***Agromyces luteolus* sp. nov., *Agromyces rhizosphaerae* sp. nov. and *Agromyces bracchium* sp. nov., from the mangrove rhizosphere**

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The taxonomic positions of four strains isolated from the mangrove rhizosphere were studied by a polyphasic approach using phenotypic, chemotaxonomic and genetic methods. The four isolates contain 2,4-diaminobutyric acid in their peptidoglycan, and rhamnose as the major cell wall sugar. The predominant menaquinones are MK-12 and MK-11. The predominant cellular fatty acids are iso-C_{16:0}, anteiso-C_{15:0} and/or anteiso-C_{17:0}. The G+C content of the DNA ranges from 70.0 to 73.3 mol%. The four strains formed a coherent cluster with *Agromyces* species in a phylogenetic inference based on 16S rDNA sequences. Interestingly, the four isolates grew well in the presence of 5% NaCl. The differences in some phenotypic and chemotaxonomic characteristics, 16S rDNA sequence similarity data and DNA–DNA relatedness data indicate that the four isolates represent three new species in the genus *Agromyces*, for which are proposed the names *Agromyces luteolus* for strain 8^T (IFO 16235^T = VKM Ac-2085^T), *Agromyces bracchium* for strain 65^T (IFO 16238^T = VKM Ac-2088^T) and *Agromyces rhizosphaerae* for strains 14^T (IFO 16236^T = VKM Ac-2086^T) and 58(5) (IFO 16237 = VKM Ac-2087).

Keywords: *Agromyces luteolus* sp. nov., *Agromyces rhizosphaerae* sp. nov., *Agromyces bracchium* sp. nov.

INTRODUCTION

The diversity and populations of micro-organisms in the mangrove rhizosphere have been studied at the Institute for Fermentation, Osaka (IFO) for several years. It has been suggested that the mangrove rhizosphere is a good source for isolating new and diverse actinomycetes (Hatano, 1997; Takeuchi & Hatano, 1998, 1999). We have previously isolated 25 actinobacteria from the rhizosphere of mangroves in the estuary of the Shiira River, Iriomote Island, and determined their taxonomic positions at the genus level on the basis of phylogenetic and chemotaxonomic traits. Four strains were determined to be members of the genus *Agromyces*, 13 strains *Cellulomonas*, 2 strains *Microbacterium*, 2 strains *Mycobacterium*, 1 strain

Micrococcus, 1 strain *Gordonia* and 1 strain *Rhodococcus*, and 1 strain which contained LL-diaminopimelic acid in the cell wall (Takeuchi & Hatano, 1999) was of undetermined taxonomic position.

The object of the present study was to determine the exact taxonomic positions of the four strains of the genus *Agromyces* (Casida, 1986), strains 8 (IFO 16235^T), 14 (IFO 16236^T), 58(5) (IFO 16237) and 65 (IFO 16238^T), on the basis of morphological, physiological and chemotaxonomic characteristics, together with phylogenetic studies based on 16S rDNA sequences and DNA relatedness.

These strains are yellow, filamentous, elementary branching, irregular rod-shaped bacteria with all of the characteristic chemotaxonomic markers of the genus *Agromyces*, including 2,4-diaminobutyric acid (DAB) in their cell walls, DNA G + C contents ranging from 70.0 to 73.3 mol%, and MK-12 with smaller amounts of MK-11 and/or MK-13 menaquinones.

Abbreviation: DAB, 2,4-diaminobutyric acid.

The DDBJ accession numbers for the 16S rDNA sequences determined in this study are AB023356–AB023359.

The genus *Agromyces* was established by Gledhill & Casida (1969) for the single species *Agromyces ramosus*, a filamentous, branching, catalase-negative actinomycete isolated from soil. Since then, two species each with two subspecies, *Agromyces cerinus* subsp. *cerinus* and *Agromyces cerinus* subsp. *nitratus*, and *Agromyces fucosus* subsp. *fucosus* and *Agromyces fucosus* subsp. *hippuratus* (Zgurskaya *et al.*, 1992), and later a further species, *Agromyces mediolanus* (Suzuki *et al.*, 1996), have been added to the genus.

