Emended Description of the Genus *Agromyces* and Description of *Agromyces cerinus* subsp. *cerinus* sp. nov., subsp. nov., *Agromyces cerinus* subsp. *nitratus* sp. nov., subsp. nov., *Agromyces fucosus* subsp. *fucosus* sp. nov., subsp. nov., and *Agromyces fucosus* subsp. *hippuratus* sp. nov., subsp. nov.

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Fourteen soil-inhabiting, fragmenting actinomycetes with diaminobutyric acid in their cell walls and MK-12 as their major menaquinone were characterized and assigned to two new *Agromyces* species; these organisms were further subdivided into four subspecies on the basis of numerical data, cell wall sugar compositions, and levels of DNA relatedness. The key characteristics which differentiated between the new species and subspecies of the genus *Agromyces* and *Agromyces ramosus* included the presence of tyvelose or fucose in the cell walls; some physiological properties, including catalase and oxidase activities; growth on media supplemented with inorganic nitrogen; reduction of nitrate; and hydrolysis of hippurate. The data which became available when we studied more strains of *Agromyces* species and subspecies suggested that the genus description should be emended. We suggest that the following characteristics should be included in the genus description: catalase and oxidase activities are variable; the majority of strains grow on inorganic media; as a rule, galactose and rhamnose are present in the cell walls; some strains contain glucose, fucose, tyvelose, mannose, ribose, and/or xylose as the major cell wall sugar(s); iso and anteiso types of fatty acids predominate (94%); and the G+C contents of the DNAs are 70 to 72 mol%, as determined by the thermal denaturation method. The type strains of the new species and subspecies are as follows: *Agromyces cerinus* subsp. *cerinus* VKM Ac-1340, *Agromyces cerinus* subsp. *nitratus* VKM Ac-1351, *Agromyces fucosus* subsp. *fucosus* VKM Ac-1345, and *Agromyces fucosus* subsp. *hippuratus* VKM Ac-1352.

The group of actinomycetes and coryneform bacteria which have two molecules of diaminobutyric acid in their peptidoglycans is represented by members of the genera *Agromyces* (10) and *Clavibacter* (5) and by individual strains of coryneform bacteria whose taxonomic positions are unclear ("Corynebacterium spp.") (5).

Soil actinomycetes belonging to the genus *Agromyces* Gledhill and Casida (10) differ from phytopathogenic bacteria belonging to the genus *Clavibacter* mainly in the following characteristics: formation of true mycelia, menaquinone composition, and a number of physiological characteristics. These actinomycetes (in particular, the genus *Agromyces* as originally described) have been characterized as being catalase and oxidase negative (10).

Only one species of the genus *Agromyces*, *Agromyces ramosus*, has been described previously. Most chemotaxonomic characteristics (the exceptions are the differential amino acids and the major cell wall sugars) have been determined only for the type strain of *A. ramosus*, strain ATCC 25173 (2, 3, 10, 15). As far as we know, at present only one strain of *A. ramosus* is kept in culture collections.

In 1985, we isolated from soils 14 strains of aerobic, catalase- and oxidase-positive actinomycetes that have yellow fragmenting mycelia and contain diaminobutyric acid in their cell walls (9). The results of a preliminary characteri-

zation of these strains in which they were compared with the previously published descriptions of several taxa showed that they are most similar to *A. ramosus* but differ from this species in a number of characteristics (2, 9).

In this paper we describe the results of a comparative study of the 14 isolates obtained from soil, as well as the type strain of *A. ramosus* (strain ATCC 25173) and representatives of the genus *Clavibacter*.

MATERIALS AND METHODS

Bacterial strains. The strains were isolated from soil samples that were obtained in the Moscow Region of Russia. The *Agromyces* and *Clavibacter* type strains were obtained from the American Type Culture Collection (ATCC), Rockville, Md., and the International Collection of Microorganisms from Plants (ICMP), Auckland, New Zealand. The strains used in this work are shown in Tables 1 through 3 and Fig. 1.

Morphology. Morphological characteristics were determined by using phase-contrast microscopy of 3- and 7-day-old cultures that were grown on peptone-yeast extract agar (5 g of peptone, 3 g of yeast extract, 5 g of glucose, 0.2 g of K₂HPO₄, 1 liter of distilled water, 15 g of agar).

