Agromyces soli sp. nov., isolated from farm soil

Myungjin Lee,[1] Leonid N. Ten,[2] Sung-Geun Woo[1,3] and Joonhong Park[3]

Correspondence
Myungjin Lee
mgeneli@dreamwiz.com

[1]Research and Development Division, H-Plus Eco Ltd, BVC 301, KRIBB, Eoeun-dong, Yuseong-gu, Daejeon, 305-333, Republic of Korea

[2]Bioenergy Research Center, Korea Institute of Energy Research, 102, Gajeong-ro, Yuseong-gu, Daejeon, 305-343, Republic of Korea

[3]School of Civil and Environmental Engineering, Yonsei University, Seoul, 120-749, Republic of Korea

A Gram-positive, aerobic to microaerophilic, non-motile bacterial strain, designated MJ21^T, was isolated from farm soil and was characterized to determine its taxonomic position by using a polyphasic approach. On the basis of 16S rRNA gene sequence analysis, strain MJ21^T was placed within the genus *Agromyces*, and exhibited relatively high levels of similarity to *Agromyces ulmi* XIL01^T (97.8 %), *Agromyces aurantiacus* YIM 21741^T (97.1 %), *Agromyces mediolanus* JCM 3346^T (96.7 %), *A. mediolanus* JCM 1376 (99.1 %), *A. mediolanus* JCM 9632 (99.1 %), *A. mediolanus* JCM 9633 (98.9 %) and *A. mediolanus* JCM 9631 (96.5 %). Chemotaxonomic data also supported the classification of strain MJ21^T within the genus *Agromyces*. The new isolate contained MK-12 as the predominant menaquinone and rhamnose, galactose and xylose as cell-wall sugars. The major cellular fatty acids (>10 % of the total) were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. Cell-wall amino acids were 2,4-diaminobutyric acid, glutamic acid, glycine and alanine. Diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids and one unidentified phospholipid were detected as polar lipids. The DNA G+C content of strain MJ21^T was 73.4 mol%. However, levels of DNA–DNA relatedness between strain MJ21^T and the seven phylogenetically closest *Agromyces* strains ranged from 14 to 56 %, showing clearly that the new isolate represents a novel genomic species. Strain MJ21^T could be differentiated clearly from its phylogenetic neighbours on the basis of phenotypic, genotypic and chemotaxonomic features. Therefore, strain MJ21^T is considered to represent a novel species of the genus *Agromyces*, for which the name *Agromyces soli* sp. nov. is proposed. The type strain is MJ21^T (=KCTC 19549^T =JCM 16247^T).

The genus *Agromyces*, with *Agromyces ramosus* as the type species, was established by Gledhill & Casida (1969) to accommodate microaerophilic to aerobic, filamentous, branching, fragmenting, catalase- and oxidase-negative actinomycetes isolated from soil. Zgurskaya *et al.* (1992) emended the description of the genus and added two species, each with two subspecies, *Agromyces cerinus* subsp. *cerinus*, *A. cerinus* subsp. *nitratus*, *Agromyces fucosus* subsp. *fucosus* and *A. fucosus* subsp. *hippuratus*, which are characterized by rapid growth on simple media and show positive catalase and oxidase reactions. At the time of writing, the genus *Agromyces* comprises 22 recognized species: *A. ramosus* (Gledhill & Casida, 1969), *A. cerinus* (Zgurskaya *et al.*, 1992), *A. mediolanus* (Suzuki *et al.*, 1996), *A. luteolus*, *A. rhizospherae* and *A. bracchium* (Takeuchi & Hatano, 2001), *A. albus* (Dorofeeva *et al.*, 2003), *A. aurantiacus* (Li *et al.*, 2003), *A. fucosus* and *A. hippuratus* (Zgurskaya *et al.*, 1992; Ortiz-Martinez *et al.*, 2004), *A. ulmi* (Rivas *et al.*, 2004), *A. neolithicus* and *A. salentinus* (Jurado *et al.*, 2005a), *A. humatus*, *A. italicus* and *A. lapidis* (Jurado *et al.*, 2005b), *A. subbeticus* (Jurado *et al.*, 2005c), *A. allii* (Jung *et al.*, 2007), *A. terreus* (Yoon *et al.*, 2008), *A. atrinae* (Park *et al.*, 2010), *A. bauzanensis* (Zhang *et al.*, 2010) and *A. tropicus* (Thawai *et al.*, 2011). Most of the above-mentioned species were isolated from soil and the rhizosphere of plants. Here we describe a Gram-positive, rod-shaped bacterial strain, designated MJ21^T, which was isolated from a soil sample. Phenotypic, chemotaxonomic and phylogenetic analyses established that this isolate was affiliated with the genus *Agromyces*. The data obtained suggest that the isolate represents a novel species of the genus.