By comparing the four isolates from the mangrove rhizosphere with previously described *Agromyces* species, we conclude that the four isolates represent three new species in the genus *Agromyces*. In this paper, we propose the new species names *Agromyces luteolus* for strain 8 (IFO 16235^T), *Agromyces brachium* for strain 65 (IFO 16238^T) and *Agromyces rhizosphaerae* for strains 14 (IFO 16236^T) and 58(5) (IFO 16237).

METHODS

Bacterial strains and cultivation. Soil samples were taken from the rhizosphere of mangroves in the estuary of the Shiira River, Iriomote Island, Japan, in July 1997. Soils were stored at 5–8 °C, dried in air at 17 °C for 6 d, and then passed through sieves of 20 and 60 mesh to separate soil and fine roots. Strain 8 (IFO 16235^T) was isolated from the surface of roots of *Sonneratia alba*, strain 14 (IFO 16236^T) was isolated from soil of the rhizosphere of *S. alba* and strains 58(5) (IFO 16237) and 65 (IFO 16238^T) were isolated from soil of the rhizosphere of *Bruguiera gymnorrhiza* by the method described by Hayakawa & Nonomura (1987, 1989). *A. cerinus* subsp. *cerinus* IFO 15780^T, *A. cerinus* subsp. *nitratus* IFO 15783^T, *A. fucosus* subsp. *fucosus* IFO 15781^T, *A. fucosus* subsp. *hippuratus* IFO 15782^T (Zgurskaya *et al.*, 1992), *A. mediolanus* IFO 15704^T (Suzuki *et al.*, 1996) and *A. ramosus* IFO 13899^T (Gledhill & Casida, 1969) were used for comparison of physiological properties and for DNA–DNA hybridization tests with the isolates. Each strain was cultivated aerobically at 30 °C in peptone-yeast extract medium (PY medium) containing 1% Polypepton (Wako Pure Chemicals), 0.2% yeast extract and 0.1% MgSO₄·7H₂O (pH 7.2). Cells used for biochemical tests were harvested by centrifugation during the stationary phase, washed with water and lyophilized.

Morphological and physiological characterization. For investigation of morphological characteristics, cultures grown on PY medium to the early growth phase (approx. 16 h) and to stationary phase at 30 °C were observed with a light microscope and a scanning electron microscope (model JSM-5400; JEOL). Samples for scanning electron microscopy were prepared by fixing in 2% glutaraldehyde in potassium phosphate buffer for 4 h at 4 °C and 1% osmic acid in potassium phosphate buffer for 16 h at 4 °C, dehydrating through a graded acetone series, critical point drying (model HCP-2; Hitachi Koki), and then sputter-coating with palladium under vacuum. Motility was determined by the hanging-drop method. Biochemical characteristics of the *Agromyces* strains were examined by using the API CORYNE system and API 50CH with AUX Medium. Other physiological characteristics were determined as described by Cowan & Steel (1965).

Chemotaxonomic characterization. Analyses of cell wall amino acid and sugar composition and the acyl type of the peptidoglycan, menaquinone composition, cellular fatty acid composition and DNA G+C content were performed as described previously (Takeuchi & Hatano, 1998).

DNA–DNA hybridization. DNA–DNA hybridization was carried out fluorometrically in microdilution wells by using biotinylated DNA (Ezaki *et al.*, 1989).

16S rDNA sequence determination and phylogenetic analysis. A 16S rDNA fragment corresponding to position 8–1540 in the *Escherichia coli* numbering system (Brosius *et al.*, 1978) was amplified by PCR, sequenced directly with a ThermoSequenase cycle sequencing kit with 7-deaza-dGTP (Amersham), and analysed with a LI-COR 4200L-2 (2-dye system) DNA sequencer, following the manufacturer's instructions. The 16S rDNA sequences were aligned with published sequences available from DDBJ, EMBL and GenBank. '*Agromyces succinolyticus*' is a DAB-containing coryneform bacterium isolated from soil (K.-I. Suzuki & J. Sasaki, unpublished data). The CLUSTAL W version 1.6 (Thompson *et al.*, 1994) software package was used to generate the evolutionary distances (K_{mc} values) (Kimura, 1980) and the similarity values. A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987), and the topology of the tree was evaluated by the bootstrap resampling method (Felsenstein, 1985) with 1000 replicates.