Physiology. Utilization of carbohydrates was tested by using modified Pridham-Gottlieb medium (23) supplemented with 0.01% yeast extract and glucose or other carbon sources at final concentrations of 1%. Modified Pridham-

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TABLE 1. Sugar compositions of whole cells of *Agromyces* strains

Strain	Presence of ^a :							
	Galactose	Glucose	Mannose	Xylose	Ribose	Fucose	Rhamnose	Tyvelose
<i>A. ramosus</i> ATCC 25173	(+)	+	+	+	+	—	+	—
<i>A. cerinus</i> subsp. <i>cerinus</i>								
VKM Ac-1340 ^T	+	+	+	+	+	—	+	+
VKM Ac-1341	+	+	+	+	+	—	+	+
VKM Ac-1342	+	+	+	(+)	+	—	+	+
VKM Ac-1343	+	+	+	(+)	+	—	+	+
VKM Ac-1344	+	+	+	(+)	+	—	+	(+)
VKM Ac-1350	+	+	+	(+)	+	—	+	+
<i>A. cerinus</i> subsp. <i>nitratus</i>	+	+	+	+	+	—	—	—
VKM Ac-1351 ^T								
<i>A. fucosus</i> subsp. <i>fucosus</i>								
VKM Ac-1345 ^T	+	+	+	—	+	+	+	—
VKM Ac-1346	+	+	+	—	+	+	+	—
VKM Ac-1347	(+)	+	+	—	+	(+)	+	—
VKM Ac-1348	+	+	+	—	+	(+)	+	—
VKM Ac-1349	+	+	+	—	+	(+)	+	—
<i>A. fucosus</i> subsp. <i>hippuratus</i>								
VKM Ac-1352 ^T	+	+	+	(+)	+	(+)	+	—
VKM Ac-1353	+	+	+	(+)	+	(+)	+	—

^a +, detected; —, not detected; (+), detection weak or questionable.

Gottlieb medium which contained 15 ml of 0.03% bromocresol purple was used to test the production of acids from carbohydrates and related carbon sources, and modified Pridham-Gottlieb medium which contained 15 ml of 0.03% phenol red was used to test the utilization of organic acids. Utilization of amino acids as N sources was tested by using previously described methods (11). Catalase production was determined by mixing a fresh colony of bacteria with 1 drop of 3% hydrogen peroxide. Production of oxidase was tested by using the method of Kovacs (16). Susceptibility to growth inhibitors and antibiotics was tested on peptone-yeast extract agar. Degradation activity and additional biochemical characteristics were determined by using previously described methods (5, 12, 13).

Analysis of phenotypic data. Similarities were calculated by using the simple matching coefficient and the Jaccard coefficient. The BIOMATRIX copackage was used for cluster analysis (22).

Chemotaxonomy. Cell wall peptidoglycan preparations were obtained by using the methods of Schleifer and Kandler (24). Peptidoglycan amino acids were assayed by using a model LC600 amino acid analyzer (Biotronic, Munich, Germany). The sugar compositions of hydrolysates of whole cells and cell walls that were purified by using enzymes (0.01 mg of DNase per ml; 0.1 mg of pronase per ml; 0.1 mg of trypsin per ml; 0.05 M sodium phosphate buffer [pH 7.0]) were analyzed by chromatographic methods (14, 17). Cellular fatty acid methyl ethers were determined by using previously described methods (8). Menaquinones were extracted and purified by using the method of Collins (4); the menaquinone compositions were determined by using a model MAT 8430 mass spectrometer (Finnigan, Bremen, Germany). Phospholipids were isolated, analyzed by thin-layer chromatography, and identified by using the method of Minnikin et al. (20).

Polyacrylamide gel electrophoresis. Gradient slab gels were used as described by Carlson and Vidaver (1). Cells were grown aerobically in peptone-yeast extract medium for 24 h at 27°C.

