Diversity of grass-associated Microbacteriaceae isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of Subtercola pratensis sp. nov., Curtobacterium herbarum sp. nov. and Plantibacter flavus gen. nov., sp. nov.

Undine Behrendt,1 Andreas Ulrich,2 Peter Schumann,3 Dieter Naumann4 and Ken-ichiro Suzuki5

A representative selection of coryneform bacteria, isolated from the phyllosphere of grasses and the litter layer after mulching the sward, was characterized by a polyphasic approach to clarify their taxonomic position in the family Microbacteriaceae, with particular reference to potentially plant-pathogenic bacteria. On the basis of 16S rDNA analysis, the isolates can be classified into six genotypes representing the genera Curtobacterium, Clavibacter, Subtercola and a subgroup, which was not affiliated to a known genus. One genotype, belonging to the genus Curtobacterium, had an identical 16S rDNA sequence to reference strains of the Curtobacterium flaccumfaciens pathovars. Another genotype, closely related to the potentially pathogenic Curtobacterium flaccumfaciens, could be distinguished from known species of the genus on the basis of phylogenetic and phenotypic characterization and is consequently proposed as a novel species, Curtobacterium herbarum sp. nov. (type strain P 420/07T = DSM 14013T = LMG 19917T). Two genotypes assigned to Clavibacter showed a close relationship to Clavibacter michiganensis subsp. tessellarius, a pathogenic bacterium causing foliar lesions on wheat. A further genotype, which clustered clearly in the genus Subtercola by comparison of 16S rDNA sequences, showed a hitherto undescribed B-type of peptidoglycan containing the diagnostic diamino acids ornithine and 2,4-diaminobutyric acid, in the cell wall; this genotype is proposed as Subtercola pratensis sp. nov. (type strain P 229/10T = DSM 14246T = LMG 21000T). For one genotype, which formed a phylogenetically separate branch in the family of Microbacteriaceae showing chemotaxonomic similarities to the genus Rathayibacter, a novel genus, Plantibacter gen. nov., is proposed; the type species is Plantibacter flavus sp. nov. (type strain P 297/02T = DSM 14012T = LMG 19919T).

Keywords: plant-associated bacteria, polyphasic analysis, Subtercola pratensis sp. nov., Curtobacterium herbarum sp. nov., Plantibacter flavus gen. nov., sp. nov.

Abbreviations: DAB, 2,4-diaminobutyric acid; FT-IR spectroscopy, Fourier-transform infrared spectroscopy.

The EMBL accession numbers for the 16S rDNA sequences analysed in this study are AJ310412 (Subtercola pratensis P 229/10T = DSM 14246T = LMG 21000T), AJ310413 (Curtobacterium herbarum P 420/07T = DSM 14013T = LMG 19917T), AJ310414 (Curtobacterium flaccumfaciens P 259/26), AJ310415 (Clavibacter michiganensis P 202/10), AJ310416 (Clavibacter michiganensis P 250/01), AJ310417 (Plantibacter flavus P 297/02T = DSM 14012T = LMG 19919T) and AJ312209 (Curtobacterium flaccumfaciens pv. flaccumfaciens LMG 3645T).
INTRODUCTION

The phyllosphere of plants is a typical habitat for coryneform bacteria displaying the B-type of peptidoglycan. Several ecological studies have shown that this group forms a significant part of bacterial communities of a number of plant species (Austin et al., 1978; Bell et al., 1995; Ercolani, 1991; Legard et al., 1994; McInroy & Kloeper, 1995; Thompson et al., 1993). Because of difficulties in taxonomic characterization on the basis of phenotypic features, most of the isolated strains have been identified at the genus level or affiliated to Curtobacterium flaccumfaciens and Clavibacter michiganensis without further specification at the subspecies or pathovar level.

Studies of the microbial communities on grasses have also revealed a high abundance of coryneform bacteria, forming up to 80% of the culturable bacterial population (Behrendt, 2001). In accordance with Harris-Baldwin & Gudmestad (1996), use of the Biolog system, which is supported by some chemotaxonomic and physiological features, for identification has led to unsatisfactory taxonomic results leaving specific classification in comparison to potentially phytopathogenic species unresolved.

A variety of methods, proven to be valuable but somewhat limited for the differentiation of this bacterial group, has been used to classify coryneform bacteria on the basis of pheno- or genotypic features. In particular, plant-pathogenic coryneform organisms form a very homogeneous group and therefore, numerical analyses on the basis of phenotypic features are often inadequate to classify them at the species level (Kämpfer & Kroppenstedt, 1996; Kämpfer et al., 1993; Seiler, 1983). Classification by molecular methods like 16S rDNA restriction analysis have been shown to be useful for species and subspecies of the genera Rathayibacter and Clavibacter (Lee et al., 1997). However, temperature-gradient gel electrophoresis of 16S rRNA genes of coryneforms revealed a genus-dependent differentiation at the species or genus level or intermediate between them (Felske et al., 1999). In contrast, recent studies on coryneform bacteria based on Fourier-transform infrared (FT-IR) spectroscopy showed the high resolution power of this method at the species level, which also allowed an infraspecific classification, as demonstrated for Brevibacterium linens, Rhodococcus erythropolis and Corynebacterium glutamicum (Oberreuter, 2001). Thus, phenotypic classification by FT-IR spectroscopy, classical methods of physiological and chemotaxonomic characterization, in comparison with phylogenetic analysis by 16S rDNA and DNA–DNA hybridization, were used to study the grass-associated isolates with particular reference to plant-associated coryneforms.

Fifty strains representing major groups were selected based on rough physiological and chemotaxonomic classification to investigate the diversity of the grass-associated coryneforms. Members of the genus Microbacterium were excluded and studied separately (Behrendt et al., 2001). Inasmuch as 16S rDNA restriction analyses of selected isolates revealed six genotypes representing more than one strain, five representative strains of each genotype were chosen for analysis following a polyphasic strategy of classification to clarify their taxonomic position.