RESULTS AND DISCUSSION

Morphological and physiological characteristics

The four strains studied in this work were Gram-positive, non-spore-forming, irregular rods. The cells measured 0.2–0.4 by 1.5–6.0 µm long (Fig. 1a); they differed in length from strain to strain, and tended to be shorter in old cultures as shown in Fig. 1(b). Filamentous elongation and branching were observed in strains IFO 16235^T, IFO 16236^T and IFO 16238^T, but were not observed obviously in strain IFO 16237. All strains formed yellow to pale yellow, entire, convex colonies on PY medium agar. Colonies sometimes penetrated into the agar. Aerial mycelia were not produced.

All strains grew optimally at a temperature of 20–30 °C, but did not grow at 10 or 37 °C. They grew well in the presence of 5% NaCl, but very little in the presence of 8% NaCl. All strains were positive for catalase, hydrolysis of aesculin, gelatin and starch (data not shown). Using the API CORYNE system, pyrazinamidase and aesculinase were positive, but alkaliphosphatase, nitrate reductase, β-glucuronidase, β-galactosidase, α-glucosidase and *N*-acetyl-β-glucosaminidase were negative for all strains. Using the API 50CH system, acid was produced from fructose, glucose, maltose and mannose, but was not produced from adonitol, D-arabinose, L-arabitol, dulcitol, erythritol, D-fucose, *N*-acetylglucosamine, α-methyl-D-glucoside, glycerol, inositol, inulin, α-methyl-D-mannoside, sorbitol, sorbose, D-tagatose, L-xylose, β-methyl-D-xyloside or xylitol. Other biochemical characteristics that showed different results

Fig. 1. Scanning electron micrographs of *A. luteolus*, *A. rhizosphaerae* and *A. brachium* grown on PY medium at 30 °C. *A. luteolus* IFO 16235^T (i), *A. rhizosphaerae* IFO 16236^T (ii), *A. rhizosphaerae* IFO 16237 (iii) and *A. brachium* IFO 16238^T (iv) in the early growth phase (a) and in the exponential growth phase (b). Bars, 1 μm.

among the four strains are shown in Table 1. Two strains, IFO 16236^T and IFO 16237, showed the same results in the API 50CH system with regard to acid production from all carbohydrates except for glycogen

and lactose. Strain IFO 16235^T was differentiated from strains IFO 16236^T and IFO 16237 by acid production from amidon, cellobiose, gentibiose, salicin and trehalose. Strain IFO 16238^T was differentiated from IFO

Table 1. Biochemical characteristics that differentiate the four new *Agromyces* strains

+, Positive reaction; +w, weakly positive reaction; —, negative reaction.

Characteristic	<i>A. luteolus</i> IFO 16235 ^T	<i>A. rhizosphaerae</i>		<i>A. braccium</i> IFO 16238 ^T
		IFO 16236 ^T	IFO 16237	
Pyrrolidonyl arylamidase*	+	+	+	—
Acid production from:†				
Amidon	—	+	+	+
Amygdalin	—	—	—	+
L-Arabinose	—	—	—	+
Arbutin	—	—	—	+
Cellobiose	—	+	+	+
Galactose	—	—	—	+
Gentiobiose	—	+w	+w	+
Gluconate	—	—	—	+
Glycogen	—	+	—	+
Lactose	+	—	+	+
Mannitol	+w	—	—	+
Melezitose	—	—	—	+w
Melibiose	+w	—	—	+
Raffinose	—	—	—	+
Rhamnose	+w	—	—	+w
Ribose	—	—	—	+
Salicin	—	+	+w	+
Sucrose	+w	—	—	+
Trehalose	+	—	—	+
D-Turanose	—	—	—	+
D-Xylose	—	—	—	+

* API CORYNE system.

