Transfer of the misnamed [*Alysiella*] sp. IAM 14971 (=ATCC 29468) to the genus *Moraxella* as *Moraxella oblonga* sp. nov.

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Phylogenetic analysis based on 16S rRNA gene sequences revealed that [*Alysiella*] sp. IAM 14971 (=ATCC 29468) is closely related to the genus *Moraxella* of the γ -Proteobacteria (96–97 % similarity). The newly obtained phenotypic data also indicate that [*Alysiella*] sp. IAM 14971 is distinct from the genus *Alysiella* and similar to the genus *Moraxella*. On the basis of these results, the strain should be classified in the genus *Moraxella*, as *Moraxella oblonga* sp. nov. The type strain is IAM 14971^T (=ATCC 29468^T).

[*Alysiella*] sp. IAM 14971 (=ATCC 29468) was isolated from the oral cavity of a sheep and identified by Kuhn (Gherna *et al.*, 1989). Rossau *et al.* (1986, 1989, 1991) argued that this strain was not at all related to the type strain of *Alysiella filiformis* (IAM 14895^T) based on the studies of rRNA cistron similarities and DNA hybridization levels. *A. filiformis* was identified as a member of the β -Proteobacteria based on phylogenetic analyses of the 16S rRNA gene sequence available from the GenBank database. The aim of this study was to identify the exact taxonomic position of [*Alysiella*] sp. IAM 14971 (=ATCC 29468). In this report, we present substantial evidence, mainly from the 16S rRNA gene sequence, fatty acid composition, quinone system analysis and biochemical tests, suggesting that [*Alysiella*] sp. IAM 14971 belongs to the genus *Moraxella* of the γ -Proteobacteria.

The genus *Moraxella* Lwoff 1939, which comprises opportunistic bacterial pathogens, includes 15 authentic species to date. The genus was reclassified into two subgenera based on the shape of the cells (coccus and rod): *Branhamella* and *Moraxella* (Bøvre, 1979). The species with coccus-shaped cells are *Moraxella* (*Branhamella*) *catarrhalis*, *Moraxella* (*Branhamella*) *caviae*, *Moraxella cuniculi* and *Moraxella* (*Branhamella*) *ovis*. The species with rod-shaped cells are *Moraxella* (*Moraxella*) *atlantae*, *Moraxella* (*Moraxella*) *bovis*, *Moraxella boeveyi*, *Moraxella canis*, *Moraxella caprae*, *Moraxella equi*, *Moraxella* (*Moraxella*) *lacunata*, *Moraxella lincolni*, *Moraxella* (*Moraxella*) *nonliquefaciens*, *Moraxella* (*Moraxella*) *osloensis* and *Moraxella saccharolytica*.

The reference strains *A. filiformis* IAM 14895^T (=NCTC

10282^T), *A. filiformis* IAM 14968^T (=ATCC 15532^T) and [*Alysiella*] sp. IAM 14971 (=ATCC 29468) were used in this study. Bacterial strains were incubated in ATCC medium 687 at 37 °C. For electron microscopy, cells grown on solid medium were fixed in a solution of 1 % glutaraldehyde in 0.01 M phosphate buffer pH 7.2 for 2 h at room temperature. Cells were spread on the slide and air-dried. Water was removed gradually by suspending the samples in an acetone series (30, 50, 70, 90, 95 and 100 %) and 100 % 2-methyl-2-propanol. Samples were freeze-dried, sputter-coated with platinum under a vacuum and observed with a model Hitachi S4500 scanning electron microscope. API 20NE and 50CHL microtest galleries were used to determine the physiological and biochemical characteristics. The API strips were incubated for 2 days at 37 °C. Cellular fatty acid methyl esters were prepared, separated and identified by using the Microbial Identification system as described by Xie & Yokota (2003). The respiratory quinone system was extracted and characterized by HPLC (Shimadzu), and the genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described previously (Xie & Yokota, 2003). The 16S rRNA gene sequence of [*Alysiella*] sp. IAM 14971 (1273 bp) was determined and compared with the sequences obtained from the DDBJ and GenBank. The sequences were aligned using the CLUSTAL W software package (Thompson *et al.*, 1994), and the evolutionary distances and K_{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when the 16S rRNA gene nucleotides were compared. The phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The similarity values were calculated using PAUP* 4.0b1 (Swofford, 1998).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Moraxella oblonga* IAM 14971^T is AB087260.