METHODS

Bacterial strains and cultivation. The bacterial strains examined in this study were isolated from grasses and surface litter as described previously (Behrendt, 2001). Numbers of the isolates and strains of reference species used for comparative studies are listed in Table 1. General laboratory cultivation was performed on nutrient agar II (SIFIN) or nutrient broth II (SIFIN) at 25 °C unless otherwise stated. Stocks of all cultures were maintained at −70 °C using the Microbank storage system (Pro-Lab Diagnostics).

16S rDNA sequence determination and phylogenetic analysis. Restriction analysis of amplified 16S rDNA was performed as described by Behrendt et al. (1999). Representative strains of genotypes A, B1, B2, C, D and F, respectively, were used in 16S rDNA sequence determinations. The 16S rDNA fragments amplified with Pfu DNA polymerase (Promega) were cloned and sequenced using a cycle sequencing protocol with a Li-Cor Sequencer (model 4200; MWG Biotech) as previously described (Behrendt et al., 2001). The nearly complete 16S rDNA sequences were compared to sequences available from the Ribosomal Database Project and EMBL/GenBank. Similarity values were based on a pairwise comparison of sequences. For phylogenetic analyses, DNA sequences were aligned using the CLUSTAL W algorithm (program version 1.74; Thompson et al., 1994) and the trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms (PHYLP, version 3.57; Felsenstein, 1993). Distance matrices were prepared using DNADIST with a transition/transversion ratio of 2.0 (Kimura, 1980). Tree topologies were evaluated by bootstrap analysis of the neighbour-joining tree using the original dataset and 1000 bootstrap datasets. The root position was estimated using the 16S rRNA sequence of Brevibacterium linens ATCC 9172 (X77451) as outgroup.

DNA–DNA hybridization. DNA–DNA similarity was examined for P 420/077 (genotype D) and Curtobacterium flaccumfaciens pv. flaccumfaciens (LMG 36457), according to the method described by Martin et al. (1997).

Determination of chemotaxonomic characteristics. Methods used for the analyses of menaquinones and G+C values were described previously by Groth et al. (1999). Elucidation of the peptidoglycan structure was accomplished as described previously (Männistö et al., 2000). Analysis of enantiomeric diamino acid isomers was performed according to Sasaki et al. (1998). Fatty acid methyl esters and 1,1-dimethoxy-alkanes were analysed by GC and GC/MS as described by Schumann et al. (1997) using lyophilized biomass obtained after incubation of strains for 48 h at 28 °C. Determination of the glycolate content was performed according to the colorimetric method of Uchida et al. (1999).

Morphological, physiological and biochemical characterization. Cell morphology was determined by light microscopy of cells grown for 24 and 72 h. Motility of cells was
Novel grass-associated Microbacteriaceae

Table 1. Reference strains and isolated strains used in this study

<table>
<thead>
<tr>
<th>Species/genotype of grass isolates</th>
<th>Collection no. (corresponding no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curtobacterium flaccumfaciens pv. flaccumfaciens</td>
<td>LMG 3645T (= ICMP 2584T = NCPPB 1446T); DSM 20129 (= ATCC 6887 = NCTC 4758)</td>
</tr>
<tr>
<td>Curtobacterium flaccumfaciens (Corynebacterium flaccumfaciens subsp. aurantiacum) pv. flaccumfaciens (Corynebacterium flaccumfaciens subsp. violaceum) pv. betae</td>
<td>DSM 20135 (= ATCC 12813)</td>
</tr>
<tr>
<td>pv. poinsettiae</td>
<td>DSM 20149 (= ATCC 9682 = CCM 1587); LMG 3713 (= ICMP 2563 = NCPPB 849)</td>
</tr>
<tr>
<td>pv. oortii</td>
<td>LMG 3702 (= ATCC 25283 = ICMP 2632 = ICPB CO101 = NCPPB 2113); LMG 3704 (= ICMP 3498 = NCPPB 2240)</td>
</tr>
<tr>
<td>Curtobacterium luteum</td>
<td>DSM 20542T (= ATCC 15830T = CCM 2298T = NCIB 11029T)</td>
</tr>
<tr>
<td>Curtobacterium citreum</td>
<td>DSM 20528T (= ATCC 15828T = CCM 2297T = IAM 1614T = NCIB 10702T)</td>
</tr>
<tr>
<td>Curtobacterium pusillum</td>
<td>DSM 20527T (= ATCC 19096T = IAM 1479T = NCIB 10354T)</td>
</tr>
<tr>
<td>Curtobacterium albidi</td>
<td>DSM 20512T (= ATCC 15831T = CCM 2296T = IAM 1631T = NCIB 11030T)</td>
</tr>
<tr>
<td>Clavibacter michiganensis subsp. tellus</td>
<td>DSM 20741T (= ATCC 33566T = CIP 10364T)</td>
</tr>
<tr>
<td>subsp. nebraskensis</td>
<td>DSM 7483T (= ATCC 27794T = CIP 10362T = ICMP 3298T = NCPPB 2581T)</td>
</tr>
<tr>
<td>subsp. michiganensis</td>
<td>DSM 46364T (= ICMP CM 177T = LMG 7333T = NCPPB 2979T)</td>
</tr>
<tr>
<td>subsp. insidiosus</td>
<td>DSM 20157T (= ATCC 10253T = NCPPB 1109T)</td>
</tr>
<tr>
<td>subsp. sepedonicus</td>
<td>DSM 20744T (= ATCC 33113T = ICMP CS101T = NCPPB 2137T)</td>
</tr>
<tr>
<td>Rathayibacter tritici</td>
<td>DSM 7486T (= ATCC 11403T = ICMP 2626T = NCPPB 1857T = VKM Ac-1603T)</td>
</tr>
<tr>
<td>Rathayibacter iranicus</td>
<td>DSM 7484T (= ICMP 3496T = NCPPB 2253T = VKM Ac-1602T)</td>
</tr>
<tr>
<td>Rathayibacter rathayi</td>
<td>DSM 7485T (= ICMP 2574T = NCPPB 2980T = VKM Ac-1601T)</td>
</tr>
<tr>
<td>Rathayibacter toxicus</td>
<td>DSM 7488T (= ATCC 49908T = ICMP 9525T = NCPPB 3552T)</td>
</tr>
</tbody>
</table>