Strain MJ21^T was isolated from a farm soil sample collected near Daejeon, South Korea. The sample was suspended and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ21^T is GQ241325.

One supplementary table and two supplementary figures are available with the online version of this paper.

spread on R2A agar (Difco) plates after being serially diluted with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 25 °C for 2 weeks. Single colonies on the plates were purified by transferring them onto fresh plates and incubating them again under the same conditions. The isolate was routinely cultured on R2A agar at 25 °C and maintained as a glycerol suspension (20 %, w/v) at −70 °C. *A. ulmi* KACC 20592^T, *A. aurantiacus* KCTC 9967^T, *A. mediolanus* JCM 3346^T, *A. mediolanus* JCM 1376, *A. mediolanus* JCM 9632, *A. mediolanus* JCM 9633 and *A. mediolanus* JCM 9631 were used as reference strains for DNA–DNA hybridization and other experiments.

The Gram reaction was determined by using a Gram-stain kit (Difco) according to the manufacturer's instructions. Additionally, the Gram reaction was tested by using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000 magnification with cells grown at 25 °C on nutrient agar. Motility was tested by using the hanging-drop technique. Additionally, motility was studied on Luria–Bertani (LB) swarming agar (0.3 %, w/v). The oxidase reaction was tested on filter paper moistened with a 1 % (w/v) aqueous solution of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine, and catalase activity was demonstrated by using 3 % (v/v) hydrogen peroxide as outlined by Cappuccino & Sherman (2002). The test for anaerobic growth in LB medium was performed in 10 ml anaerobic jars with rubber septa (Sigma) that were evacuated with hydrogen gas and then incubated at 25 °C for 7 days. Microaerophilic growth was tested in semi-liquid LB medium containing 0.4 % agar. Assimilation of single carbon sources, enzyme activities, acid production from substrates and other physiological characteristics were determined with the API ID 32 GN (AUX medium, 48 h, 28 °C), API ZYM (0.85 % NaCl medium, 4 h, 28 °C), API 20NE (AUX medium, 48 h, 28 °C) and API 50 CH (API 50 CHB medium, 48 h, 28 °C) galleries according to the manufacturer's instructions (bioMérieux). Cells for inoculation of the strips were grown for 2 days at 28 °C in LB broth. Hydrolysis of casein was examined on nutrient agar supplemented with 1.5 % dry skimmed milk (Tindall *et al.*, 2007). Chitinase activity was tested by using R2A agar medium supplemented with colloidal chitin (4 g l^{-1}) (Hsu & Lockwood, 1975). Hydrolysis of starch was tested on nutrient agar plates supplemented with soluble starch (10 g l^{-1}). Starch hydrolysis was detected by flooding the plates with iodine solution (Tindall *et al.*, 2007). Tests for degradation of DNA [DNase agar (Scharlau), with DNase activity detected by flooding plates with 1 M HCl] were performed according to Collins & Lyne (1984). Hydrolysis of xylan and HE-cellulose was determined with a nutrient agar medium supplemented with a mixture of chromogenic substrates (xylan-red and HE-cellulose-blue), as described previously (Ten *et al.*, 2004). Growth at 4, 10, 15, 20, 25, 30, 37 and 42 °C was assessed on R2A agar, nutrient agar, LB agar, trypticase soy agar (TSA; Difco) and MacConkey agar after 5 days of incubation. Growth at pH 5.0–10.0 (at intervals of 0.5 pH units) was assessed after incubation for 5 days at 25 °C. The effect of pH on growth was determined in R2A broth by using three different buffers (final concentration, 50 mM): sodium acetate buffer (for pH 5.0–5.5), potassium phosphate buffer (for pH 6.0–8.0) and Na_2CO_3/$NaHCO_3$ buffer (for pH 8.5–10.0).