The scanning electron micrograph of [*Alysiella*] sp. IAM 14971 shows that the cells are cocci that are linked in chains of $\geq 30\ \mu\text{m}$ in length. The cells are $0.48\text{--}1.2\ \mu\text{m}$ in diameter (Fig. 1a). The morphology of the chains of cocci is quite different from that of other species of the genus *Moraxella*, which are rod- or coccus-shaped, and also different from that of the multicellular filamentous *A. filiformis*, which has palisade organization in the middle of individual cells (Fig. 1b). [*Alysiella*] sp. IAM 14971 has similar biochemical and chemotaxonomic characteristics to other species of the genus *Moraxella* (shown in Table 1): the quinone system (Q-8); the presence of catalase and oxidase activity; and the absence of indole production and urea hydrolysis. [*Alysiella*] sp. IAM 14971 and *Moraxella* species cannot produce acid from glucose, but *A. filiformis* can. [*Alysiella*] sp. IAM 14971 can produce acid from mannitol, adonitol, inositol, rhamnose, arabinose, sucrose and maltose, which is different from *A. filiformis* (data not shown). The major cellular fatty acids of [*Alysiella*] sp. IAM 14971 are $16:0$ ($26.7\text{--}27.0\%$), $16:0\ N$ alcohol ($22.4\text{--}23.2\%$) and $16:1\omega 7c$ ($10.2\text{--}12.6\%$),

$18:1\omega 9c$ ($7.9\text{--}8.1\%$), $18:3\omega 6c$ ($6.9, 12$) ($6.7\text{--}9.8\%$), $12:0$ ($4.2\text{--}4.3\%$), $18:2\omega 6,9c$ ($3.9\text{--}4.1\%$), $10:0$ ($2.9\text{--}3.7\%$), $14:0$ ($2.1\text{--}2.2\%$) and $18:0$ ($2.4\text{--}3.5\%$); the major hydroxy fatty acid is $3\text{OH-}12:0$ ($3.0\text{--}3.7\%$). [*Alysiella*] sp. IAM 14971 and *Moraxella* species possess $10:0$ and $16:0\ N$ alcohols, but *A. filiformis* does not. The presence of long-chain alcohols in relatively high amounts also seems to be of taxonomic value for *Moraxella* (Rossau *et al.*, 1991). On the other hand, [*Alysiella*] sp. IAM 14971 does not contain significant $18:1\omega 7c$, which is different from other species of the genus *Moraxella*. The G+C content the DNA of [*Alysiella*] sp. IAM 14971 is $40.5\ \text{mol}\%$ (Rossau *et al.*, 1991), which is 5% lower than that of the genus *Alysiella* but similar to that of the genus *Moraxella*.

The 16S rRNA gene sequences of 12 species of the genus *Moraxella* and related genera of the family *Moraxellaceae* were analysed and a phylogenetic tree was constructed using the neighbour-joining method with 1181 nucleotides positions calculated (Fig. 2). These comparisons revealed that [*Alysiella*] sp. IAM 14971 should be included within the genus *Moraxella* ($96\text{--}97\%$ sequence similarity compared with 93% similarity to members of the genus *Psychrobacter*). The phylogenetic analysis of 16S rRNA gene sequences was consistent with the results of DNA-rRNA hybridization, indicating that [*Alysiella*] sp. IAM 14971 clustered together with the *M. (M.) lacunata* subgroup within the genus *Moraxella* (Rossau *et al.*, 1991). The significant node (100% bootstrap value) positioned at the branch point between [*Alysiella*] sp. IAM 14971 and the main *Moraxella* cluster indicated a close phylogenetic relationship. Therefore, [*Alysiella*] sp. IAM 14971 is suggested to represent a novel species of the genus *Moraxella*, *Moraxella oblonga* sp. nov. (type strain, IAM 14971^T=ATCC 29468^T). The cellular morphology of cocci in chains clearly differentiates this novel species from other species of the genus *Moraxella*.

Similar to other species of the genus *Moraxella*, which can be isolated from the mucous membranes of man and/or other animals and can typically grow on ordinary blood agar medium at $37\ ^\circ\text{C}$, *M. oblonga* IAM 14971^T was isolated from the oral cavity of a sheep. *M. oblonga* IAM 14971^T has characteristics similar to those of other members of the genus *Moraxella* (Bøvre, 1984). Its cells are aerobic, oxidase-positive, catalase-positive cocci. No acid is produced from glucose, and negative reactions result with indole and urea. The cells have a signature fatty acid composition with $16:0\ N$ alcohol as one of the major fatty acids, and the major hydroxy fatty acid is $3\text{OH-}12:0$. The G+C content of the DNA is $40.5\ \text{mol}\%$, which is within the typical range of *Moraxella* species ($40\text{--}45.0\%$; Bøvre, 1984).

Description of *Moraxella oblonga* sp. nov.

Moraxella oblonga (ob.lon'ga. L. fem. adj. *oblonga* elongated, stretched out).

Cells are cocci that are organized in chains of $\geq 30\ \mu\text{m}$ in length. Cells are $0.48\text{--}1.2\ \mu\text{m}$ in diameter. Catalase- and

Fig. 1. Scanning electron micrographs of (a) *Moraxella oblonga* sp. nov. IAM 14971^T and (b) *A. filiformis* IAM 14968^T. (a) The cocci are linked in chains. Bar, $2.4\ \mu\text{m}$. (b) The individual cells are of the same width, while those in the middle filament are sunk and appear ribbon-like. The ends of the individual filaments are square. Bar, $4\ \mu\text{m}$.

