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Leifsonia poae gen. nov., sp. nov., isolated from nematode galls on *Poa annua*, and reclassification of '*Corynebacterium aquaticum*' Leifson 1962 as *Leifsonia aquatica* (*ex* Leifson 1962) gen. nov., nom. rev., comb. nov. and *Clavibacter xyli* Davis *et al.* 1984 with two subspecies as *Leifsonia xyli* (Davis *et al.* 1984) gen. nov., comb. nov.

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The new genus *Leifsonia* gen. nov. with two new species, *Leifsonia poae* sp. nov. (type strain VKM Ac-1401^T) and *Leifsonia aquatica* (*ex* Leifson 1962) nom. rev., comb. nov. (the type species, with VKM Ac-1400^T = DSM 20146^T = JCM 1368^T as type strain), is proposed to accommodate bacteria found in *Poa annua* root gall, induced by the nematode *Subanguina radicicola*, and '*Corynebacterium aquaticum*' Leifson 1962. Further, it is proposed to reclassify *Clavibacter xyli* Davis *et al.* 1984 with two subspecies in the new genus as *Leifsonia xyli* (Davis *et al.* 1984) comb. nov., *Leifsonia xyli* subsp. *xyli* (Davis *et al.* 1984) comb. nov. and *Leifsonia xyli* subsp. *cynodontis* (Davis *et al.* 1984) comb. nov. Members of the proposed genus are characterized by coryneform morphology, peptidoglycans based upon 2,4-diaminobutyric acid, the major menaquinone MK-11, phosphatidylglycerol and diphosphatidylglycerol as principal phospholipids, the high content of anteiso- and iso-branched saturated fatty acids, and a DNA G+C base composition of 66–73 mol%. They form a distinct phylogenetic branch attached to the line of descent of *Agromyces* spp. The new and reclassified species of the new genus clearly differ from each other phylogenetically and phenetically and can be recognized by their morphologies, the cell wall sugar composition, the requirement of complex media for growth, and numerous physiological characteristics, including the oxidase reaction.

Keywords: *Actinomycetales*, *Leifsonia* gen. nov., *Clavibacter xyli*, '*Corynebacterium aquaticum*', *Subanguina radicicola*

INTRODUCTION

Until recently, six genera of Gram-positive bacteria with 2,4-diaminobutyric acid (DAB) in their cell walls have been described, i.e. *Agromyces* (Gledhill & Casida, 1969), *Clavibacter* (Davis *et al.*, 1984), *Rathayibacter* (Zgurskaya *et al.*, 1993), *Agrococcus* (Groth *et al.*, 1996), *Leucobacter* (Takeuchi *et al.*, 1996) and *Cryobacterium* (Suzuki *et al.*, 1997). The following salient characteristics were proposed to differentiate the above genera at the phenetic level: morphology, major menaquinones, amino acid composition of the peptidoglycan, phospholipid pattern, fatty acid profile and growth temperature. Recently, Sasaki *et al.* (1998) demonstrated that the D- and L-

Abbreviation: DAB, 2,4-diaminobutyric acid.

The GenBank accession number for the 16S rDNA sequence of strain *Leifsonia poae* gen. nov., sp. nov. VKM Ac-1401^T is AF116342.

isomers of DAB in the peptidoglycans were also indicative of these genera: *Agromyces*, *Agrococcus*, *Cryobacterium*, *Leucobacter* and *Rathayibacter* were characterized by the L-DAB exclusively, whereas organisms of the genus *Clavibacter* contained D- and L-DAB in their peptidoglycan in almost equal proportions. Additionally, Altenburger *et al.* (1997) showed differences in some of the above genera in the polyamine pattern. Some other bacteria known to have DAB in their peptidoglycan were misclassified or not assigned to validly described taxa (Leifson, 1962; Iizuka & Komagata, 1964;Yamada & Komagata, 1970a; Schleifer & Kandler, 1972; Fiedler & Kandler, 1973; Collins & Jones, 1980, 1981; Hensel, 1984; Bendinger *et al.*, 1992; Evtushenko *et al.*, 1994). Subsequently, based on comparative analysis of 16S rDNA, '*B. helvolum*' and '*Corynebacterium aquaticum*' were shown to form two separate sublines of descent within the radiation of actinomycetes belonging to the family *Microbacteriaceae* (Rainey *et al.*, 1994; Takeuchi & Yokota, 1994), and they were suggested to be nuclei of novel genera (Rainey *et al.*, 1994). Furthermore, '*Corynebacterium aquaticum*' was determined to be a phylogenetic neighbour of *Clavibacter xyli* subsp. *cynodontis*, and these organisms formed an independent cluster positioned close to the *Agromyces* branch (Takeuchi & Yokota, 1994; Sasaki *et al.*, 1998). Based on the phylogenetic data and similarity in salient chemotaxonomic characteristics of '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis*, Sasaki *et al.* (1998) suggested that a new genus should be established to accommodate these organisms. In addition, a high level of phylogenetic similarity of *Clavibacter xyli* subsp. *cynodontis* and *Clavibacter xyli* subsp. *xyli* was shown (Lee *et al.*, 1997), supporting the latter subspecies to be another member of the genus.