DNA base composition and DNA-DNA hybridization. DNA

was prepared as described previously (6, 8). The G+C content of each DNA was determined from its thermal denaturation midpoint (T_m) (18) in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by using a Beckman model DU-8B spectrophotometer equipped with a thermoprogrammer. DNA base composition was calculated by using the equation of Owen and Pitcher (21). DNA from *Micrococcus luteus* VKM B-1314^T (T = type strain), which has a G+C content of 72 mol% (21), was used as a reference. The levels of DNA reassociation between strains were determined by using the membrane filter method (19, 25), as described previously (8).

RESULTS

Morphological and chemotaxonomic characteristics, such as peptidoglycan amino acid composition (2,4-diaminobutyric acid, alanine, glutamic acid, and glycine, 2:1:1:1), diagnostic phospholipids (phosphatidylglycerol and diphosphatidylglycerol), and predominant menaquinones (MK-12), were similar in the 14 soil isolates which we studied and were the same as the characteristics of the type strain of *A. ramosus*, strain ATCC 25173.

All of the strains which we studied except the type strain of *A. ramosus* grew well aerobically and were catalase and oxidase positive.

The following sugars were found in the whole-cell hydrolysates: galactose, glucose, mannose, ribose, rhamnose, xylose, fucose, and tyvelose (7, 17) (Table 1). Not all of the sugars were present in the cell walls of all of the strains. Galactose, glucose, rhamnose, tyvelose, fucose, mannose, and ribose were detected in all or some preparations of purified cell walls (Table 2); xylose was also present in the native cell walls (17). The majority of the strains could be grouped on the basis of the presence of fucose and tyvelose in whole-cell hydrolysates or in purified cell walls (Tables 1 and 2). Xylose was detected in all of the hydrolysates containing tyvelose except the strain VKM Ac-1351^T hydrolysate. Strains VKM Ac-1352^T and VKM Ac-1353, which contained fucose, also contained xylose.

TABLE 2. Cell wall sugars of *Agromyces* strains

Strain	Molar ratio ^a						
	Galactose	Glucose	Mannose	Ribose	Fucose	Rhamnose	Tyvelose
<i>A. cerinus</i> subsp. <i>cerinus</i>							
VKM Ac-1340 ^T	1		0.3			3.4	3.5
VKM Ac-1341	1					1.2	2.1
VKM Ac-1342	1					2.5	0.8
VKM Ac-1343	1					2.3	1.3
VKM Ac-1344	1	Tr	0.2			3.5	0.6
VKM Ac-1348	1					1.7	1.1
<i>A. cerinus</i> subsp. <i>nitratus</i>	1	0.3	Tr	0.2			
VKM Ac-1351 ^T							
<i>A. fucosus</i> subsp. <i>fucosus</i>							
VKM Ac-1345 ^T	1		Tr		0.9	0.6	
VKM Ac-1346	1	4.6			2.5	3.8	
VKM Ac-1347	1					7.0	
VKM Ac-1349	1		Tr			2.3	
<i>A. fucosus</i> subsp. <i>hippuratus</i>							
VKM Ac-1352 ^T	1					13.8	
VKM Ac-1353	1		Tr			8.5	

^a The galactose level was defined as 1. Tr, trace.

The whole-cell protein patterns appeared to be rather similar in all of the strains which we studied. The type strain of *A. ramosus* differed from the other strains to the greatest extent. The protein patterns of the strains that had similar cell wall sugar compositions were most similar to each other.

The DNA G+C contents of the strains which we studied, including the type strain of *A. ramosus*, ranged from 70 to 72 mol%.

The level of DNA-DNA relatedness between the type strain of *A. ramosus* and reference strain VKM Ac-1340^T was 12%. At least two groups among all of the isolates were identified on the basis of levels of DNA-DNA relatedness (Table 3). Strains belonging to the first group (which as a rule contained tyvelose in their cell walls) exhibited rather high levels of DNA-DNA relatedness (68 to 92%) and were considered to be members of one genospecies (19). It should

be noted that this genospecies also contained strain VKM Ac-1351^T, which differed from the majority of the isolates by lacking rhamnose and tyvelose in its cell wall and by some physiological characteristics (Tables 1, 2, and 4).

