Leucobacter komagatae gen. nov., sp. nov., a New Aerobic Gram-Positive, Nonsporulating Rod with 2,4-Diaminobutyric Acid in the Cell Wall

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A new aerobic, gram-positive, nonsporulating rod-shaped organism is described. Strain IFO 15245^T (T = type strain) has the following characteristics: the menaquinone contains a side chain with 11 isoprenyl units; the guanine-plus-cytosine content of the DNA is 66.2 mol%; 2,4-diaminobutyric acid, glutamic acid, alanine, glycine, and γ -aminobutyric acid are present in the cell wall at a molar ratio of ca. 1:1:2:1:1; and glucose and galactose are also present in the cell wall. A comparison of partial 16S rRNA sequences revealed that IFO 15245^T represents a distinct line of descent within the gram-positive bacteria with high guanine-plus-cytosine contents. The taxonomic characteristics of this organism are different from those of previously described aerobic, gram-positive, nonsporulating, rod-shaped bacteria. The name *Leucobacter komagatae* gen. nov., sp. nov., is proposed for this organism. The type strain is strain IFO 15245.

The following three genera of gram-positive, aerobic, irregular rods with 2,4-diaminobutyric acid (DAB) in their cell walls have been described previously: the genera *Clavibacter* (3), *Rathayibacter* (32), and *Agromyces* (33). Recently, the new genus *Agrococcus* (7), whose members also contain DAB in their peptidoglycans, has been proposed. These genera are differentiated by their menaquinone types, the compositions of their cell wall sugars, their DNA G+C contents, their pigmentation, and selected physiological features (1, 3, 4). The menaquinone found in the genus *Clavibacter* is MK-9, the menaquinone found in the genus *Rathayibacter* is MK-10, the menaquinone found in the genus *Agromyces* is MK-12, and the menaquinones found in the genus *Agrococcus* are MK-11 and MK-12.

Strain IFO 15245^T (T = type strain), which was a contaminant in an ampoule labeled "*Pseudomonas riboflavina*" (18), is a gram-positive, nonmotile, rod-shaped organism. This strain has DAB in its cell wall, and its predominant menaquinone is MK-11. The presence of MK-11 reliably distinguishes strain IFO 15245^T from the members of the genera *Agrococcus*, *Agromyces*, *Clavibacter*, and *Rathayibacter* that have been described, but the taxonomic position of strain IFO 15245^T has remained uncertain. To clarify the taxonomic position of this organism and its intergeneric relationships with the previously described gram-positive, high-G+C-content bacteria, we determined a partial 16S rRNA sequence of IFO 15245^T and compared it with sequences of 20 representative actinobacteria and related organisms (11).

In this paper, we describe the taxonomic features of strain IFO 15245^T, and on the basis of morphological, physiological, and chemotaxonomic characteristics and 16S rRNA sequence analysis data we propose that this organism should be classified in the new genus *Leucobacter*, as *Leucobacter komagatae* gen. nov., sp. nov.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strain IFO 15245^T was isolated as a contaminant in an ampoule labeled "*P. riboflavina*" IFO 13584, which was recently reidentified as *Devosia riboflavina* (18), and maintained in the Institute for Fermentation, Osaka. Strain IFO 15245^T was cultivated aerobically at 28°C in a peptone-yeast extract medium supplemented with brain heart infusion (Difco Laboratories, Detroit, Mich.), which contained 1% peptone, 0.2% yeast extract, 0.2% brain heart infusion, 0.2% NaCl, and 0.2% D-glucose (pH 7.0) (PY-BHI medium). Cells used for biochemical tests were harvested by centrifugation during the stationary phase, washed with water, and lyophilized.

Morphological, physiological, and biochemical characteristics. Cell morphology was determined by phase-contrast microscopy following cell growth on PY-BHI agar. Motility was determined by the hanging drop method. Unless otherwise indicated, all tests were performed at 28°C. Catalase activity was determined by the presence of bubbles in a 3% hydrogen peroxide solution. Oxidase activity was determined by the oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates was studied in a medium containing 0.3% peptone, 0.25% NaCl, 0.003% bromocresol purple, and 0.5% carbohydrate (pH 7.2) (27). Organic acid assimilation was studied in a medium containing 0.5% organic acid (sodium salt), 0.02% D-glucose, 0.01% yeast extract, 0.01% Trypticase (BBL), 0.1% K₂HPO₄, 0.5% NaCl, 2% agar, and 12 mg of phenol red per liter (pH 7.0) (27). Nitrate reduction and hydrolysis of starch, gelatin, casein, esculin, Tween 20, Tween 40, Tween 60, and Tween 80 were determined by the methods described by Cowan and Steel (2).

