Note

Agromyces aurantiacus sp. nov., isolated from a Chinese primeval forest

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A catalase-negative actinomycete, strain YIM 21741^T, was isolated from a soil sample collected from a primeval forest at Xishuangbanna, Yunnan Province, China. Analysis of 16S rDNA showed the strain to be related to members of the genus *Agromyces*, with which it also shares morphological characteristics, e.g. branching hyphae breaking into diphtheroid and rod-like, irregular, non-motile fragments and a peptidoglycan containing the diagnostic amino acid 2,4-diamino-*n*-butyric acid. Whole-cell hydrolysates of strain YIM 21741^T contained rhamnose and small quantities of glucose, galactose and mannose. The major menaquinone was MK-12, while MK-13 and MK-12 were minor components. Diagnostic phospholipids were phosphatidylglycerol and diphosphatidylglycerol. The G + C content of the DNA was 72.8 mol%. Physiological and biochemical characteristics reveal strain YIM 21741^T to be different from all validly described species of the genus *Agromyces*. As DNA–DNA similarity values between this isolate and the phylogenetically neighbouring type strains of *Agromyces brachium* and *Agromyces luteolus* are only moderate, the novel species *Agromyces aurantiacus* sp. nov. is proposed with strain YIM 21741^T (= CCTCC 001012^T = AS 4.1717^T = DSM 14598^T) as the type strain.

The genus *Agromyces* was proposed by Gledhill & Casida (1969) for the soil organism *Agromyces ramosus*, a filamentous, branching, catalase-negative actinomycete. The emended genus (Zgurskaya *et al.*, 1992) includes seven species and two subspecies, which are characterized chemotaxonomically by menaquinone MK-12 and a peptidoglycan with 2,4-diamino-*n*-butyric acid (DAB) as the principal amino acid. Here, we report the description of a novel member of the genus, *Agromyces aurantiacus* sp. nov., based upon the description of strain YIM 21741^T.

Strain YIM 21741^T, isolated on humic acid-vitamin (HV) agar medium (Hayakawa & Nonomura, 1987) after about 2 weeks incubation at 28 °C, originates from a soil sample collected from Xishuangbanna in Yunnan Province, China. The strain was maintained on agar slants of International *Streptomyces* Project (ISP) media 2 (yeast extract-malt extract) and 5 (glycerol-asparagine) at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C. Biomass for chemical

and molecular-systematic studies was obtained following growth in shake flasks (about 200 r.p.m.) of ISP2 broth, supplemented with the vitamin mixtures of the HV medium (Hayakawa & Nonomura, 1987) at 28 °C for 2 weeks.

Morphological features were determined on ISP2 after 3 weeks at 28 °C. Strain YIM 21741^T had morphological characteristics typical of the genus *Agromyces* (Fig. 1), in that branching hyphae broke up into diphtheroid and rod-like, irregular, non-motile fragments. Cell dimensions are 0.5–0.9 × 1.0–1.4 µm. Cultural characteristics were determined after 2 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966). Strain YIM 21741^T showed good growth on all media tested (Table 1). Colour determinations (Table 1) were made by comparing the pure cultures with colour chips from the ISCC-NBS Color Charts Standard Samples no. 2106 (Kelly, 1964). Media and procedures used for examination of physiological and biochemical features and carbon-source utilization were those described by Shirling & Gottlieb (1966) and Williams *et al.* (1989). The results are indicated in Table 2 in comparison with those of phylogenetically related *Agromyces* species. On tryptone soy broth agar (Oxoid), 16 h and 4 day old cultures were catalase-negative.

Abbreviations: DAB, 2,4-diamino-*n*-butyric acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain YIM 21741^T is AF389342.

Fig. 1. Morphology of strain YIM 21741^T grown on ISP2. Bar, 1 µm.

Metabolic properties were determined using the API Coryne, API 50CHE and API 50CHL test kits (bioMérieux), the latter processed under aerobic conditions. Acid production from fructose, glucose, arabinose, mannitol and xylose was also analysed aerobically by colour change of bromocresol purple (0.04 %) in medium containing (l^{-1}) 1.0 g $(NH_4)_2HPO_4$, 0.2 g KCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g yeast extract, 15.0 g agar and sterile-filtered substrate (final concentration 1 %). Under these conditions, growth was positive on fructose, glucose, arabinose, mannitol and xylose but acid was not produced. Similarly, acid was not produced using the API Coryne substrate panel. Acid production was tested by the API 50CHE substrate panel. Reactions are listed in either Table 3 or the species description.

Cell walls were purified and analysed using the TLC method of Lechevalier & Lechevalier (1980). The procedures of Becker *et al.* (1964) and Lechevalier & Lechevalier (1980) were used for analysis of principal amino acids: the principal component was DAB. The main component of whole-cell hydrolysates, determined according to Takahashi & Egusa (1992), was rhamnose. Phospholipid analysis was carried out using the method of Minnikin *et al.* (1979) and Collins

& Jones (1980). Phosphatidylglycerol and diphosphatidylglycerol were detected as the diagnostic phospholipids. Menaquinones were extracted using the procedures of Collins *et al.* (1977) and identified as described by Groth *et al.* (1996); the principal menaquinone was MK-12. Fatty acids (Table 3) were determined following the procedure of Takeuchi & Hatano (1998). On a variety of different media, *Agromyces* type strains synthesize anteiso-C15:0, anteiso-C17:0 and iso-C16:0 as predominant fatty acids.