The levels of DNA-DNA relatedness between strain VKM Ac-1340^T and strains belonging to the other group (strains VKM Ac-1345^T, VKM Ac-1348, VKM Ac-1349, VKM Ac-1352^T, and VKM Ac-1353) ranged from 40 to 48% (the whole-cell protein patterns of these strains were similar).

Our numerical analysis of strains of *Agromyces* spp. and *Clavibacter* strains based on 135 physiological and biochemical characteristics showed that all of the *Agromyces* strains which we studied formed one cluster (simple matching coefficient, 80%; Jaccard coefficient, 68%) (Fig. 1). Within the *Agromyces* spp. cluster, three subclusters (subclusters A, B, and C) stood out clearly. Subcluster A contained strains that had DNA complementarity levels ranging from 71 to 92% and tyvelose in their cell walls. Subcluster B consisted of five strains that had fucose in their whole-cell hydrolysates and exhibited levels of DNA-DNA relatedness with strain VKM Ac-1340^T of 40 to 48%. Subcluster C contained three strains which belonged to various genospecies and had different cell wall sugar compositions.

The differential physiological properties of the strains which we studied are summarized in Table 4. Detailed characterizations of the physiological properties are given below in the descriptions of the new species and the emended description of the genus *Agromyces*.

DISCUSSION

Chemotaxonomic characteristics and the levels of DNA relatedness (Table 3) supported the suggestion that the strains which we studied and the type strain of *A. ramosus* (strain ATCC 25173) belong to a single genus.

Taking into account phenotypic differences, we believe that the newly described strains belong to species that are different from *A. ramosus*.

We propose that the majority of the strains should be classified in two species, *Agromyces cerinus* sp. nov. and *Agromyces fucosus* sp. nov., on the basis of their inclusion

TABLE 3. Levels of DNA-DNA relatedness between strain VKM Ac-1340^T and *Agromyces* spp. strains

Strain	% Relatedness to [³ H]DNA from strain VKM Ac-1340 ^T
<i>A. cerinus</i> subsp. <i>cerinus</i>	
VKM Ac-1340 ^T	100
VKM Ac-1341.....	92
VKM Ac-1342.....	88
VKM Ac-1343.....	71
<i>A. cerinus</i> subsp. <i>nitratus</i>	
VKM Ac-1351 ^T	68
<i>A. fucosus</i> subsp. <i>fucosus</i>	
VKM Ac-1345 ^T	40
VKM Ac-1348.....	45
VKM Ac-1349.....	47
<i>A. fucosus</i> subsp. <i>hippuratus</i>	
VKM Ac-1352 ^T	48
VKM Ac-1353.....	45
<i>A. ramosus</i> ATCC 25173.....	12
<i>Clavibacter michiganensis</i> subsp. ICMP 2550 ^T	10
<i>Clavibacter rathayi</i> ^a ICMP 2574 ^T	12

^a It has been proposed that this organism should be transferred to *Rathayibacter* gen. nov. (26).

TABLE 4. Characteristics that differentiate species and subspecies of the genus *Agromyces*

Characteristic	<i>A. ramosus</i> (60 strains) ^a	<i>A. cerinus</i> subsp. <i>cerinus</i> (6 strains)	<i>A. cerinus</i> subsp. <i>nitratius</i> (1 strain)	<i>A. fucosus</i> subsp. <i>fucosus</i> (5 strains)	<i>A. fucosus</i> subsp. <i>hippuratus</i> (2 strains)
Sugars in whole-cell hydrolysates					
Tyvelose	ND ^b	100 ^c	—	0 ^c	0 ^c
Xylose	77 ^d	100	+	0	100
Fucose	0	0	—	100	100
Rhamnose	100	100	—	100	100
Catalase activity	0	100	+	100	100
Benzidine test	0	100	+	100	100
Utilization of the following compounds as C sources:					
Inositol	ND	83	—	0	0
Starch	ND	0	+	100	100
Lysin	ND	16	+	80	50
Acid production from:					
L-Arabinose	97 ^d	0	—	100	100
Xylose	95 ^d	0	—	60 ^d	100
Lactose	10	100	—	80 ^d	0
Raffinose	98 ^d	0	—	60	0
Salicin	13	100	—	60 ^d	0
Sucrose	92 ^d	0	—	100	0
Fructose	100	50	—	100	0
Utilization of the following organic acids:					
Aconitate	ND	0	+	0	50
Formate	ND	0	+	0	0
Citrate	5	100	+	20	0
Succinate	ND	83	+	80	0
Degradation of:					
Hippurate	ND	0	+	20 ^d	100
Tyrosine	0	0	+	20	100
NO ₃ reduction	15	0	+	0	100
Growth at 37°C	ND ^d	0	+	0	0
Growth in the presence of 4% NaCl	57	0	—	0	0
Antibiosis to <i>Bacillus subtilis</i>	ND	0	—	0	50 ^d