Genotype

| A | P 259/26; P 412/02; P 420/01; P 425/08; P 429/02 |
| B1 | P 202/10; P 211/03; P 219/02; P 221/03; P 231/03 |
| B2 | P 250/01; P 251/03; P 251/08; P 251/06; P 251/07 |
| C | P 297/02T (= DSM 14012T = LMG 19919T); P 309/02; P 259/33; P 294/03; P 334/04 |
| D | P 420/07T (= DSM 14013T = LMG 19917T); P 220/02; P 445/13; P 407/01; P 433/01 |
| F | P 229/10T (= DSM 14246T = LMG 21000T); P 209/03; P 209/05; P 243/03; P 243/10 |

tested by the hanging drop method of Rudolph & Marvidis (1990). The Gram reaction was tested by the classical staining procedure as described by Süßmuth et al. (1987), the rapid KOH string test (Ryu, 1938), growth on MacConkey agar (Merck) and by the presence of l-alanine aminopeptidase (Bactident test strips; Merck). Most tests for characterizing the biochemical profiles of studied strains were performed as described previously (Behrendt et al., 1999). Hydrolysis of Tween and starch was assayed according to Sands (1990). The ability to grow anaerobically was tested using Anaerocult A (Merck).

Fourier-transform infrared (FT-IR) spectroscopy of bacterial cells. Spectral analyses were performed on cells grown for 72 h on CASO agar (Merck) at 25 °C. A small amount of late-exponential-phase cells (approx. 10–60 μg) was removed with a platinum loop from confluent colonies in the second or third quadrant of growth on the agar surface, depending on intensity of growth, and suspended in 30 μl

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distilled water. An aliquot (25 μl) was transferred to a zinc-
selenite optical plate and dried under moderate vacuum
between 2.5 and 7.5 kPa to a transparent film suitable for
absorbance/transmission measurement. All spectra were
recorded between 4000 and 500 cm⁻¹ (wave numbers) on an
IFS-48 FT-IR spectrometer (Bruker) equipped with an MCT
(mercury-cadmium-telluride) detector by co-addition and
averaging 256 scans. Spectral resolution was 8 cm⁻¹.

RESULTS
Phylogenetic characterization
All isolates yielded a single band of about 1500 bp
after amplification with the universal eubacterial
primers used (Weisburg et al., 1991). Digestion of the
PCR products led to three to five different restriction
patterns per enzyme, which were grouped into nine
16S rDNA restriction patterns of the grass isolates and
and, thus, the inability to differentiate them by this
nearly identical 16S rDNA sequences of the pathovars
Curtobacterium flaccumfaciens
pathovars showed identical patterns for
F, each represented by one strain.

Restriction analysis of the Curtobacterium flaccum-
faciens pathovars showed identical patterns for
each endonuclease used (Table 2). This result points to
nearly identical 16S rDNA sequences of the pathovars
and, thus, the inability to differentiate them by this
method. Furthermore, grass isolates of genotype A
revealed the same restriction patterns as pathovars of
Curtobacterium flaccumfaciens indicating that they
also belong to this genus. Sequencing of the type
strain of Curtobacterium flaccumfaciens
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Curtobacterium flaccumfaciens
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nearly identical 16S rDNA sequences of the pathovars
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and, thus, the inability to differentiate them by this
method. Furthermore, grass isolates of genotype A
revealed the same restriction patterns as pathovars of
Curtobacterium flaccumfaciens indicating that they
also belong to this genus. Sequencing of the type
strain of Curtobacterium flaccumfaciens
LMG 3645T) confirmed 16S rDNA identity with genotype A (strain P 259/26).

The phylogenetic relationship of genotypes A–F, as
deetermined using sequences of representative isolates,
is shown in Fig. 1. Isolate P 420/07T (genotype D)
unequivocally clustered into the genus Curtobacterium,
supported by a high bootstrap value (100). Within the
genus, however, the strain formed a separate branch
that was found in both the neighbour-joining and
maximum-likelihood trees. Optimum conformity of
genotype D was found with Curtobacterium flaccum-
faciens pv. flaccumfaciens LMG 3645T (similarity of
98.2%). However, analysis of DNA–DNA re-
association of both strains resulted in a similarity of
35.6%, clearly suggesting the novel species status of
strain P 420/07T (genotype D).

Reference strains of genotypes B1 and B2 clustered
with Clavibacter michiganensis on the basis of 16S
rDNA sequence similarity (Fig. 1). Genotype B1 (P
202/10) showed the highest similarity to Clavibacter
michiganensis subsp. tessellarius (99.8%). Genotype
B2 (P 250/01) displayed a similarity of 99.6% to
Clavibacter michiganensis subsp. tessellarius and to
Clavibacter michiganensis subsp. insidiosus. These
results suggest that isolates of genotypes B1 and B2
belong to the species Clavibacter michiganensis.

Genotype F, represented by P 229/10T, clustered with
Subtercola using both neighbour-joining and
maximum-likelihood algorithms. This branch was also
supported by a high bootstrap value (96). Sequence
similarities of P 229/10T to Subtercola boreus and
Subtercola frigoramans were 96.7 and 95.3%, respec-
tively, which was in the same range as the similarity
between both Subtercola species (95.7%). These results
indicated that genotype F should be assigned to the
species Subtercola as a novel species.