Genomic DNA, used in the determination of G + C content and 16S rDNA amplification, was extracted using a procedure (Hopwood *et al.*, 1985) slightly modified by Cui *et al.* (2001). The base composition of strain YIM 21741^T was determined by the thermal denaturation method (Mandel & Marmur, 1968). 16S rDNA was amplified by PCR using TaKaRa ExTaq as described previously (Cui *et al.*, 2001). Following a BLAST search (Altschul *et al.*, 1997), the sequences with the highest scores were retrieved from various databases. Calculations of levels of sequence similarity were carried out using CLUSTALW 1.74 (Higgins *et al.*, 1992). The phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980) and the algorithm of De Soete (1983). The topology of the phylogenetic tree was evaluated by bootstrap resampling (Felsenstein, 1985) with 1000 replicates. The almost complete 16S rDNA sequence of strain YIM 21741^T (1464 bp) revealed the highest similarity to members of *Agromyces* (95.1–97.6 %). Dendrograms of relatedness obtained with different algorithms were identical with respect to the position of strain YIM 21741^T, which formed a distinct branch with its closest relatives *Agromyces luteolus* IFO 16235^T (97.9 % similarity) and *Agromyces brachium* IFO 16238^T (97.2 %). *Agromyces mediolanus* DSM 20152^T branched slightly deeper. A phylogenetic dendrogram, based on the algorithms of De Soete (1983), is depicted in Fig. 2.

DNA–DNA reassociation was performed to determine the relatedness between strain YIM 21741^T, *A. brachium* IFO 16238^T and *A. luteolus* IFO 16235^T, which appeared as the closest relatives as judged from 16S rDNA analysis. DNA was isolated as described by Cashion *et al.* (1977) and Escara &

Table 1. Cultural characteristics of strain YIM 21741^T

Medium	Growth	Colour		
		Aerial mycelium	Substrate mycelium	Diffusible pigment
Czapek agar	Good	Absent	Pink-white	Pale orange-yellow
Glycerol-asparagine (ISP5)	Good	Light yellow-brown	Pink-white	Deep yellow-brown
Inorganic salts-starch (ISP4)	Good	Absent	Light orange-yellow	Light orange-yellow
Yeast extract-malt extract (ISP2)	Good	Absent	Deep orange-yellow	Deep orange-yellow
Oatmeal (ISP3)	Good	Absent	Pink-grey	Pink-grey
Potato-extract agar	Good	Absent	Brill-yellow	Light orange-yellow
Nutrient agar	Moderate	Absent	Light orange-yellow	Light orange-yellow

Table 2. Characteristics that differentiate YIM 21741^T from the type strains of its phylogenetic neighbours

All strains are negative for nitrate reduction and urease; all strains are positive for starch hydrolysis and produce acid from aesculin, fructose, glucose, maltose, mannitol, mannose, melibiose, rhamnose and sucrose. Acid production from fructose, glucose and mannitol was negative using the non-commercial test described in the text. Data for reference strains were taken from Takeuchi & Hatano (2001), based on API 50CH tests.

Characteristic	YIM 21741 ^T	<i>A. brachium</i> IFO 16238 ^T	<i>A. luteolus</i> IFO 16235 ^T
Catalase production	—	+	+
Pyrrolidonyl arylamidase*	—	—	+
Acid production from:†			
Amygdalin	—	+	—
Arbutin	—	+	—
Cellobiose	+	+	—
Galactose	+	+	—
β -Gentiobiose	—	+	—
Gluconate	—	+	—
Glycogen	+	+	—
Glycerol	+	—	—
Raffinose	+	+	—
Trehalose	—	+	+
D-Turanose	w	+	—
Melezitose	+	w	—
D-Xylose	—	+	—
Ribose	—	+	—
Salicin	—	+	+
Starch	+	+	—
Growth at 37 °C	+	—	—
Cell-wall sugars‡	Rha (Glc, Gal, Man)	Rha (Glc, Gal, Man)	Rha (Glc, Man)

*Determined with API Coryne system.

†Determined with API 50CHE system.

‡Rha, Rhamnose; Glc, glucose; Gal, galactose; Man, mannose. Data for reference strains were determined according to Mikami & Ishida (1983). Minor components are given in parentheses.

Hutton (1980) and DNA–DNA reassociation (Huß *et al.*, 1983; Jahnke, 1992), performed under optimal conditions ($2 \times$ SSC with 10% dimethyl sulphoxide at 70 °C), was

Table 3. Cellular fatty acid compositions of strain YIM 21741^T and related strains of the genus *Agromyces*

Strains: 1, YIM 21741^T; 2, *A. luteolus* IFO 16235^T; 3, *A. brachium* IFO 16238^T; 4, *A. rhizosphaerae* IFO 16236^T; 5, *A. mediolanus* JCM 3346^T; 6, *A. ramosus* JCM 3108^T. Values are percentages of the total fatty acids. Data for reference strains were taken from Suzuki *et al.* (1996) and Takeuchi & Hatano (2001).