In the course of studying micro-organisms from root galls induced by the grass root gall nematode *Subanguina radicicola* in annual meadow grass (*Poa annua*), we found a new set of coryneform bacteria which were phenotypically close to '*Corynebacterium aquaticum*'. In this paper, we present the results of the taxonomic study of a representative of these organisms. Based on data published previously and obtained in this study, we propose a new genus, *Leifsonia* gen. nov., to accommodate bacteria from *P. annua* root galls, '*Corynebacterium aquaticum*' Leifson 1962 and *Clavibacter xyli* Davis *et al.* 1984.

METHODS

Isolation of bacteria. The plants of *Poa annua* infected by the grass root gall nematode *Subanguina radicicola* were collected in Moscow Region in June 1991. The bacteria were isolated from root galls of these herbarium samples in 1993. Surfaces of the root galls were sterilized with 75% (v/v) ethanol for 1 min and dried; the galls were pre-incubated in sterile tap water at 24 °C for 2 h, cut into pieces, added to 2 ml NaCl solution at a concentration of 0·85% (w/v), and ground using a pestle. Then, 10% (w/v) KOH solution was added to this ground gall suspension at a final concentration of 0·1% (w/v) for inhibition of most Gram-negative bacteria. One drop of this suspension was plated onto corynebacterial (CB) agar (Zgurskaya *et al.*, 1993) and incubated for 3 weeks at room temperature (18–24 °C). Representative colonies were selected and regrown on CB agar. The isolated strains were stored at 8 °C or under freeze-dried conditions.

Strains and their cultivation. The novel isolate VKM Ac-1401^T and the following type and reference strains were used in this comparative study: '*Corynebacterium aquaticum*' VKM Ac-1400^T (= DSM 20146^T = JCM 1368^T), *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T (= ICMP 8790^T = JCM 1376^T), *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2032 (= ICMP 8792), *Agromyces mediolanus* VKM Ac-1388^T (= DSM 20152^T = JCM 3346^T), *Agromyces cerinus* subsp. *cerinus* VKM Ac-1340^T and *Agromyces fucosus* subsp. *fucosus* VKM Ac-1345^T (VKM, All-Russian Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; ICMP, International Collection of Microorganisms from Plants, Manaaki Whenua Landcare Research, Auckland, New Zealand; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Japan). All strains were grown on CB agar, except *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T and *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2032, which were cultivated on complex SC medium containing (l^{-1}) 17 g cornmeal agar (Difco), 8 g peptone from soy meal, 1 g K_2HPO_4, 1 g KH_2PO_4, 0·2 g $MgSO_4 . 7H_2O$, 0·5 g glucose, 1 g cysteine-free base, 2 g bovine serum albumin and 15 mg bovine haemin chloride, pH to 6·6 (Davis *et al.*, 1980).

Cell chemistry. Shake cultures of VKM Ac-1401^T and '*Corynebacterium aquaticum*' used for chemical analysis were grown in liquid CB media. For strains *Clavibacter xyli* subsp. *cynodontis*, SC medium was used. Cell walls were prepared from wet cells which were disrupted by sonification, separated from unbroken cells by centrifugation and purified using trypsin and 2% SDS as described by Streshinskaya *et al.* (1979). The cell wall preparations were hydrolysed with 6 M HCl at 105 °C for 6 h (Schleifer & Kandler, 1972). The presence of DAB was detected by TLC (Bousfield *et al.*, 1985). Quantitative determination of the amino acids was performed with an LC 600 amino acid analyser (Biotronic). Whole-cell sugars were determined by the method of Hasegawa *et al.* (1983). Cell wall sugars were studied in acid hydrolysates (3 M trifluoroacetic acid, 100 °C, 6 h) as described previously (Maltsev *et al.*, 1992). Menaquinones were extracted and purified as described by Collins *et al.* (1977), and their composition was determined using a model MAT 8430 mass spectrometer (Finnigan). Isolation and analysis of phospholipids were performed by TLC using the method of Minnikin *et al.* (1978). Fatty acid composition was analysed as described by Evtushenko *et al.* (1989).

Morphology and physiology. Morphology and life cycle were studied in cultures grown on CB or SC agar by phase-contrast microscopy. Physiological features were examined as described previously (Zgurskaya *et al.*, 1993). CB agar was used to check the strains for their tolerance to antibiotics and growth inhibitors. Oxidase activity was tested using 1% (w/v) tetramethyl-*p*-phenylenediamine solution on filter paper discs as reported by Groth *et al.* (1996).

DNA isolation and hybridization. Genomic DNA was isolated from thawed cells resuspended in TE buffer (10 mM Tris, 1 mM EDTA; pH 8·0) by the technique of Bradley *et al.* (1973). [H^3]DNAs from reference strains were obtained by *in vitro* nick translation. DNA–DNA hybridization was performed by using the membrane filter method (Meyer & Schleifer, 1978; Stackebrandt *et al.*, 1981) as described previously (Evtushenko *et al.*, 1989).

