Taxonomic Study of Bacteria Isolated from Plants: Proposal of *Sphingomonas rosa* sp. nov., *Sphingomonas pruni* sp. nov., *Sphingomonas asaccharolytica* sp. nov., and *Sphingomonas mali* sp. nov.

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The taxonomic positions of 10 strains of 3-ketolactose-forming bacteria which were isolated from the roots of plants (*Rosa* sp., *Psychotria nairobiensis*, *Ardisia crispa*, *Prunus persica*, and apple trees) were investigated. The DNA base compositions of these strains ranged from 64.0 to 65.7 mol%, the isoprenoid quinone of each strain was ubiquinone 10, 3-hydroxy fatty acids were lacking in the cellular fatty acids of these organisms, and all of the strains contained a sphingolipid with the long-chain base dihydrosphingosin. These are characteristics of the genus *Sphingomonas*. On the basis of morphological, physiological, and chemotaxonomic characteristics, together with DNA-DNA hybridization and 16S ribosomal DNA sequence comparison data, we propose the following four new species of the genus *Sphingomonas*: *Sphingomonas rosa* (type strain, IFO 15208) for the strains isolated from rose plants and formerly named [*Agrobacterium rhizogenes*]; *Sphingomonas pruni* (type strain, IFO 15498) for the strains isolated from *Prunus persica*; and *Sphingomonas asaccharolytica* (type strain, IFO 15499) and *Sphingomonas mali* (type strain, IFO 15500) for the strains isolated from apple trees. Two strains which were isolated from *Psychotria nairobiensis* and formerly named [*Chromobacterium lividum*] were identified as *Sphingomonas yanoikuyae* strains.

3-Ketolactose-forming activity (2) has been found only in biovar 1 strains of the genus *Agrobacterium* (3, 10), and this characteristic has proven to be a useful taxonomic marker for agrobacteria. Holmes and Roberts (8) proposed an identification scheme for agrobacteria based on 84 phenotypic tests. Fifty *Agrobacterium* strains, mostly strains maintained in the National Collection of Plant Pathogenic Bacteria, were separated into four clusters on the basis of the results of a numerical analysis. Holmes and Roberts suggested that a small group of "yellow-pigmented, 3-ketolactose positive bacteria," including [*Agrobacterium rhizogenes*] NCPPB 2661, NCPPB 2662, and NCPPB 2663, which were isolated from hairy roots of *Rosa* sp., and [*Chromobacterium lividum*] NCTC 10590 and NCTC 10591, which were isolated from *Psychotria nairobiensis* or *Ardisia crispa*, may constitute another distinct group of agrobacteria (brackets indicate taxa that are misnamed). The results of rRNA cistron similarity studies indicated that these strains are more closely related to *Flavobacterium capsulatum* than to the genus *Agrobacterium* (1). *F. capsulatum* was subsequently transferred to the genus *Sphingomonas* as *Sphingomonas capsulata* by Yabuuchi et al. in 1990 (27).

The genus *Sphingomonas* was proposed by Yabuuchi et al.

(27), and the description of this taxon has recently been emended by us (20). Members of the genus *Sphingomonas* are yellow-pigmented, nonfermentative, gram-negative, nonmotile or motile rods with a single polar flagellum and are characterized by the presence of a large amount of a unique sphingoglycolipid with the long-chain base dihydrosphingosin, by the presence of 2-hydroxymyristic acid (2-OH 14:0), by the absence of 3-hydroxy fatty acids, and by the presence of ubiquinone 10 (Q-10). Therefore, we examined the taxonomic positions of the five strains of yellow-pigmented 3-ketolactose-positive bacteria described by Holmes and Roberts (8). An additional five strains of 3-ketolactose-positive bacteria, which were isolated from plants and were designated agrobacterium-like strains, were also studied.

In this paper, we report both the phenotypic and chemotaxonomic characteristics of 3-ketolactose-positive bacteria and the results of a comparison of the 16S rRNA gene (rDNA) sequences of these organisms with the sequences of previously described species of the genus *Sphingomonas* (20, 27) and *Rhizomonas suberifaciens* (23). On the basis of phenotypic data, DNA-DNA hybridization data, and the results of the 16S rDNA sequence analysis, we propose four new species of the genus *Sphingomonas*.

MATERIALS AND METHODS

Microorganisms and cultures. The strains which we used are listed in Table 1. The type strains of *Sphingomonas* species and two strains of *Rhizomonas suberifaciens* (23) were used for a comparison of taxonomic characteristics. All of the strains except the *Rhizomonas suberifaciens* strains were cultured at 28°C with aerobic shaking in PY medium containing 1% peptone, 0.2% yeast extract, 0.2% NaCl, and 0.2% glucose (pH 7.0). *Rhizomonas suberifaciens* IFO 15211^T (T = type strain) and IFO 15212 were cultured at 28°C with aerobic shaking in PG medium containing 0.5% peptone, 0.25% glucose, 0.13% K₂HPO₄, 0.05% MgSO₄ · 7H₂O, 0.05% KNO₃, and 0.006% Ca(NO₃)₂ · 4 H₂O (pH 7.2).