The representative isolate of genotype C (strain P

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Strain</th>
<th>Restriction pattern using endonuclease:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ala</td>
</tr>
<tr>
<td>A</td>
<td>P 259/26; P 412/02; P 420/01; P 425/08; P 429/02; Curtobacterium flaccumfaciens pv. flaccumfaciens (LMG 3645T); Curtobacterium flaccumfaciens pv. betae (LMG 3596); Curtobacterium flaccumfaciens pv. poinsettiae (DSM 20149); Curtobacterium flaccumfaciens pv. oortii (LMG 3702; DSM 3704)</td>
<td>A</td>
</tr>
<tr>
<td>B1</td>
<td>P 202/10; P 211/03; P 219/02; P 221/03; P 231/03</td>
<td>B</td>
</tr>
<tr>
<td>B2</td>
<td>P 250/01; P 251/03; P 251/08; P 251/06; P 251/07</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>P 297/02T (= DSM 14012T); P 294/03; P 334/04; P 309/02</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>P 259/33</td>
<td>C</td>
</tr>
<tr>
<td>D</td>
<td>P 420/07T (= DSM 14013T); P 445/13; P 407/01; P 433/01</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>P 220/02</td>
<td>D</td>
</tr>
<tr>
<td>F</td>
<td>P 229/10T (= DSM 14642T); P 209/03; P 243/03; P 243/10</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>P 209/05</td>
<td>F</td>
</tr>
</tbody>
</table>
Novel grass-associated Microbacteriaceae

Fig. 1. Phylogenetic tree showing the relationship of the isolated genotypes 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 9172T; X77451) was used as an outgroup. The values are the number of times that a branch appeared in 100 bootstrap replications. Strains characterized in this study are in bold. Bar, relative sequence divergence.

297/02T) showed highest 16S rDNA similarity to Microbacterium testaceum (95.6%), followed by Mycetocola saprophilus, Microbacterium keratanolyticum, Mycetocola tolaasinivorans and Microbacterium lacticum at a 95% similarity level. Clustering on the basis of the neighbour-joining algorithm, as well as the maximum-likelihood method (Fig. 1), clearly revealed that strains of genotype C formed a separate branch connected with the cluster of genera Microbacterium and Mycetocola. This separate phylogenetic position indicates that genotype C deserves the status of a novel genus.
Chemotaxonomic characterization

As shown in Table 3, an extensive chemotaxonomic characterization was performed using type strains of the restriction types proposed to represent novel taxa characterized was performed using type strains of the 16S rRNA restriction groups representing a new taxon. As revealed by GC, GC/MS, HPLC and an automatic amino acid analyser. Two additional strains of genotype F (P 209/05 and P 243/03) were included in the analyses. All analytical techniques revealed the combined occurrence of Orn and DAB and both strains showed the same patterns as strain P 229/10T, indicating that this peptidoglycan composition was typical of this group. Predominant menaquinones were MK-10 and MK-11. As shown in Table 3, fatty acid analyses revealed anteiso-methyl-branched and iso-methyl-branched fatty acids in major amounts. Straight-chain saturated fatty acids were also found in a higher proportion than in isolates of genotypes C and D. As revealed by GC/MS analysis, the fatty acid methyl ester extract of strain P 229/10T contained approximately 42% 1,1-dimethoxy-anteiso-pentadecane (ai-15:0 DMA). The G+C contents of strains P 229/10T and P 209/05 were 65 and 66 mol%, re-

Table 3. Chemotaxonomic characteristics of type strains of the 16S rRNA restriction groups representing a new taxon

<table>
<thead>
<tr>
<th>Feature</th>
<th>Genotype D (P 420/07T = DSM 14013T)</th>
<th>Genotype C (P 297/02T = DSM 14012T)</th>
<th>Genotype F (P 229/10T = DSM 14642T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menaquinone composition</td>
<td>MK-7</td>
<td>MK-9</td>
<td>MK-9</td>
</tr>
<tr>
<td>(ratio of peak area)</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Fatty acid composition (%)</td>
<td>14:0</td>
<td>16:0</td>
<td>14:0</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>71</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Diagnostic diamino acid</td>
<td>Orn</td>
<td>L-DAB; d-Orn</td>
<td>L-DAB; d-Orn</td>
</tr>
<tr>
<td>Acyl type</td>
<td>Acetyl</td>
<td>Acetyl</td>
<td>Acetyl</td>
</tr>
</tbody>
</table>
| Noteworthy values are shown in bold. DMA, 1,1-Dimethoxy-anteiso-pentadecane.

Strain P 229/07T (genotype F) showed the peptidoglycan amino acids Gly, Ala and threo-3-hydroxyglutamic acid, but only traces of Glu and a hitherto unknown combination of diagnostic diamino acids in the peptidoglycan, L-DAB and d-Orn. To confirm this cell wall composition, hydrolysates were subjected to analyses by TLC, GC, GC/MS, HPLC and an automatic amino acid analyser. Two additional strains of genotype F (P 209/05 and P 243/03) were included in the analyses. All analytical techniques revealed the combined occurrence of Orn and DAB and both strains showed the same patterns as strain P 229/10T, indicating that this peptidoglycan composition was typical of this group. Predominant menaquinones were MK-10 and MK-11. As shown in Table 3, fatty acid analyses revealed anteiso-methyl-branched and iso-methyl-branched fatty acids in major amounts. Straight-chain saturated fatty acids were also found and in a higher proportion than in isolates of genotypes C and D. As revealed by GC/MS analysis, the fatty acid methyl ester extract of strain P 229/10T contained approximately 42% 1,1-dimethoxy-anteiso-pentadecane (ai-15:0 DMA). The G+C contents of strains P 229/10T and P 209/05 were 65 and 66 mol%, re-
Table 4. Physiological and morphological characteristics of the 16S rRNA restriction types in comparison to reference strains of the genera Curtobacterium, Clavibacter and Rathayibacter