Chemical analyses. Cell walls were prepared from ca. 500 mg (dry weight) of bacterial cells as described by Schleifer and Kandler (21). Amino acids in an acid hydrolysate of the cell walls were identified by two-dimensional descending chromatography on cellulose thin-layer chromatography plates (Tokyo Kasei Co., Ltd., Tokyo, Japan) by the method of Harper and Davis (8) and by high-performance liquid chromatography (HPLC) as their phenylthiocarbamoyl derivatives with a model LC-6AD HPLC apparatus (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturer's instructions (27). The molar ratio of amino acids was determined with an amino acid analyzer (Hitachi Co., Ltd., Tokyo, Japan). An unknown amino acid was identified by electrospray ionization mass spectrometry. The optical configurations of the amino acids were determined as described by Kandler and König (12). Cell wall sugars were analyzed as described by Mikami and Ishida (15). Polar lipids and fatty acids were extracted from dried cells, purified (17, 22), and examined as described previously (29, 30). Mycolic acids were analyzed by the method of Minnikin et al. (16). The glycolyl test was performed by the method of Uchida and Aida (26).

DNA base composition. DNA was obtained by the method of Saito and Miura (19). The G+C content of the DNA was determined by a modification of the method of Mesbah et al. (14, 29, 30).

16S ribosomal DNA sequence determination and phylogenetic analysis. Wet cells (1 to 3 mg) were suspended in 300 μ l of InstaGene purification matrix (Bio-Rad Co., Ltd.), and the resulting preparation was incubated at 56°C for 15 to 30 min, sonicated at 100 W for 30 s, and heated at 100°C for 5 min. After centrifugation at 1,000 $\times$ g for 2 to 3 min, the supernatant was subjected to PCR amplification (9). Amplification was carried out with a *Taq* polymerase kit (Ce-

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TABLE 1. Phenotypic and chemotaxonomic characteristics of strain IFO 15245^T

Characteristic	Strain IFO 15245 ^T
Phenotypic characteristics	
Catalase activity	+
Oxidase activity	-
Hydrolysis of:	
Tween 20	+
Tween 40	+
Tween 60	+
Tween 80	+
Gelatin	-
Starch	-
Esculin	-
Urease formation	+
H ₂ S formation	+
Indole formation	-
Nitrate reduction	-
Voges-Proskauer reaction	-
Methyl red test	+
Arginine dihydrolase activity	-
Growth in the presence of 2.0% NaCl	+
Growth in the presence of 5.0% NaCl	+
Growth at pH 5	-
Growth at pH 10	+
Assimilation of:	
Acetate	+
Citrate	-
Formate	-
D- and L-Lactate	+
Malate	+
Succinate	+
Formate	+
Oxalate	+
Hippurate	+
Chemotaxonomic characteristics	
Alanine-glutamic acid-DAB-glycine- γ -aminobutyric acid ratio in cell wall	1.90:1.00:0.85:0.89:0.72
Galactose-glucose ratio in cell wall	1.00:0.60
Cell wall acyl type	acetyl
Mycolic acid	-
Quinone system	MK-11
Polar lipids	DPG, PG, GL ^a
Cellular fatty acids	anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{16:0}
G+C content (mol%)	66.2

^a Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, glycolipid.

tus, Inc.), and the purified PCR products were sequenced by using a Sequenase kit for ³⁵S-dATP (United Biochemical Inc.) and 12 primers as previously described (24). DNA sequences were aligned by using the ODN system (10). Nucleotide substitution rates (K_{nuc} values) were calculated (13), and a phyloge-

netic tree was constructed by the neighbor-joining method (20). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (5) with 1,000 replicates.