Determination of phenotypic characteristics. Cultures were grown on PY or

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TABLE 1. Bacterial strains studied

Species or isolate	IFO no. ^a	Other designation(s) ^b	Source	Reclassified as:
[<i>Agrobacterium rhizogenes</i>]	15208 ^T	NCPBP 2661 ^T , IAM 14222 ^T	<i>Rosa</i> sp. roots	<i>Sphingomonas rosa</i>
[<i>Agrobacterium rhizogenes</i>]	15209	NCPBP 2662	<i>Rosa</i> sp. roots	<i>Sphingomonas rosa</i>
[<i>Agrobacterium rhizogenes</i>]	15210	NCPBP 2663	<i>Rosa</i> sp. roots	<i>Sphingomonas rosa</i>
[<i>Chromobacterium lividum</i>]	15163	NCTC 10590, IAM 14225	<i>Psychotria nairobiensis</i> roots	<i>Sphingomonas yanoikuyae</i>
[<i>Chromobacterium lividum</i>]	15164	NCTC 10591	<i>Ardisia crispa</i> roots	<i>Sphingomonas yanoikuyae</i>
Y-250 ^{Tc}	15498 ^T		<i>Prunus persica</i> roots	<i>Sphingomonas pruni</i>
Y-345 ^{Tc}	15499 ^T		Apple tree roots	<i>Sphingomonas asaccharolytica</i>
Y-347 ^{Tc}	15500 ^T		Apple tree roots	<i>Sphingomonas mali</i>
Y-348 ^c	15501		Apple tree roots	<i>Sphingomonas mali</i>
Y-351 ^c	15502		Apple tree roots	<i>Sphingomonas mali</i>
<i>Sphingomonas paucimobilis</i>	13935 ^T	JCM 7516 ^T		
<i>Sphingomonas parapaucimobilis</i>	15100 ^T	JCM 7510 ^T		
<i>Sphingomonas yanoikuyae</i>	15102 ^T	JCM 7371 ^T		
<i>Sphingomonas adhaesiva</i>	15099 ^T	JCM 7370 ^T		
<i>Sphingomonas capsulata</i>	12533 ^T	JCM 7508 ^T		
<i>Sphingomonas sanguis</i>	13937 ^T	JCM 7514 ^T		
<i>Sphingomonas macrogoltabidus</i>	15033 ^T			
<i>Sphingomonas terrae</i>	15098 ^T	JCM 7513 ^T		
<i>Rhizomonas suberifaciens</i>	15211 ^T			
<i>Rhizomonas suberifaciens</i>	15212			

^a IFO, Institute for Fermentation, Osaka, Osaka, Japan.

^b NCPBP, National Collection of Plant Pathogenic Bacteria, Harpenden, England; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Japan; IAM, Microbial and Microalgal Research Center, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan.

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PG medium containing 1.5% agar. API 20NE tests (API, La Balme les Grottes, Montalieu Vercieu, France) were used to determine physiological and biochemical characteristics. API AUX medium containing 0.2% carbohydrates was used to determine carbohydrate assimilation, and peptone-yeast extract-phenol red medium (26) containing 0.2% carbohydrates was used to determine acid production. The 3-ketolactose test (formation of 3-ketolactose from lactose) was carried out by the method of Bernaerts and De Ley (3). Quantitative determination of 3-ketolactose was performed by high-performance liquid chromatography (HPLC) (12) as described previously (22). The method used to identify 3-ketolactose from *Sphingomonas* strains is described in the accompanying paper (15).

Determination of chemotaxonomic characteristics. Analyses to determine cellular fatty acids, sphingolipids, isoprenoid quinones, and G+C contents of DNAs and DNA-DNA hybridization experiments were performed as described previously (20). Polyamines were analyzed by HPLC as described previously (6).

16S rDNA sequence determination and analysis. Wet cells (1 to 3 mg) were suspended in 300 µl of InstaGene purification matrix (Bio-Rad Co., Ltd., Richmond, Calif.), incubated at 56°C for 15 to 30 min, sonicated at 100 W for 30 s, and heated for 5 min. After centrifugation at 1,000 × g for 2 to 3 min, the supernatant was subjected directly to PCR amplification (7) by using a *Taq* polymerase kit (Cetus, Inc., Norwalk, Conn.). The purified PCR products of isolates Y-250^T (= IFO 15498^T), Y-345^T (= IFO 15499^T), and Y-347^T (= IFO 15500^T) were sequenced by using a Sequenase kit for ³⁵S-dATP (United Biochemical, Inc., Cleveland, Ohio) (16) and the following four primers: primer 350R, covering the sequence from position 342 to position 357 (*Escherichia coli* numbering [4]); primer 520R, covering the sequence from position 517 to position 531; primer 920R, covering the sequence from position 907 to position 924; and primer 1400R, covering the sequence from position 1392 to position 1406. The 16S rDNA sequences of [*A. rhizogenes*] IFO 15208 (= IAM 14222) and [*C. lividum*] IFO 15163 (= IAM 14225) were determined with a model 373A automated fluorescent-DNA sequencer (Applied Biosystems Co., Ltd., Foster City, Calif.) and a Dye Primer Cycle sequencing kit, using dye primer -21M13 (Applied Biosystems) as described previously (28). DNA sequences were aligned by using the ODEN system (9). Nucleotide substitution rates (K_{sub} values) were calculated (11), and a phylogenetic tree was constructed by the neighbor-joining method (14). The sequences were aligned with previously published sequences obtained from DNA databases.