Abbreviations: +, positive reaction; +w, weakly positive; −, negative; d, reaction differs among strains; y, yellow; i, ivory; ly, light yellow; o, orange; y/b, yellow/orange; w, white. Species/genotype: 1, Curtobacterium flaccumfaciens pv. flaccumfaciens; 2, Curtobacterium flaccumfaciens pv. flaccumfaciens (Corynebacterium flaccumfaciens subsp. auranticum); 3, Curtobacterium flaccumfaciens pv. flaccumfaciens (Corynebacterium flaccumfaciens subsp. violaceum); 4, Curtobacterium flaccumfaciens pv. betae; 5, Curtobacterium flaccumfaciens pv. poinsettiae; 6, Curtobacterium flaccumfaciens pv. oortii; 7, genotype A; 8, genotype D; 9, Curtobacterium luteum; 10, Curtobacterium citreum; 11, Curtobacterium psiiulium; 12, Curtobacterium albidum; 13, Clavibacter michiganensis subsp. tensellarius; 14, Clavibacter michiganensis subsp. nebraskensis; 15, Clavibacter michiganensis subsp. michiganensis; 16, Clavibacter michiganensis subsp. insidiosus; 17, Clavibacter michiganensis subsp. sepedonicus; 18, genotype B1; 19, genotype B2; 20, genotype C; 21, Rathayibacter tritici; 22, Rathayibacter iranicus; 23, Rathayibacter rathayi; 24, Rathayibacter toxicus; 25, genotype F.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
| Number of strains | 2 | 1 | 1 | 2 | 2 | 2 | 5 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 5 | 5 | 1 | 1 | 1 | 1 | 5 |
| Colony colour | y | o | y | y | o | o | y | y | ly | i | o | o | y | y | y | y | y | y | y | y | y | y | y |
| Motility | + | + | + | d | d | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hydrolysis of: | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gelatin | + | w | + | d | d | d | d | d | d | d | d | d | w | + | + | + | + | + | + | + | + | + | + |
| Aesculin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Starch | - | - | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| Tween 80 | w | + | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| DNase | w | w | w | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Levan | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Oxidative acid production from: | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adonitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Inositol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Limon | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | d | d | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| d-Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| d-Melezitose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Melibiose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| l-Sorbose | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Raffinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Rhamnose | + | + | + | + | + | + | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d | d |
| Ribose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

* Reaction of the type strain P 420/07 (= DSM 14013 = LMG 19917).
† Reaction of the type strain P 297/02 (= DSM 14012 = LMG 19919).
‡ Reaction of the type strain P 229/10 (= DSM 14642 = LMG 21000).

spectively. This range is in accordance with that of the family Microbacteriaceae.

Analysis of diagnostic diamino acids of genotype A, phylogenetically highly related to Curtobacterium flaccumfaciens, revealed Orn as being typical of the genus. The acetyl residue of the peptidoglycan and the predominant menaquinone, MK-9, observed for the representative strain, P 259/26, also corresponded with Curtobacterium. Strains of genotypes B1 and B2 had DAB in their cell wall, corresponding with the genus Clavibacter.

Morphological and physiological characterization

Strains listed in Table 1 were studied with respect to their phenotypic features. Reference species compared with the grass-associated isolates were chosen for their affiliation to genera known for their potential occurrence in the phyllosphere of plants.

In spite of the taxa they belong to, all strains were negative for spore formation, anaerobic growth, oxidase and the Voges–Proskauer reaction, as well as hydrolysis of cellulose; however, they were catalase-positive and able to form acid from D-mannitol, D-galactose, D-fructose, sucrose, L-arabinose and D-trehalose (excluding strain P 251/07). Furthermore, they oxidatively produced acid from D-mannose, D-xylose and D-glucose, with the exception of Clavibacter michiganensis subsp. sepedonicus, which was negative for these characteristics. The tested strains were Gram-positive but, by the rapid KOH test, a lot of grass isolates behaved like Gram-negative strains. Furthermore, the classical staining procedure gave uncertain results as most of the isolates decolourized easily. The absence of L-alanine aminopeptidase was also difficult to determine because borderline reactions often occurred. Thus, cell wall analysis was the method of choice to make certain of the Gram assignment. To select these problematic strains for cell wall analyses from a collection of isolates also containing Gram-negative strains, a comparison of results of the KOH

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Fig. 2. Classification of coryneform bacteria by FT-IR spectroscopy. Cluster analysis (Wards algorithm) was performed using the mean spectrum of the first derivatives of four spectra determined per strain considering the equally weighted spectral ranges (windows) 901–699 cm\(^{-1}\) and 1201–900 cm\(^{-1}\) for all the tested strains and window 1111–1089 cm\(^{-1}\) to separate genotypes F and C. Abbreviations: C., Curtobacterium; f., flaccumfaciens; b., betae; p., poinsettiae; o., oortii; a., aurantiacum; v., violaceum; Cl., Clavibacter; m., michiganensis; i., insidiosus; s., sepedonicus; t., tessellarius; n., nebraskenis; R., Rathayibacter.

Physiological properties which were appropriate for classification are shown in Table 4. The orange-pigmented strains of genotypes A and D could only be differentiated from one another by acid formation from adonitol. In particular, genotype A was physiologically highly similar to the pathovars of Curtobacterium flaccumfaciens. Separation on the basis of tested features was not possible. Pathovars of Curtobacterium flaccumfaciens were also highly similar to each other and no feature was evident for a corresponding classification. In contrast, the remaining species of the genus Curtobacterium could be differentiated from genotypes A and D by comparison of the following characteristics: hydrolysis of aesculin and Tween 80 in combination with acid production from adonitol and raffinose.

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Strains of genotypes B1 and B2, which were phylogenetically highly related to subspecies of Clavibacter michiganensis, differed from one another only in acid formation from d-melezitose and colony colour. Apart from the slow growing Clavibacter michiganensis subsp. sepedonicus, which was mainly physiologically inactive, comparison between type strains of the subspecies and the grass-associated isolates revealed more differences in acid production from carbohydrates than when the subspecies were compared to each other (Table 4). Accordingly, the grass isolates of genotype B formed a physiologically separate group which cannot be affiliated to a known subspecies of Clavibacter on the basis of tested characteristics.

