Agromyces terreus sp. nov., isolated from soil

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A Gram-positive, non-motile, cream-coloured, curved or straight rod-shaped bacterial strain, DS-10^T, was isolated from soil of Dokdo, Korea, and its taxonomic position was investigated by using a polyphasic approach. Strain DS-10^T grew optimally at 25 °C and pH 7.0–8.0 in the presence of 0.5–1.0 % (w/v) NaCl. Strain DS-10^T had peptidoglycan type B2_γ [D-Glu–D-Dab (diaminobutyric acid)] and galactose and ribose as whole-cell sugars. Strain DS-10^T contained MK-11, MK-12 and MK-10 as predominant menaquinones. Major cellular fatty acids (>10 % of total) were anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and iso-C_{15:0}. Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The DNA G + C content was 71.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-10^T belonged to the genus *Agromyces*. Similarity values between the 16S rRNA gene sequence of strain DS-10^T and those of the type strains of recognized *Agromyces* species ranged from 95.8 to 99.3 %. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain DS-10^T could be differentiated from recognized *Agromyces* species. On the basis of the data presented, strain DS-10^T is considered to represent a novel species of the genus *Agromyces*, for which the name *Agromyces terreus* sp. nov. is proposed. The type strain is DS-10^T (=KCTC 19216^T =JCM 14581^T).

The genus *Agromyces* was proposed by Gledhill & Casida (1969) and, at the time of writing, comprises 18 species with validly published names: *Agromyces ramosus* (Gledhill & Casida, 1969), *A. cerinus* (Zgurskaya *et al.*, 1992), *A. mediolanus* (Suzuki *et al.*, 1996), *A. luteolus*, *A. rhizospherae* and *A. brachium* (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) and *A. allii* (Jung *et al.*, 2007). Here we report on the taxonomic characterization of an *Agromyces*-like bacterial strain, DS-10^T, which was isolated from soil of Dokdo, an island of Korea.

Abbreviation: Dab, diaminobutyric acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-10^T is EF363711.

A table detailing the cellular fatty acid compositions of *Agromyces terreus* sp. nov. DS-10^T and the type strains of phylogenetically related *Agromyces* species is available as supplementary material with the online version of this paper.

Strain DS-10^T was isolated by using the standard dilution plating technique at 30 °C on 10-fold-diluted nutrient agar (NA; Difco). The type strains of four recognized *Agromyces* species were used as reference strains for DNA–DNA hybridization experiments: *A. salentinus* DSM 16198^T, *A. italicus* DSM 16388^T and *A. lapidis* DSM 16390^T were obtained from the DSMZ and *A. allii* UMS-62^T was described by Jung *et al.* (2007). The morphological, physiological and biochemical characteristics of strain DS-10^T were investigated by using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. The presence of flagella was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on TSA. Growth in the absence of NaCl and at various NaCl concentrations (0.5 and 1.0–10.0 %, w/v, at intervals of 1.0 %) was investigated in trypticase soy broth prepared

according to the formula of the Difco medium. The pH range for growth was determined in nutrient broth (Difco) that was adjusted prior to sterilization to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically under nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied as described by Lányi (1987). Susceptibility to antibiotics was tested on TSA plates by using discs containing the following antibiotics: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) or carbenicillin (100 µg). Assimilation of various substrates, acid production from various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50CH and API ZYM systems (bioMérieux). Assimilation of various substrates was determined by inoculating API 50CH strips with cell suspension in AUX medium (bioMérieux). Acid production by fermentation was determined by using API 50CHB medium according to the manufacturer's instructions.

Cell biomass for DNA extraction and for analyses of cell-wall components, isoprenoid quinones and polar lipids was obtained from cultures grown by shaking at 150 r.p.m. in trypticase soy broth (Difco) for 3 days at 25 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values. Preparation of cell walls and determination of peptidoglycan structure were carried out by using the methods of Schleifer & Kandler (1972), MacKenzie (1987) and Groth *et al.* (1996). Whole-cell sugars were determined as

described by Komagata & Suzuki (1987). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed by using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For fatty acid methyl ester analysis, cell mass of strain DS-10^T was harvested from TSA plates after incubation for 3 days at 25 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990).

