently lost in laboratory cultures (12); this could lead to classification of these red-pigmented organisms as color variants of the pastel-red- to orange-red-pigmented organism *Micrococcus roseus* (12). Decades later, strains of *M. agilis* deposited in the American Type Culture Collection (ATCC 98 and ATCC 9814) were also found to be nonmotile (9), and loss of motility by *M. agilis* strains has also been observed in the DSM-German Collection of Microorganisms (18a). Thus, in *M. agilis* motility is not a stable taxonomic marker.

M. agilis is related to *Arthrobacter* group I, and phylogenetic evidence and key chemotaxonomic characteristics indicate that *M. agilis* should be reclassified in the genus *Arthrobacter* as *Arthrobacter agilis* Ali-Cohen 1889. The transfer of a coccoid species to the genus *Arthrobacter* means that the genus description must be emended, as follows: a marked rod-coccus growth cycle occurs during growth in complex media; stationary-phase cultures (generally after 2 to 7 days) are composed entirely or largely of coccoid cells that are ~0.6 to 1.0 μm in diameter; one species forms only spherical cells.

Description of *Arthrobacter agilis* (Ali-Cohen) comb. nov. The species description below is based on information derived from several sources (2, 8, 9) and our own observations. Spheres that are 0.8 to 1.2 μm in diameter occur in pairs and tetrads. Agar colonies are circular, entire, slightly convex, smooth, and matte. Sediment is formed in nutrient broth. No

growth occurs on Simmons citrate medium. Good dark rose-red-pigmented growth occurs on agar slants. The pigment is water insoluble.

Motile by means of one to three flagella. Nonmotile strains may occur. Spores are not formed. Gram positive.

The cell wall peptidoglycan type is type L-Lys–L-Thr–L-Ala (variation A3α). The predominant menaquinone isoprenolog is MK-9(H_2); MH-8(H_2) is a minor component. Contains major amounts of anteiso methyl-branched acids (~65.5% anteiso-$C_{15:0}$) and smaller amounts of iso methyl-branched acids (~13% iso-$C_{15:0}$ and ~6% iso-$C_{16:1}$). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown ninhydrin-negative glycolipid. The amino sugar in the cell wall polysaccharide is glucosamine.

Chemoorganotrophic. Metabolism is respiratory. Aerobic.

Acid and gas are not produced from glucose, mannose, lactose, galactose, and glycerol. Catalase positive. Porphyrin respiratory enzymes are produced. Oxidase positive. Acetylmethylcarbinol is not produced. β-Galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test) positive. Methyl red negative. Indole and hydrogen sulfide are not produced. Nitrate is not reduced. Gelatin, starch, and esculin are hydrolyzed. Arginine dihydrolase, ornithine and lysine decarboxylases, and phenylalanine deaminase are not produced. Tween 80 may be split. DNase may be produced. Urease, tyrosinase, and phosphatase are not produced.

Beta-hemolysis does not occur.

Good growth occurs at temperatures between 20 and 30°C. No growth occurs at 37°C.

No growth occurs on medium containing 5% NaCl.

Susceptible to penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, novobiocin, ampicillin, carbenicillin, and gentamicin. Resistant to lysozyme.

Saprophytic. Habitat: water, soil, and human skin.

The G+C content of the DNA is 67.0 to 69.0 mol%.

Closely related phylogenetically to *A. globiformis*, *A. ramosus*, *Arthrobacter pascens*, *A. citreus*, *A. oxydans*, *Arthrobacter polychromogenes*, *A. aurescens*, *A. ilicis*, *Arthrobacter ureafaciens*, *A. histidinolovorans*, and *Arthrobacter nicotinovorans* as determined by a 16S rDNA analysis.

The type strain is ATCC 966 (= DSM 20550 = CCM 2390).

Nucleotide sequence accession numbers. The EMBL accession numbers for the 16S rDNA sequences which we determined are as follows: *A. aurescens* DSM 20116^T, X83405; *A. histidinolovorans* DSM 20115^T, X83406; *A. ilicis* DSM 20138^T, X83407; *A. oxydans* DSM 20119^T, X83408; *A. sulfureus* DSM 20167^T, X83409; and *A. uratoxydans* DSM 20647^T, X83410.

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