Promicromonospora xylanilytica sp. nov., an endophytic actinomycete isolated from surface-sterilized leaves of the medicinal plant *Maytenus austroyunnanensis*

Sheng Qin,^{1,2} Ji-Hong Jiang,² Hans-Peter Klenk,³ Wen-Yong Zhu,¹ Guo-Zhen Zhao,¹ Li-Xing Zhao,¹ Shu-Kun Tang,¹ Li-Hua Xu¹ and Wen-Jun Li¹

Correspondence

Wen-Jun Li
wjli@ynu.edu.cn or
liact@hotmail.com

¹Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education, and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China

²The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province, Xuzhou Normal University, Xuzhou, Jiangsu 221116, PR China

³DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7b, D-38124 Braunschweig, Germany

A novel xylan-degrading actinomycete, strain YIM 61515^T, was isolated from surface-sterilized leaves of the medicinal plant *Maytenus austroyunnanensis*. Cells were Gram-positive and non-spore-forming, produced primary branches and formed white to yellowish white colonies on the media tested. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 61515^T was most similar to *Promicromonospora aerolata* V54A^T and *Promicromonospora vindobonensis* V45^T (99.4 and 99.1 % sequence similarity, respectively). The isolate formed a separate lineage in a cluster containing *P. aerolata* V54A^T. 16S rRNA gene sequence similarity between the isolate and other members of the genus *Promicromonospora* ranged from 96.3 to 98.4 %. Chemotaxonomic data, including major menaquinones, fatty acid compositions and polar lipid profiles, supported the placement of strain YIM 61515^T in the genus *Promicromonospora*. DNA–DNA relatedness, physiological and biochemical data showed that strain YIM 61515^T could be distinguished from members of all known species of the genus *Promicromonospora* and therefore represented a novel species. The name *Promicromonospora xylanilytica* sp. nov. is proposed, with YIM 61515^T (=DSM 21603^T=CCTCC AA 208046^T) as type strain.

The genus *Promicromonospora* was proposed by Krasil'nikov *et al.* (1961) as a member of the family *Promicromonosporaceae*. Members of the genus are aerobic, Gram-positive, non-spore-forming actinomycetes, which form an extensively branched substrate mycelium that fragments into bacillary or coccoid elements. At the time of writing, the genus *Promicromonospora* comprised seven species with validly published names: *Promicromonospora citrea* (Krasil'nikov *et al.*, 1961), *P. sukumoe* (Takahashi *et al.*, 1987), *P. aerolata* and *P. vindobonensis* (Busse *et al.*, 2003), *P. kroppenstedtii* (Alonso-Vega *et al.*, 2008), *P. flava* (Jiang *et al.*, 2009) and *P. umidemergens* (Martin *et al.*, 2010), isolated from soil, air,

sediment and moisture from indoor wall material. During a study of rare actinobacteria associated with the tropical rainforest medicinal plants of Xishuangbanna, Yunnan Province, south-west China and the screening of actinomycetes that degrade plant polymers, a *Promicromonospora*-like strain, designated YIM 61515^T, was isolated from healthy leaves of a traditional Chinese medicinal plant, *Maytenus austroyunnanensis*. This is the first endophytic actinomycete identified as a member of the genus *Promicromonospora*.

Strain YIM 61515^T was isolated from healthy leaf samples as follows. Samples were air dried for 48 h at room temperature and then washed ultrasonically (160 W, 15 min) to completely remove surface soil and adherent epiphytes. After drying, the samples were subjected to a five-step surface-sterilization procedure, described by Qin *et al.* (2008). After being thoroughly dried under sterile conditions, the samples were heated for 15 min at 100 °C

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 61515^T is FJ214352.

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

and pretreated by the combined enzymic hydrolysis and differential centrifugation method of Qin *et al.* (2009). Strain YIM 61515^T was isolated after incubation at 28 °C for 2 weeks on xylan-arginine agar [per litre: 2.5 g xylan, 1.0 g arginine, 1.0 g (NH₄)₂SO₄, 2.0 g CaCl₂, 1.0 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 10 mg FeSO₄·7H₂O, 15.0 g agar; pH 7.2]. The isolate was maintained as mycelial fragments suspended in 20 % (v/v) glycerol at –80 °C.

The morphological and cultural characteristics were observed on yeast extract-malt extract agar (International *Streptomyces* Project medium 2; ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4) and glycerol-asparagine agar (ISP 5) (Shirling & Gottlieb, 1966), as well as potato-glucose agar (Difco), Czapek's agar and nutrient agar (Waksman, 1967) for 3 weeks at 28 °C. Colours were determined according to Kelly (1964). The mycelium was observed by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM 5600LV; JEOL). Growth at pH 4.0–11.0 (at intervals of 0.5 pH units) and at 0, 4, 7, 10, 12, 15, 18, 20, 22, 25, 28, 30, 32, 35, 37, 40, 45, 50, 55 and 60 °C was tested on ISP 2. Growth with 0–20 % (w/v) NaCl (at intervals of 0.5 %) was tested on ISP 2. Growth was assessed after incubation for 14 days at 28 °C. Strain YIM 61515^T was examined for a range of phenotypic properties using standard procedures (Gordon *et al.*, 1974) and the API Coryne, API ZYM and API 50CH systems (bioMérieux), according to the manufacturer's instructions. *P. aerolata* V54A^T and *P. vindobonensis* V45^T were used as reference strains under the same conditions.