Analysis of 16S rDNA sequences. The 16S rRNA gene was amplified by using the PCR method and prokaryotic 16S rDNA universal primers fD1 and rP1 (Weisburg *et al.*, 1991) as described by Zhou *et al.* (1997). PCR products were detected by agarose gel electrophoresis and visualized by UV fluorescence after ethidium bromide staining, and were then purified and concentrated by using a Wizard PCR Preps DNA purification system (Promega), according to the manufacturer's instructions. The 16S rDNA sequences were analysed directly using the purified PCR products as the sequencing template. The sequencing reactions were performed by automated fluorescent *Taq* cycle sequencing using the ABI Catalyst 800 and a model ABI 373A automatic DNA sequencer (Applied Biosystems), according to the manufacturer's protocol. Nucleotide substitution rates were calculated as described by Kimura & Ohta (1972), and the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with CLUSTAL W software (Thompson *et al.*, 1994). Tree topologies were evaluated by bootstrap analysis of the sequence data with the same software. GenBank, DDBJ and EMBL accession numbers for reference 16S rDNA sequences of the strains used in this analysis are given in Fig. 1. *Brevibacterium linens* DSM 20425^T (X77451) was used as an outgroup member.

RESULTS AND DISCUSSION

Isolation

Most of the colonies that developed from the ground gall suspension plated on CB agar were yellow, orange–yellow or orange. Numerous coryneform isolates forming colonies of different tints of yellow were found to be motile. One representative of the last group, strain VKM Ac-1401^T, was studied in detail in this work.

Morphology

Colonies of strain VKM Ac-1401^T on CB agar were yellowish to deep yellow with age, circular, convex, opaque, glistening and butyrous. The young culture (16–24 h) consisted of Gram-positive, curved long rods (4–6 × 0·6–0·9 μm) or filaments with primary branching. In a 3–4-d-old culture, the rods and filaments fragmented into shorter (about 2·0–2·5 μm) elements which were usually motile by peritrichous flagella. Cells with clubbed ends were frequently seen. In old cultures, irregular rods predominated and occurred singly, in pairs or in short chains with diphtheroid arrangements. Colonies of '*Corynebacterium aquaticum*' were yellow, circular, convex, opaque, glistening and butyrous. The young cells (16–24 h) were Gram-positive, non-spore-forming, pleomorphic motile rods (1·2–2·5 × 0·4–0·7 μm) with long peritrichous flagella (length 10 μm or more), as reported by Leifson (1962). Primary branching was observed occasionally. In 3–4 d and older cultures, shorter pleomorphic rods and coccoid cells were predominant and occurred singly, in pairs or in short chains with diphtheroid arrangements. The above rapidly growing organisms differed morphologically from the slowly growing *Clavibacter xyli* subsp. *cynodontis* (SC medium), whose cells were considerably thinner (0·2–0·3 μm). Both of the *Clavibacter xyli* subsp. *cynodontis* strains examined, VKM Ac-2032 and VKM Ac-2041^T, were weakly motile in living cultures, although the flagellated cells were not observed previously (Davis *et al.*, 1984).

Cell chemistry

The cell wall of strain VKM Ac-1401^T contained glycine, glutamic acid, DAB and alanine in a molar ratio close to 1:1:2:1. The qualitative sugar composition of the whole cells was similar in all strains examined (glucose, galactose, mannose, fucose, ribose, rhamnose and traces of xylose) except for strain VKM Ac-1401^T, which lacked fucose. Quantitative cell wall sugar analysis showed a predominant amount of rhamnose and minor amounts of glucose, galactose and mannose in strain VKM Ac-1401^T. In the cell wall of '*Corynebacterium aquaticum*' VKM Ac-1400^T and *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T, fucose was found additionally, in significant or trace amounts (Table 1). In addition, members of *Clavibacter xyli* subsp. *cynodontis* and *Clavibacter xyli* subsp. *xyli* were previously shown to be similar in the composition of cell wall sugars, although fucose was determined as a main sugar along with rhamnose, and mannose was not found (Davis *et al.*, 1984).

Cell wall teichoic acids were not found in the two tested strains VKM Ac-1401^T and '*Corynebacterium aquaticum*' VKM Ac-1400^T (I. B. Naumova, personal

Table 1. Cell wall sugars of the strains studied (molar ratio)

Strain	Glucose	Galactose	Mannose	Fucose	Rhamnose
VKM Ac-1401^T	0·7	1·0	0·7	–	19·4
'*Corynebacterium aquaticum*' VKM Ac-1400^T	1·3	1·0	2·7	3·9	7·6
Clavibacter xyli subsp. *cynodontis* VKM Ac-2041^T	0·6	1·0	Trace	Trace	0·6

Table 2. Phenotypic differences between strains VKM Ac-1401^T, '*Corynebacterium aquaticum*' VKM Ac-1400^T and *Clavibacter xyli* subspecies