Fatty acid	1	2	3	4	5	6
14:0	0.3	4.1	—	—	—	—
iso-15:0	8.5	6.1	4.3	7.7	2.0	3.0
anteiso-15:0	37.3	32.5	41.6	26.1	33.0	38.0
16:0	4.2	—	—	—	2.0	1.0
iso-16:0	20.1	47.9	28.1	53.3	20.0	16.0
iso-17:0	2.0	—	—	—	—	2.0
anteiso-17:0	23.6	9.4	26.0	12.9	40.0	38.0

recorded with a Gilford 2600 spectrophotometer. Relatedness values determined for strain YIM 21741^T were respectively 52 and 50% with the type strains of *A. brachium* and *A. luteolus*. The interspecies similarity value

Fig. 2. 16S rDNA dendrogram (De Soete, 1983) displaying the phylogenetic position of strain YIM 21741^T and some related members of the genus *Agromyces*. Numbers indicate percentages of bootstrap support, derived from 1000 resamplings. Bar, 1% sequence divergence.

of the latter two species was 67 %. This value was significantly higher than that reported by Takeuchi & Hatano (2001) (13 %) using the fluorometric hybridization method of Ezaki *et al.* (1989). These authors also determined the degree of binding of *A. luteolus* and *A. brachium* to *A. mediolanus*. As the binary values were low (respectively 21 and 24 %), *A. mediolanus* was also not considered to be related to strain YIM 21741^T at the strain level.

Taxonomic conclusions

Based on the morphology, chemotaxonomy, base composition of DNA and 16S rDNA sequence, strain YIM 21741^T is a member of the genus *Agromyces*, where it forms a distinct branch together with *A. luteolus*, *A. brachium* and *A. mediolanus*. Based on differences in phenotypic properties, we suggest that strain YIM 21741^T represents a novel species of the genus. This is substantiated by DNA–DNA reassociation values of significantly less than 70 %, determined for strain YIM 21741^T and *A. luteolus* (50 %) and *A. brachium* (52 %). These three taxa differ from each other in acid production from various carbohydrates, as determined by the API 50C system (Table 2). While *A. luteolus* IFO 16235^T shows mainly negative reactions (7 of 25), *A. brachium* IFO 16238^T forms acid from almost all carbohydrates (21 of 25). Strain YIM 21741^T shows an intermediate reaction, in that acid is produced from 16 of 25 sources. The acid production pattern of strain YIM 21741^T is also different from those of other type strains of *Agromyces* species listed by Takeuchi & Hatano (2001). The close phylogenetic neighbour *A. mediolanus* IFO 15704^T differs from strain YIM 21741^T in nitrate reduction, urease production, starch hydrolysis and acid production from amygdalin, ribose, rhamnose and salicin. The name *Agromyces aurantiacus* sp. nov. is proposed for the novel species.

Description of *Agromyces aurantiacus* sp. nov.

Agromyces aurantiacus (au.ran.ti.a'cus. N.L. fem. adj. *aurantiacus* orange-coloured).

Gram-positive, non-acid-fast and aerobic. Catalase-negative. Branching hyphae break up into diphtheroid and rod-like, irregular, non-motile fragments. Colony pigmentation and formation of aerial mycelium are indicated in Table 1. The cell wall contains DAB. Whole-cell hydrolysates contain rhamnose and small quantities of galactose, glucose and mannose. Optimal growth temperature is 28 °C. Milk coagulation is positive, gelatin liquefaction, milk peptonization, H₂S production and melanin production are negative. The following substrates are utilized: glucose, galactose, fructose, sucrose, xylose, raffinose, D-arabinose, inulose, sorbitol, mannitol and inositol. Lactose and rhamnose are not utilized. According to the API Coryne system, positive for α -glucosidase and aesculin, weakly positive for β -galactosidase and negative for alkaline phosphatase, β -glucuronidase, *N*-acetyl- β -glucosaminidase and gelatin hydrolysis. According to the API 50CHE test

system, positive for inulin, weakly positive for L-arabinose and negative for erythritol, D-arabinose, adonitol, L-sorbose, dulcitol, sorbitol, lactose, xylitol, L-lyxose, D-tagatose, D- and L-fucose and D- and L-arabitol. Other physiological and biochemical properties are indicated in Table 2. The predominant cellular fatty acids are iso-C_{16:0}, anteiso-C_{15:0} and anteiso-C_{17:0}. Phosphatidylglycerol and diphosphatidylglycerol are the predominant phospholipids. The major menaquinone is MK-12; minor components are MK-13 and MK-11. Fatty acids are indicated in Table 3. The G+C content of the DNA of the type strain is 72.8 mol%.

The type strain, YIM 21741^T, was isolated from a soil sample collected from Xishuangbanna in Yunnan Province, China, and has been deposited in the Chinese Center of Type Culture Collection as strain CCTCC 001012^T (= AS 4.1717^T = DSM 14598^T).

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