For phylogenetic analysis, genomic DNA was extracted by using a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the program CLUSTAL X (Thompson *et al.*, 1997). Gaps were edited in the program BioEdit (Hall, 1999). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods within the program MEGA4 (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For measurement of the chromosomal DNA G+C content, genomic DNA was extracted and purified as described by Moore & Dowhan (1995) and was degraded enzymically into nucleosides; the DNA G+C content was determined as described by Mesbah *et al.* (1989) by using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1 : 1, v/v). The crude quinone in n-hexane was purified by using Sep-Pak Vac silica cartridges (Waters) and was then analysed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acids were analysed in cells grown on TSA for 24 h at 28 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acid methyl esters were then analysed by GC (model 6890; Hewlett Packard) by using the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted according to the procedure described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents as described previously (Lee *et al.*, 2008). Purified cell-wall preparations were obtained by using the method of Schleifer (1985). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates with the solvent systems described by Schleifer & Kandler (1972). Sugar analysis of the purified cell wall was performed as described by Staneck & Roberts (1974).

Cells of strain MJ21^T were Gram-positive, non-motile, straight or curved rods (0.3–0.4 μm wide and 0.8–1.2 μm long). Cells exhibited aerobic or microaerophilic growth,

but no growth was observed under anaerobic conditions. Colonies grown in the light on R2A agar plates for 2 days at 25 °C were 0.5–1.8 mm in diameter, circular, shiny, of low convexity, transparent and pale yellow. Colonies grown in the dark were colourless, indicating that pigment production was induced by light. Strain MJ21^T grew at 10–30 °C; the optimum temperature for growth was 25 °C. Growth also occurred on nutrient agar, TSA and LB agar but not on MacConkey agar. Pigmentation was observed on all agar media tested and its intensity was dependent slightly on growth temperature. Filamentous elongation and branching were not observed on any growth media. Strain MJ21^T did not exhibit a rod–coccus growth cycle. The isolate was positive for catalase but showed a negative oxidase reaction with *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine. Other morphological, physiological and biochemical characteristics of strain MJ21^T are given in the species description below. Phenotypic and chemotaxonomic characteristics that differentiate strain MJ21^T from its closest phylogenetic relatives are listed in Table 1. In particular, in contrast to all reference strains the new isolate was positive for alkaline phosphatase and acid production from L-fucose and gluconate. Furthermore, strain MJ21^T differed from its phylogenetically closest neighbours, *A. mediolanus* JCM 1376, *A. mediolanus* JCM 9632 and *A. mediolanus* JCM 9633 in 29, 24 and 30, respectively, of 113 characteristics examined.

The almost-complete 16S rRNA gene sequences of strain MJ21^T and *A. mediolanus* JCM 3346^T determined in this study comprised 1409 and 1372 bp, respectively. As expected, an unusual 43 nt insertion in the 16S rRNA gene sequence of *A. mediolanus* DSM 20152^T (GenBank accession number X77449) was not detected. The 16S rRNA gene sequence of *A. mediolanus* JCM 3346^T (GenBank accession number HM641754) obtained here was used for phylogenetic analysis. 16S rRNA gene sequence similarity calculations after neighbour-joining analysis indicated that among recognized members of the family *Microbacteriaceae* the closest relatives of strain MJ21^T were *A. ulmi* XIL01^T (97.8 %), *A. aurantiacus* YIM 21741^T (97.1 %) and *A. mediolanus* JCM 3346^T (96.7 %). Higher levels of 16S rRNA gene sequence similarity were found with other strains of *A. mediolanus*, namely *A. mediolanus* JCM 1376 (99.1 %), *A. mediolanus* JCM 9632 (99.1 %), *A. mediolanus* JCM 9633 (98.9 %) and *A. mediolanus* JCM 9631 (96.5 %). This relationship between strain MJ21^T and other members of the genus *Agromyces* was also evident in the phylogenetic trees (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The generally accepted criteria for delineating bacterial species state that strains with 16S rRNA gene sequence dissimilarity above 3 % or strains with a DNA–DNA relatedness value of less than 70 % (as measured by hybridization) are considered to belong to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). In view of this definition, the above-mentioned data indicate that strain MJ21^T can be clearly separated from other members of the genus *Agromyces* with the exception of the strains given above. For further verification of the taxonomic position of strain MJ21^T, DNA–DNA hybridization was performed with its phylogenetically closest relatives.