^a Data from reference 10.^b ND, no data available.^c Percentage of positive strains.^d The reaction of the type strain is positive.

in subclusters A and B (Fig. 1) and belong to different genospecies (Table 3).

Strain VKM Ac-1351^T differed from the other strains in its cell wall sugars and in a number of phenotypic characteristics (Table 4) but exhibited a level of DNA relatedness of 68% with other representatives of *A. cerinus*; this strain is a member of a subspecies of *A. cerinus*, *A. cerinus* subsp. *nitratius* subsp. nov. (named for its ability to reduce nitrate).

Two strains, strains VKM Ac-1352^T and VKM Ac-1353, differed from the major group of strains on the basis of the results of a numerical analysis (Fig. 1 and Table 4) but were closely related to *A. fucosus* on the basis of whole-cell sugar compositions and whole-cell protein patterns and are classified as members of *A. fucosus* subsp. *hippuratus* subsp. nov. (named for its ability to hydrolyze hippurate).

Characteristics of the new species and subspecies are given below in their descriptions.

Below we also give an emended description of the genus *Agromyces*, which is based on the results of a study of 14 original strains and *A. ramosus* ATCC 25173^T and on previously described data (2, 3, 10, 15).

Most of the strains studied by Gledhill and Casida and assigned to *A. ramosus* are heterogeneous in physiological properties and cell wall sugar composition. It is possible that some of these strains do not belong to the species *A. ramosus*. However, since these strains were not deposited in

accessible culture collections, we could not confirm this hypothesis.

Emended description of the genus *Agromyces*. *Agromyces* (Ag.ro.my'ces. Gr. n. *agros*, field or soil; Gr. n. *myces*, fungus; M.L.n. *Agromyces*, soil fungus). Branching hyphae (width, 0.3 to 0.6 µm) break up into diphtheroid and rodlike, irregular, nonmotile fragments. No endo- and exospores are formed. Colonies on peptone-yeast extract medium are opaque, entire, and convex and sometimes penetrate into the agar. Rough colony variants occur. Produces a yellow carotenoid pigment.

Gram positive. Not acid fast. Aerobic to microaerophilic. Catalase and oxidase activities are variable for different species. Chemoorganotrophic. Glucose is metabolized oxidatively. Utilizes a wide range of C and N sources. Grows on media supplemented with inorganic nitrogen; organic nitrogen is required for growth of some strains. Mesophilic. Optimal growth occurs at temperatures between 26 and 30°C; some strains grow at 37°C. The Voges-Proskauer test is negative.

The peptidoglycan contains 2,4-diaminobutyric acid, alanine, glutamic acid, and glycine (molar ratio, 2:1:1:1). Galactose and rhamnose occur in the cell walls of most strains; fucose, tyvelose, glucose, mannose, xylose, or ribose may also be present. Mycolic acids are not present. Diagnostic polar lipids are represented by diphosphatidylglycerol and

FIG. 1. Dendrogram showing the relationships among strains belonging to *Agromyces* and *Clavibacter* species. The dendrogram was based on 135 physiological characteristics and was drawn by using Jaccard coefficient values.

phosphatidylglycerol. The major menaquinone type is MK-12. Iso- and anteiso-branching fatty acids predominate (up to 94% of the total fatty acids). The G+C content of the DNA is 70 to 72 mol% (as determined by the T_m method).

Found mainly in soils.

The type species of the genus *Agromyces* is *Agromyces ramosus* Gledhill and Casida 1969, 365^{AL}.