Character	VKM Ac-1401^T	'*Corynebacterium aquaticum*' VKM Ac-1400^T	*Clavibacter xyli* subsp. *xyli**	*Clavibacter xyli* subsp. *cynodontis**
Cell width (μm)	0·6–0·9	0·4–0·7	0·2	0·2–0·3
Cell length (μm)	8·0–15·0	1·2–2·5	5·0	3·0–6·0
Visible colonies (day)	2	2	7–10	5–7
Colony colour	Yellow	Yellow	White	Yellow
Growth on CB agar	+	+	−	−
Fucose in the cell wall	−	+	+	+
Oxidase	−	+	−	−
Production of H_2S	−	+	−	−
Voges–Proskauer test	+	−	−	+
Methyl red test	+	−	−	+
Utilization of:				
Citrate	−	+	−	+
Gluconate	−	+	−	−
Propionate	+	+	−	−
Hydrolysis of:				
Starch	−	+	−	+
Aesculin	+	−	−	−
Gelatin	+	−	−	−
Growth in 5% NaCl	−	+	−	−
Acid from:				
D-Arabinose	+	+	−	−
D-Galactose	+	+	−	−
Salicin	+	+	−	−
D-Sucrose	+	+	−	−
Used as C-source for growth:				
Adonitol	−	+	ND	ND
L-Arabinose	+	−	ND	ND
Inositol	−	+	ND	ND
Melezitose	−	+	ND	ND
Raffinose	+	−	ND	ND
L-Sorbose	−	+	ND	ND
Tolerance to antibiotics (μg ml^{-1}):				
Chloramphenicol (10)	−	+	ND	ND
Doxycycline (5)	−	+	ND	ND
Erycycline (10)	−	+	ND	ND
Gramicidin (50)	−	+	ND	ND
Rifampicin (30)	+	−	ND	ND
Major menaquinones	MK-11	MK-11, 10†	ND	MK-11, 12†
Source of isolation	Nematode gall on *P. annua* roots	Distilled water	*Saccharum*, interspecific hybrid	*Cynodon dactylon*

* Data from Davis *et al.* (1984).

† Data from Sasaki *et al.* (1998).

communication). The predominant menaquinone was MK-11; a minor amount of MK-10 and traces of MK-12 were also detected. The principal phospholipids were phosphatidylglycerol and diphosphatidylglycerol. Among cellular fatty acids of strain VKM Ac-1401^T, large proportions of anteiso- and iso-branched acids were determined (36·3% anteiso-15:0, 45·4% anteiso-17:0 and 15·6% iso-16:0). Other fatty acids (16:0, iso-15:0, 15:0, iso-17:0, 18:1 and iso-18:0) were present in small amounts, 1% or less. Mycolic acids were not found. In '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis*, the lipid characteristics were similar as a whole (Collins & Jones, 1980; Suzuki *et al.*, 1996; Sasaki *et al.*, 1998), including the predominance of anteiso-branched fatty acids as reported by Collins & Jones (1980),

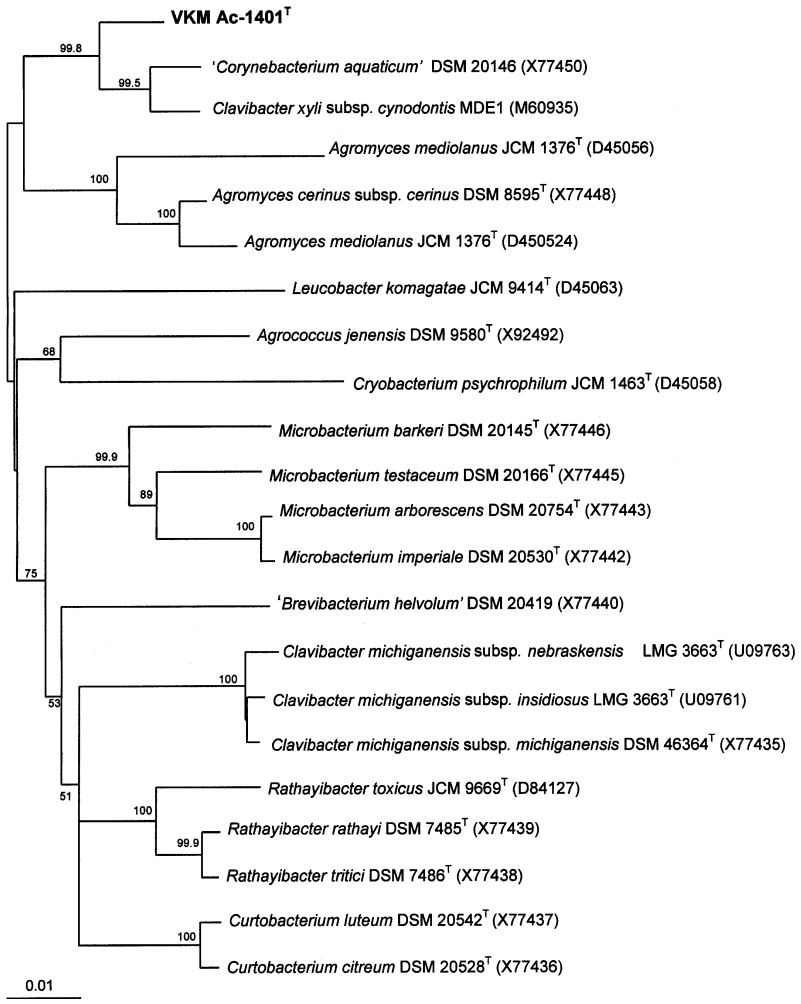

Fig. 1. Phylogenetic tree showing the position of strain VKM Ac-1401^T based on 16S rDNA analysis. The sequence of strain *Brevibacterium linens* DSM 20425^T (X77451) served as an outgroup sequence (not presented). Accession numbers of nucleotide sequences are given in parentheses. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 1 nucleotide substitution per 100 nucleotides.