† Carbohydrate assimilation reactions: API 50CH and AUX medium, incubated at 30 °C.

16235^T, IFO 16236^T and IFO 16237 by pyrrolidonyl arylamidase activity and by acid production from amygdalin, L-arabinose, arbutin, galactose, gluconate, melezitose, raffinose, ribose, D-turanose and D-xylose.

Chemotaxonomic characteristics

The molar ratio of glycine, alanine and DAB to glutamic acid in the cell wall peptidoglycan isolated from cells of strains IFO 16235^T, IFO 16236^T, IFO 16237 and IFO 16238^T was (0.85–1.31):(0.55–0.62):(1.38–1.77):1.00, as shown in Table 2. This ratio is the same as that obtained for *A. ramosus* ATCC 25173^T (0.98:0.71:1.73:1.00) (Fiedler & Kandler, 1973). These cell walls were of an acetyl type. The main sugar component of the cell wall of all strains was rhamnose, and small amounts of glucose and mannose were also detected in the cell walls of all the strains studied. In addition, fructose and galactose were present in the cell walls of strains IFO 16235^T and IFO 16238^T, respectively.

All the four strains had cellular fatty acids of the iso- and anteiso-branched type. All except IFO 16238^T contained iso-C_{16:0} as the primary cellular fatty acid

and anteiso-C_{15:0} as the second most common type. IFO 16238^T contained anteiso-C_{15:0} as the primary cellular fatty acid and iso-C_{16:0} and anteiso-C_{17:0} as the second most common types. All strains except for IFO 16238^T contained MK-12 and MK-11 as the major menaquinones. IFO 16238^T contained MK-12 as the major menaquinone and MK-13 as the second most common type.

Phylogenetic analysis

The 16S rDNA sequences of the four strains IFO 16235^T, IFO 16236^T, IFO 16237 and IFO 16238^T were compared with representative members of the genus *Agromyces* and related micro-organisms. A phylogenetic tree (Fig. 2) showed that they formed a coherent cluster with species of the genus *Agromyces* (Takeuchi & Hatano, 1999). Strains IFO 16236^T and IFO 16237 had a 16S rDNA sequence similarity value of 99.2% with each other, and they represented a distinct lineage within the genus *Agromyces* with similarity values of 96.5–97.8% among the representative strains of previously described *Agromyces* species (data not shown). Strains IFO 16235^T and IFO

Table 2. Chemotaxonomic characteristics of the four new *Agromyces* strains

Characteristic	<i>A. luteolus</i> IFO 16235 ^T	<i>A. rhizosphaerae</i>		<i>A. braccium</i> IFO 16238 ^T
		IFO 16236 ^T	IFO 16237	
Amino acid composition of cell wall (molar ratio to glutamic acid)				
Glutamic acid	1.00	1.00	1.00	1.00
Glycine	1.15	0.85	1.25	1.31
Alanine	0.55	0.60	0.56	0.62
DAB	1.77	1.38	1.56	1.47
Sugar composition of cell wall (%)				
Galactose	—	—	—	6
Glucose	2	5	4	7
Fructose	25	—	—	—
Mannose	6	6	7	7
Rhamnose	67	89	89	80
Cellular fatty acid composition (%)				
iso-C _{14:0}	4.1	—	1.0	—
iso-C _{15:0}	6.1	7.7	9.3	4.3
iso-C _{16:0}	47.9	53.3	43.9	28.1
anteiso-C _{15:0}	32.5	26.1	27.0	41.6
anteiso-C _{17:0}	9.4	12.9	18.9	26.0
Major menaquinone (%)				
MK-11	35	48	19	8
MK-12	65	52	81	78
MK-13	—	—	—	14

16238^T had a 16S rDNA sequence similarity value of 98.4% with each other. The closest species to these two strains was *A. mediolanus*, but the levels of 16S rDNA sequence similarity between these strains and *A. mediolanus* was 97.5 and 97.7%, respectively (data not shown).

