

Reclassification of *Subtercola pratensis* Behrendt *et al.* 2002 as *Agreia pratensis* comb. nov.

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Comparative analysis of 16S rDNA sequences revealed a close phylogenetic relationship (99.6% similarity) between *Subtercola pratensis* Behrendt *et al.* 2002 and *Agreia bicolorata* Evtushenko *et al.* 2001. The two species were found to share genus-specific chemotaxonomic characteristics such as the occurrence of D-ornithine and L-2,4-diaminobutyric acid in the peptidoglycan and the profile of cellular fatty acids and 1,1-dimethoxy-alkanes. DNA–DNA relatedness of only 47.8% and differences in phenotypic features such as the menaquinone profile and oxidase and Voges–Proskauer reactions confirmed the distinct species status of *S. pratensis* and *A. bicolorata*. On the basis of the data from phylogenetic and phenotypic analyses, the reclassification of *S. pratensis* as *Agreia pratensis* comb. nov. is proposed. As a result of this reclassification, the two genera are coherent, in that the cell wall composition and 1,1-dimethoxy-alkane spectrum are significant genus-specific characteristics.

Studies of bacterial communities in various environments are now being performed increasingly fast by the application of techniques and approaches for determining genetic polymorphism. Novel taxa can be detected rapidly by the comparison of 16S rDNA sequences with those of type strains available from comprehensive public databases. As result of this development, a multitude of new taxa are currently being proposed, and the probability of coincident submissions of manuscripts describing highly similar novel organisms is increasing.

Without being aware of the similarity of their research subjects, two research groups isolated strains of coryneform bacteria that contained a hitherto novel B-type peptidoglycan containing ornithine and 2,4-diaminobutyric acid (DAB). One group decided in favour of proposing the new genus *Agreia* to accommodate the isolates (Evtushenko *et al.*, 2001), whereas the other submitted a proposal for a novel species of the phylogenetically closest genus *Subtercola*,

Subtercola pratensis (Behrendt *et al.*, 2002), during the review process of the description of *Agreia*. For obvious reasons, the type strains of *Agreia bicolorata* and *S. pratensis* could not be compared at that time. The taxonomic status of *A. bicolorata* and *S. pratensis* had to be reconsidered after valid publication of both names. The type strains *A. bicolorata* DSM 14575^T and *S. pratensis* DSM 14246^T were therefore subjected to supplementary chemotaxonomic studies, detailed phylogenetic analyses and DNA–DNA hybridization.

To clarify whether *S. pratensis* and *A. bicolorata* indeed display the same type of peptidoglycan, enantiomeric diamino acid isomers were determined according to Sasaki *et al.* (1998). The type strain of *A. bicolorata* and the type strain of *S. pratensis* contained both D-Orn and L-DAB in almost equal amounts in their cell walls and thus can be clearly differentiated from *Subtercola boreus* and *Subtercola frigoramans*, which contain only DAB in their peptidoglycan. *S. pratensis* and *A. bicolorata* display identical two-dimensional TLC patterns of peptides and amino acids resulting from partial hydrolysis (Schleifer, 1985) of the peptidoglycan (data not shown). Dinitrophenylation (Schleifer, 1985) of peptidoglycan samples of *S. pratensis* and *A. bicolorata* revealed free amino groups at Orn and

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Abbreviations: DAB, 2,4-diaminobutyric acid; a-15:0 DMA, 1,1-dimethoxy-anteiso-pentadecane; i-16:0 DMA, 1,1-dimethoxy-iso-hexadecane; a-17:0 DMA, 1,1-dimethoxy-anteiso-heptadecane.

DAB. Since position 3 of the peptide subunit of all known B-type peptidoglycan structures contains an L-isomeric amino acid (Schleifer & Kandler, 1972; DSMZ, 2001), it may be concluded that L-DAB occupies position 3 of the peptide subunit and D-Orn is involved in the interpeptide bridge. Muramic acid residues of the peptidoglycans of both *S. pratensis* and *A. bicolorata* are acetylated, as determined according to Uchida *et al.* (1999). These results suggest that *S. pratensis* and *A. bicolorata* display the same peptidoglycan structure.