Strains of genotype C that phylogenetically represent a novel genus could be differentiated from the chemo-taxonomically similar species of Rathayibacter by the hydrolyses of Tween 80 and DNase. Furthermore, the genotype C organisms oxidatively formed acid from d-melezitose, raffinose, l-rhamnose and ribose, in contrast to Rathayibacter species. Higher physiological similarity was revealed to the yellow-pigmented strains of genotype B2. As shown in Table 4, genotype C strains differed only in Tween hydrolyses and production of acid from inositol and ribose. Similar results were obtained for a comparison with genotype F.
which can be distinguished by DNase and acid formation from lactose and D-melezitose.

Genotype F could be differentiated from the remaining 16S rDNA genotypes, as well as from Clavibacter and Curtobacterium, by acid production from inulin. Species of Rathayibacter were dissimilar in hydrolyses of Tween 80 and acid production from l-raffinose and ribose.

Finally, it can be concluded that the tested grass isolates and reference strains of related species were physiologically very similar. There were only a few differentiating characteristics, which were useful for a grouping corresponding to the phylogenetic classification.

FT-IR spectroscopy

Classification on the basis of FT-IR spectra was performed using three representative isolates of each restriction type and reference strains listed in Table 1. As shown in Fig. 2, cluster analysis on the basis of the selected spectral regions, 901–699 cm⁻¹ and 1201–900 cm⁻¹, showed a phenetic classification where most of the tested strains were grouped according to the genus affiliation based on 16S rDNA similarity. Only strains of genotypes C and F formed a mixed cluster close to some Curtobacterium species. Analysing this cluster by another spectral window (1111–1089 cm⁻¹) allowed a clear classification according to phylogenetic typing (Fig. 2).

Strains belonging to the genus Curtobacterium were divided up into two clusters in the spectral range shown in Fig. 2. In particular, the pathovars of Curtobacterium flaccumfaciens were subdivided. One cluster included Curtobacterium flaccumfaciens pv. flaccumfaciens, Curtobacterium flaccumfaciens pv. poinsettiae and the tested isolates of genotype A. In the other group, Curtobacterium flaccumfaciens pv. betae, Curtobacterium flaccumfaciens pv. oortii and the genotype D isolates clustered together. Furthermore, Curtobacterium flaccumfaciens pv. flaccumfaciens, formerly assigned to Corynebacterium flaccumfaciens subsp. violaceum, was found in this group, whereas the strain of Curtobacterium flaccumfaciens pv. flavescens, formerly assigned to Corynebacterium flaccumfaciens subsp. aurantiaci, clustered in the first group. Separated analysis of further spectral regions of the Curtobacterium flaccumfaciens pathovars revealed no differences in clustering according to pathovar classification. Strain-specific dissimilarities were higher than pathovar-specific spectral vibrations. Accordingly, genotype A organisms could not be distinguished from this group, whereas genotype D strains and representatives of further Curtobacterium species could be separated by analysing additional spectral windows.

DISCUSSION

Taxonomic analyses of coryneform bacteria associated with the phyllosphere of grasses and the surface litter after mulching the sward revealed a phylogenetically highly diverse assemblage. In addition to groups closely related to potentially phytopathogenic species, bacteria representing novel taxa have been isolated.

Characterization of strains belonging to 16S rDNA genotype A showed that they are epiphytically living strains of the species Curtobacterium flaccumfaciens, which comprises phytopathogenic pathovars of several plant species (Collins & Jones, 1983). In contrast to the subspecies of Clavibacter michiganensis, which can be grouped on the basis of 16S rDNA restriction patterns as shown by Lee et al. (1997), genotype A and Curtobacterium flaccumfaciens pathovars displayed no dissimilarities in 16S rDNA sequence. As with results of other studies (Dye & Kemp, 1977; Vidaver & Davis, 1949), analysis of morphological and physiological properties revealed no phenotypic markers for a valid classification of pathovars. Davis et al. (1984) showed a combination of differential physiological characteristics, which are partly in contrast to the results of the studies mentioned above. Evaluation of the reliability of the Biolog identification system to classify pathovars of Curtobacterium by utilization of 95 carbon sources (Harris-Baldwin & Gudmestad, 1996) confirmed the difficulties in distinguishing pathovars on the basis of physiological features. Furthermore, comparison of FT-IR spectra, a method proven to show infraspecific diversity between strains possessing high 16S rDNA conformity (Oberreuter, 2001), enabled differentiation between a subset of two pathovars only. Further specific pathovar differences to enable complete classification were not detected as strain-specific spectral vibrations were dominant. These results indicate that all four pathovars, as well as the grass isolates of genotype A, are highly similar genetically and their biochemical and physiological traits reflect these close relationships.

The representative strain of genotype D was assigned to the genus Curtobacterium on the basis of 16S rDNA sequence comparison. Chemotaxonomic features like respiratory quinones, fatty acid composition, G+C content and peptidoglycan type of the cell wall also matched the genus description. Phylogenetic analysis significantly demonstrated the separate position within the monophyletic branch of Curtobacterium, indicating novel species status (Fig. 1). DNA–DNA hybridization studies supported this finding. Analysis of FT-IR spectra confirmed the separate position of genotype D on the basis of phenotypic characteristics. However, physiological properties of this group are very similar to those of grass isolates of genotype A, as well as the Curtobacterium flaccumfaciens pathovars, a general problem becoming more and more noticeable in the description of novel taxa (Behrendt et al., 2001; Pukall et al., 1999). The only effective feature to distinguish them from each other was the ability to
form acid from adonitol (Table 4). Furthermore, genotype D could also be distinguished from the other described species of Curtobacterium (Curtobacterium luteum, Curtobacterium citreum, Curtobacterium pusillum and Curtobacterium albidum). On the basis of physiological characterization (Table 4), differential properties like acid formation from adonitol and raffinose or hydrolyses of aesculin and Tween 80 are revealed. As a consequence of this phenotypic and phylogenetic characterization, it is concluded that genotype D isolates deserve a separate species status, for which the name Curtobacterium herbarum sp. nov. is proposed.