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA hybridization values. Strain MJ21^T showed less than 57 % DNA–DNA relatedness with all assayed *Agromyces* strains (see Supplementary Table S1), indicating that it is not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

Strain MJ21^T contained large amounts of the branched-chain fatty acids anteiso-$C_{15:0}$ (46.9 % of the total), anteiso-$C_{17:0}$ (23.7 %) and iso-$C_{16:0}$ (18.1 %), a smaller amount of iso-$C_{15:0}$ (7.4 %), and minor amounts of iso-$C_{17:0}$ (1.7 %), iso-$C_{14:0}$ (1.1 %) and $C_{16:0}$ (1.1 %). This fatty acid profile was similar to those of recognized species of the genus *Agromyces*, producing anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$ as predominant components on a variety of different media (Suzuki *et al.*,1996; Takeuchi & Hatano, 2001; Li *et al.*, 2003; Rivas *et al.*, 2004; Yoon *et al.*, 2008). The amino acids 2,4-diaminobutyric acid, glutamic acid, glycine and alanine were detected in total hydrolysates of the cell-wall preparation of strain MJ21^T. This composition is consistent with peptidoglycan type B2γ, which has been reported for most members of the genus *Agromyces*. Cell-wall sugars found in strain MJ21^T were rhamnose, galactose and xylose. Galactose and rhamnose have been reported for most species of the genus *Agromyces* (Takeuchi & Hatano, 2001; Dorofeeva *et al.*, 2003; Li *et al.*, 2003; Jurado *et al.*, 2005a), while xylose has been found only in a few *Agromyces* species (Zgurskaya *et al.*, 1992). The major polar lipids detected in strain MJ21^T were diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids and one unidentified phospholipid (see Supplementary Fig. S2). This result is in good agreement with other species of the genus *Agromyces*; in particular, all of them produced diphosphatidylglycerol and phosphatidylglycerol as diagnostic phospholipids (Suzuki *et al.*, 1996; Li *et al.*, 2003; Jurado *et al.*, 2005a; Zhang *et al.*, 2010). Strain MJ21^T contained MK-12 (85.8 %) as the major menaquinone, with minor amounts of MK-11 (8.9 %) and MK-10 (5.3 %). The DNA G+C content of strain MJ21^T was 73.4 mol%, which is slightly higher than values reported for recognized species of the genus *Agromyces* (65.3–72.8 mol%) (Li *et al.*, 2003; Jurado *et al.*, 2005a). However, the value still lies within the range expected for members of the same genus and the DNA G+C content range of the genus *Agromyces* should be extended taking this into account.

The phenotypic and phylogenetic data presented here indicate that strain MJ21^T belongs to the genus *Agromyces*. The phylogenetic distinctiveness of strain MJ21^T, together

Table 1. Differential phenotypic characteristics between strain MJ21^T and its phylogenetically closest relatives in the genus *Agromyces*

Strains: 1, MJ21^T; 2, *A. ulmi* KACC 20592^T; 3, *A. aurantiacus* KCTC 9967^T; 4, *A. mediolanus* JCM 3346^T; 5, *A. mediolanus* JCM 1376 (CNF134); 6, *A. mediolanus* JCM 9632 (CNF186); 7, *A. mediolanus* JCM 9633 (CNF208); 8, *A. mediolanus* JCM 9631 (CNF183). All data are from this study except where indicated. All strains are positive for aesculin hydrolysis but negative for Gram-stain reaction, H_2S and indole production. In API ID 32 GN, API 20NE (assimilation of carbon sources), API ZYM and API 50 CHB tests, all strains showed identical biochemical characteristics except those indicated here (see species description). W, Weakly positive reaction.