Additional descriptive information. The majority of the strains of the genus grow on media containing D-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, methyl- α -D-glucoside, methyl- β -D-glucopyranoside, inulin, maltose, D-mannose, melezitose, raffinose, L-rhamnose, salicin, sucrose, or trehalose as a carbon source. Dulcitol, meso-erythritol, D-mannitol, methyl- β -D-arabinopyranoside, and sorbose are not used as sole carbon sources. Acids are produced from cellobiose, D-galactose, D-mannose, and L-rhamnose; no acids are formed from meso-inositol and D-mannitol. An alkaline reaction occurs with fumarate. *trans*-Aconitate, ascorbate, gluconate, oxalate, salicylate, and tartrate are not utilized. L-Asparagine is used as a sole N source; arginine, methionine, norleucine, phenylalanine, threonine, tryptophan, tyrosine, and valine are not utilized as N sources. Phenylalanine is not deaminated.

Esculin and arbutin are hydrolyzed; casein, elastin, guanine, hypoxanthine, pectin, testosterone, Tween 21, Tween 60, Tween 80, urea, and xanthine usually are not decomposed. All strains are susceptible to bacitracin (10 μ g/ml), tetracycline (30 μ g/ml), novobiocin (30 μ g/ml), erythromycin (15 μ g/ml), neomycin (30 μ g/ml), rifampin (5 μ g/ml), kanamycin (30 μ g/ml), and vancomycin (30 μ g/ml) and are resistant to clindamycin (2 μ g/ml) and nalidixic acid (30 μ g/ml). No growth occurs in the presence of 4% NaCl, 0.01% sodium azide, and 0.0175% potassium tellurite.

The following tests are variable: utilization of adonitol, L-arabinose, meso-inositol, melibiose, starch, and D-xylose; acid production from L-arabinose, cellobiose, D-fructose, D-glucose, meso-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, salicin, sucrose, trehalose,

and D-xylose; alkaline reaction with fumarate, citrate, α -ketoglutarate, succinate, or valerate; use of asparagine, lysine, or serine as a sole N source; hydrolysis of hippurate, starch, Tween 40, and tyrosine; NO₃ reduction; susceptibility to ampicillin (10 μ g/ml), gentamicin (10 μ g/ml), penicillin (10 μ g/ml), rifampin (50 μ g/ml), and streptomycin (10 μ g/ml); and growth at 37°C.

Description of *Agromyces cerinus* sp. nov. *Agromyces cerinus* (ce.ri'nus. M.L. adj. *cerinus*, honey yellow). Branching hyphae (width, 0.2 to 0.6 μ m) break up into diphtheroid and rodlike, irregular, nonmotile fragments. Colonies on peptone-yeast extract medium are opaque, entire, and convex and penetrate into the agar. Produces a yellow carotenoid pigment.

In most strains the cell wall contains galactose, rhamnose, tyvelose, and xylose.

Ammonium ions are usually assimilated as a sole N source. Organic nitrogen is required for good growth of only some strains. D-Arabinose, cellobiose, D-fructose, D-galactose, D-glucose, inulin, maltose, D-mannose, melezitose, methyl- α -D-glucoside, methyl- β -D-glucoside, raffinose, L-rhamnose, salicin, sucrose, and trehalose are used as sole C sources. Does not utilize dulcitol, i-erythritol, D-mannitol, methyl- β -D-arabinopyranoside, or sorbose as a sole C source. Acids are produced from cellobiose, D-galactose, D-mannose, and L-rhamnose, and no acids are formed from L-arabinose, meso-inositol, melibiose, raffinose, sucrose, and D-xylose.

Alkaline reactions occur with citrate, fumarate, α -ketoglutarate, and succinate, but aconitate, ascorbate, gluconate, salicylate, oxalate, tartrate, and usually formate are not utilized. Arginine, methionine, norleucine, phenylalanine, threonine, tryptophan, tyrosine, and valine are not utilized as N sources. Deamination of phenylalanine is negative.

Usually nitrate reduction does not occur. The Voges-Proskauer test is negative.

