Agromyces allii sp. nov., isolated from the rhizosphere of *Allium victorialis* var. *platyphyllum*

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Two Gram-positive, non-motile, yellow-pigmented, slightly curved and rod-shaped bacterial strains, UMS-62^T and UMS-101, were isolated from the rhizosphere of *Allium victorialis* var. *platyphyllum*, a variety of wild edible greens grown on Ulleung island, Korea. The taxonomic position of the strains was investigated by a polyphasic approach. Strains UMS-62^T and UMS-101 grew optimally at 30 °C and at pH 6.5–7.5. The novel strains contained MK-11 and MK-12 as the predominant menaquinones and rhamnose, ribose and galactose as the major cell-wall sugars. The major cellular fatty acids (> 10 % of the total fatty acids) were anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The DNA G + C contents were 71.1–71.3 mol%. Phylogenetic trees based on 16S rRNA gene sequences showed that strains UMS-62^T and UMS-101 belong to the genus *Agromyces*. Strains UMS-62^T and UMS-101 showed a 16S rRNA gene sequence similarity value of 99.9 % and a mean DNA–DNA relatedness level of 91.1 %. Similarity values between the 16S rRNA gene sequences of the two novel strains and the type strains of recognized *Agromyces* species ranged from 95.2 to 99.1 %. The levels of DNA–DNA relatedness between the two novel strains and the type strains of five phylogenetically related *Agromyces* species were in the range of 13.4 to 54.2 %. On the basis of phenotypic properties, phylogenetic distinctiveness and genetic data, strain UMS-62^T (=KCTC 19181^T =JCM 13584^T) and strain UMS-101 are classified as representing a novel species in the genus *Agromyces*, for which the name *Agromyces allii* sp. nov. is proposed.

The genus *Agromyces*, as proposed by Gledhill & Casida (1969) and emended by Zgurskaya *et al.* (1992), currently includes the following species: *Agromyces ramosus* (Gledhill & Casida, 1969), *Agromyces cerinus* (Zgurskaya *et al.*, 1992), *Agromyces mediolanus* (Suzuki *et al.*, 1996), *Agromyces luteolus*, *Agromyces rhizospherae* and *Agromyces brachium* (Takeuchi & Hatano, 2001), *Agromyces albus* (Dorofeeva *et al.*, 2003), *Agromyces aurantiacus* (Li *et al.*, 2003), *Agromyces fucosus* and *Agromyces hippuratus* (Zgurskaya *et al.*, 1992; Ortiz-Martinez *et al.*, 2004), *Agromyces ulmi* (Rivas *et al.*, 2004), *Agromyces neolithicus* and *Agromyces salentinus* (Jurado *et al.*, 2005a), *Agromyces humatus*, *Agromyces italicus* and *Agromyces lapidis* (Jurado *et al.*, 2005b) and *Agromyces subbeticus* (Jurado *et al.*, 2005c). Species of this genus are characterized chemotaxonomically by having MK-12 as the common predominant menaquinone, anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} as the

major fatty acids and by DNA G + C contents of 65.3–73.3 mol% (Gledhill & Casida, 1969; Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Sasaki *et al.*, 1998; Takeuchi & Hatano, 2001; Dorofeeva *et al.*, 2003; Li *et al.*, 2003; Jurado *et al.*, 2005a, b, c). Here, we report the detailed taxonomic characterization of two *Agromyces*-like bacterial strains, UMS-62^T and UMS-101, which were isolated from the rhizosphere of *Allium victorialis* var. *platyphyllum*, a type of wild edible greens grown on Ulleung island, Korea.

Strains UMS-62^T and UMS-101 were isolated by a standard dilution plating technique at 30 °C on nutrient agar (NA; Difco). To investigate their morphological, physiological and biochemical characteristics, the strains were routinely cultivated at 30 °C on NA. Growth at various temperatures from 4 to 40 °C was measured on NA and tolerance to various NaCl concentrations was measured in nutrient broth (NB; Difco). The optimal pH and pH range for growth were determined in NB that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on NA and on NA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Cell morphology and presence

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strains UMS-62^T and UMS-101 are DQ673873 and DQ673874, respectively.