Nucleotide sequence accession numbers. The accession numbers of the sequences used for comparison with the sequences which we determined are as follows: *Erythrobacter longus*, M59062; *Roseobacter denitrificans*, M96746; *Porphyrobacter neustonensis*, M96745; *Sphingomonas paucimobilis* IFO 13935^T, D13725; *Sphingomonas parapaucimobilis* IFO 15100^T, D13724; *Sphingomonas adhaesiva*, G1FU11458, D16146; *Sphingomonas sanguis* IFO 13937^T, D13726; *S. capsulata*, M59296; *Sphingomonas yanoikuyae* IFO 15102^T, D13728; *Sphingomonas terrae* IFO 15098^T, D13727; *Sphingomonas macrogoltabidus* IFO 15033^T, D13723; and *Rhizomonas suberifaciens* IFO 15211^T, D13737. The nucleotide sequence data for new species of the genus *Sphingomonas* and other sequences

which we determined have been deposited in the DNA Data Bank of Japan database under the following accession numbers: *Sphingomonas asaccharolytica* IFO 15499^T, D28571, D28572, and D28573; *Sphingomonas mali* IFO 15500^T, D28574, D28575, and D28576; *Sphingomonas pruni* IFO 15498^T, D28568, D28569, and D28570; *Sphingomonas rosa* [*A. rhizogenes*] IFO 15208, D13945; and *S. yanoikuyae* [*C. lividum*] IFO 15163, D13946.

RESULTS

Morphological, physiological, and biochemical characteristics. All of the strains which we studied were gram-negative, motile rods. As shown in Table 2, all of the strains gave positive results in tests for 3-ketolactose production, β-galactosidase activity, esculin hydrolysis, and assimilation of maltose and xylose, and all of the strains gave negative results in tests for arginine dihydrolase activity, urease activity, production of indole, and assimilation of adipate, adonitol, inositol, malonate, mannitol, oxalate, sorbitol, and tartrate. The 10 strains were separated into five groups on the basis of their phenotypic characteristics. The first group consisted of three [*A. rhizogenes*] strains; the second group consisted of two [*C. lividum*] strains; the third and fourth groups each contained a single agrobacterium-like isolate (Y-250^T [= IFO 15498^T] and Y-345^T [= IFO 15499^T]); and the fifth group consisted of strains Y-347^T (= IFO 15500^T), Y-348 (= IFO 15501), and Y-351 (= IFO 15502).

Chemical characteristics. The chemical characteristics of the test strains are summarized in Tables 3 and 4. All of the strains contained ubiquinone Q-10, and the DNA base compositions ranged from 64.0 to 65.9 mol%. All of the strains contained sphingolipids, and the long-chain bases in the sphingolipids of [*C. lividum*] IFO 15163 were identified as dihydrosphingosins (d18:0, d20:1, and d21:1) by gas chromatography-mass spectrometry (data not shown). In the three strains of [*A. rhizogenes*], octadecenoic acid (18:1) and hexadecenoic acid (16:1) were major nonpolar fatty acids, while in the [*C. lividum*] strains and the agrobacterium-like isolates, 18:1 fatty acids were the major fatty acids; similarly, small amounts of 16:1 fatty acids were also present, and in agrobacterium-like

TABLE 2. Physiological characteristics of 3-ketolactose-producing strains

Characteristic	[<i>A. rhizogenes</i>] IFO 15208 ^T , IFO 15209, and IFO 15210	[<i>C. lividum</i>] IFO 15163 and IFO 15164	Strain Y-250 ^T	Strain Y-345 ^T	Strains Y-347 ^T , Y-348, and Y-351
3-Ketolactose production	+	+	+	+	+
β-Galactosidase	+	+	+	+	+
Reduction of nitrate	w	+	—	—	—
Deamination of phenylalanine	+	—	—	—	—
Arginine dihydrolase	—	—	—	—	—
Urease	—	—	—	—	—
Liquefaction of gelatin	—	+	—	—	—
Hydrolysis of esculin	+	+	+	+	+
Production of indole	—	—	—	—	—
Acid production from:					
Glucose	+	+	w	w	—
Glycerol	—	w	—	—	—
Rhamnose	+	+	—	—	+
Salicin	+	—	—	—	—
Assimilation of:					
Acetate	w	+	w	—	—
<i>N</i> -acetylglucosamine	w	+	+	+	+
Adipate	—	—	—	—	+
Adonitol	—	—	—	—	—
Arabinose	+	+	+	w	w
Caprate	—	+	—	—	—
Cellobiose	+	+	+	—	+
Citrate	—	+	—	—	—
Fructose	+	w	+	—	w
Fumarate	—	+	+	—	+
Galactose	+	+	+	—	+
Gluconate	+	+	+	—	+
Glucose	+	+	+	w	+
Glycerol	w	+	—	—	—
Inositol	—	—	—	—	—
Lactate	w	+	—	—	—
Lactose	+	+	+	w	+
Malate	—	+	w	—	—
Malonate	—	—	—	—	—
Maltose	+	+	+	+	+
Mannitol	—	—	—	—	—
Mannose	+	w	+	w	+
Melezitose	—	w	—	—	—
Melibiose	w	+	w	w	+
α-Methylglucoside	—	+	—	—	+
Oxalate	—	—	—	—	—
Propionate	w	+	—	—	—
Raffinose	+	+	—	—	+
Rhamnose	+	+	+	+	+
Salicin	+	+	+	—	+
Sorbitol	—	—	—	—	—
Starch	—	w	w	—	w
Sucrose	+	+	+	—	+
Tartrate	—	—	—	—	—
Trehalose	+	+	+	—	+
Xylose	+	+	+	+	+

^a +, positive; w, weakly positive; —, negative.

isolates Y-250^T and Y-345^T heptadecenoic acids (17:1) were present instead of 16:1 fatty acid. On the other hand, the major 2-hydroxy fatty acid was 2-hydroxymyristic acid (2-OH 14:0) in the five [*A. rhizogenes*] and [*C. lividum*] strains. Four of the other five isolates, Y-250^T, Y-347^T, Y-348, and Y-351, contained 2-hydroxypentadecanoic acid (2-OH 15:0) in addition to the major components 2-OH 14:0, and isolate Y-345^T contained 2-OH 15:0 as a major component. No strain contained 3-hydroxy fatty acids. The major component of polyamine was spermidine in the three [*A. rhizogenes*] strains, the two [*C.*

lividum] strains, and Y-345^T and homospermidine in the other isolates.