Characteristic	1	2	3	4	5	6	7	8
Growth at 37 °C	−	+	+	+	−	+	+	+
Hydrolysis of:								
Casein	−	−	+	+	+	+	+	+
Gelatin	−	−	+	+	−	−	−	−
Starch	−	−	+	−	+	+	+	+
Nitrate reduction to nitrite	−	−	+	+	+	−	−	−
Enzyme activities (API ZYM and API 20NE)								
N-Acetyl-β-glucosaminidase	+	−	−	+	+	+	+	+
Acid phosphatase	+	−	+	+	−	−	+	+
Alkaline phosphatase	+	−	−	−	−	−	−	−
Cystine arylamidase	+	+	−	+	+	+	+	+
α-Glucosidase	−	−	+	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	−	−	+	−
Valine arylamidase	+	+	−	+	+	+	+	+
Assimilation of (API ID 32 GN and API 20NE):								
Acetate	+	+	+	+	+	+	−	+
L-Alanine	−	−	−	+	+	−	−	+
L-Arabinose	+	+	−	−	−	−	−	−
Citrate	−	−	+	−	−	−	−	−
L-Fucose	+	−	−	−	+	+	−	+
Gluconate	−	+	+	−	−	−	−	+
L-Histidine	+	−	−	+	+	+	−	−
DL-3-Hydroxybutyrate	−	+	+	−	−	−	−	−
Lactate	+	−	−	+	+	+	+	+
Malate	+	−	−	−	−	−	−	−
D-Mannitol	+	−	+	−	−	+	+	+
Melibiose	+	−	−	−	−	−	−	−
Propionate	+	+	+	−	−	−	−	−
L-Rhamnose	+	+	−	+	+	+	+	+
D-Ribose	+	−	−	+	+	+	+	−
Salicin	+	−	+	+	+	+	+	+
D-Sorbitol	−	−	+	−	−	−	−	−
Valerate	−	+	+	−	−	−	−	−
Acid production from (API 50 CHB):								
N-Acetylglucosamine	+	+	+	−	−	−	−	−
Amygdalin	+	+	−	W	+	W	−	+
D-Arabinose	+	−	−	W	−	W	W	−
L-Arabinose	+	−	+	−	−	−	−	−
Arbutin	+	+	−	+	+	+	+	+
D-Fructose	+	+	+	−	−	+	−	−
L-Fucose	+	−	−	−	−	−	−	−
β-Gentiobiose	+	+	−	−	−	−	−	−
Gluconate	+	−	−	−	−	−	−	−
Glycogen	+	+	+	−	−	−	−	−
Inulin	−	+	−	−	−	−	−	−
5-Ketogluconate	−	−	+	−	−	−	−	+
Lactose	−	+	−	−	−	−	−	−
D-Lyxose	−	+	−	−	−	−	−	−
Maltose	+	+	+	+	−	+	+	−

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
D-Mannitol	+	−	+	−	−	−	−	−
D-Mannose	+	+	+	+	−	+	−	−
Melezitose	+	+	+	−	−	−	−	−
Melibiose	−	+	+	−	−	−	−	−
Methyl α-D-glucopyranoside	+	+	+	−	−	−	−	−
Methyl α-D-mannopyranoside	+	+	−	−	−	−	−	−
Raffinose	−	+	+	−	−	−	−	−
D-Ribose	+	+	−	+	−	+	−	−
Salicin	+	+	−	+	+	−	−	+
L-Sorbose	−	+	−	−	−	−	−	−
Starch	+	−	+	−	−	−	−	−
Sucrose	+	+	+	+	−	+	−	−
Trehalose	+	+	−	+	+	w	w	w
Turanose	+	−	+	+	+	+	+	+
Xylitol	−	+	−	−	−	−	−	−
D-Xylose	+	+	−	−	−	−	−	−
Cell-wall sugars*	Rha, Gal, Xyl	Rha, Fuc, Glu[a]†	Rha, Gal, Glu, Man[b]	Rha, Man, Gal	Rha, Man, Gal	Rha, Man, Gal	Rha, Man, Gal	Rha, Man, Glu
Menaquinone(s)§	MK-12 (-11, -10)	MK-12 (-11, -10)[a]	MK-12[b]	MK-12 (-11, -10)[c]	MK-12 (-11, -10)[c]	MK-12 (-11, -10)[c]	MK-12 (-11, -10)[c]	MK-12 (-11, -10)[c]
DNA G+C content (mol%)	73.4	72.0[a]	72.8[b]	72.3[c]	72.3[c]	72.3[c]	73.3[c]	72.8[c]

*Fru, fructose; Fuc, fucose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.
† Data from: *a*, Rivas *et al.* (2004); *b*, Li *et al.* (2003); *c*, Suzuki *et al.* (1996).
§Menaquinones in parentheses are present as the next most common components.

with DNA–DNA hybridization data, confirmed that this strain represents a species that is distinct from recognized species of the genus *Agromyces*. Strain MJ21^T can be differentiated from phylogenetically related *Agromyces* strains based on several phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strain MJ21^T should be classified within the genus *Agromyces* as the type strain of a novel species, for which the name *Agromyces soli* sp. nov. is proposed.