A supplementary table detailing the phenotypic characteristics that differentiate species of the genus *Agromyces* is available in IJSEM Online.

of flagella were examined by light microscopy (E600; Nikon) and transmission electron microscopy by using cells cultivated on NA. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase and oxidase activities and hydrolysis of casein, starch, hypoxanthine, xanthine, tyrosine and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied as described by Lanyi (1987). Acid production from carbohydrates was determined according to Leifson (1963). Utilization of various substrates for growth was investigated as described by Yurkov *et al.* (1994). The API ZYM system (bioMérieux) was used to characterize enzyme activity. The API 20E system (bioMérieux) was used to examine other physiological and biochemical properties. Susceptibility to antibiotics was tested on NA plates using antibiotic discs containing the following concentrations of antibiotic; ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (100 µg), gentamicin (30 µg), lincomycin (15 µg), kanamycin (30 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin (15 µg), penicillin G (20 U), polymyxin B (100 IU), streptomycin (50 µg) and tetracycline (30 µg).

Strains UMS-62^T and UMS-101 were cultivated for 3 days in NB at 30 °C to obtain the cell biomass required for DNA extraction and analyses of isoprenoid quinones, polar lipids and whole-cell sugars. Chromosomal DNA was extracted and purified according to a previously described procedure (Yoon *et al.*, 1996). 16S rRNA gene amplification was conducted according to the method described previously using two universal primers (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). A phylogenetic analysis was conducted using the CLUSTAL W software package (Thompson *et al.*, 1994). Isoprenoid quinones were analysed as described previously (Komagata & Suzuki, 1987) using reverse-phase HPLC. For fatty acid methyl ester analysis, cell mass of strains UMS-62^T and UMS-101 was harvested from NA plates after cultivation for 3 days at 30 °C. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to Minnikin *et al.* (1984) and were identified using two dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). Whole-cell sugars were analysed as described previously (Staneck & Roberts, 1974). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA–DNA relatedness was determined by the microplate hybridization method (Ezaki *et al.*, 1989) using photobiotin-labelled DNA probes. The type strains of five *Agromyces* species were used as reference strains for DNA–DNA hybridization. Samples of *A. salentinus* DSM 16198^T, *A. lapidis* DSM 16390^T, *A. italicus* DSM 16388^T, *A. ramosus* DSM 43045^T and *A. neolithicus* DSM 16197^T were obtained from the Deutsche Sammlung

von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Morphological, cultural, physiological and biochemical characteristics of strains UMS-62^T and UMS-101 are given in the species description (see below) or are shown in Table 1 and Supplementary Table S1 in IJSEM Online. The almost complete 16S rRNA gene sequences of strains UMS-62^T and UMS-101 determined in this study each comprised 1483 nt. The 16S rRNA gene sequences of the two novel strains were 99.9% similar, showing only two nucleotide differences. Comparative 16S rRNA gene sequence analyses showed that strains UMS-62^T and UMS-101 fell within the radiation of the cluster comprising the recognized *Agromyces* species (Fig. 1).

The chemotaxonomic properties of the two novel strains were in agreement with their phylogenetic affiliation to the genus *Agromyces*. Strains UMS-62^T and UMS-101 contained MK-11 and MK-12 as the predominant menaquinones at peak area ratios of approximately 45–47% and 35–38%, respectively. The cellular fatty acid profiles of strains UMS-62^T and UMS-101 are shown in Table 2, together with those of some phylogenetically related *Agromyces* species. Strains UMS-62^T and UMS-101 contained large amounts of straight-chain and branched fatty acids; the major components (>10% of total fatty acids) were anteiso-C_{15:0} (34.3–39.1%), iso-C_{16:0} (11.8–28.7%) and anteiso-C_{17:0} (17.0–18.3%). C_{16:0} (6.4–24.1%) was detected as a major component only in strain UMS-101. The fatty acid profiles of the two novel strains were similar to those of recognized *Agromyces* species, although there were differences in the proportions of some fatty acids, perhaps because of differences in cultivation conditions and extraction procedures (Takeuchi & Hatano, 2001; Li *et al.*, 2003; Jurado *et al.*, 2005a, b, c). The major polar lipids detected in strains UMS-62^T and UMS-101 were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The whole-cell sugars detected in the novel strains were rhamnose, ribose and galactose; xylose was detected only in strain UMS-62^T. Minor amounts of mannose and glucose were also detected. The DNA G+C contents of strains UMS-62^T and UMS-101 were 71.1 and 71.3 mol%, respectively.