DNA-DNA homology. As shown in Table 5, the levels of DNA-DNA homology among the three [*A. rhizogenes*] strains and between the two [*C. lividum*] strains were 92 to 100 and 86 to 100%, respectively. The levels of homology between the three [*A. rhizogenes*] strains and all of the other strains tested (Table 5) were less than 28%. The levels of homology between the two [*C. lividum*] strains and *S. yanoikuyae* were 62 and 68%. Isolates Y-347^T, Y-348, and Y-351 exhibited high levels of

TABLE 3. Chemotaxonomic characteristics of 3-ketolactose-producing strains

Species or isolate	IFO no.	G+C content (mol%)	Isoprenoid quinone	Sphingolipid	Polyamine content ^a (μmol/g [wet wt] of cells)			
					put	spd	hspd	agm
[<i>Agrobacterium rhizogenes</i>]	15208 ^T	64.7	Q-10	+	0	1.30	0	0
[<i>Agrobacterium rhizogenes</i>]	15209	65.0	Q-10	+	0	1.70	0	0
[<i>Agrobacterium rhizogenes</i>]	15210	64.9	Q-10	+	0	0.65	0	0
[<i>Chromobacterium lividum</i>]	15163	64.0	Q-10	+	0	1.45	0	0.01
[<i>Chromobacterium lividum</i>]	15164	64.9	Q-10	+	0	2.05	0	0.03
Y-250 ^T	15498 ^T	65.4	Q-10	+	0	0	1.42	0
Y-345 ^T	15499 ^T	64.8	Q-10	+	0	0.61	0	0.02
Y-347 ^T	15500 ^T	65.9	Q-10	+	0	0	0.75	0
Y-348	15501	65.4	Q-10	+	0	0	0.57	0
Y-351	15502	65.7	Q-10	+	0	0	0.56	0
<i>Sphingomonas paucimobilis</i>	13935 ^T	63.7 ^b	Q-10 ^b	+	0	0	2.55	0
<i>Sphingomonas parapaucimobilis</i>	15100 ^T	65.1 ^b	Q-10 ^b	+	0	0	2.35	0
<i>Sphingomonas yanoikuyae</i>	15102 ^T	61.6 ^b	Q-10 ^b	+	0	1.57	0	0.03
<i>Sphingomonas adhaesiva</i>	15099 ^T	67.8 ^b	Q-10 ^b	+	0	1.90	0	0.01
<i>Sphingomonas capsulata</i>	12533 ^T	63.8 ^b	Q-10 ^b	+	0.10	1.80	0	0.10
<i>Sphingomonas sanguis</i>	13937 ^T	61.8 ^b	Q-10 ^b	+	0	0	1.97	0
<i>Sphingomonas macrogoltabidus</i>	15033 ^T	63.9 ^b	Q-10 ^b	+	0.02	2.10	0	0
<i>Sphingomonas terrae</i>	15098 ^T	63.0 ^b	Q-10 ^b	+	0.02	2.24	0	0.01
<i>Rhizomonas suberifaciens</i>	15211 ^T	59.0 ^c	Q-10 ^c	+	0.01	0.58	0	0
<i>Rhizomonas suberifaciens</i>	15212	58.7 ^c	Q-10 ^c	+	0.01	1.25	0	0

^a put, putrescine; spd, spermidine; hspd, homospermidine; agm, agmatine.^b Data from reference 20.^c Data from reference 21.

homology (98 to 121%) with each other, but lower levels of homology with Y-250^T (24 to 65%) and Y-345^T (57 to 61%). Similarly, the levels of homology between Y-250^T and the other four new isolates and the levels of homology between Y-345^T and the other four new isolates were around 60%. The levels of homology between the five new isolates and the reference strains (three [*A. rhizogenes*] strains, one [*C. lividum*] strain, eight *Sphingomonas* strains, and one *Rhizomonas suberifaciens* strain) were less than 36%.

Phylogenetic analysis. The sequences of 1372- and 1369-nucleotide fragments of the 16S rDNAs of [*A. rhizogenes*] IFO 15208 and [*C. lividum*] IFO 15163, respectively, were determined, and the primary structures were aligned and compared with each other and with the primary structures of 12 reference strains, including strains of *Sphingomonas* species, *Erythrobacter longus* (18), *Rhizomonas suberifaciens*, *Porphyrobacter neustonensis* (5), and *Roseobacter denitrificans* (17), which are

members of the alpha subdivision of the *Proteobacteria* (19, 21, 25). Calculations of levels of sequence similarity were based on the data for 1,244 nucleotides because of deletions. A phylogenetic tree based on K_{nuc} values is shown in Fig. 1. As shown in Table 6, the levels of similarity between [*A. rhizogenes*] and [*C. lividum*], *Rhizomonas suberifaciens*, and eight strains of *Sphingomonas* species ranged from 92.8% (*S. capsulata*) to 95.6% (*Rhizomonas suberifaciens*), while the levels of similarity between [*C. lividum*] and [*A. rhizogenes*], *Rhizomonas suberifaciens*, and eight strains of *Sphingomonas* species ranged from 91.5% (*S. capsulata*) to 99.4% (*S. yanoikuyae*).