Henningson & Gudmestad (1991) and Suzuki *et al.* (1996).

Physiology

The rapidly growing strains VKM Ac-1401^T and '*Corynebacterium aquaticum*' VKM Ac-1400^T were aerobic, catalase-positive and mesophilic, with a growth optimum at 24–28 °C. These strains differed from each other in the oxidase reaction, utilization of adonitol, D-arabinose, inositol, melezitose, raffinose and L-sorbose as sole carbon sources, hydrolysis of aesculin, gelatin and starch, production of H_2S, Voges–Proskauer and methyl red tests, tolerance to 5% NaCl and some antibiotics, utilization of citrate and gluconate and some other characteristics (Table 2). Most of these characteristics were previously found to be negative in the slowly growing organisms of the species *Clavibacter xyli* (Davis *et al.*, 1984).

16S rDNA sequence analysis

More than 1480 bases of the 16S rDNA sequence were determined in strain VKM Ac-1401^T. Comparison of the sequence of this strain with available reference sequences indicated that our strain was most similar to '*Corynebacterium aquaticum*' DSM 20146^T (= VKM Ac-1400^T), *Clavibacter xyli* subsp. *cynodontis* JCM 9733^T (= VKM Ac-2041^T) and *Clavibacter xyli* subsp. *xyli* F1A. All above organisms formed a separate phylogenetic branch with a 99·8% bootstrap replication value attached to the *Agromyces* cluster (Fig. 1). Strain VKM Ac-1401^T exhibited 98·4 and 98·1% 16S rDNA sequence similarity to the sequences of '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis*, respectively, whereas the similarity between '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis* was 98·8%. A high level of phylogenetic similarity of *Clavibacter xyli* subsp. *xyli* to *Clavibacter xyli* subsp. *cynodontis* was shown previously by Lee *et al.* (1997).

DNA–DNA hybridization study

Strains VKM Ac-1401^T, '*Corynebacterium aquaticum*' VKM Ac-1400^T and *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T were hybridized against reference [H^3]DNA of strains VKM Ac-1401^T and '*Corynebacterium aquaticum*' VKM Ac-1400^T. DNA–DNA homology between VKM Ac-1401^T and '*Coryne-*

Table 3. DNA relatedness (%) between studied strains

Strain	VKM Ac-1401^T	'*Corynebacterium aquaticum*' VKM Ac-1400^T
VKM Ac-1401^T	100·0	44·6
'*Corynebacterium aquaticum*' VKM Ac-1400^T	43·6	100·0
Clavibacter xyli subsp. *cynodontis* VKM Ac-2041^T	40·9	40·1
Agromyces cerinus subsp. *cerinus* VKM Ac-1340^T	5·0	7·7

bacterium aquaticum' VKM Ac-1400^T was on average 44·1%, while each of these organisms exhibited 40·9 and 40·1% DNA relatedness with *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T, respectively (Table 3).

Taxonomic affiliation of strains

As mentioned above, our isolate from *P. annua* root galls induced by the grass root gall nematode *Subanguina radicicola* was most similar to '*Corynebacterium aquaticum*' and *Clavibacter xyli* both phylogenetically and in salient chemotaxonomic characteristics. These organisms form a distinct phylogenetic branch close to the *Agromyces* spp. cluster. At the chemotaxonomic level, strains VKM Ac-1401^T, '*Corynebacterium aquaticum*' VKM Ac-1400^T and *Clavibacter xyli* subsp. *cynodontis* VKM Ac-2041^T are distinguished from *Agromyces* spp. by the major menaquinone, which is the essential differentiating characteristic of genera containing DAB in their peptidoglycan (Zgurskaya *et al*., 1992, 1993; Groth *et al*., 1996; Takeuchi *et al*., 1996; Suzuki *et al*., 1997; Sasaki *et al*., 1998). Furthermore, members of the '*Corynebacterium aquaticum*' group differ from agromycetes by their cell wall composition: '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis* contained both D- and L-DAB isomers in their peptidoglycan (Sasaki *et al*., 1998), whereas *Agromyces* spp. had L-DAB almost exclusively. Also, our isolate, VKM Ac-1401^T, and '*Corynebacterium aquaticum*' do not contain cell wall teichoic acids (I. B. Naumova, personal communication), in contrast to most members of the genus *Agromyces*, which possess these polymers (Shashkov *et al*., 1993, 1995; Gnilozub *et al*., 1994). Altenburger *et al*. (1997) found that the total polyamine content in strain VKM Ac-1401^T (designated '*Agromyces* sp.' DL 89 in the respective publication) was 0·62 μmol g^{-1}, while the true *Agromyces* species contain only 0·21–0·28 μmol g^{-1} of these compounds. In addition, all members of the genus *Agromyces* are non-motile (Gledhill & Casida, 1969; Zgurskaya *et al*., 1992; Suzuki *et al*., 1996), whereas our isolate, '*Corynebacterium aquaticum*' and *Clavibacter xyli* subsp. *cynodontis* were motile. It is interesting that most of the organisms of the group under consideration, except strain '*Corynebacterium aquaticum*' VKM Ac-1400^T, were isolated from plants and that they are responsible for or associated with plant diseases (Davis *et al*., 1980, 1984; Metzler *et al*., 1997), while the natural habitat of *Agromyces* spp. is mainly soil (Gledhill & Casida, 1969; Zgurskaya *et al*., 1992; Suzuki *et al*., 1996).