Nucleotide sequence accession numbers. The sequences which we determined were aligned with previously published sequences that have been deposited in the DDBJ, GenBank, and EMBL data libraries under the following accession numbers: *Agrococcus jenensis*, X92492; *Agromyces cerinus* subsp. *cerinus*, D45060; *Agromyces fucosus* subsp. *hippuratus*, D45061; *Agromyces mediolanum*, X77449; *Agromyces ramosus*, X77434; *Arthrobacter globiformis*, M23411; *Aureobacterium liquefaciens*, X77437; *Aureobacterium testaceum*, X77438; *Brevibacterium linens*, X77440; *Clavibacter michiganense* subsp. *insidiosus*, D45051; *Clavibacter michiganense* subsp. *michiganense*, X77441; "*Corynebacterium aquaticum*," X77443; *Curtobacterium citreum*, X77445; *Kineococcus aurantiacus* (25, 31), D17527; *Microbacterium arborescens*, D21339; *Microbacterium lacticum*, D21343; *Micrococcus luteus*, M38242; *Rathayibacter rathayi*, X77450; *Rathayibacter tritici*, X77438; and *Renibacterium salmoninarum*, X51601. The nucleotide sequence of IFO 15245^T has been deposited in the DDBJ database under accession number D17751.

RESULTS

Phenotypic and chemotaxonomic characteristics. Strain IFO 15245^T was a gram-positive, nonmotile, rod-shaped organism. The cells were 0.2 to 0.3 μm wide and 1.0 to 1.5 μm long. On PY-BHI agar, this strain formed rough, round, convex, pale brown colonies. The biochemical and chemotaxonomic characteristics of strain IFO 15245^T are summarized in Table 1. The amino acids found in the cell wall hydrolysate were alanine, glutamic acid, DAB, glycine, and an unknown amino acid; the molar ratio of these compounds was 1.90:1.00:0.85:0.89:0.72. The unknown amino acid was analyzed by thin-layer chromatography, by HPLC, with an amino acid analyzer, and by electrospray ionization mass spectrometry and was identified as γ -aminobutyric acid. The optical rotations of alanine, glutamic acid, and DAB were D- and L-alanine, D-glutamic acid, and L-DAB. D-DAB was not detected. However, a more detailed structure of the peptidoglycan was not deduced.

Only a small amount of glycolate was found in the acid hydrolysate of bacterial cells, which suggested that muramic acid occurred in the N-acetyl form. The major sugars were galactose and glucose. The isoprenoid quinones were menaquinones MK-11 (72%), MK-10 (16%), and MK-12 (12%) (Table 2). The major fatty acids were anteiso-C_{15:0} (65%), iso-C_{16:0} (13%), and anteiso-C_{17:0} (22%). Diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid were identified by thin-layer chromatography and their staining characteristics. The G+C content of the DNA was 66.2 mol%.

Phylogenetic analysis. A total of 1,524 nucleotides of the 16S ribosomal DNA of IFO 15245^T were sequenced, and the primary structure was aligned with the primary structures of 20 reference actinobacteria. Because only partial sequences were available for some of the reference strains, sequence similarity calculations were based on 1,196 nucleotides. The phylogenetic

TABLE 2. Menaquinone compositions of strains

Strain	Menaquinone composition (%)						
	MK-7	MK-8	MK-9	MK-10	MK-11	MK-12	MK-13
IFO 15245 ^T				16	72	12	
<i>Agrococcus jenensis</i> DSM 9580 ^{Ta}				7	37	49	5
<i>Agrococcus jenensis</i> DSM 9996				4	41	41	13
<i>Agromyces mediolanum</i> IFO 15704 ^T				8	20	67	5
<i>Agromyces cerinus</i> subsp. <i>cerinus</i> JCM 9083 ^{Tb}			1	7	8	60	21
<i>Agromyces fucosus</i> subsp. <i>hippuratus</i> JCM 9086 ^{Tb}			1	7	9	57	24
<i>Agromyces ramosus</i> JCM 3168 ^b				7	7	56	14
<i>Clavibacter michiganense</i> subsp. <i>michiganense</i> JCM 3345 ^b	9	15	65	11			
<i>Rathayibacter rathayi</i> JCM 9307 ^{Tb}			15	74	6		

^a Data from reference 7.

^b Data from reference 23.

FIG. 1. Unrooted phylogenetic tree showing the relationship of strain IFO 15245^T to reference organisms. The numbers indicate the percentages of bootstrap samples, derived from 1,000 samples, that supported the internal branches (5). Bar = 0.01 K_{nuc} unit.

dendrogram in Fig. 1 shows that IFO 15245^T belongs to the radiation of actinobacteria that contain group B peptidoglycans and represents a distinct lineage. The levels of sequence similarity between strain IFO 15245^T and *Agrococcus jenensis*, *Agromyces* species, *Clavibacter* species, and *Rathayibacter* species were 95.2, 93.7 to 94.6, 94.4 to 94.8, and 94.6 to 94.9%, respectively. Thus, strain IFO 15245^T is equidistant from all lineages of DAB-containing strains.