Esculin and arbutin are hydrolyzed; casein, elastin, gua-

nine, hypoxanthine, pectin, testosterone, Tween 21, Tween 60, Tween 80, and xanthine are not decomposed. Tyrosine and Tween 40 are decomposed by most strains. No growth occurs on media supplemented with 7% NaCl, 0.01% sodium azide, or 0.0175% potassium tellurite. Susceptible to ampicillin (10 µg/ml), bacitracin (10 µg/ml), erythromycin (15 µg/ml), kanamycin (30 µg/ml), neomycin (30 µg/ml), novobiocin (30 µg/ml), rifampin (5 µg/ml), tetracycline (30 µg/ml), and vancomycin (30 µg/ml) but resistant to clindamycin (2 µg/ml), nalidixic acid (30 µg/ml), and penicillin (10 µg/ml).

The following tests are variable: utilization of L-arabinose, *meso*-inositol, and melibiose as sole C sources; acid production from D-fructose, D-glucose, lactose, maltose, D-mannitol, salicin, and trehalose; utilization of formate, *trans*-aconitate, or valerate; assimilation of asparagine, lysine, and serine as N sources; hydrolysis of hippurate and starch; susceptibility to gentamicin (10 µg/ml), rifampin (50 µg/ml), and streptomycin (10 µg/ml); and growth at 37°C.

The G+C content of the DNA is 72 mol% (as determined by the T_m method).

Isolated from soil.

The type strain of *A. cerinus* is strain VKM Ac-1340.

The description given above was based on seven strains.

Description of *Agromyces cerinus* subsp. *cerinus* sp. nov., subsp. nov. Strains of *A. cerinus* subsp. *cerinus* are distinguished by a specific cell wall sugar composition; tyvelose, xylose, and rhamnose are present.

Most strains grow on medium containing *meso*-inositol. Acids are produced from lactose, salicin, or trehalose. Acids are not produced from L-arabinose, inulin, or D-mannitol. *trans*-Aconitate and formate are not utilized. Nitrate is not reduced. Tyrosinase activity is negative. Hippurate is not hydrolyzed. Organic nitrogen is not required for good growth. No growth occurs at 37°C.

The G+C content of the DNA is 72 mol% (as determined by the T_m method).

The type strain of *A. cerinus* subsp. *cerinus* is strain VKM Ac-1340.

The description given above was based on six strains.

Description of *Agromyces cerinus* subsp. *nitratus* sp. nov., subsp. nov. *Agromyces cerinus* subsp. *nitratus* (ni.tra'tus. N.L. n. *nitratum*, nitrate; N.L. adj. *nitratus*, decomposed nitrate) is distinguished from *A. cerinus* subsp. *cerinus* by the absence of rhamnose, tyvelose, and xylose and the presence of glucose and ribose in the cell walls. Organic nitrogen is required for good growth. Grows on media containing L-arabinose, maltose, melibiose, and starch as C sources. Produces acid from maltose or mannitol; acids are not produced from D-fructose, *meso*-inositol, lactose, salicin, and trehalose. Utilizes formate and *trans*-aconitate. Valerate is not utilized. Starch and Tween 40 are decomposed. Reduces nitrate and produces tyrosinase. Grows at 37°C. The G+C content of the DNA is 72 mol% (as determined by the T_m method).

The type strain of *A. cerinus* subsp. *nitratus* is strain VKM Ac-1351.

The description given above was based on one strain.

Description of *Agromyces fucosus* sp. nov. *Agromyces fucosus* (fu.co'sus. N.L. n. *fucosum*, fucose; N.L. adj. *fucosus*, containing fucose in the cell wall). Branching hyphae (width, 0.2 to 0.6 µm) break up into diphtheroid and rodlike, irregular, nonmotile fragments. Colonies on peptone-yeast extract medium are opaque, entire, and convex and sometimes penetrate into the agar. Rough colony variants occur. Produces a yellow carotenoid pigment.

The whole-cell hydrolysates contain galactose, rhamnose, fucose, mannose, and glucose as major sugars.

Organic nitrogen is not required for good growth.