Thus the evidence at hand suggests that the bacteria under discussion comprise a new genus of the family *Microbacteriaceae* (Park *et al*., 1993). This conclusion is in line with the opinion expressed earlier by Rainey *et al*. (1994) and Sasaki *et al*. (1998). We propose the name *Leifsonia* for this new genus, in recognition of Einar Leifson, who isolated and described '*Corynebacterium aquaticum*' (Leifson, 1962). The phenotypic properties differentiating the new genus *Leifsonia* from other genera of the family *Microbacteriaceae* with DAB in the cell wall are summarized in Table 4.

The DNA–DNA homology between the rapidly growing '*Corynebacterium aquaticum*' VKM Ac-1400^T and isolate VKM Ac-1401^T and their phenotypic differences in cell wall sugar composition (Table 1), the oxidase reaction, the tolerance to 5% NaCl, the Voges–Proskauer test, the production of H_2S and quite a number of other physiological characteristics (Table 2) indicate that these strains belong to different species (Wayne *et al*., 1987). The names *Leifsonia aquatica* (*ex* Leifson 1962) nom. rev., comb. nov. and *Leifsonia poae* sp. nov. are proposed. The species *Leifsonia aquatica* nom. rev., comb. nov. and the strains *Leifsonia aquatica* VKM Ac-1400^T and *Leifsonia poae* VKM Ac-1401^T are proposed as types of the respective taxa.

In turn, both slowly growing subspecies of *Clavibacter xyli*, *Clavibacter xyli* subsp. *cynodontis* and *Clavibacter xyli* subsp. *xyli*, causing bermudagrass (*Cynodon dactylon*) stunting disease and ratoon stunting disease of sugar cane (*Saccharum*, interspecies hybrid), are markedly distinguished from *Leifsonia poae* sp. nov. and *Leifsonia aquatica* nom. rev., comb. nov. in morphology, physiological features, including the requirement of complex media for growth, and in their sources of isolation (Table 2). Furthermore, these subspecies have a lower DNA G+C content (66 mol%) (Davis *et al*., 1984) than *Leifsonia aquatica* nom. rev., comb. nov. VKM Ac-1400^T (about 70 mol%) (Yamada & Komagata, 1970b; Dopfer *et al*., 1982). These differences between *Clavibacter xyli*

Table 4. Salient phenotypic characteristics that differentiate *Leifsonia* gen. nov. from other genera containing DAB in their peptidoglycan

The table is based on the data obtained in this study and extracted from relevant references (see text). R, Rods; F, filaments; C, cocci; +, positive; −, negative; DL-DAB, D and L isomers of diaminobutyric acid; GABA, γ-aminobutyric acid; Asp, asparagine; Thr, threonine; PUT, putrescine; SPM, spermine; SPD, spermidine; ND, no data.

Genus	Morphology	Motility	Major menaquinone	Peptidoglycan amino acid*	Polyamine pattern	Total polyamine (μmol g^{-1})	Growth at 18 °C
Leifsonia gen. nov.	R, F	+	MK-11, 10	DL-DAB	PUT	0·6	+
Agromyces	F, R	−	MK-12	L-DAB	PUT	0·2–0·3	+
Clavibacter	R	−	MK-9	DL-DAB	SPD, SPM	2·0–7·0	+
Rathayibacter	R	−	MK-10	L-DAB	SPD, SPM	4·8–18·0	+
Cryobacterium	R	−	MK-10	L-DAB	ND	ND	−
Leucobacter	R	−	MK-11	L-DAB, GABA	ND	ND	+
Agrococcus	C	−	MK-12, 11	L-DAB, Asp, Thr	SPM	0·9	+

* All organisms also contain alanine, glutamic acid and glycine.

subsp. *cynodontis* and the rapidly growing species *Leifsonia aquatica* nom. rev., comb. nov. and *Leifsonia poae* sp. nov. were demonstrated to be of the species level by DNA–DNA homology values, 40·1 and 40·9 %, respectively. The phylogenetic similarity of members of different subspecies of *Clavibacter xyli* was shown previously (Lee *et al.*, 1997). Based on the above data, we propose to reclassify the species *Clavibacter xyli* with two subspecies into the new genus *Leifsonia* as *Leifsonia xyli* (Davis *et al.* 1984) comb. nov., *Leifsonia xyli* subsp. *xyli* (Davis *et al.* 1984) comb. nov. and *Leifsonia xyli* subsp. *cynodontis* (Davis *et al.* 1984) comb. nov.