Description of *Agromyces soli* sp. nov.

Agromyces soli (so′li. L. gen. n. *soli* of soil).

Cells are Gram-positive, aerobic to microaerophilic, straight or curved rods (0.3–0.4 μm wide and 0.8–1.2 μm long). Growth occurs at 10–30 °C and at pH 5.0–9.0 (optimum pH 7.0–7.5). Nitrate is not reduced to nitrite. Hydrolyses DNA, but not casein, HE-cellulose, chitin, starch or xylan. In API ID 32 GN and API 20NE tests, utilizes acetate,

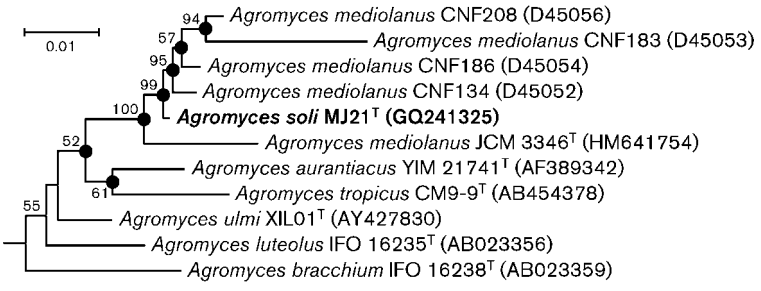

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain MJ21^T among selected recognized members of the genus *Agromyces*. Bootstrap values (expressed as percentages of 1000 replications) of >50 % are shown at branch points. *Arthrobacter globiformis* DSM 20124^T (GenBank accession number M23411) was used as an outgroup (not shown). Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position. An expanded version of this tree is available as Supplementary Fig. S1.

N-acetylglucosamine, L-arabinose, L-fucose, D-glucose, L-histidine, lactate, malate, maltose, D-mannitol, D-mannose, melibiose, propionate, L-rhamnose, D-ribose, salicin and sucrose for growth, but not adipate, L-alanine, caprate, citrate, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, malonate, phenylacetate, L-proline, L-serine, D-sorbitol, suberate or valerate. In API ZYM and API 20NE tests, positive for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), β-galactosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for arginine dihydrolase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and urease. In API 50 CHB tests, acid is produced from *N*-acetylglucosamine, aesculin, amygdalin, D-arabinose, L-arabinose, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, β-gentiobiose, D-glucose, gluconate, glycerol, glycogen, maltose, D-mannitol, D-mannose, melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, L-rhamnose, D-ribose, salicin, starch, sucrose, trehalose, turanose and D-xylose, but not from adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, melibiose, methyl β-D-xylopyranoside, raffinose, sorbitol, L-sorbose, D-tagatose, xylitol or L-xylose. MK-12 is the predominant menaquinone. Major cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. Cell-wall sugars are rhamnose, galactose and xylose. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids and one unidentified phospholipid. Cell-wall amino acids are 2,4-diaminobutyric acid, glutamic acid, glycine and alanine. The DNA G+C content of the type strain is 73.4 mol%.

The type strain, MJ21^T (=KCTC 19549^T =JCM 16247^T), was isolated from a farm soil near Daejeon, South Korea.

Acknowledgements

This work was supported by the Eco-Technopia 21 Project, Ministry of Environment, Republic of Korea (071-081-133).

References

Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. San Francisco: Benjamin Cummings.

Collins, C. H. & Lyne, P. M. (1984). *Microbiological Methods*, 5th edn. London: Butterworth.

Dorofeeva, L. V., Krausova, V. I., Evtushenko, L. I. & Tiedje, J. M. (2003). *Agromyces albus* sp. nov., isolated from a plant (*Androsace* sp.). *Int J Syst Evol Microbiol* **53**, 1435–1438.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1985). Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gledhill, W. E. & Casida, L. E., Jr (1969). Predominant catalase negative soil bacteria. III. *Agromyces* gen. nov., microorganisms intermediary to *Actinomyces* and *Nocardia*. *Appl Microbiol* **18**, 340–349.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.

Hsu, S. C. & Lockwood, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl Microbiol* **29**, 422–426.

