Agromyces albus sp. nov., isolated from a plant (*Androsace* sp.)

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Agromyces albus sp. nov. is proposed for an aerobic, oxidase- and catalase-positive actinomycete that was isolated from the above-ground part of a plant (*Androsace* sp., in the family *Primulaceae*). The strain is characterized by white colonies, fragmenting hyphae that penetrate into agar media and chemotaxonomic properties that are typical of the genus *Agromyces*. Analysis of 16S rDNA sequences confirmed that the strain belongs to the genus *Agromyces* and revealed its close phylogenetic relationship with *Agromyces ramosus*. DNA–DNA pairing studies showed that the strain belongs to a separate genomic species; this is consistent with its distinction from other *Agromyces* species at the phenotypic level. The G + C content of the DNA was 69.0 mol%. The type strain is VKM Ac-1800^T (= UCM Ac-623^T).

The genus *Agromyces* was established by Gledhill & Casida (1969) for microaerophilic to aerobic, filamentous, branching, fragmenting, catalase-negative actinomycetes that showed a negative oxidase reaction and inhabited soil; it originally contained a single species, *Agromyces ramosus*. Later, strictly aerobic, catalase- and oxidase-positive and non-filamentous agromycetes were described (Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Takeuchi *et al.*, 2001; Li *et al.*, 2003). Members of the genus are characterized by B2γ-type peptidoglycan [based on L-DAB (2,4-diaminobutyric acid) (Schleifer & Kandler, 1972)], the major isoprenoid quinone MK-12 and the predominant cellular fatty acids anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} (Gledhill & Casida, 1969; Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Sasaki *et al.*, 1998; Takeuchi *et al.*, 2001; Li *et al.*, 2003). Currently, the genus harbours eight species and four subspecies with validly published names, which are presented in Table 1; they were all isolated from soil or the mangrove rhizosphere. In this work, we report the characterization of *Agromyces albus* sp. nov., which was isolated from the above-ground part of a plant (*Androsace* sp., in the family *Primulaceae*) collected at the seed-formation stage in the Central-Chernozem Biosphere Park, Belgorod region, Russia.

For isolation of micro-organisms, the surface-sterilized part of the plant (stem top with several leaves and inflorescence) was cut into pieces, added to 1 ml 0.85 % NaCl

(w/v) and ground with a pestle. One drop of this suspension was plated onto modified corynebacterium agar that contained 5 g glucose, 3 g yeast extract, 5 g casein peptone, 2 g Casamino acids, 3 g beef extract, 5 g NaCl, 15 g agar, 100 ml skimmed milk and 900 ml distilled water (pH 7.2–7.4) and incubated for 3 weeks at room temperature (18–24 °C). Morphology and life cycle were studied in cultures grown on corynebacterium (CB) agar (Zgurskaya *et al.*, 1992) by phase-contrast microscopy. Gram-reaction was tested by the rapid test with KOH (Ryu, 1938). Physiological and chemotaxonomic features were examined as described previously (Evtushenko *et al.*, 2000). For chemotaxonomic study, shaken cultures had been grown in liquid CB medium and harvested at the exponential-growth phase (20–24 h). Methods used for extraction and purification of DNA and 16S rDNA amplification and analyses were described by Evtushenko *et al.* (2000). GenBank, DDBJ and EMBL accession numbers of the sequences of *Agromyces* species that were used in phylogenetic analysis are shown in Fig. 1. The DNA G + C content was determined by thermal denaturation and DNA–DNA relatedness was studied by the membrane filter method as reported previously (Evtushenko *et al.*, 2002). ³H-labelled DNA was obtained by using deoxy-[1',2',5'-³H]CTP and a Nick Translation kit (N5500; Amersham Biosciences).

Colonies of strain VKM Ac-1800^T on CB agar were visible after 24–36 h and were white, circular and opaque. Branching substrate hyphae (0.3–0.6 µm in width) that broke up into diphtheroid and rod-shaped, irregular, non-motile fragments and penetrated into agar were formed.

Abbreviation: L-DAB, 2,4-diaminobutyric acid.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Agromyces albus* VKM Ac-1800^T is AF503917.