Description of *Leifsonia* gen. nov.

Leifsonia (Leif.so'ni.a. M.L. fem. n. *Leifsonia* named after Einar Leifson, who isolated and described the first organism of this genus).

The colonies are yellow or white, circular, somewhat convex, glistening, opaque and butyrous. The cells are Gram-positive, non-spore-forming, irregular rods or filaments which usually fragment into shorter rods or coccoid elements. Primary branching occurs in young cultures of some species. Usually motile. Non-acid-fast. Mesophilic. Obligately aerobic. Catalase-positive. Oxidase test is variable among species. Cell wall peptidoglycan of B-type contains glycine, glutamic acid, DAB and alanine in a molar ratio close to 1:1:2:1; both isomers, L-DAB and D-DAB, are usually present in almost equal proportions. The major menaquinone is MK-11; MK-10 is present. Cell wall sugars consist of a predominant amount of rhamnose and minor amounts of glucose, galactose and mannose; some species contain fucose. Cell wall teichoic acids and mycolic acids are not present. The principal phospholipids are phosphatidylglycerol and diphosphatidylglycerol. Among fatty acids, anteiso-15:0, anteiso-17:0 and iso-16:0 predominate. Concentrations of polyamines are low; putrescine is the predominant compound. The DNA G+C content ranges from 66·0 to 73 mol%. Some species distinguished by tolerance to antibiotics. Forms a coherent phylogenetic cluster attached to the branch of *Agromyces* spp. The type species of this genus is *Leifsonia aquatica*. The genus description is based on the data cited above and obtained in this study.

Description of *Leifsonia aquatica* (*ex* Leifson 1962) nom. rev., comb. nov.

Leifsonia aquatica; basonym, '*Corynebacterium aquaticum*' Leifson 1962.

The colonies on CB agar are yellowish to deep yellow with age, circular, somewhat convex, glistening, opaque and butyrous. Cells are Gram-positive, non-spore-forming, pleomorphic rods (length 1·2–2·5 μm, width 0·4–0·7 μm); motile by long peritrichous flagella (10 μm or more). Primary branching is observed occasionally. In older cultures, short rods or coccoid cells predominate and occur singly, in pairs or in short chains with diphtheroid arrangements. Rod–coccus cycle is observed. Aerobic. Catalase- and oxidase-positive. Growth occurs between 7 and 37 °C; optimum is 24–28 °C. Adonitol, cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, L-rhamnose, salicin, L-sorbose, sucrose, trehalose, turanose and D-xylose are used for growth as carbon sources in mineral medium supplemented with 0·1% (w/v) yeast extract and 0·1% (w/v) casitone. D-Arabinose, D-fucose, inulin, lyxose, *meso*-erythritol, raffinose, ribose and sorbitol are not used as carbon source in the same medium. Acids are produced from D-arabinose, D-fructose, D-galactose, mannose and sucrose, but not from melibiose. An alkaline reaction is observed with acetate, citrate, formate, fumarate, gluconate, α-keto-1-glutarate, malonate and

propionate, but no reaction occurs with oxalate, succinate or tartrate. Methyl red and Voges–Proskauer tests are negative. H_2S is produced. Tween 40 and starch are decomposed; aesculin, gelatin, hypoxanthine, tyrosine, xanthine, casein, Tween 60, Tween 80 and urea are not hydrolysed. Tolerates 5% (w/v) NaCl, 0·02% (w/v) potassium tellurite and the following antibiotics: ampicillin (50 μg ml^{-1}), chloramphenicol (10 μg ml^{-1}), doxycycline (5 μg ml^{-1}), erycycline (10 μg ml^{-1}), gentamicin (50 μg ml^{-1}), gramicidin (50 μg ml^{-1}), lincomycin (50 μg ml^{-1}), neomycin (50 μg ml^{-1}), penicillin G (50 μg ml^{-1}), rifampicin (10 μg ml^{-1}), streptomycin (50 μg ml^{-1}) and tetracycline (10 μg ml^{-1}). Sensitive to rifampicin (30 μg ml^{-1}). DNA G+C content is about 70 mol%. Cell wall sugars consist of a predominant amount of rhamnose and minor amounts of fucose, glucose, galactose and mannose. No teichoic acids are present in the cell wall. The major menaquinones are MK-11 and MK-10. Other salient chemotaxonomic characteristics are as given above in the genus description. The type strain is VKM Ac-1400^T = DSM 20146^T = JCM 1368^T isolated from distilled water. The above description is based on the data of Leifson (1962), Yamada & Komagata (1970b), Collins & Jones (1980), Dopfer *et al*. (1982) and our study.