Jung, S.-Y., Lee, S.-Y., Oh, T.-K. & Yoon, J.-H. (2007). *Agromyces allii* sp. nov., isolated from the rhizosphere of *Allium victorialis* var. platyphyllum. *Int J Syst Evol Microbiol* **57**, 588–593.

Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L. & Saiz-Jimenez, C. (2005a). *Agromyces salentinus* sp. nov. and *Agromyces neolithicus* sp. nov. *Int J Syst Evol Microbiol* **55**, 153–157.

Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L., Schuetze, B. & Saiz-Jimenez, C. (2005b). *Agromyces italicus* sp. nov., *Agromyces humatus* sp. nov. and *Agromyces lapidis* sp. nov., isolated from Roman catacombs. *Int J Syst Evol Microbiol* **55**, 871–875.

Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L. & Saiz-Jimenez, C. (2005c). *Agromyces subbeticus* sp. nov., isolated from a cave in southern Spain. *Int J Syst Evol Microbiol* **55**, 1897–1901.

Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β-glucosidase-producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Lee, M., Ten, L. N., Lee, H. W., Oh, H. W., Im, W. T. & Lee, S.-T. (2008). *Sphingopyxis ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **58**, 2342–2347.

Li, W.-J., Zhang, L.-P., Xu, P., Cui, X.-L., Xu, L.-H., Zhang, Z., Schumann, P., Stackebrandt, E. & Jiang, C.-L. (2003). *Agromyces aurantiacus* sp. nov., isolated from a Chinese primeval forest. *Int J Syst Evol Microbiol* **53**, 303–307.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.

Ortiz-Martinez, A., Gonzalez, J. M., Evtushenko, L. I., Jurado, V., Laiz, L., Groth, I. & Saiz-Jimenez, C. (2004). Reclassification of *Agromyces fucosus* subsp. *hippuratus* as *Agromyces hippuratus* sp. nov., comb. nov.

and emended description of *Agromyces fucosus*. *Int J Syst Evol Microbiol* **54**, 1553–1556.

Park, E.-J., Kim, M.-S., Jung, M.-J., Roh, S. W., Chang, H.-W., Shin, K.-S. & Bae, J.-W. (2010). *Agromyces atrinae* sp. nov., isolated from fermented seafood. *Int J Syst Evol Microbiol* **60**, 1056–1059.

Rivas, R., Trujillo, M. E., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2004). *Agromyces ulmi* sp. nov., a xylanolytic bacterium isolated from *Ulmus nigra* in Spain. *Int J Syst Evol Microbiol* **54**, 1987–1990.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria through fatty acid analysis. In *Methods in Phytobacteriology*, pp. 199–204. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kaido.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

Suzuki, K., Sasaki, J., Uramoto, M., Nakase, T. & Komagata, K. (1996). *Agromyces mediolanus* sp. nov., nom. rev., comb. nov., a species for ''*Corynebacterium mediolanum*'' Mamoli 1939 and for some aniline-assimilating bacteria which contain 2,4-diaminobutyric acid in the cell wall peptidoglycan. *Int J Syst Bacteriol* **46**, 88–93.

Takeuchi, M. & Hatano, K. (2001). *Agromyces luteolus* sp. nov., *Agromyces rhizospherae* sp. nov. and *Agromyces bracchium* sp. nov., from the mangrove rhizosphere. *Int J Syst Evol Microbiol* **51**, 1529–1537.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.

Thawai, C., Tanasupawat, S., Suwanborirux, K. & Kudo, T. (2011). *Agromyces tropicus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **61**, 605–609.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tindall, J. B., Sikorski, J., Simbert, A. R. & Krieg, R. N. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yoon, J.-H., Schumann, P., Kang, S.-J., Park, S. & Oh, T.-K. (2008). *Agromyces terreus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **58**, 1308–1312.

Zgurskaya, H. I., Evtushenko, L. I., Akimov, V. N., Voyevoda, H. V., Dobrovolskaya, T. G., Lysak, L. V. & Kalakoutskii, L. V. (1992). 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. *Int J Syst Bacteriol* **42**, 635–641.

Zhang, D.-C., Schumann, P., Liu, H.-C., Xin, Y.-H., Zhou, Y.-G., Schinner, F. & Margesin, R. (2010). *Agromyces bauzanensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **60**, 2341–2345.