Agromyces ulmi sp. nov., a xylanolytic bacterium isolated from *Ulmus nigra* in Spain

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Two xylan-degrading bacterial strains were isolated from a decayed *Ulmus nigra* tree in Spain. The isolates were Gram-positive, non-motile, aerobic and formed substrate mycelium which fragmented into irregular rods. 16S rRNA gene sequence analysis indicated that the isolates form a separate branch within the genus *Agromyces* phylogenetic cluster, with *Agromyces mediolanus* DSM 20152^T being their closest relative (97.7 and 97.6% sequence similarity). Catalase, nitrate reduction and urease tests differentiated these strains from *A. mediolanus*. Cell-wall peptidoglycan composition, major menaquinone, predominant fatty acids and phospholipid pattern were typical of the genus *Agromyces*. The DNA G + C content determined for the type strain XIL01^T was 72 mol%. Based on the data presented, a novel species *Agromyces ulmi* sp. nov. is proposed. The type strain is XIL01^T (=LMG 21954^T=DSM 15747^T).

The genus *Agromyces* proposed by Gledhill & Casida (1969) currently harbours ten species, including both non-filamentous and filamentous, microaerophilic to aerobic, catalase- and oxidase-variable actinomycetes (Zgurskaya *et al.*, 1992; Suzuki *et al.*, 1996; Sasaki *et al.*, 1998; Takeuchi & Hatano, 2001; Li *et al.*, 2003; Ortiz-Martinez *et al.*, 2004). During a study of xylanolytic bacteria from decayed stumps of elm trees we isolated two strains designated XIL01^T and XIL05. The isolates showed the ability to hydrolyse xylan, a polymer of xylose present in plant cell walls.

The isolation of strains XIL01^T and XIL05 was performed on XED agar (7 g xylan, 3 g yeast extract and 25 g agar l⁻¹) as described by Rivas *et al.* (2003). Cells grew as opaque white colonies on nutrient and XED agar media. Young cultures (15 h) produced branching substrate hyphae that broke into non-motile, diphtheroid and irregular rod-shaped cells (1.3–2.4 × 0.8–1.0 µm) at later stages. Such cells usually appeared as branching rods when observed by phase-contrast microscopy. Aerial mycelium was not formed. The two strains stained Gram-positive (Doetsch, 1981).

Amplification of the 16S rRNA gene and its sequencing was performed according to Rivas *et al.* (2003). The MEGA2

package (Kumar *et al.*, 2001) was used to calculate phylogenetic distances according to the Kimura two-parameter method (Kimura, 1980) and the tree was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987); bootstrap analyses were based on 1000 resamplings.

Two 16S rRNA gene sequences of 1519 nucleotides were obtained for isolates XIL01^T and XIL05; two differences were found between the sequences of the two strains, which formed a separate branch within the genus *Agromyces* (Fig. 1). The sequences of strains XIL01^T and XIL05 showed 97.7 and 97.6% similarity, respectively, with the sequence of *Agromyces mediolanus* DSM 20152^T, which was the closest related species.

Biochemical and physiological properties of the two isolates investigated included carbon source assimilation, temperature growth rates and degradation of organic compounds (Rivas *et al.*, 2003). Amylase, caseinase, catalase, oxidase and cellulase activities were detected as described by Rivas *et al.* (2003). The two strains grew between 15 and 37 °C, were negative for oxidase reaction and showed very similar results for the other characteristics studied; however, strain XIL05 could be differentiated from XIL01^T on the basis of assimilation of malate and arbutin degradation. Differential phenotypic characteristics between strains XIL01^T and XIL05 and their phylogenetically closest relatives are presented in Table 1. Other results are reported under the species description.

Isolates XIL01^T and XIL05 were grown on trypticase soy broth (Becton Dickinson, BBL) for 4 days at 28 °C for chemotaxonomic characterization, which included analyses

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Abbreviation: DAB, 2,4-diaminobutyric acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains XIL01^T and XIL05 are AY427830 and AY427831, respectively.

Fig. 1. Neighbour-joining tree derived from analysis of 16S rRNA gene sequences (Kimura's two-parameter method) of isolates XIL01^T and XIL05 and related *Agromyces* species. Bootstrap percentages are indicated at branch points. Bar, 1 nucleotide substitution per 100 nucleotides. *Microbacterium lacticum* IFO 14135^T was used as the outgroup.

of peptidoglycan composition, cell-wall sugars, cellular fatty acids, menaquinones and polar lipids. Studies were carried out according to the methods described by Schleifer & Kandler (1972), Stanek & Roberts (1974), Schleifer (1985) and Zimmermann *et al.* (1998).

Both strains contained 2,4-diaminobutyric acid (DAB), glycine and glutamic acid in their cell walls, but alanine was not found. This composition is consistent with the B2 γ -type peptidoglycan, which has been reported for all strains currently representing the genus *Agromyces*. Cell-wall sugars found in strains XIL01^T and XIL05 were rhamnose, fucose and glucose. Rhamnose and glucose have been reported for most species of the genus *Agromyces*, whereas fucose has been found only in *Agromyces fucosus* (Zgurskaya *et al.*, 1992; Ortiz-Martinez *et al.*, 2004).