Table 1. Phenotypic features of *Moraxella oblonga* sp. nov. IAM 14971^T and related bacteria

Taxa: 1, *M. oblonga* IAM 14971^T; 2, *A. filiformis* IAM 14895^T; 3, *M. (M.) bovis* CCUG 2133^T; 4, *M. (M.) lacunata* CCUG 4441^T; 5, *M. (B.) catarrhalis* ATCC 25238^T. Data for *Moraxella* species are from Bøvre (1984), Jannes *et al.* (1993) and Vandamme *et al.* (1993). +, Positive reaction; –, negative reaction; ND, no data. All the strains are catalase- and oxidase-positive, and do not hydrolyse urea or produce indole; their major hydroxy fatty acid is 3OH-12:0, and they have ubiquinone Q-8.

Characteristics	1	2	3	4	5
Isolation	Sheep, oral	Sheep, oral	Horse	Human	Human
Morphology	Cocci in chains	Filamentous	Rods	Rods	Cocci
Nitrate reduction	–	–	+	+	–
Acid production from:					
Glucose	–	+	–	–	–
Hydrolysis of:					
Lysine	–	–	+	+	+
Ornithine	–	–	ND	ND	ND
Arginine	+	–	+	–	–
Gelatin	+	–	+	–	–
Major fatty acid composition	16:0 N alcohol, 16:0	16:1ω7c, 16:0	18:1 <i>cis</i> 9, 16:1 <i>cis</i> 9	18:1 <i>cis</i> 9, 16:1 <i>cis</i> 9	18:1 <i>cis</i> 9, 16:1 <i>cis</i> 9
G + C content (mol%)	40.5	45.4	42.5	43.9	41.0

oxidase-positive. Nitrate is not reduced. Acid is produced from mannitol, adonitol, inositol, rhamnose, sucrose, arabinose and maltose, but not from glucose. Hydrolyses

arginine and liquefies gelatin. Does not hydrolyse urea or lysine, decarboxylate ornithine, or produce indole. The major cellular fatty acids are 16:0, 16:0 *N* alcohol and

Fig. 2. Neighbour-joining tree, constructed from 16S rRNA gene sequences, showing the phylogenetic position of *Moraxella oblonga* sp. nov. IAM 14971^T compared with species of the genus *Moraxella* and related bacteria. Numbers at the nodes indicate bootstrap values (1000 replicates); only values greater than 60% are shown.

16:1 ω 7c, and the major hydroxy fatty acid is 3OH-12:0. The major quinone system is ubiquinone Q-8. The G+C content of the DNA is 40.5 mol%.

The type strain is IAM 14971^T (= ATCC 29468^T), which was isolated from the oral cavity of a sheep.

References

- Bøvre, K. (1979).** Proposal to divide the genus *Moraxella* Lwoff 1939 emend. Henriksen and Bøvre 1968 into two subgenera, subgenus *Moraxella* (Lwoff 1939) Bøvre 1979 and subgenus *Branhamella* (Catlin 1970) Bøvre 1979. *Int J Syst Bacteriol* **29**, 403–406.
- Bøvre, K. (1984).** Genus II. *Moraxella* Lwoff 1939, 173 emend. Henriksen and Bøvre 1968, 391^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 296–302. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gherna, R., Pienta, P. & Cote, R. (1989).** *American Type Culture Collection (ATCC) Catalogue of Bacteria and Phages*, 17th edn, p.15.
- Jannes, G., Vanechoutte, M., Lannoo, M., Gillis, M., Vancanneyt, M., Vandamme, P., Verscharegen, G., Van Heuverswyn, H. & Rossau, R. (1993).** Polyphasic taxonomy leading to the proposal of *Moraxella canis* sp. nov. for *Moraxella catarrhalis*-like strains. *Int J Syst Bacteriol* **43**, 438–449.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Rossau, R., Van Landschoot, A., Mannheim, W. & De Ley, J. (1986).** Inter- and intrageneric similarities of ribosomal ribonucleic acid cistrons of the *Neisseriaceae*. *Int J Syst Bacteriol* **36**, 323–332.
- Rossau, R., Vandenbussche, G., Thielemans, S., Segers, P., Grosch, H., Göthe, E., Mannheim, W. & De Ley, J. (1989).** Ribosomal ribonucleic acid cistron similarities and deoxyribonucleic acid homologies of *Neisseria*, *Kingella*, *Eikenella*, *Simonsiella*, *Alysiella*, and Centers for Disease Control groups EF-4 and M-5 in the emend family *Neisseriaceae*. *Int J Syst Bacteriol* **39**, 185–198.
- Rossau, R., Van Landschoot, A., Gillis, M. & De Ley, J. (1991).** Taxonomy of *Moraxellaceae* fam. nov., a new bacterial family to accommodate the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* and related organisms. *Int J Syst Bacteriol* **41**, 310–319.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Swofford, D. L. (1998).** PAUP* – Phylogenetic Analysis Using Parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Vandamme, P., Gillis, M., Vancanneyt, M., Hoste, B., Kersters, K. & Falsen, E. (1993).** *Moraxella lincolnii* sp. nov., isolated from the human respiratory tract, and reevaluation of the taxonomic position of *Moraxella osloensis*. *Int J Syst Bacteriol* **43**, 474–481.
- Xie, C.-H. & Yokota, A. (2003).** Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.