DNA base composition and DNA–DNA hybridization

The DNA base composition of the four strains ranged from 70.0 to 73.3 mol%, as shown in Table 3. The levels of DNA–DNA relatedness between IFO 16236^T and IFO 16237 were 69 and 73%, but levels of DNA–DNA relatedness among these two strains and other strains studied in the genus *Agromyces* were 11–22%. These results show that the two strains should be considered as a new species in the genus *Agromyces*. In addition, strains IFO 16235^T and IFO 16238^T also showed levels of DNA–DNA relatedness of 13 and 36% with each other, and 8 to 36%, and 13 to 39%, respectively, with other strains studied. These results show that strains IFO 16235^T and IFO 16238^T also each represent a new species in the genus *Agromyces*.

The high values of 16S rDNA sequence similarity and DNA–DNA relatedness between strains IFO 16236^T and IFO 16237, and the low levels of DNA–DNA relatedness among these strains and previously described species in the genus *Agromyces*, confirmed that the two strains IFO 16236^T and IFO 16237 represent a

new species in the genus *Agromyces*. Similarly, the low levels of DNA–DNA relatedness between strains IFO 16235^T and IFO 15238, and among these strains and other strains used in this study confirmed that strains IFO 16235^T and IFO 16238^T each represents a new species in the genus *Agromyces*. From the above results, we propose three new species in the genus *Agromyces*, *Agromyces rhizosphaerae* for strains IFO 16236^T and IFO 16237, *A. luteolus* for strain IFO 16235^T and *A. braccium* for strain IFO 16238^T.

The phenotypic characteristics also distinguish these three new species from the previously described species and subspecies in the genus *Agromyces*, as shown in Table 4. *A. mediolanus* is the closest species to *A. braccium* and *A. luteolus* on the basis of phylogenetic analysis (Fig. 2), but they can be differentiated by nitrate reduction, urease production, hydrolysis of starch and acid production from glycerol, fructose, rhamnose and mannitol. The finding that all strains of the new species studied grew well in the presence of 4% NaCl, in contrast to almost all *Agromyces* species described previously, is very interesting from the viewpoint of ecology, because the soils of the mangrove rhizosphere have higher salinity than terrestrial soils (Wakushima *et al.*, 1994).

The sugar components of the cell walls are also useful to distinguish the three new species from the previously described *Agromyces* species, as shown in Table 4. All strains of the new species studied contained rhamnose

Fig. 2. Unrooted phylogenetic tree showing the relationship of *A. luteolus*, *A. rhizosphaerae* and *A. brachium* to related micro-organisms. The numbers indicate the percentages of bootstrap samplings, derived from 1000 samplings of the internal branches (Felsenstein, 1985).

as the major cell wall sugar; galactose was not found as a major component. *A. luteolus* was also characterized by having fructose in the cell wall. In contrast, all the previously described species or subspecies in the genus

Agromyces contain galactose as the major cell wall sugar. *A. ramosus* also contains xylose (Gledhill & Casida, 1969), and *A. cerinus* subsp. *cerinus* also contains tyvelose in their cell walls, and *A. cerinus*

Table 3. DNA base compositions and levels of DNA–DNA homology of *Agromyces* species

Species	G + C content (mol %)	% DNA–DNA reassociation with:			
		IFO 16235 ^T	IFO 16236 ^T	IFO 16237	IFO 16238 ^T
<i>A. luteolus</i> IFO 16235 ^T	71.1	100	27	27	13
<i>A. rhizosphaerae</i> IFO 16236 ^T	71.2	13	100	69	31
<i>A. rhizosphaerae</i> IFO 16237	73.3	8	73	100	39
<i>A. brachium</i> IFO 16238 ^T	70.0	36	13	14	100
<i>A. mediolanus</i> IFO 15704 ^T	72.3	21	11	18	24
<i>A. ramosus</i> IFO 13899 ^T	68.9	15	19	13	18
<i>A. cerinus</i> subsp. <i>cerinus</i> IFO 15780 ^T	70.5	24	20	18	13
<i>A. cerinus</i> subsp. <i>nitratus</i> IFO 15783 ^T	70.9	28	15	17	26
<i>A. fucosus</i> subsp. <i>fucosus</i> IFO 15781 ^T	70.6	10	18	13	31
<i>A. fucosus</i> subsp. <i>hippuratus</i> IFO 15782 ^T	70.8	31	14	22	24