For agrobacterium-like isolates Y-250^T, Y-345^T, and Y-347^T, partial sequences consisting of a 604-bp 16S rDNA fragment extending from nucleotides 227 to 501, 720 to 894, and 1180 to 1383 (*E. coli* numbering) were determined, and similarity values were calculated on the basis of this 604-bp sequence (Table 7). The levels of similarity between Y-250^T

TABLE 4. Cellular fatty acid contents of 3-ketolactose-producing strains^a

Species or isolate	IFO no.	Fatty acid content (%)										
		Nonpolar fatty acids ^b								2-OH fatty acids ^c		
		14:1	15:0	16:0	16:1	17:0	17:1	18:0	18:1	14:0	15:0	16:0
[<i>Agrobacterium rhizogenes</i>]	15208 ^T	5		15	26		2		50	100	tr	
[<i>Agrobacterium rhizogenes</i>]	15209	7		15	32		3		43	100	tr	
[<i>Agrobacterium rhizogenes</i>]	15210	4		16	27		2		51	100	tr	
[<i>Chromobacterium lividum</i>]	15163			12	12		1		76	94	tr	6
[<i>Chromobacterium lividum</i>]	15164			10	11		1		70	93		7
Y-250 ^T	15498 ^T		4	10	tr	4	17	tr	68	47	45	8
Y-345 ^T	15499 ^T		4	8	tr	9	29	tr	51	34	66	
Y-347 ^T	15500 ^T	tr		11	1		4	2	82	82	13	5
Y-348	15501	tr		16	1		4	1	79	78	14	8
Y-351	15502	tr		11	1		4	1	83	78	15	7

^a None of the organisms contained 3-OH fatty acids.^b Percentage of total nonpolar acids.^c Percentage of total 2-hydroxy acids.

TABLE 5. Levels of DNA-DNA homology for *Sphingomonas* strains

Species or isolate	IFO no.	G+C content (mol%)	% Homology with labeled DNA from:											
			[<i>A. rhizogenes</i>] strains			[<i>C. lividum</i>] strains		<i>S. yanoikuyae</i> IFO 15102 ^T	Strain Y-250 ^T	Strain Y-345 ^T	Strain Y-347 ^T	Strain Y-348	Strain Y-351	<i>Rhizomonas suberifaciens</i> IFO 15211 ^T
			IFO 15208 ^T	IFO 15209	IFO 15210	IFO 15163	IFO 15164							
[<i>Agrobacterium rhizogenes</i>]	15208 ^T	64.7	100	95	95				9	5	13	2	2	1
[<i>Agrobacterium rhizogenes</i>]	15209	65.0	100	100	96				2	2	2	1	1	
[<i>Agrobacterium rhizogenes</i>]	15210	64.9	100	92	100				4	1	2	1	3	
[<i>Chromobacterium lividum</i>]	15163	64.0	5			100	96	62	1	6	9			13
[<i>Chromobacterium lividum</i>]	15164	64.9				86	100							4
Y-250 ^T	15498 ^T	65.4	7	5	6	5	6		100	23	24	65	32	1
Y-345 ^T	15499 ^T	64.8	11	1	1				46	100	61	57	57	1
Y-347 ^T	15500 ^T	65.9	10	1	1				39	40	100	98	98	1
Y-348	15501	65.4	4	1	1				47	46	101	100	99	
Y-351	15502	65.7	5	1	2	3	4		61	63	121	104	100	
<i>Sphingomonas paucimobilis</i>	13935 ^T	63.7	19	1	7	9	10	7	5	21	16	3	5	8
<i>Sphingomonas parapaucimobilis</i>	15100 ^T	65.1	9			11		7	1	10	13			9
<i>Sphingomonas yanoikuyae</i>	15102 ^T	61.6	14			68		100	12	20	28			18
<i>Sphingomonas adhaesiva</i>	15099 ^T	67.8	28			6		9	26	26	22			5
<i>Sphingomonas capsulata</i>	12533 ^T	63.8	8			8		7	20	21	20			33
<i>Sphingomonas sanguis</i>	13937 ^T	61.8	12			10		6	7	1	11			2
<i>Sphingomonas macrogoltabidus</i>	15033 ^T	63.9	1			7		12	11	5	36			1
<i>Sphingomonas terrae</i>	15098 ^T	63.0	13			8		9	1	1	18			3
<i>Rhizomonas suberifaciens</i>	15211 ^T	59.0	11			6	7		4	13	13			100
<i>Rhizomonas suberifaciens</i>	15212	58.7				5	13							83

and members of 11 other species, including 8 species of the genus *Sphingomonas*, *Rhizomonas suberifaciens*, [*A. rhizogenes*], and [*C. lividum*], ranged from 95.7% (*S. capsulata*) to 100% (isolate Y-347^T).

Our 16S rRNA sequence comparison confirmed that [*A. rhizogenes*], [*C. lividum*], and isolates Y-250^T, Y-345^T, and Y-347^T all belong to the genus *Sphingomonas*.

As mentioned above, chemotaxonomic and physiological characteristics and phylogenetic data showed that all of the strains which we studied are members of the genus *Sphingomonas*.