The morphological, cultural, physiological and biochemical characteristics of strain DS-10^T are given in the species description below or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain DS-10^T determined in this study comprised 1449 nt (approximately 96% of the *Escherichia coli* 16S rRNA gene sequence). 16S rRNA gene sequence analyses showed that strain DS-10^T was phylogenetically related most closely to members of the genus *Agromyces* (Fig. 1). In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-10^T fell within the cluster comprising *Agromyces* species, particularly by forming a coherent cluster with *A. allii*, *A. salentinus*, *A. italicus* and *A. lapidis* (Fig. 1). Strain DS-10^T exhibited 16S rRNA gene sequence similarity values of 95.8 (*A. brachium*) to 99.3% (*A. salentinus*) with respect to the type strains of recognized *Agromyces* species.

Chemotaxonomic data confirmed the phylogenetic affiliation of strain DS-10^T as a member of the genus *Agromyces*. The total hydrolysate of the peptidoglycan (4 M HCl, 16 h at 100 °C) of strain DS-10^T contained the amino acids diaminobutyric acid (Dab), alanine, glycine and glutamic acid in an approximate ratio of 1.0:0.8:1.0:1.4. The peptide Gly–D–Glu was detected by two-dimensional TLC after hydrolysis under milder conditions (4 M HCl, 45 min at 100 °C). Dinitrophenylation of the peptidoglycan revealed that Dab was the only N-terminal amino acid in the interpeptide bridge. From these data, it was concluded that strain DS-10^T had peptidoglycan type B2γ, D–Glu–D–Dab, as described by Schleifer & Kandler (1972). The amino acids in positions 1 and 3 were glycine and L–Dab, respectively. Cell-wall sugars were galactose and ribose. Strain DS-10^T contained MK-11, MK-12 and MK-10 at peak area ratios of approximately 49, 31 and 16%, respectively, as the predominant menaquinones. Strain DS-10^T contained large amounts of branched-chain fatty acids; the major components (>10% of total fatty acids) were anteiso-C_{15:0} (40.5%), anteiso-C_{17:0} (20.5%), iso-C_{16:0} (19.4%) and iso-C_{15:0} (11.8%) (see Supplementary Table S1 in IJSEM Online). This fatty acid profile was similar to those of recognized *Agromyces* species (Takeuchi & Hatano, 2001; Li *et al.*, 2003; Jurado *et al.*, 2005a, b, c; Jung *et al.*, 2007). Major polar lipids detected in strain

Table 1. Differential phenotypic characteristics between strain DS-10^T and phylogenetically related *Agromyces* species

Taxa: 1, strain DS-10^T; 2, *A. allii* (data from Jung *et al.*, 2007); 3, *A. salentinus* (Jurado *et al.*, 2005a); 4, *A. italicus* (Jurado *et al.*, 2005b); 5, *A. lapidis* (Jurado *et al.*, 2005b). +, Positive; –, negative; v, variable (data in parentheses are for the type strain); w, weakly positive; ND, not determined or not described. All are positive for catalase, hydrolysis of aesculin, acid production from glycerol, D-arabinose, arbutin, L-fucose, glycogen and starch (not determined for *A. allii*), cellobiose, galactose, D-glucose, maltose and mannose, β -glucosidase and susceptibility to ampicillin, novobiocin (not determined for *A. salentinus*) and penicillin G. All are negative for indole production, acid production from mannitol, melibiose (not determined for *A. allii*), utilization of citrate, lipase (C14) and α -mannosidase.