Most strains grow on media containing D-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, inulin, maltose, D-mannose, melezitose, methyl-β-D-glucoside, methyl-α-D-glucoside, raffinose, L-rhamnose, salicin, starch, sucrose, trehalose, or D-xylose as a sole carbon source; dulcitol, i-erythritol, *meso*-inositol, D-mannitol, methyl-β-D-arabinopyranoside, and L-sorbose are not used as sole carbon sources. Acids are produced from L-arabinose, cellobiose, D-galactose, D-mannose, L-rhamnose, and D-xylose. No acids are formed from D-glucose and *meso*-inositol. Ascorbate, *trans*-aconitate, citrate, formate, gluconate, oxalate, salicylate, and tartrate are not utilized. Arginine, methionine, norleucine, phenylalanine, threonine, tryptophan, tyrosine, and valine are not utilized as N sources. Does not deaminate phenylalanine. Esculin, arbutin, and starch are decomposed; casein, elastin, guanine, hypoxanthine, pectin, testosterone, Tween 21, Tween 40, Tween 60, Tween 80, urea, and xanthine are not decomposed. All strains are susceptible to bacitracin (10 µg/ml), erythromycin (15 µg/ml), gentamicin (10 µg/ml), kanamycin (30 µg/ml), neomycin (30 µg/ml), novobiocin (30 µg/ml), rifampin (5 µg/ml), rifampin (50 µg/ml), tetracycline (30 µg/ml), and vancomycin (30 µg/ml) and resistant to clindamycin (2 µg/ml) and nalidixic acid (30 µg/ml). No growth occurs on media supplemented with 7% NaCl, 0.01% sodium azide, or 0.0175% potassium tellurite. No growth occurs at 37°C.

The Voges-Proskauer test is negative.

The following tests are variable: utilization of adonitol, *meso*-inositol, and melibiose; production of acids from cellobiose, D-fructose, inulin, D-glucose, lactose, maltose, D-mannitol, melibiose, raffinose, salicin, sucrose, and trehalose; utilization of fumarate, α-ketoglutarate, succinate, *trans*-aconitate, and valerate; decomposition of hippurate and tyrosine; nitrate reduction; and susceptibility to ampicillin (10 µg/ml), penicillin (10 µg/ml), and streptomycin (10 µg/ml). The G+C contents of the DNAs are 70 to 72 mol% (as determined by the T_m method).

Isolated from soil.

The type strain of *A. fucosus* is strain VKM Ac-1345.

The description given above was based on seven strains.

Description of *Agromyces fucosus* subsp. *fucosus* sp. nov., subsp. nov. Members of *A. fucosus* subsp. *fucosus* are distinguished from strains belonging to the other subspecies by the presence of fucose and glucose in their cell walls and by the absence of xylose in their whole-cell hydrolysates.

Most strains grow on media containing L-arabinose, starch, and D-xylose as sole C sources and produce acids from inulin, lactose, and maltose. Acids are not produced from melibiose, *meso*-inositol, or D-mannitol by most strains. An alkaline reaction occurs with α-ketoglutarate and succinate. Formate is not utilized. Starch and Tween 40 are not hydrolyzed. Nitrate is not reduced. Tyrosinase is not produced.

The G+C contents of the DNAs are 70 to 72 mol% (as determined by the T_m method).

The type strain of *A. fucosus* subsp. *fucosus* is strain VKM Ac-1345.

The description given above was based on five strains.

Description of *Agromyces fucosus* subsp. *hippuratus* sp. nov., subsp. nov. *Agromyces fucosus* subsp. *hippuratus* (hip.pu.ra'tus. N.L. n. *hippuratum*, hippurate; N.L. adj. *hippuratus*, decomposes hippurate). Strains are distinguished from *A. fucosus* subsp. *fucosus* strains by the

presence of xylose in whole-cell hydrolysates and by some physiological characteristics. Strains grow on media supplemented with melibiose, and no growth occurs when meso-inositol is the sole carbon source. Acids are not produced from lactose, maltose, raffinose, salicin, sucrose, and trehalose. Succinate is not utilized. Hippurate is hydrolyzed. Reduces nitrate and produces tyrosinase.

One strain utilizes α -ketoglutarate and *trans*-aconitate and exhibits antibiotic activity against some gram-positive bacteria.

The G+C content of the DNA is 72 mol% (as determined by the T_m method).

The type strain of *A. fucosus* subsp. *hippuratus* is strain VKM Ac-1352.

The description given above was based on two strains.

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