Strains UMS-62^T and UMS-101 exhibited 16S rRNA gene sequence similarity values of 97.0–99.1% with respect to the type strains of *A. salentinus*, *A. lapidis*, *A. italicus*, *A. ramosus* and *A. neolithicus* and of 95.2–96.9% with the type strains of the other recognized *Agromyces* species (Fig. 1). Strains UMS-62^T and UMS-101 exhibited a mean DNA–DNA relatedness value of 91.1% when their DNAs were used individually as labelled DNA probes for cross-hybridization. This value indicates that strains UMS-62^T and UMS-101 are members of the same genomic species (Wayne *et al.*, 1987). Levels of DNA–DNA relatedness between strains UMS-62^T and UMS-101 and the type strains of *A. salentinus*, *A. lapidis*, *A. italicus*, *A. ramosus* and *A. neolithicus*, which showed 16S rRNA gene sequence similarity values of >97.0% to the two novel strains, were in the range 13.4–54.2%, indicating that the two

Table 1. Differential phenotypic characteristics of *Agromyces allii* sp. nov. and some phylogenetically related *Agromyces* species

Species: 1, *A. allii* sp. nov.; 2, *A. neolithicus* (data from Jurado *et al.*, 2005a); 3, *A. salentinus* (Jurado *et al.*, 2005a); 4, *A. italicus* (Jurado *et al.*, 2005b); 5, *A. lapidis* (Jurado *et al.*, 2005b). All species are positive for catalase activity, hydrolysis of casein, acid production from cellobiose, galactose, D-glucose, maltose and mannose and are susceptible to ampicillin, novobiocin (not determined for *A. neolithicus* and *A. salentinus*) and penicillin G. All species are negative for hydrolysis of Tween 80, indole production and utilization of benzoate (not determined for *A. italicus* and *A. lapidis*) and citrate. +, Positive; –, negative; v, variable; w, weakly positive; ND, not determined. Data in parentheses refer to the type strain.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	4–33	15–37	10–37	10–37	10–37
Growth in/at:					
37 °C	–	+	+	+	+
10 °C	+	–	+	w	w
NaCl 4.0% (w/v)	–	–	+	+	+
Nitrate reduction	+	+	–	+	+
Oxidase activity	+	v	v	+	v
Hydrolysis of:					
Gelatin	+	+	–	+	v
Hypoxanthine	–	–	–	+	–
Tyrosine	–	+	+	+	+
Urea	–	–	+	–	–
Xanthine	–	–	–	+	–
H ₂ S production	–	+	+	+	+
Acid production from:					
L-Arabinose	+	+	+	+	–
Lactose	+	–	–	w	–
Mannitol	–	+	–	–	–
D-Raffinose	–	+	w	–	w
Rhamnose	+	–	+	–	–
Ribose	+	–	+	–	–
Salicin	+	–	+	+	+
Sucrose	+	v	+	–	+
Trehalose	+	–	+	–	–
D-Xylose	+	–	+	+	–
Utilization of:					
Acetate	v(–)	–	+	ND	ND
Malate	+	–	–	–	–
Succinate	+	–	–	–	–
Enzyme activity (API ZYM):					
α -Chymotrypsin	v(+)	+	–	–	+
α -Galactosidase	–	+	–	–	–
β -Galactosidase	+	–	+	+	+
β -Glucuronidase	+	+	–	–	–
Antibiotic susceptibility:					
Kanamycin	+	–	+	–	–
Polymyxin B	+	+	+	–	–
DNA G + C content (mol%)	71.1–71.3	65.3	72.3	70.8	70.4

novel strains represent a different genomic species to the five recognized *Agromyces* species tested (Wayne *et al.*, 1987). Strains UMS-62^T and UMS-101 also differed from the recognized *Agromyces* species in several phenotypic characteristics (Table 1; Supplementary Table S1 in IJSEM Online). Phylogenetic distinctiveness, DNA–DNA relatedness

data and differential phenotypic properties suggest that strains UMS-62^T and UMS-101 represent a species that is distinct from the recognized *Agromyces* species. Therefore, on the basis of the data presented, strains UMS-62^T and UMS-101 should be placed in the genus *Agromyces* as a novel species, for which the name *Agromyces allii* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains UMS-62^T, UMS-101 and other recognized *Agromyces* species based on 16S rRNA gene sequences. Only bootstrap values (expressed as percentages of 1000 replications) of greater than 50% are shown at the branching points. *Arthrobacter globiformis* DSM 20124^T (M23411) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Description of *Agromyces allii* sp. nov.

Agromyces allii (al.li'i. N.L. gen. n. *allii* of *Allium*, referring to the source of isolation of the micro-organisms, the rhizosphere of *Allium victorialis* var. *platyphyllum*).