A further chemotaxonomic feature, the profiles of fatty acid methyl esters and 1,1-dimethoxy-alkanes of *A. bicolorata* and *S. pratensis* type strains, was analysed by GC and GC/MS according to Schumann *et al.* (1997). As shown in Table 1, similar patterns were found for the two strains. Supplementary to the report of Evtushenko *et al.* (2001), it was found that *A. bicolorata* also contained 1,1-dimethoxy-anteiso-pentadecane (a-15:0 DMA). Comparison of profiles with those of *S. boreus* and *S. pratensis* (Männistö *et al.*, 2000) revealed a similar spectrum for iso- and anteiso-branched fatty acids, but differences were found in the composition of 1,1-dimethyl-alkanes. In addition to a-15:0 DMA, 1,1-dimethoxy-iso-hexadecane (i-16:0 DMA) and 1,1-dimethoxy-anteiso-heptadecane (a-17:0 DMA) constitute the predominant 1,1-dimethyl-alkanes of *S. boreus* and *S. frigoramans* (Table 1). Accordingly, the 1,1-dimethoxy-alkane profile is an additional chemotaxonomic feature useful for discrimination of the genera *Agreia* and *Subtercola*.

Table 1. Fatty acid composition of *A. bicolorata*, *S. pratensis*, *S. frigoramans* and *S. boreus*

Values are percentages of total fatty acids. Strains: 1, *A. bicolorata* VKM Ac-1804^T; 2, *S. pratensis* DSM 14642^T; 3, *S. frigoramans* DSM 13057^T; 4, *S. boreus* DSM 13056^T. Data for *S. frigoramans* and *S. boreus* were taken from Männistö *et al.* (2000). The growth temperature was 25 °C. tr, Trace amount (<1%).

Fatty acid	1	2	3	4
14:0	–	0.25	–	–
16:0	2.66	5.22	–	–
i-14:0	1.60	0.53	6.7	tr
i-15:0	1.02	1.00	tr	4.3
i-16:0	30.09	17.23	10.2	4.2
i-17:0	–	0.51	–	–
a-15:0	48.07	46.71	46.1	51.6
a-17:0	14.43	22.15	6.8	3.5
a-15:1	–	–	–	tr
16:0 DMA	–	–	–	tr
i-15:0 DMA	–	–	–	1.7
i-16:0 DMA	–	–	13.3	11.9
a-15:0 DMA	2.13	4.17	10.3	11.0
a-17:0 DMA	–	–	2.9	4.0
Unknown	–	2.22	2.1	4.6

Analysis of the phylogenetic relationship between species of the genera *Agreia* and *Subtercola*, on the basis of 16S rDNA sequences, was performed as described by Behrendt *et al.* (2002). *S. pratensis* clustered with *A. bicolorata* with both the neighbour-joining and maximum-likelihood methods, displaying the high sequence similarity of 99.6% (Figs 1 and 2). This branch was supported by a high bootstrap value, as was the clustering of both with *S. boreus* and *S. frigoramans*. However, the originally described species *S. boreus* and *S. frigoramans* (Männistö *et al.*, 2000) did not form a monophyletic branch. The similarity of *S. boreus* to *S. pratensis* and *A. bicolorata* was up to 1% higher than its similarity to *S. frigoramans*. Moreover, clustering of *S. frigoramans* and *S. boreus*, as shown in Fig. 1, was not supported by bootstrap analysis and could not be confirmed by either the maximum-likelihood method (Fig. 2) or the consensus tree, according to Felsenstein (1993). These results show that the genera *Subtercola* and *Agreia* cannot be differentiated unambiguously on the basis of phylogenetic analyses. A comprehensive phylogenetic comparison of the two genera could not be performed by Evtushenko *et al.* (2001) since the publication of the genus *Subtercola* and the review process of the description of the genus *Agreia* coincided.

On the basis of the aforementioned results, it appears reasonable to affiliate strain DSM 14246^T to the genus *Agreia*. However, it remained to be investigated whether strain DSM 14246^T represents an individual species or whether it is a strain of *A. bicolorata*. The intraspecific relationship of the type strains of *S. pratensis* and *A. bicolorata* was examined by DNA–DNA hybridization using 2 × SSC containing 10% (v/v) DMSO as hybridization buffer at 68 °C (Escara & Hutton, 1980; Huß *et al.*, 1983; Jahnke, 1992). The DNA–DNA relatedness of the type strains DSM 14246^T and DSM 14575^T was found to be 47.8%. The finding that the two type strains represent different genomospecies according to Wayne *et al.* (1987) is supported by several differentiating phenotypic features. In contrast to *S. pratensis*, cells of *A. bicolorata* are motile and positive for the oxidase and Voges–Proskauer reactions. Furthermore, the isoprenoid quinones of *A. bicolorata* are dominated by MK-10, whereas those of *S. pratensis* predominantly comprise MK-10 and MK-11.