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

1, *A. luteolus*; 2, *A. rhizosphaerae*; 3, *A. brachium*; 4, *A. mediolanus*; 5, *A. ramosus*; 6, *A. cerinus* subsp. *cerinus*; 7, *A. cerinus* subsp. *nitratus*; 8, *A. fucosus* subsp. *fucosus*; 9, *A. fucosus* subsp. *hippuratus*. Data for all species except *A. luteolus*, *A. rhizosphaerae* and *A. brachium* are from Gledhill & Casida (1969), Zguraskaya *et al.* (1992) and Suzuki *et al.* (1996). +, 90 % or more of strains positive; –, 90 % or more of strains negative; +w, weakly positive reaction; v, variable reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Catalase production	+	+	+	+	–	+	+	+	+
Nitrate reduction	–	–	–	+	–	–	+	–	+
Urease production	–	–	–	+	v	–	–	–	–
Starch hydrolysis	+	+	+	–	+	+	+	+	+
Acid production from:									
Glycerol	–	–	–	+	+	+	–	–	+
Ribose	–	–	+	+	+	–	–	–	–
Galactose	–	–	+	+	v	+	–	–	–
Glucose	+	+	+	+	+	–	–	–	–
Fructose	+	+	+	–	v	+	–	–	–
Mannose	+	+	+	+	v	–	–	–	–
Rhamnose	+w	–	+w	–	+	+	+	–	+
Mannitol	+w	–	+	–	+	–	–	–	–
Amygdalin	–	–	+	–	ND	+	–	–	–
Aesculin	+	+	+	+	v	+	+	+	–
Salicin	–	+	+	+w	v	+	–	–	–
Maltose	+	+	+	+	+	+	–	–	–
Sucrose	+w	–	+	+	+	–	–	–	–
Growth in 4 % NaCl	+	+	+	ND	v	–	–	–	v
Growth at 37 °C	–	–	–	ND	v	–	+	–	–
Cell wall sugars*	Rha, Fruc, (Glc, Man)	Rha, (Glc, Man)	Rha, (Gal, Glc, Man)	ND	Gal, Glc, Man, Rha, Xyl	Gal, Rha, Tyv, (Man)	Gal, (Glc, Man)	Gal, Rha, (Glc, Man)	Gal, Rha, (Man)

* Rha, rhamnose; Fruc, fructose; Glc, glucose; Man, mannose; Xyl, xylose; Tyv, tyvelose. Parentheses indicate that a compound may or may not be present.

subsp. *nitratus* is characterized by the absence of rhamnose.

On the basis of the phenotypic, chemotaxonomic and phylogenetic studies, as well as DNA–DNA relatedness data, we conclude that the four isolates represent three new species in the genus *Agromyces*.

Description of *Agromyces luteolus* sp. nov.

Agromyces luteolus (lu.te'olus. N.L. adj. *luteus* yellow; M.L. dim. adj. *luteolus* yellowish, somewhat yellow).

Cells are aerobic, Gram-positive, irregular rods that form filaments (0.2–0.4 by 3.0–5.0 µm) or show el-