DISCUSSION

On the basis of chemotaxonomic and physiological characteristics and the results of DNA-DNA hybridization studies and a 16S rDNA sequence comparison, we propose the following four new species of the genus *Sphingomonas*: *Sphingomonas rosa* (type strain, IFO 15208) for the strains isolated from rose plants and formerly named [*A. rhizogenes*]; *Sphingomonas pruni* (type strain, IFO 15498) for the strains isolated

from *Prunus persica*; and *Sphingomonas asaccharolytica* (type strain, IFO 15499) and *Sphingomonas mali* (type strain, IFO 15500) for the isolates obtained from apple trees. Two strains which were isolated from *Psychotria nairobiensis* and formerly named [*C. lividum*] were identified as *S. yanoikuyae* strains. *S. rosa* was isolated from the hairy roots of rose plants, but it did not contain either Ti or Ri plasmids and did not exhibit plant pathogenicity (data not shown).

Recently, on the basis of 16S rRNA genes sequencing data, it was found that all of the species belonging to the genera *Sphingomonas* and *Rhizomonas* are phylogenetically related and can be divided into several subgroups (13, 24), although it is not appropriate at the present time to transfer these species to other genera, because they have similar chemotaxonomic characteristics and there are no remarkable morphological and physiological differences which distinguish these species at the generic level. We also suggest that the genus *Sphingomonas* sensu stricto should be restricted to the species *S. paucimobilis*, *S. parapaucimobilis* and *S. sanguis* and that four other species (but not *S. capsulata*), *S. macrogoltabidus*, *S. terrae*, *S.*

FIG. 1. Unrooted phylogenetic tree showing the relationships of *Sphingomonas* species. Bar = 0.01 K_{nuc} unit.

TABLE 6. Levels of nucleotide similarity for 16S rDNA sequences of *Sphingomonas* species and other members of the alpha-4 subgroup of the *Proteobacteria*^a

Species	% Similarity												
	<i>Erythrobacter longus</i>	<i>Porphyrobacter neustonensis</i>	<i>Rhizomonas suberifaciens</i>	<i>Roseobacter denitrificans</i>	[<i>Agrobacterium rhizogenes</i>]	[<i>Chromobacterium lividum</i>]	<i>Sphingomonas adhaesiva</i>	<i>Sphingomonas capsulata</i>	<i>Sphingomonas macrogoltabidus</i>	<i>Sphingomonas parapaucimobilis</i>	<i>Sphingomonas paucimobilis</i>	<i>Sphingomonas sanguis</i>	<i>Sphingomonas terrae</i>
<i>Porphyrobacter neustonensis</i>	94.2												
<i>Rhizomonas suberifaciens</i>	90.3	91.5											
<i>Roseobacter denitrificans</i>	83.5	83.3	82.4										
[<i>Agrobacterium rhizogenes</i>]	91.6	92.7	95.6	83.0									
[<i>Chromobacterium lividum</i>]	90.7	91.8	96.1	82.7	94.6								
<i>Sphingomonas adhaesiva</i>	91.2	92.2	94.7	82.6	94.5	94.7							
<i>Sphingomonas capsulata</i>	90.3	91.2	91.6	85.1	93.7	91.5	91.2						
<i>Sphingomonas macrogoltabidus</i>	91.8	92.7	95.4	82.3	94.7	94.1	93.3	92.1					
<i>Sphingomonas parapaucimobilis</i>	89.7	90.8	92.8	82.2	92.5	93.2	95.8	89.7	92.2				
<i>Sphingomonas paucimobilis</i>	90.3	91.2	92.8	82.3	92.8	93.1	95.8	89.6	92.4	98.6			
<i>Sphingomonas sanguis</i>	90.0	90.8	93.0	82.0	92.8	93.2	96.3	89.9	92.5	98.8	98.2		
<i>Sphingomonas terrae</i>	91.3	92.3	96.0	82.5	95.1	94.5	93.8	91.3	97.0	93.1	93.5	93.6	
<i>Sphingomonas yanoikuyae</i>	90.4	91.5	96.0	82.4	94.1	99.4	94.2	91.2	94.1	92.8	92.8	92.8	94.5

^a Data for a 1,244-bp sequence.

yanoikuyae, and *Rhizomonas suberifaciens*, should be removed from this genus (21).

The results of our 16S rDNA comparison based on 1,244 bases support the results described above and also support the

finding that *S. adhaesiva* belongs to the genus *Sphingomonas* sensu stricto, while *S. rosa* represents a distinct line of descent. [*C. lividum*] strains were identified as *S. yanoikuyae* strains, although the levels of DNA-DNA hybridization between [*C.*

TABLE 7. Levels of nucleotide similarity for 16S rDNA sequences of *Sphingomonas* species and other members of the alpha-4 subgroup of the *Proteobacteria*^a