Table 1. Characteristics that differentiate *Agromyces albus* sp. nov. from other species of the genus *Agromyces*

Strains: 1, *A. albus* VKM Ac-1800^T; 2, *A. aurantiacus* YIM 21741^T; 3, *A. brachium* VKM Ac-2088^T; 4, *A. cerinus* subsp. *cerinus* VKM Ac-1340^T; 5, *A. cerinus* subsp. *nitratu*s VKM Ac-1351^T; 6, *A. fucosus* subsp. *fucosus* VKM Ac-1345^T; 7, *A. fucosus* subsp. *hippuratus* VKM Ac-1352^T; 8, *A. luteolus* VKM Ac-2085^T; 9, *A. mediolanus* VKM Ac-1388^T; 10, *A. ramosus* VKM Ac-1198^T; 11, *A. rhizosphaerae* VKM Ac-2086^T. Data from Gledhill & Casida (1969), Zgurskaya *et al.* (1992), Suzuki *et al.* (1996), Takeuchi & Hatano (2001), Li *et al.* (2003) and this study. Abbreviations: W, white; O, orange; PW, pink-white; PG, pink-grey; Y, yellow; +, positive; –, negative; D, weakly positive or variable among tests; ND, not determined; Fruc, fructose; Fuc, fucose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Tyv, tyvelose; Xyl, xylose.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour	W	O, PW, PG, Y	Y	Y	Y	Y	Y	Y	Y	W	Y
Hyphae	+	+	+	+	+	+	+	+	–	+	+
Catalase production	+	–	+	+	+	+	+	+	+	–	+
Oxidase test	+	ND	+	+	+	+	+	+	+	–	D
Microaerophilic	–	–	–	–	–	–	–	–	–	+	–
Acid from:											
D-Arabinose	+	–	–	–	–	+	+	–	–	+	–
D-Galactose	+	+	+	+	+	+	+	–	+	–	–
D-Glucose	+	–	+	+	+	+	+	+	+	–	+
Inulin	+	+	–	–	–	+	–	–	–	+	–
Salicin	+	–	+	+	D	+	–	–	+	–	+
D-Sucrose	+	+	+	+	+	+	+	D	+	+	–
Hydrolysis of:											
Casein	+	ND	–	–	–	–	–	–	–	–	–
Starch	+	+	+	+	+	+	+	+	–	ND	+
Hypoxanthine	+	ND	–	+	+	+	+	–	+	–	–
Xanthine	–	ND	–	–	–	–	–	–	+	–	–
Growth at 4% NaCl	–	ND	+	–	+	–	–	+	+	D	+
DNA G + C content (mol%)	69.0	72.8	70.0	70.5	70.9	70.6	70.8	71.1	72.3	68.9	71.2
Cell-wall sugars*	Rha (Gal, Glu, Man)	Rha (Gal, Glu, Man)	Rha (Gal, Glu, Man)	Gal, Rha, Tyv (Man)	Gal (Glu, Man, Rib)	Gal, Rha, Fuc (Glu, Man)	Gal, Rha (Man)	Rha, Fruc (Glu, Man)	ND	Gal, Glu, Man, Rha, Xyl	Rha (Glu, Man)
Menaquinones†	MK-12 (11)	MK-12	MK-12 (13)	MK-12 (13)	MK-12 (13)	MK-12 (13)	MK-12 (13)	MK-12 (11)	(11, 10)	MK-12 (13)	MK-12, 11
Source	Above-ground part of <i>Androsace</i> sp.	Soil	Rhizosphere of <i>Bruguera gymnorhiza</i>	Soil	Soil	Soil	Soil	Rhizosphere of <i>Sonneratia alba</i>	Soil	Soil	Rhizosphere of <i>Sonneratia alba</i>

*Components given in parentheses are present in minor amounts; no available data on sugar amounts in *A. ramosus*. Sugar composition in *A. aurantiacus* was determined in whole-cell hydrolysates (Li *et al.*, 2003).

†Designation of Collins & Jones (1981). Menaquinones in parentheses are present as the second most common component.

Fig. 1. Phylogenetic tree showing the position of *Agromyces albus* sp. nov., based on 16S rDNA analysis. *Brevibacterium linens* DSM 20425^T (GenBank no. X77451) served as an outgroup (not shown). Bootstrap values, expressed as a percentage of 500 replications, are shown at branching points. Bar, 1 nucleotide substitution per 100 nucleotides.

Aerial hyphae and spores were not observed. The strain was aerobic, catalase- and oxidase-positive and mesophilic (optimum growth temperature, 26–28 °C). It was Gram-positive and contained DAB, alanine, glutamic acid and glycine (molar ratio, 1.7:0.9:1.0:1.1) and also muramic acid and glucosamine in the peptidoglycan. The amino acid ratio was consistent with peptidoglycan type B2γ. Cell-wall sugars included rhamnose as the predominant component and minor amounts of glucose, galactose and mannose. No mycolic acids were present. The major isoprenoid quinone was menaquinone MK-12, although MK-11 occurred in small quantities. The DNA G + C content was 69.0 mol%. The strain used a wide spectrum of carbon and nitrogen sources for growth and degraded some organic compounds, including casein (see species description).