Characteristic	1	2	3	4	5
Maximum temperature for growth (°C)	35	33	37	37	37
Growth at 10 °C	+	+	+	w	w
Growth at 4.0 % (w/v) NaCl	+	–	+	+	+
Nitrate reduction	+	+	–	+	+
Oxidase activity	+	+	v	+	v
Hydrolysis of:					
Casein	–	+	+	+	+
Gelatin	+	+	–	+	v
Hypoxanthine	+	–	–	+	–
Starch	+	–	+	+	+
Tyrosine	+	–	+	+	+
Tween 80	+	–	–	–	–
Xanthine	–	–	–	+	–
Urea	–	–	+	–	–
H ₂ S production	–	–	+	+	+
Acid production from:					
L-Arabinose	+	+	+	+	–
Fructose	+	v (+)	+	+	+
Gentiobiose	–	ND	w	–	–
Lactose	–	+	–	w	–
Raffinose	w	–	w	–	w
Rhamnose	+	+	+	–	–
Ribose	–	+	+	–	–
Salicin	w	+	+	+	+
Sucrose	+	+	+	–	+
Trehalose	–	+	+	–	–
Turanose	–	ND	+	–	–
D-Xylose	+	+	+	+	–
N-Acetylglucosamine	–	ND	–	–	+
Amygdalin	–	ND	+	w	+
Inulin	+	ND	+	–	+
Utilization of malate	–	+	–	–	–
Enzyme activity (API ZYM)					
Alkaline phosphatase	–	–	+	+	+
Valine arylamidase	–	+	ND	+	+
Cystine arylamidase	–	+	ND	+	+
α -Chymotrypsin	–	v (+)	–	–	+
α -Galactosidase	–	–	–	–	–
β -Galactosidase	w	+	+	+	+
β -Glucuronidase	–	+	–	–	–
Antibiotic susceptibility					
Kanamycin	w	+	+	–	–
Polymyxin B	w	+	+	–	–
Streptomycin	+	+	+	–	–
DNA G + C content (mol%)	71.1	71.1–71.3	72.3	70.8	70.4

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain DS-10^T and *Agromyces* species based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are given at branch points; only values >50% are shown. *Arthrobacter globiformis* DSM 20124^T (GenBank accession no. M23411) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

DS-10^T were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The DNA G + C content of strain DS-10^T was 71.1 mol%.

Levels of DNA–DNA relatedness between strain DS-10^T and the type strains of four *Agromyces* species, *A. allii*, *A. salentinus*, *A. italicus* and *A. lapidis*, its closest phylogenetic neighbours, were in the range 12–40%, indicating that strain DS-10^T represents a genomic species different from these four recognized *Agromyces* species (Wayne *et al.*, 1987). Strain DS-10^T differed from these species based on several phenotypic characteristics (Table 1). Despite the small phylogenetic divergence between the novel strain and its closest neighbour (99.3% 16S rRNA gene sequence similarity), DNA–DNA relatedness data and differential phenotypic properties show that strain DS-10^T can be distinguished from this and all other recognized *Agromyces* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-10^T is considered to represent a novel species of the genus *Agromyces*, for which the name *Agromyces terreus* sp. nov. is proposed.

Description of *Agromyces terreus* sp. nov.

Agromyces terreus (ter're.us. L. masc. adj. *terreus* of the earth).

Cells are Gram-positive, curved or straight rods, 0.2–0.5 × 0.7–5.0 µm. Non-motile. Strict aerobe. Colonies on TSA are circular, convex, smooth, glistening, cream-coloured

and 0.8–1.2 mm in diameter after 3 days incubation at 25 °C. Growth occurs at 10 and 35 °C with an optimum temperature of 25 °C, but not at 4 or 36 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.0 and 10.0, but not at pH 4.5 or 10.5. Growth occurs in the presence of 0–6.0% (w/v) NaCl with optimum growth in the presence of 0.5–1.0% (w/v) NaCl. Tweens 20, 40 and 60 are hydrolysed. Arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. The following substrates are assimilated: glycerol, phenylacetate, D-arabinose, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, rhamnose, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose, inulin, raffinose, starch, glycogen, gentiobiose, turanose and L-fucose. The following substrates are not assimilated: caprate, adipate, erythritol, ribose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, lactose, melibiose, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. Acid is produced from aesculin and 5-ketogluconate, but not from erythritol, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. In assays with the API ZYM system, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase are present and acid phosphatase and N-acetyl-β-glucosaminidase are weakly present, but trypsin and α-fucosidase are absent. Susceptible to chloramphenicol, cephalothin, gentamicin, tetracycline, carbenicillin, neomycin and oleandomycin, but not to lincomycin. The cell-wall peptidoglycan type is B2γ, D-Glu–D-Dab. Cell-wall sugars are galactose and ribose. The predominant menaquinones are MK-11, MK-12 and MK-10. Major cellular fatty acids (>10% of total fatty acids) are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and iso-C_{15:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The DNA G + C content is 71.1 mol%.

The type strain, DS-10^T (=KCTC 19216^T =JCM 14581^T), was isolated from a soil of Dokdo, Korea.

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