Species or isolate	% Similarity													
	<i>Erythrobacter longus</i>	<i>Porphyrobacter neustonensis</i>	<i>Rhizomonas suberifaciens</i>	<i>Roseobacter denitrificans</i>	Strain Y-250 ^T	Strain Y-345 ^T	Strain Y-347 ^T	[<i>Agrobacterium rhizogenes</i>]	[<i>Chromobacterium lividum</i>]	<i>Sphingomonas adhaesiva</i>	<i>Sphingomonas capsulata</i>	<i>Sphingomonas macrogoltabidus</i>	<i>Sphingomonas parapaucimobilis</i>	<i>Sphingomonas paucimobilis</i>
<i>Porphyrobacter neustonensis</i>	96.5													
<i>Rhizomonas suberifaciens</i>	93.2	94.5												
<i>Roseobacter denitrificans</i>	87.8	87.1	86.3											
Y-250 ^T	93.9	94.9	97.8	86.6										
Y-345 ^T	93.7	95.0	96.7	85.9	98.8									
Y-347 ^T	93.9	94.9	97.8	86.6	100	98.8								
[<i>Agrobacterium rhizogenes</i>]	94.7	96.4	96.4	87.1	97.2	96.0	97.2							
[<i>Chromobacterium lividum</i>]	93.5	94.9	96.2	86.4	97.0	97.2	97.0	96.4						
<i>Sphingomonas adhaesiva</i>	94.7	95.0	96.2	86.8	97.4	97.8	97.4	97.4						
<i>Sphingomonas capsulata</i>	93.9	95.2	94.9	86.3	95.7	94.5	95.8	97.8	94.9	95.9				
<i>Sphingomonas macrogoltabidus</i>	94.5	95.9	97.5	86.9	97.4	96.2	97.4	96.7	95.5	95.9	95.2			
<i>Sphingomonas parapaucimobilis</i>	94.0	94.4	94.7	86.9	96.9	97.0	96.9	95.7	96.5	97.5	94.5	95.2		
<i>Sphingomonas paucimobilis</i>	94.0	94.7	95.0	86.9	97.2	97.4	97.2	96.0	96.9	97.7	94.5	95.5	99.3	
<i>Sphingomonas sanguis</i>	94.4	94.5	94.9	86.9	97.0	97.2	97.0	96.2	96.4	98.0	94.7	95.4	99.5	99.1
<i>Sphingomonas terrae</i>	94.4	95.8	97.7	87.3	98.9	97.7	98.8	96.9	94.9	96.7	95.0	98.0	96.7	97.0
<i>Sphingomonas yanoikuyae</i>	93.2	94.5	96.5	86.1	97.7	97.8	97.0	96.0	99.3	97.0	94.5	95.5	96.5	96.9
														97.2

^a Data for a 604-bp sequence.

TABLE 8. Differential characteristics of 13 *Sphingomonas* species

Characteristic	<i>S. paucimobilis</i>	<i>S. parapaucimobilis</i>	<i>S. adhaesiva</i>	<i>S. capsulata</i>	<i>S. sanguis</i>	<i>S. terrae</i>	<i>S. macrogolubidus</i>	<i>S. yanoikuyae</i> ^a	<i>S. rosa</i>	<i>S. pruni</i>	<i>S. asaccharolytica</i>	<i>S. mali</i>
3-Ketolactose	+ ^b	+	-	-	+	-	-	+	(+)	+	+	+
Liquefaction of gelatin	-	+	-	-	+	-	-	-	(+)	-	-	-
Reduction of nitrate	-	+	-	-	-	-	-	-	(+)	w	-	-
Deamination of phenylalanine	+	-	-	+	+	-	-	-	(-)	+	-	-
Acid produced from:												
Glycerol	-	-	-	+	+	-	-	-	(w)	-	-	-
Rhamnose	-	+	-	+	+	-	-	+	(+)	+	-	+
Salicin	-	-	-	-	+	-	-	-	(-)	+	-	-
Assimilation of:												
Adipate	-	-	-	+	-	+	-	-	(-)	-	-	+
Caprate	-	-	-	-	-	-	-	+	(+)	-	-	-
Citrate	+	+	-	-	-	-	-	+	(+)	-	-	-
Fructose	+	+	+	+	+	-	-	+	(w)	+	-	w
Fumarate	+	+	+	+	+	+	-	+	(+)	-	+	+
Gluconate	-	+	-	+	-	-	-	+	(+)	+	+	+
Rhamnose	-	+	-	+	-	-	-	+	(+)	+	+	+
Xylose	+	+	-	+	+	-	-	+	(+)	+	+	+
Major polyamine ^c	hspd	hspd	spd	spd	hspd	spd	spd	spd (spd)	spd	hspd	spd	hspd
Major cellular 2-hydroxy fatty acid(s)	14:0	14:0	14:0 (15:0)	14:0	14:0	14:0, 15:0	14:0, 16:0	14:0 (14:0)	14:0	14:0, 16:0	15:0, 14:0	14:0 (15:0)

^a The data in parentheses are data for strains IFO 15163 and IFO 15164.^b +, most strains are positive; -, most strains are negative; w, all strains are weakly positive.^c See Table 3, footnote a.

lividum] and *S. yanoikuyae* strains were less than 70% (62 to 68%). However, these strains shared many chemotaxonomic and physiological features, and the high levels of 16S rDNA similarity (99.8%) between these two taxa support this conclusion. On the other hand, in our phylogenetic study based on a 604-bp 16S rDNA sequence, *S. pruni* and *S. mali* exhibited a high level of similarity (100%), but these two organisms could be differentiated by DNA-DNA hybridization data and many physiological characteristics.

Differential characteristics of the new species and of previously described species of the genus *Sphingomonas* are shown in Table 8. *S. pruni* does not produce acid from rhamnose and does not assimilate adipate, α -methylglucoside, and raffinose, but *S. mali* produces acid from rhamnose and assimilates these sugars (Table 2). These organisms also differ in their cellular fatty acid patterns (Table 4).

Strains of four of the species which we studied (*S. paucimobilis*, *S. parapaucimobilis*, *S. sanguis*, and *S. yanoikuyae*) were found to produce 3-ketolactose from lactose, as shown in Table 8. 3-Ketolactose-forming activity has been found previously only in strains belonging to *Agrobacterium* biovar 1, and this characteristic has proven to be a useful taxonomic marker for *agrobacteria*. However, in this study, we found that many species of the genus *Sphingomonas* contain the 3-ketolactose biosynthetic pathway. The results of a study of the distribution of 3-ketolactose-forming activity in members of the alpha subdivision of the *Proteobacteria* are described in the accompanying paper (15).