Cells are Gram-positive, slightly curved, rod-shaped and 0.2–0.4 × 0.5–1.5 µm. Non-motile. Strictly aerobic. Colonies are circular, convex, smooth and yellow. Growth

occurs at 4 and 33 °C, with an optimum temperature of 30 °C. Optimal pH for growth is between 6.5 and 7.5; growth occurs at pH 5.5, but not at 5.0. Growth occurs at <3% (w/v) NaCl. Aesculin is hydrolysed, but starch and Tweens 20, 40 and 60 are not. Arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. D-Glucose, D-galactose, D-cellobiose, D-mannose, D-trehalose, D-xylose, L-arabinose, sucrose,

Table 2. Cellular fatty acid content (%) of strains UMS-62^T, UMS-101 and some other recognized species of the genus *Agromyces*

Strains: 1, *A. allii* sp. nov. strain UMS-62^T (results for strain UMS-101 are given in parentheses); 2, *A. brachium* IFO 16238^T (data from Takeuchi & Hatano, 2001); 3, *A. luteolus* IFO 16235^T (Takeuchi & Hatano, 2001); 4, *A. rhizosphaerae* IFO 16236^T (Takeuchi & Hatano, 2001); 5, *A. aurantiacus* YIM 21741^T (Li *et al.*, 2003); 6, *A. ulmi* XIL01^T (Rivas *et al.*, 2004); 7, *A. neolithicus* 23-23^T (Jurado *et al.*, 2005a); 8, *A. salentinus* 20-5^T (Jurado *et al.*, 2005a); 9, *A. humatus* CD5^T (Jurado *et al.*, 2005b); 10, *A. italicus* CD1^T (Jurado *et al.*, 2005b); 11, *A. lapidis* CD55^T (Jurado *et al.*, 2005b); 12, *A. subbeticus* Z33^T (Jurado *et al.*, 2005c). Values are percentage of total fatty acids. –, Not detected or not described.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
Saturated fatty acids:												
C _{14:0}	0.4 (1.6)	—	—	—	0.3	—	—	—	—	—	—	—
C _{15:0}	1.5 (0.8)	—	—	—	—	—	—	—	—	—	—	—
C _{16:0}	6.4 (24.1)	—	—	—	4.2	—	—	—	1.3	1.7	5.9	1.0
C _{17:0}	0.7 (0.8)	—	—	—	—	—	—	—	—	—	—	—
C _{18:0}	— (1.5)	—	—	—	—	—	—	—	—	—	—	—
Branched fatty acids:												
iso-C _{14:0}	2.0 (1.0)	—	4.1	—	—	—	—	—	—	—	—	—
iso-C _{15:0}	3.2 (3.8)	4.3	6.1	7.7	8.5	—	12.8	6.0	5.4	14.2	10.5	16.3
anteiso-C _{15:0}	39.1 (34.3)	41.6	32.5	26.1	37.3	46.2	37.8	52.0	41.5	50.9	48.1	45.6
iso-C _{16:0}	28.7 (11.8)	28.1	47.9	53.3	20.1	25.3	13.0	12.9	15.5	14.7	16.5	12.4
iso-C _{17:0}	1.1 (2.0)	—	—	—	2.0	—	3.4	2.2	—	—	—	3.6
anteiso-C _{17:0}	17.0 (18.3)	26.0	9.4	12.9	23.6	19.6	31.9	24.8	34.0	15.3	13.0	20.4

maltose and salicin are utilized as sole carbon and energy sources, but D-fructose, pyruvate, formate and L-glutamate are not. Acid is produced from melibiose, but not from D-sorbitol, *myo*-inositol and D-melezitose. Acid production from D-fructose is variable (positive for type strain). Using the API ZYM system, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI phosphohydrolase, α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase activities are present, but alkaline phosphatase, lipase (C14), trypsin, α -mannosidase and α -fucosidase activities are absent. Susceptible to streptomycin, chloramphenicol, cephalothin, gentamicin, tetracycline, lincomycin, oleandomycin, neomycin and carbenicillin. The predominant menaquinones are MK-11 and MK-12. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. Cell-wall sugars are rhamnose, ribose and galactose; xylose is detected only in the type strain as the major component. The DNA G+C content is 71.1–71.3 mol%.

The type strain, strain UMS-62^T (=KCTC 19181^T=JCM 13584^T), was isolated from the rhizosphere of *Allium victorialis* var. *platyphyllum*, a type of wild edible greens grown on Ulleung island, Korea.

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