Description of *Leifsonia poae* sp. nov.

Leifsonia poae (po'a.e. M.L. gen. n. *poae* of *Poa*, generic name of the annual meadow grass, *Poa annua*, the source of the type strain of this species).

The colonies on CB agar are yellowish to deep yellow with age, circular, somewhat convex, glistening, opaque and butyrous. The young culture (16–24 h) consists of Gram-positive, curved long cells or filaments (length 8–15 μm, width 0·6–0·9 μm) with primary branching; after 3–4 d cultivation on CB agar, they break up into shorter irregular fragments (length 2·0–2·5 μm), which are usually motile by peritrichous flagella. Cells with clubbed ends are frequently seen. In old cultures, pleomorphic rods are predominant and occur singly, in pairs or in short chains with diphtheroid arrangements. Growth occurs between 7 and 37 °C; the optimum is 24–28 °C. Aerobic. Catalase-positive. Oxidase, tested by using 1% (w/v) tetramethyl-*p*-phenylenediamine solution, is negative. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, dulcitol, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-glucopyranoside, raffinose, L-rhamnose, salicin, sucrose and D-xylose are used as sole carbon sources in mineral medium supplemented with 0·1% (w/v) yeast extract and 0·1% (w/v) casitone. Adonitol, dextran, D-fucose, inositol, inulin, lyxose, melezitose, *meso*-erythritol, sorbitol, sorbose, ribose and trehalose are not utilized as the only carbon source in the same medium. Acids are produced from D-arabinose, D-fructose, D-galactose, mannose and sucrose, but not from melibiose. An alkaline reaction is observed with α-keto-1-glutarate, malate, malonate and propionate, but no reaction occurs with citrate, formate, fumarate, gluconate, oxalate, succinate or tartrate. Methyl red and Voges–Proskauer tests are positive. H_2S is not produced. Aesculin and gelatin are decomposed. Hypoxanthine, starch, tyrosine, xanthine, casein, Tween 40, Tween 60 and Tween 80 are not hydrolysed. Sensitive to 5% (w/v) NaCl, doxycycline (5 μg ml^{-1}) and erycycline (10 μg ml^{-1}), but tolerant to potassium tellurite at 0·02% (w/v) and the following antibiotics: ampicillin (50 μg ml^{-1}), chloramphenicol (10 μg ml^{-1}), gentamicin (50 μg ml^{-1}), gramicidin G (10 μg ml^{-1}), lincomycin (50 μg ml^{-1}), neomycin (50 μg ml^{-1}), penicillin G (50 μg ml^{-1}), rifampicin (30 μg ml^{-1}), streptomycin (50 μg ml^{-1}) and tetracycline (10 μg ml^{-1}). Cell wall sugars consist of a predominant amount of rhamnose and minor amounts of glucose, galactose and mannose. No teichoic acids are present in the cell wall. The major menaquinone is MK-11. Other salient chemotaxonomic characteristics are as given above in the genus description. The type strain is VKM Ac-1401^T. Isolated from *Poa annua* root gall induced by the grass root gall nematode *Subanguina radicicola*.

Description of *Leifsonia xyli* (Davis *et al.* 1984) comb. nov.

Leifsonia xyli (Davis *et al*. 1984); basonym, *Clavibacter xyli* Davis *et al*. 1984.

The description of this species is as presented by Davis *et al*. (1984) and supplemented by Sasaki *et al*. (1998). Phenotypic characteristics differentiating *Leifsonia xyli* (Davis *et al*. 1984) comb. nov. from other species of the genus *Leifsonia* are presented in Table 2. The type subspecies is *Leifsonia xyli* subsp. *xyli* (Davis *et al*. 1984).

Description of *Leifsonia xyli* subsp. *xyli* (Davis *et al.* 1984) comb. nov.

Leifsonia xyli subsp. *xyli* (Davis *et al*. 1984); basonym, *Clavibacter xyli* subsp. *xyli* Davis *et al*. 1984.

The description of this subspecies is as presented by Davis *et al*. (1984). The additional phylogenetic characteristics of the subspecies are as given by Lee *et al*. (1997). Phenotypic properties differentiating the subspecies from other intrageneric taxa of the genus *Leifsonia* are presented in Table 2. The type strain is ICMP 7127^T = LMG 7352^T.

Description of *Leifsonia xyli* subsp. *cynodontis* (Davis *et al.* 1984) comb. nov.

Leifsonia xyli subsp. *cynodontis* (Davis *et al*. 1984); basonym, *Clavibacter xyli* subsp. *cynodontis* (Davis *et al*. 1984).

The description of this subspecies is as presented by Davis *et al*. (1984) and supplemented by Sasaki *et al*. (1998). Phenotypic characteristics differentiating the subspecies from other intrageneric taxa of the genus *Leifsonia* are presented in Table 2. The type strain is

VKM Ac-2041^T (= ATCC 33973^T = DSM 46306^T = ICMP 8790^T = JCM 1376^T = NCIB 11927^T).

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