The new *Sphingomonas* species are described below.

Description of *Sphingomonas rosa* sp. nov. *Sphingomonas rosa* (ro'sa. M. L. n. *rosa*, rose, the source of the organism) is a gram-negative, nonsporing, motile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and whitish yellow. Indole, urease, and arginine dihydrolase are not

produced. 3-Ketolactose is produced. β -Galactosidase positive. Deamination of phenylalanine is positive. Gelatin is not liquefied. Reduction of nitrate is weak. Esculin, arabinose, cellobiose, fructose, lactose, maltose, mannose, raffinose, rhamnose, salicin, sucrose, trehalose, and xylose are assimilated, but adonitol, fumarate, malate, malonate, melezitose, sorbitol, starch, and trehalose are not assimilated. Acid is produced from glucose, rhamnose, and salicin but not from glycerol.

The G+C content of the DNA is 64.7 to 65.0 mol%. The major isoprenoid quinone is Q-10. The major nonpolar fatty acids are 16:1, 18:1, and 16:0, and the major 2-hydroxy fatty acid is 2-OH 14:0. Sphingolipid is present. Source: isolated from the hairy roots of *Rosa* sp. (rose).

The type strain is NCPPB 2661 (= IFO 15208).

Description of *Sphingomonas pruni* sp. nov. *Sphingomonas pruni* (pru'ni. M. L. gen. n. *pruni*, of *Prunus* [*Prunus persica*, peach], the source of the organism) is a gram-negative, nonsporing, motile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and light yellow. β -Galactosidase positive. Indole, urease, and arginine dihydrolase are not produced. Deamination of phenylalanine is negative. 3-Ketolactose is produced. Reduction of nitrate is negative. Gelatin is not liquefied. Esculin, arabinose, cellobiose, fructose, fumarate, galactose, gluconate, glucose, lactose, maltose, mannose, rhamnose, salicin, sucrose, trehalose, and xylose are assimilated, but adonitol, glycerol, inositol, lactate, malonate, manitol, raffinose, sorbitol, and tartrate are not assimilated. Acid is produced from glucose weakly, but not from glycerol, rhamnose, and salicin.

The G+C content of the DNA is 65.4 mol%. The major isoprenoid quinone is Q-10. The major nonpolar fatty acids are 17:1, 18:1, 16:0, and the major 2-hydroxy fatty acids are 2-OH 14:0 and 2-OH 15:0. Sphingolipid is present. Source: isolated

from the roots of *Prunus persica* (peach) in Tsukuba City, Japan.

The type strain is IFO 15498 (= Y-250).

Description of *Sphingomonas asaccharolytica* sp. nov. *Sphingomonas asaccharolytica* (a.sac.cha.ro.ly'ti.ca. Gr. pref. *a*, not; Gr. n. *sacchar*, sugar; Gr. adj. *lytica*, able to loosen; M. L. adj. *asaccharolytica*, not digesting sugar) is a gram-negative, non-spore, motile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and light yellow. β -Galactosidase positive. Indole, urease, and arginine dihydrolase are not produced. Deamination of phenylalanine is negative. 3-Ketolactose is produced. Reduction of nitrate is negative. Gelatin is not liquefied. Esculin, maltose, rhamnose, and xylose are assimilated, but adonitol, cellobiose, fructose, fumarate, galactose, gluconate, glycerol, inositol, lactose, malate, malonate, mannitol, raffinose, salicin, sorbitol, starch, sucrose, and trehalose are not assimilated. Acid is produced from glucose weakly, but not from glycerol, rhamnose, and salicin.

The G+C content of the DNA is 64.8 mol%. The major isoprenoid quinone is Q-10. The major nonpolar fatty acids are 17:1 and 18:1, and the major 2-hydroxy fatty acids are 2-OH 14:0 and 2-OH 15:0. Sphingolipid is present. Source: isolated from the roots of *Malus* spp. (apple) in Tsukuba City, Japan.

The type strain is IFO 15499 (= Y-345).

Description of *Sphingomonas mali* sp. nov. *Sphingomonas mali* (mal'i. M. L. gen. n. *mali*, of *Malus*, the apple genus, the source of the organism) is a gram-negative, non-spore, motile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and light yellow. β -Galactosidase positive. Indole, urease, and arginine dihydrolase are not produced. Deamination of phenylalanine is negative. 3-Ketolactose is produced. Reduction of nitrate is negative. Gelatin is not liquefied. Esculin, cellobiose, fumarate, galactose, gluconate, glucose, lactose, maltose, mannose, raffinose, rhamnose, salicin, sucrose, trehalose, and xylose are assimilated, but adonitol, citrate, glycerol, inositol, lactate, malate, malonate, mannitol, sorbitol, and tartrate are not assimilated. Acid is produced from rhamnose, but not from glucose, glycerol, and salicin.

The G+C content of the DNA is 65.4 to 65.9 mol%. The major isoprenoid quinone is Q-10. The major nonpolar fatty acids are 16:0 and 18:1, and the major 2-hydroxy fatty acids are 2-OH 14:0 and 2-OH 15:0. Sphingolipid is present. Source: isolated from the roots of *Malus* spp. (apple) in Tsukuba City, Japan.

The type strain is IFO 15500 (= Y-351).

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