elementary branching in the early growth phase and fragment into short rods (0.2–0.4 by 2.0–3.0 µm) during exponential growth phase. Colonies on PY agar medium are yellow to whitish yellow, opaque, entire and convex, and sometimes penetrate into the agar. Grows optimally at 20–30 °C, but does not grow at 10 or 37 °C. Catalase-positive. Acid is produced from glucose, fructose, lactose, maltose, mannose and trehalose, but is not produced from adonitol, amidon, amygdalin, arabinose, arbutin, cellobiose, dulcitol, erythritol, fucose, galactose, gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, melezitose, raffinose, ribose, salicin, sorbitol, sorbose, tagatose, turanose, xylose or xylitol. Aesculin, gelatin and starch are hydrolysed. Urease is not produced. DAB is the principal amino acid of the cell wall peptidoglycan. The cell wall is of an acetyl type. The predominant isoprenoid quinone is MK-12, with small amounts of MK-11. The predominant cellular fatty acids are iso-C_{16:0} and anteiso-C_{15:0}. The G+C content of the DNA is 71.1 mol%. Isolated from soil of rhizosphere of mangrove on Iriomote Island, Japan. The type strain is IFO 16235^T (= VKM Ac-2085^T).

Description of *Agromyces rhizosphaerae* sp. nov.

Agromyces rhizosphaerae (rhi.zo'spher.æ. M.L. *rhizo* root; Gr. adj. *sphaera* sphere; M.L. n. *rhizosphaerae* within the sphere of the root).

Cells are aerobic, Gram-positive, irregular rods that form filaments (0.2–0.4 by 1.5–3.0 µm) or show elementary branching in the early growth phase and fragment into short rods (0.2–0.4 by 0.8–1.5 µm) during exponential growth phase. Colonies on PY agar medium are yellow, opaque, entire and convex, and sometimes penetrate into the agar. Grows optimally at 20–30 °C, but does not grow at 10 or 37 °C. Catalase-positive. Acid is produced from amidon, cellobiose, fructose, gentiobiose, glucose, maltose, mannose, salicin, but is not produced from adonitol, amygdalin, arabinose, arbutin, cellobiose, dulcitol, erythritol, fucose, galactose, gluconate, glycerol, inositol, mannitol, melezitose, raffinose, rhamnose, ribose, sorbitol, sorbose, sucrose, tagatose, trehalose, turanose, xylose or xylitol. Aesculin, gelatin and starch are hydrolysed. Urease is not produced. DAB is the principal amino acid of the cell wall peptidoglycan. The cell wall is of an acetyl type. The predominant isoprenoid quinones are MK-12 and MK-11. The predominant cellular fatty acids are iso-C_{16:0} and anteiso-C_{15:0}. The G+C content of the DNA is 71.2–73.3 mol%. Isolated from soil of or roots in rhizosphere of mangrove on Iriomote Island, Japan. The type strain is IFO 16236^T (= VKM Ac-2086^T). The other strain is IFO 16237 (= VKM Ac-2087).

Description of *Agromyces brachium* sp. nov.

Agromyces brachium (bracchi.um. M.L. adj. *brachium* like a twig).

Cells are aerobic, Gram-positive, irregular rods that form filaments (0.2–0.4 by 5.0–6.0 µm) or show elementary branching in the early growth phase and fragment into short rods (0.2–0.4 by 1.5–3.0 µm) during exponential growth phase. Colonies on PY agar medium are yellow, opaque, entire and convex, and sometimes penetrate into the agar. Grows optimally at 20–30 °C, but does not grow at 10 or 37 °C. Catalase-positive. Acid is produced from amidon, amygdalin, L-arabinose, arbutin, cellobiose, fructose, galactose, gentiobiose, gluconate, glucose, glycogen, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sucrose, trehalose, D-turanose and D-xylose, but is not produced from adonitol, D-arabinose, arabitol, dulcitol, erythritol, fucose, glycerol, inositol, inulin, sorbitol, sorbose, tagatose, L-xylose or xylitol. Aesculin, gelatin and starch are hydrolysed. Urease is not produced. DAB is the principal amino acid of the cell wall peptidoglycan. The cell wall is of an acetyl type. The predominant isoprenoid quinone is MK-12 with small amounts of MK-13 and MK-11. The predominant cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}. The G+C content of the DNA is 70.0 mol%. Isolated from soil of rhizosphere of mangrove on Iriomote Island, Japan. The type strain is IFO 16238^T (= VKM Ac-2088^T).

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