The major fatty acids found for strains XIL01^T and XIL05 were anteiso-C_{15:0} (46.2 and 45.6 %), iso-C_{16:0} (25.3 and 24.3 %) and anteiso-C_{17:0} (19.6 % and 22.0 %). This result is in agreement with other *Agromyces* species described (Suzuki *et al.*, 1996; Takeuchi & Hatano, 2001; Li *et al.*, 2003).

HPLC analysis of the menaquinones revealed major amounts of MK-12 and MK-11 for both strains, a result that is also reported for many *Agromyces* species (Takeuchi & Hatano, 2001; Dorofeeva *et al.*, 2003). Isolate XIL01^T contained the menaquinones MK-12, -11, -10, -13 and -9 (50:35:11:2:2), whereas XIL05 contained MK-12, -11, -13, -9 and -10 (61:23:7:4:3). Polar lipids were only studied for strain XIL01^T and were composed of diphosphatidylglycerol, phosphatidylglycerol and glycolipid.

Table 1. Characteristics that differentiate *Agromyces ulmi* sp. nov. from other phylogenetically close *Agromyces* species

Data are from this study and Takeuchi & Hatano (2001), Li *et al.* (2003) and Dorofeeva *et al.* (2003). +, Positive; -, negative; w, weak; ND, not determined.

Characteristic	<i>A. ulmi</i> sp. nov.	<i>A. luteolus</i>	<i>A. mediolanus</i>	<i>A. aurantiacus</i>	<i>A. brachium</i>
Colony colour*	w	y	y	O, Y, PG, PW	y
Hyphae	+	+	-	+	+
Nitrate reduction	-	-	+	-	-
Urease	-	-	+	-	-
Gelatinase	-	+	ND	+	+
Starch hydrolysis	-	+	-	+	+
Catalase	-	+	+	-	+
Growth at 37 °C	+	-	ND	+	-
Acid from:					
Glycerol	+	-	+	+	-
Ribose	+	-	+	-	+
Galactose	+	-	+	+	+
Mannitol	-	w	-	+	+
Salicin	+	-	+	-	+
Sucrose	+	w	+	+	+
DNA G + C content (mol%)	72.0	71.1	72.3	72.8	70.0
Cell-wall sugars†	Rha, Fuc, Glc	Rha, Fru, Glc, Man	ND	Rha, Gal, Glc, Man	Rha, Gal, Glc, Man
Menaquinones‡	MK-12 (-11, -10)	MK-12 (-11)	MK-12 (-11, -10)	MK-12	MK-12 (-13)

*O, Orange; PG, pink-grey; PW, pink-white; w, white; y, yellow.
†Fru, Fructose; Fuc, fucose; Glc, glucose; Man, mannose; Rha, rhamnose.
‡Menaquinones in parentheses are present as the next most common components.

Determination of DNA base composition and DNA–DNA hybridization analyses were carried out as previously described by Rivas *et al.* (2003). The G + C contents of strains XIL01^T and XIL05 were 72.0 and 71.7 mol%, respectively. DNA–DNA relatedness values between strains XIL01^T and XIL05, *A. mediolanus* DSM 20152^T and *Agromyces luteolus* DSM 14595^T were 100, 48.3 and 53.4 %, respectively, which clearly indicates the strains to be members of a separate genomic species (Wayne *et al.*, 1987)

Thus, the results presented in this paper show that strains XIL01^T and XIL05 should be classified as a novel species within the genus *Agromyces*, for which the name *Agromyces ulmi* sp. nov. is proposed.

Description of *Agromyces ulmi* sp. nov.

Agromyces ulmi (ul' mi. L. fem. gen. n. *ulmi* of the elm tree, referring to the isolation source of this micro-organism).

Gram-positive and non-spore-forming bacterium. Young cultures produce branching substrate hyphae that break up into non-motile, diphtheroid and irregular-shaped cells after 15 h. Aerial mycelium absent. Colonies on XED and nutrient agar are circular convex, white, opaque and usually 1–2 mm in diameter within 7 days at 28 °C. Aerobic; oxidase- and catalase-negative. Optimal growth temperature is 28 °C. Chemo-organotrophic. The following substrates are used as carbon sources: L-arabinose, cellulose, mannose, N-acetylglucosamine, gentiobiose, maltose, starch and xylan. By contrast, adipate, caproate, citrate, malate, mannitol and phenylacetate are not used. Produces acid from glucose, glycerol, D-xylose, galactose, fructose, mannose, L-sorbose, rhamnose, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, maltose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, β -gentiobiose and D-lyxose. Aesculin is hydrolysed; caseinase, β -galactosidase and gelatinase are produced. Arginine dehydrolase, indole, tryptophan deaminase and urease are not produced. Nitrate is not reduced to nitrite. The peptidoglycan type is B2 γ . Other chemotaxonomic properties are described in the text and in Table 1.

The type strain, XIL01^T (=LMG 21954^T=DSM 15747^T), was isolated from the decayed stump of an elm tree, *Ulmus nigra*.

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