DISCUSSION

Characteristics that differentiate strain IFO 15245^T from four other genera with DAB in their peptidoglycans are shown in Table 3. Strain IFO 15245^T hydrolyzes Tween 20, Tween 40, Tween 60, and Tween 80, but species belonging to the genera *Clavibacter*, *Rathayibacter*, *Agromyces*, and *Agrococcus* do not. In contrast to *Agrococcus* species, starch and esculin are not hydrolyzed by strain IFO 15245^T. The major menaquinones of the genera *Clavibacter*, *Rathayibacter*, and *Agromyces* are MK-9, MK-10, and MK-12, respectively, while the major menaquinone of IFO 15245^T is MK-11. *Agrococcus jenensis* contains nearly equimolar concentrations of MK-11 and MK-12. In *Agrococcus jenensis*, the whole-cell sugars include rhamnose. Rhamnose, xylose, fucose, and/or tyvelose are present in the cell walls of *Clavibacter*, *Rathayibacter*, and *Agromyces* species,

TABLE 3. Some diagnostic and differentiating characteristics of strain IFO 15245^T and the genera *Clavibacter*, *Rathayibacter*, *Agromyces*, and *Agrococcus*

Characteristic	IFO 15245 ^T	<i>Clavibacter</i> ^a	<i>Rathayibacter</i> ^b	<i>Agromyces</i> ^c	<i>Agrococcus</i> ^d
Utilization of:					
Acetate	+	—	+	ND	—
Formate	—	—	+	ND	—
Hippurate	+	ND	—	v	—
Hydrolysis of:					
Tween 20	+	—	—	—	ND
Tween 40	+	ND	—	v	ND
Tween 60	+	ND	—	—	ND
Tween 80	+	—	—	—	—
Starch	—	—	—	v	+
Esculin	—	v	—	+	+
Urea	+	—	—	—	—
Nitrate reduction	—	—	—	v	—
H ₂ S formation	+	—	ND	ND	+
Growth in the presence of:					
2.0% NaCl	+	+	+	+	+
4.0% NaCl	+	ND	+	—	+
5.0% NaCl	+	—	v	—	+
Usual source(s) of isolation	Air	Plants, plant substrates	Plants	Soil	Soil, sandstone surfaces
Murein type	B	B2γ ^f	B2γ	B2γ	B
Cell wall diamino acid	L-DAB	D- and L-DAB ^g	D- and L-DAB ^h	D- and L-DAB ^h	DAB
Cell wall sugars					
Glucose	+	+	+	v	+
Galactose	+	+	v	+	—
Mannose	—	+	+	v	—
Rhamnose	—	+	+	+	+
Xylose	—	—	v	v	—
Fucose	—	v	—	v	—
Tyvelose	—	—	—	v	—
Major menaquinone(s)	MK-11	MK-9	MK-10	MK-12	MK-11, MK-12
G+C content (mol%)	66	65–75	63–72	70–72	74

^a Data from reference 3.

^b Data from reference 32.

^c Data from references 23 and 33.

^d Data from reference 7.

^e +, positive reaction; —, negative reaction; v, reaction variable among strains; ND, not determined.

^f Data from reference 21.

^g Data from reference 6.

^h Data from references 6 and 21.

but these sugars are not present in the cell walls of strain IFO 15245^T. The peptidoglycan type of strain IFO 15245^T also differentiates this organism from members of other DAB-containing genera. The peptidoglycan type of the genera *Agromyces*, *Clavibacter*, and *Rathayibacter* is type B2 γ , which includes D-DAB as well as L-DAB. It has been suggested that D-DAB is present in the interpeptide bridge (1, 6, 32, 33). In strain IFO 15245^T, D-DAB was not detected in the peptidoglycan.

The low levels of similarity of the 16S rRNA gene sequence indicated that strain IFO 15245^T represents a distinct lineage within the coryneform bacteria. Thus, strain IFO 15245^T can be readily distinguished from all previously described taxa and warrants a new taxon.

On the basis of the distinct peptidoglycan features, 16S ribosomal DNA sequence, and nutritional properties of strain IFO 15245^T, we propose that this strain should be placed in a new genus, the genus *Leucobacter*, as a member of the type species, *Leucobacter komagatae*. Moreover, this is the first report of the unusual amino acid γ -aminobutyric acid, which is present in the peptidoglycan of *L. komagatae* IFO 15245^T, as a major component of the cell wall of a bacterium.

Descriptions of the new genus and new species are given below.