The polyphasic approach to the definition of a new genus is based on consideration of both phylogenetic relationships and phenotypic properties. When 16S rDNA sequence comparison and chemotaxonomic analyses provide discordant results, selection of the decisive dataset requires taxonomic expertise. The unification of the genera *Microbacterium* (containing Lys in the peptidoglycan) and *Aureobacterium* (containing Orn in the peptidoglycan) by Takeuchi & Hatano (1998) is an example of attributing priority to analyses of partial 16S rDNA sequence similarity for genus allocation. On the other hand, the recent re-evaluation of the genus status of *Oerskovia* (unified earlier with the phylogenetically related genus *Cellulomonas*) by

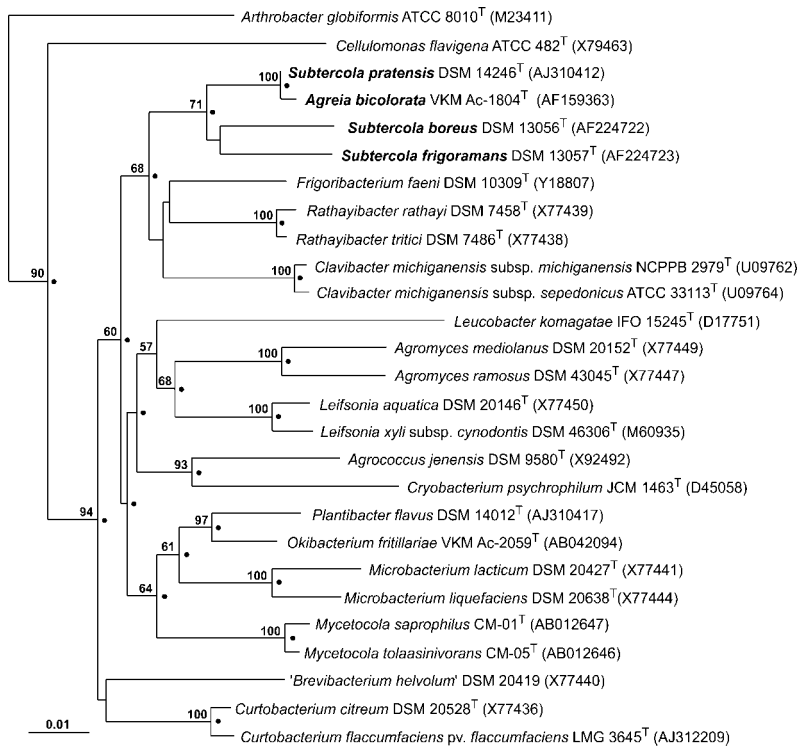


Fig. 1. Phylogenetic tree showing the relationships of *Subtercola* and *Agreia* species within the family *Microbacteriaceae*. The tree is based on a 1486 bp alignment of 16S rDNA sequences and was constructed using the neighbour-joining method (Saitou & Nei, 1987). Dots indicate branches of the tree that were also formed using the maximum-likelihood method (Felsenstein, 1981). To estimate the root position of the tree, *Brevibacterium linens* ATCC 9172^T (X77451) was used as an outgroup. Each value is the number of times that a branch appeared in 100 bootstrap replications. The bar indicates the relative sequence divergence.

Stackebrandt *et al.* (2002) showed that genus-specific chemotaxonomic features can also be of greater concern when defining a genus. Thus, the close phylogenetic relationship but low DNA–DNA relatedness of *S. pratensis* and *A. bicolorata* and the clear differentiation of both from the original psychrophilic species of *Subtercola* on the basis of chemotaxonomic features support the separate genus status of *Agreia* and, hence, we propose the reclassification of *S. pratensis* as *Agreia pratensis* comb. nov.

Description of *Agreia pratensis* (Behrendt *et al.* 2002) comb. nov.

Agreia pratensis (pra.ten' sis. L. fem. adj. *pratensis* pertaining to meadows/grassland).

Basonym: *Subtercola pratensis* Behrendt *et al.* 2002.

The description is as given by Behrendt *et al.* (2002). The type strain is DSM 14246^T (= P 229/10^T = LMG 21000^T).

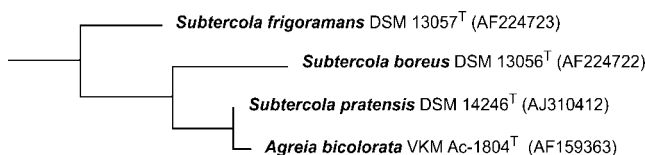


Fig. 2. Part of the maximum-likelihood tree showing the relationship between *Subtercola* and *Agreia* species. The tree is based on 1486 bp and was constructed using the DNAML program (Felsenstein, 1993).

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