Phylogenetic analyses of genotypes B1 and B2 revealed their assignment to the species Clavibacter michiganensis (Fig. 1). Both genotypes were highly related to Clavibacter michiganensis subsp. textellarius but, on the basis of slight differences in their 16S rDNA sequences, they could be separated by restriction analyses, a method that has been proven by Lee et al. (1997) to be effective in classification of Clavibacter michiganensis subspecies. This result indicates that both genotypes form a separate group of strains that should not be assigned to a known subspecies. Differences in physiological properties support this assumption.

The representative strain of genotype C constituted a separate phylogenetic branch in the family Microbacteriaceae. The nearest phylogenetic neighbours are the genera Microbacterium and Mycetococca, which clustered with genotype C by both neighbour-joining and maximum-likelihood methods, supported by high bootstrap values (Fig. 1). Phylogenetic distances between Mycetococca and Microbacterium are much smaller than to genotype C, indicating novel genus status. Chemotaxonomic properties commonly used for differentiation at the genus level also differed for both genera and genotype C (Table 5). Thus, the type of peptidoglycan is a prominent feature to distinguish them from one another. Comparison of the cell wall composition of genotype C, which corresponds to type B2γ of Schleifer & Kandler (1972), with those of the remaining genera of the family Microbacteriaceae revealed agreement with Clavibacter, Leifsonia, Agromyces, Cryobacterium and Rathayibacter (Etvushenko et al., 2000; Sasaki et al., 1998). Whereas Clavibacter and Leifsonia represent a subgroup displaying l- and d-isomers of DAB in almost equal amounts, genotype C isolates, Rathayibacter, Cryobacterium and Agromyces shared the same amino acid ratio, with l-DAB as the predominant isomer (Sasaki et al., 1998), and these species form another subgroup. Within this subgroup, Agromyces species are characterized by filamentous cells fragmenting to yield coccoid forms in older cultures and the predominant menaquinones MK-12 and MK-11 and thus, they can easily be distinguished from genotype C by phenotypic features without requiring phylogenetic studies. In contrast, the genus Rathayibacter shows similar phenotypic properties in terms of chemotaxonomic and physiological characteristics (Tables 4 and 5). Only acid production from raffinose, l-rhamnose and ribose, hydrolysis of DNA and Tween 80 are features that can be used to discriminate genotype C strains and all currently described Rathayibacter species. In spite of this phenotypic similarity, assignment to Rathayibacter is not possible because of the high phylogenetic distance of at least 5%, based on 16S rDNA sequences. Cryobacterium psychrophilum is obligately psychrophilic (Suzuki et al., 1997) and represents a phylogenetic lineage separate from that of genotype C strains (Fig. 1). A further genus containing l-DAB in the peptidoglycan, but incorporated in an unknown structure is Agrococcus (Groth et al., 1996; Wieser et al., 1999). In addition to the amino acids found in genotype C strains, threonine and aspartic acid can be shown in the peptidoglycan. The coccoid cell form and menaquinone composition are additional features for discrimination on the basis of phenotypic characteristics (Table 5).

In conclusion, regarding the separate phylogenetic position of the genotype C, which is also reflected by phenotypic differences to the nearest related genera, it is proposed that this grass-associated genotype should
be placed in a novel genus of the family Microbacteriaceae, Plantibacter gen. nov., with the type species Plantibacter flavus sp. nov.

The last grass-associated genotype under investigation, genotype F, was closely related to species of the genus Subtercola (Fig. 1). Sequence conformity to S. boreus and S. frigoramans below 97% clearly revealed a demarcation from species of this genus (Stackebrandt & Goebel, 1994). The chemotaxonomic traits of Subtercola recently described by Männistö et al. (2000) are characterized by peptidoglycan type B2γ of Schleifer & Kandler (1972), although nearly all of the glutamic acid residues were replaced by hydroxy-glutamic acid. In contrast, the peptidoglycan of genotype F contained a hitherto undescribed combination of the diagnostic amino acids L-DAB and D-Orn, and the glutamic acid is also replaced by hydroxy-glutamic acid. Furthermore, the isoprenoid quinones of Subtercola are dominated by MK-9 and MK-10; those of genotype F comprised predominantly MK-10 and MK-11. These chemotaxonomic properties commonly used in the past for generic assignment are drastically different. However, following the taxonomic strategy to attribute most importance to analyses of partial 16S rDNA sequence similarity for determining evolutionary relationships at the genus level, as shown for unification of the genera Microbacterium and Aureobacterium (Takeuchi & Hatano, 1998), it is proposed that genotype F be assigned to the genus Subtercola.

The fatty acid composition of genotype F was predominantly anteiso- and iso-branched acids, which is similar to the situation in genera of the family Microbacteriaceae (Table 5). In contrast to the described species of Subtercola, a relatively high level of saturated fatty acids is found. On the other hand, strains of genotype F and Subtercola correspond in all containing 1,1-dimethoxy-anteiso-pentadecane.

Species of the genus Subtercola are particularly characterized by optimal growth temperatures 10 °C lower than the majority of Microbacteriaceae and the ability to grow partially down to −2 °C, which is similar to the genus Frigobacterium (Kämpfer et al., 2000; Männistö et al., 2000). Both genera, along with Cryobacterium psychrophilum (Suzuki et al., 1997), have been assigned as a psychrophilic group of Microbacteriaceae. Strains of genotype F also grow in this temperature range, but optimal growth was observed at room temperature. Thus, strains of genotype F should not be considered as psychrophilic.

As a consequence of phylogenetic and chemotaxonomic analyses, which clearly indicate a separate species status for genotype F strains in the genus Subtercola, a novel species, Subtercola pratensis sp. nov., is proposed.

Application of FT-IR spectroscopy to the classification of grass-associated coryneforms, with regard to bacteria of the genera Curtobacterium, Clavibacter and Rathayibacter associated with plants, showed that this method is reliable to distinguish coryneform bacteria at the species level, which is similar to results presented by Oberreuter (2001).