Description of *Leucobacter* gen. nov. *Leucobacter* (Leu.co.bac'ter. Gr. adj. *leucus*, clear, light; Gr. masc. m. *bacter*, rod; M.L. masc. n. *Leucobacter*, colorless rod) cells are gram-positive, nonsporulating, nonmotile rods. No mycelium is produced. Catalase is produced, but oxidase, arginine dihydrolase, hydrogen sulfide, and urease are not produced.

The G+C content of the DNA is 66 mol%. The cell wall peptidoglycan contains L-DAB as a diamino acid. The glycan moiety of the peptidoglycan contains acetyl residues. The major isoprenoid quinone is menaquinone MK-11. Mycolic acid is not present. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, and anteiso-C_{17:0}. Diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid are present.

The type species is *Leucobacter komagatae*.

Description of *Leucobacter komagatae* sp. nov. *Leucobacter komagatae* (koma.ga'tae. N.L. adj. *komagatae*, of Komagata, in honor of Kazuo Komagata, the Japanese microbiologist who first recognized this strain). Cells are gram-positive, irregular rods that are 0.2 to 0.3 μ m wide and 1.0 to 1.5 μ m long. The cells are nonsporulating and nonmotile in 2-day-old cultures on PY-BHI agar. No mycelium is produced. Colonies are circular, entire, low convex, smooth, opaque, and whitish brown. Catalase is produced, but oxidase, arginine dihydrolase, hydrogen sulfide, and urease are not produced. Gelatin, starch, and esculin are not hydrolyzed, but Tween 20, Tween 40, Tween 60, and Tween 80 are hydrolyzed. Nitrate is not reduced. Growth occurs in the presence of 2.0 to 5.0% NaCl and at pH 10.0. Acetate, D- and L-lactate, malate, succinate, propionate, oxalate, and hippurate are assimilated, but citrate and formate are not assimilated. The G+C content of the DNA is 66 mol%. The cell wall peptidoglycan contains L-DAB, alanine, glycine, γ -aminobutyric acid, and glutamic acid. The acyl type of the cell wall is an acetyl type. The major isoprenoid quinone is menaquinone MK-11. Mycolic acids are not present. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, and anteiso-C_{17:0}. No diagnostic sugar is present in the cell wall. Diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid are present. Isolated as a contaminant on an agar plate.

The type strain is strain IFO 15245 (= IAM 1093).

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REFERENCES

- Bendinger, B., R. M. Kroppenstedt, S. Klatte, and K. Altendorf. 1992. Chemotaxonomic differentiation of coryneform bacteria isolated from biofilters. *Int. J. Syst. Bacteriol.* 42:472-486.
- Cowan, S. T., and K. J. Steel. 1965. Manual for the identification of medical bacteria. Cambridge University Press, London.
- Davis, M. J., A. G. Gillaspie, Jr., A. K. Vidaver, and R. W. Harris. 1984. *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. *Int. J. Syst. Bacteriol.* 34:107-117.
- Döpfner, H., E. Stackebrandt, and F. Fiedler. 1982. Nucleic acid hybridization studies on *Microbacterium*, *Curtobacterium*, *Agromyces* and related taxa. *J. Gen. Microbiol.* 126:1697-1708.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Fiedler, E., and O. Kandler. 1973. Die Aminosäuresequenz von 2,4-Diaminobuttersäure enthaltenden Mureinen bei verschiedenen coryneformen Bakterien und *Agromyces ramosus*. *Arch. Mikrobiol.* 89:51-66.
- Groth, I., P. Schumann, N. Weiss, K. Martin, and F. A. Rainey. 1996. *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminoibutyric acid in the cell wall. *Int. J. Syst. Bacteriol.* 46:234-239.
- Harper, J. J., and G. H. G. Davis. 1979. Two-dimensional thin-layer chromatography for amino acid analysis of bacterial cell walls. *Int. J. Syst. Bacteriol.* 29:56-58.
- Hiraishi, A. 1992. Direct automated sequencing of 16S rRNA amplified by polymerase chain reaction from bacterial culture without DNA purification. *Let. Appl. Microbiol.* 15:210-213.
- Ina, Y. 1991. Molecular evolutionary analysis system for DNA and amino acid sequences (ODEN), version 1.1. DNA Data Bank of Japan, DNA Research Center, National Institute of Genetics, Mishima, Japan.
- Jones, D., and M. D. Collins. 1986. Section 15. Irregular, nonsporulating Gram-positive rods, p. 1261-1434. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
- Kandler, O., and H. König. 1978. Chemical composition of the peptidoglycan-free cell walls of methanogenic bacteria. *Arch. Microbiol.* 118:141-152.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111-120.
- Mesbah, M., U. Premachandran, and W. E. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39:159-167.
- Mikami, H., and Y. Ishida. 1983. Post-column fluorometric detection of reducing sugars in high-performance liquid chromatography using arginine. *Bunseki Kagaku* 32:E207-E210.
- Minnikin, D. E., L. Alshamaany, and M. Goodfellow. 1975. Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *J. Gen. Microbiol.* 88:200-206.
- Minnikin, D. E., M. D. Collins, and M. Goodfellow. 1979. Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J. Appl. Bacteriol.* 47:87-95.
- Nakagawa, Y., T. Sakane, and A. Yokota. 1996. Transfer of "*Pseudomonas riboflavina*" (Foster 1944), a gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* 46:16-22.
- Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* 72:619-629.
- Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.
- Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* 36:407-477.
- Suzuki, K., and K. Komagata. 1983. Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. *Int. J. Syst. Bacteriol.* 33:188-200.
- Suzuki, K., J. Sasaki, M. Uramoto, T. Nakase, and K. Komagata. 1996. *Agromyces mediolanus* sp. nov., nom. rev., comb. nov., a species for "*Corynebacterium mediolanum*" Mamoli 1939 and for some aniline-assimilating bacteria which contain 2,4-diaminobutyric acid in the cell wall peptidoglycan. *Int. J. Syst. Bacteriol.* 46:88-93.
- Takeuchi, M., F. Kawai, Y. Shimada, and A. Yokota. 1993. Taxonomic study