Phylogenetic analysis of the 16S rDNA sequence (1486 bp) of VKM Ac-1800^T revealed that this strain belonged to the *Agromyces* phylogenetic cluster, formed a tight group with *A. ramosus*, *Agromyces cerinus*, *Agromyces fucosus* and '*Agromyces succinolyticus*' with 99 % bootstrap support and was closest to *A. ramosus* (Fig. 1). The binary sequence similarity of our strain to *A. ramosus* was 98.8 %. Subsequent DNA–DNA hybridization studies revealed 34 % reassociation of VKM Ac-1800^T DNA with its phylogenetic neighbour *A. ramosus* VKM Ac-1198^T and 30–32 % reassociation with *A. cerinus* subsp. *cerinus* VKM Ac-1340^T, *A. fucosus* subsp. *fucosus* VKM Ac-1345^T and *A. fucosus* subsp. *hippuratus* VKM Ac-1352^T. DNA homology values of our strain with *Agromyces rhizosphaerae* VKM Ac-2086^T, *Agromyces brachium* VKM Ac-2088^T and *Agromyces luteolus* VKM Ac-2085^T were 27, 29 and 31 %, respectively; *Agromyces mediolanus* VKM Ac-1388^T has the lowest DNA homology value (13 %).

The phylogenetic data obtained indicate that strain VKM Ac-1800^T is genomically distinct and represents a separate genomic species (Wayne *et al.*, 1987). This is consistent with its differentiation from other *Agromyces* species at the phenotypic level (Table 1). The most striking features that distinguish it from all rapidly growing, yellow-pigmented

species are the white colour of its colonies and its ability to hydrolyse casein. At the same time, it can be differentiated from the non-pigmented, microaerophilic species *A. ramosus* by rapid growth under aerobic conditions, positive catalase and oxidase tests, a number of other physiological characteristics and the absence of xylose in the cell wall. Thus, on the basis of both molecular genetic and phenotypic findings, we propose that strain VKM Ac-1800^T, isolated from *Androsace* sp., is classified as a novel species, *Agromyces albus* sp. nov.

Description of *Agromyces albus* sp. nov.

Agromyces albus (al'bus. L. adj. *albus* white, referring to the white colour of colonies).

Colonies on CB agar are white, circular, somewhat convex, opaque and penetrate into agar media. Forms branching hyphae (width, 0.3–0.6 μm) that break up into diphtheroid and rod-like, irregular and non-motile fragments. No aerial mycelium or spore formation. Gram-positive. Aerobic; catalase- and oxidase-positive. Mesophilic; growth occurs between 7 and 37 °C (optimum, 26–28 °C). Adonitol, D-arabinose, cellobiose, D-fructose, D-fucose, D-galactose, D-glucose, glycerol, inositol, inulin, lactose, lyxose, maltose, D-mannitol, D-mannose, melibiose, L-rhamnose, raffinose, salicin, L-sorbose, sucrose, trehalose, turanose and D-xylose are used as carbon sources for growth in salt medium supplemented with 0.1 % (w/v) yeast extract and 0.1 % (w/v) casitone. Dextran, dulcitol, melezitose, *meso*-erythritol, ribose and sorbitol are not used as carbon sources in the same medium. Acids are produced from D-arabinose, D-galactose, D-glucose, inulin, mannose, melibiose, salicin and sucrose. Alkaline reactions are observed with acetate, fumarate, malate and maleinate, but no reaction occurs with citrate, formate, oxalate, propionate, succinate or tartrate. Methyl red test is positive and Voges–Proskauer test is negative. H₂S is produced. Casein, hypoxanthine, Tween 40 and starch are decomposed; gelatin, tyrosine, xanthine, Tween 60, Tween 80 and urea are not hydrolysed. Sensitive to 4 % (w/v) NaCl. Tolerates the following antibiotics (μg ml⁻¹): amikacin (30), clindamycin (30), lincomycin

(30), rubomycin (30) and streptomycin (30). Sensitive to ampicillin (10), cefazolin (10), doxycycline (5), erythromycin (10), gentamicin (30), karbomicillin (30), levomycetin (10), metacycline (10), oletetrin (10), oxacillin (30) and rifampicin (10). DNA G+C content is 69.0 mol%. Predominant cell-wall sugar is rhamnose; minor amounts of glucose, galactose and mannose are present. Major menaquinone is MK-12, with a small amount of MK-11. Cell-wall peptidoglycan amino acids are L-DAB, alanine, glutamic acid and glycine in a molar ratio consistent with peptidoglycan type B2 γ .

The type strain, VKM Ac-1800^T, has been deposited in the All-Russian Collection of Microorganisms (VKM) and Ukrainian Collection of Microorganisms (UCM Ac-623^T). Isolated from a sample of leaves and inflorescence of *Androsace* sp. in the family Primulaceae.

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