Summarizing the results of this study and the characterization of grass-associated strains of the genus Microbacterium (Behrendt et al., 2001) isolated from the same habitat, it was shown that coryneform bacteria living in the phyllosphere of grasses are relatively similar in their physiological properties, which may be due to adaptation to environmental conditions (Table 4). In contrast, phylogenetic analysis reveals a high diversity between grass-associated coryneforms. It has also to be taken into account that the isolates studied only represent a part of the coryneform community as genotypes standing for the majority of isolated strains were analysed, indicating that the phyllosphere is a reservoir of more coryneform genotypes. Comparing phylogenetic relationships and phenotypic properties revealed contradictory results. On the one hand, at the genus level, a strain group (genotype C) was found showing similar chemotaxonomic traits to other genera, but was phylogenetically highly divergent. This was also observed at the species level for strains that can be hardly differentiated by means of phenotypic characterization, whereas DNA–DNA hybridization studies clearly reveal a separate species status, as shown for genotype D and Microbacterium spp. (Behrendt et al., 2001). On the other hand, a genotype (F) was found showing a high phylogenetic relationship to known species, but some chemotaxonomic properties that generally prove useful to discriminate between genera were extremely different.

**Description of Plantibacter gen. nov.**

Plantibacter (plan.ti.bac’ter. L. fem. n. planta plant; N.L. masc. n. bacter equivalent of Gr. neut. n. baktron rod; N.L. masc. n. Plantibacter rod of/from plants).

Cells are Gram-positive, pleomorphic, non-motile rods that are sometimes arranged at an angle to give V-formations. Endospores are not formed. Growth is obligately aerobic. Cell wall peptidoglycan contains L-DAB, D-Glu, D-Ala and Gly, corresponding to type B2γ, and muramic acid is acetylated. Non-hydroxylated fatty acids are predominantly anteiso- and iso-methyl-branched. Minor amounts of straight-chain saturated acids are found. Respiratory quinones are MK-10 and MK-11. DNA G + C composition is about 70 mol %. Type species is Plantibacter flavus.

**Description of Plantibacter flavus sp. nov.**

Plantibacter flavus (fla’vus. L. adj. flavus yellow, referring to the colony colour).

Morphological and physiological description of the species is based on five strains. Cells are Gram-positive, strictly aerobic, non-spor-forming, non-motile, irregularly shaped rods that sometimes form V-shapes. Colonies are yellow, shiny, slightly convex and round with entire margins. Oxidase and Voges–Proskauer
reaction are negative, whereas catalase is positive. Aesculin, DNA, Tween 60 and Tween 80 are hydrolysed. Utilization of starch is strain dependent; the type strain is positive for hydrolysis of starch. None of the strains hydrolyses casein, gelatin or cellulose. Formation of levans from sucrose is negative. Strains produce acid oxidatively from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, D-mannose, D-melezitose, raffinose, L-rhamnose, sucrose, D-trehalose, ribose and D-xyllose. Acid formation from inulin, melibiose, D-sorbitol and L-sorbose is strain dependent, whereas the type strain is negative for these carbohydrates. All strains are negative for adonitol and inositol. Optimum growth temperature is about 25 °C. At 4 °C, grows slowly; at −2 °C, growth also occurs, but is even slower. Major menaquinones and structure of peptidoglycan are the same as described for the genus. The major cellular fatty acids are 12-methyl tetradecanoic acid (a-15:0), 14-methyl pentadecanoic acid (i-15:0), 14-methyl hexadecanoic acid (a-17:0) and 13-methyl pentadecanoic acid (i-16:0). DNA G+C composition for the type strain is 70 mol%. Strains were isolated from the phyllosphere of grasses and from the litter layer after mulching the sward. The type strain, isolated from the litter layer, is DSM 14013T (= P 420/07T = LMG 19917T).

Description of Curtobacterium herbarum sp. nov.

*Curtobacterium herbarum* (her.ba’rum. L. gen. pl. fem. n. *herbarum* of plants).

Morphological and physiological description of the species is based on five strains. Cells are Gram-positive, strictly aerobic, non-spore-forming, non-motile, irregularly shaped rods that sometimes form V-shapes. Colonies are yellow, shiny, slightly convex and round with entire margins. Oxidase and Voges–Proskauer reaction are negative, whereas catalase is positive. Aesculin, Tween 60 and Tween 80 are hydrolysed. None of the strains hydrolyses starch, casein, gelatin, DNA or cellulose. Formation of levans from sucrose is negative. Strains produce acid oxidatively from L-arabinose, D-fructose, D-galactose, D-glucose, inulin, D-maltose, D-mannitol, D-mannose, raffinose, sucrose, D-trehalose, ribose and D-xyllose. Acid formation from melibiose is weak and is strain dependent from inositol and L-rhamnose. Type strain is negative for inositol and positive for L-rhamnose. Formation of acid from adonitol, lactose, D-melezitose, D-sorbitol and L-sorbose is negative for all strains. Optimum growth temperature is about 25 °C. At 4 °C, grows slowly; at −2 °C, growth also occurs, but is even slower. Diagnostic diamino acids of the peptidoglycan are L-DAB and D-Orn; glutamic acid is 3-hydroxylated to a high degree and muramic acid is acetylated. Major isoprenoid quinones are MK-10 and MK-11. Predominant cellular fatty acids are 12-methyl tetradecanoic acid (a-15:0), 14-methyl hexadecanoic acid (a-17:0), 14-methyl pentadecanoic acid (i-16:0) and hexadecanoic acid (16:0). 1,1-Dimethoxy-anteiso-pentadecane occurs in the whole-cell methanolysate. DNA G+C composition of the type strain is 65 mol%. Isolated from the phyllosphere of grasses. Type strain is DSM 14246T (= P 229/10T = LMG 21000T).

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Note added in proof

While this paper was being reviewed, Evtushenko et al. (2001) proposed a novel genus, *Agrieia*, which showed chemotaxonomic features corresponding to those of *Sub-
tercola pratensis. As a consequence, a necessary reclassification of Subtercola pratensis is in preparation. Thus, the authors refrain from emending the genus Subtercola in the present paper to avoid a revocation after valid reclassification of Subtercola pratensis.

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1453

Novel grass-associated Microbacteriaceae