- of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new description of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. Syst. Appl. Microbiol. **16**:227–238.
25. Takeuchi, M., and A. Yokota. 1994. Phylogenetic analysis of *Kineococcus aurantiacus* based on 16S rRNA gene sequences. FEMS Microbiol. Lett. **116**: 7–12.
 26. Uchida, K., and K. Aida. 1977. Acyl type of bacterial cell wall: its simple identification by colorimetric method. J. Gen. Appl. Microbiol. **23**:249–260.
 27. Wako Pure Chemical Industries, Ltd. 1989. Technical note on the system of PTC-amino acid analysis. Wako Pure Chemical Industries, Ltd., Osaka, Japan. (In Japanese.)
 28. Yamada, K., and K. Komagata. 1972. Taxonomic studies on coryneform bacteria. IV. Morphological, cultural, biochemical, and physiological characteristics. J. Gen. Appl. Microbiol. **198**:399–416.
 29. Yokota, A., M. Takeuchi, T. Sakane, and N. Weiss. 1993. Proposal of six new species in the genus *Aureobacterium* and transfer of *Flavobacterium esteraromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteraromaticum* comb. nov. Int. J. Syst. Bacteriol. **43**:555–564.
 30. Yokota, A., M. Takeuchi, and N. Weiss. 1993. Proposal of two new species in the genus *Microbacterium*: *Microbacterium dextranolyticum* sp. nov. and *Microbacterium aurum* sp. nov. Int. J. Syst. Bacteriol. **43**:549–554.
 31. Yokota, A., T. Tamura, T. Nishii, and T. Hasegawa. 1992. *Kineococcus aurantiacus* gen. nov., sp. nov., a new aerobic gram-positive, motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall. Int. J. Syst. Bacteriol. **43**:52–57.
 32. Zgurskaya, H. I., L. I. Evtushenko, V. N. Akimov, and L. V. Kalakoutskii. 1993. *Rathayibacter* gen. nov., including the species *Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov., *Rathayibacter iranica* comb. nov., and six strains from annual grasses. Int. J. Syst. Bacteriol. **43**:143–149.
 33. Zgurskaya, H. I., J. I. Evtushenko, V. N. Akimov, H. V. Voyevoda, T. G. Dobrovolskaya, L. V. Lysak, and L. V. Kalakoutskii. 1992. Emended description of the genus *Agromyces* and description of *Agromyces cerinus* subsp. *cerinus* sp. nov., subsp. nov., *Agromyces cerinus* subsp. *nitratu* sp. nov., subsp. nov., *Agromyces fucosus* subsp. *fucosus* sp. nov., subsp. nov., and *Agromyces fucosus* subsp. *hippuratus* sp. nov., subsp. nov. Int. J. Syst. Bacteriol. **42**:635–641.