Strain YIM 61515^T was an aerobic, Gram-positive, non-spore-forming actinomycete. It grew well on ISP 2–5 and nutrient agar and moderately well on potato-glucose agar and Czapek's media. No diffusible pigments or aerial mycelium were produced. The substrate mycelium was well developed and fragmented into non-motile, coccoid, Y-shaped, V-shaped or curved bacillary elements (Supplementary Fig. S1, available in IJSEM Online). Colonies were white to yellowish white and the substrate mycelium was white or yellowish white to orange–yellow. The physiological and biochemical properties of strain YIM 61515^T are given in Table 1 and the species description.

Cell walls were purified and the amino acids of the peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980; Jiang *et al.*, 2001). Sugar analysis of whole-cell hydrolysates was performed according to the procedure described by Hasegawa *et al.* (1983). Menaquinones were prepared and analysed by HPLC (Groth *et al.*, 1997). Polar lipids were extracted, examined using two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from tryptic soy agar after incubation for 2 days at 28 °C. The fatty acids were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification system. The fatty acid methyl esters were analysed by using Microbial Identification software

Table 1. Differential phenotypic characteristics of strain YIM 61515^T and closely related type strains of species of the genus *Promicromonospora*

Strains: 1, *Promicromonospora xylanilytica* sp. nov. YIM 61515^T; 2, *P. vindobonensis* V45^T; 3, *P. aerolata* V54A^T; 4, *P. kroppenstedtii* RS16^T; 5, *P. umidemergens* 09-Be-007^T; 6, *P. sukumoe* DSM 44121^T; 7, *P. citrea* DSM 43110^T. Data for columns 1–3 were taken from this study and for columns 4–7 from Martin *et al.* (2010) and Alonso-Vega *et al.* (2008). +, Positive; w, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Colony colour on ISP 2*	WH/YW	WH/C	WH/C	C/LY	ND	Y	LY
Oxidase	+	+	+	+	ND	–	–
Nitrate reduction	+	+	+	+	ND	–	+
Hydrolysis of:							
Gelatin	–	–	w	+	ND	+	+
Xylan	+	–	+	+	ND	–	+
Enzymes (API ZYM)							
Acid phosphatase	+	+	–	+	ND	+	+
α-Chymotrypsin	–	–	w	–	ND	–	–
Trypsin	–	w	w	w	ND	+	+
Assimilation (API 50 CH)							
Adonitol	–	–	+	–	ND	–	–
D-Fructose	w	w	+	–	+	+	+
Galactose	–	w	w	–	ND	+	+
D-Glucose	+	+	w	+	+	+	+
Glycerol	+	+	+	w	ND	+	+
Inulin	–	+	–	–	ND	–	+
2-Ketogluconate	–	w	+	+	ND	w	+
5-Ketogluconate	–	+	+	+	ND	w	+
Lactose	w	+	+	+	ND	w	+
Maltose	+	+	+	–	+	+	+
Mannitol	–	–	+	+	ND	+	+
D-Mannose	+	+	+	–	+	+	+
Melezitose	–	+	–	+	ND	–	–
Raffinose	+	+	+	–	ND	–	+
Rhamnose	–	+	+	+	+	–	+
Ribose	–	+	+	+	+	+	w
Sucrose	+	w	–	+	+	+	+
Salicin	+	+	+	–	+	+	+
Sorbitol	–	–	–	+	+	w	–
L-Sorbose	–	w	–	+	ND	w	–
Starch	+	+	+	–	ND	+	+
Growth at/with:							
37 °C	+	+	+	+	ND	–	+
9 % (w/v) NaCl	+	–	+	–	ND	–	+

*C, Cream; LY, light yellow; WH, white; Y, yellow; YW, yellowish white.

(Sherlock version 6.1; MIDI database TSBA6). Determination of the DNA G+C content was performed according to Mesbah *et al.* (1989).

Whole-cell hydrolysates of strain YIM 61515^T contained galactose and glucose. The peptidoglycan hydrolysate contained

Table 2. Differential chemotaxonomic characteristics of strain YIM 61515^T and type strains of species of the genus *Promicromonospora*

Strains: 1, *Promicromonospora xylanilytica* sp. nov. YIM 61515^T; 2, *P. vindobonensis* V45^T; 3, *P. aerolata* V54A^T; 4, *P. kroppenstedtii* RS16^T; 5, *P. umidemergens* 09-Be-007^T; 6, *P. sukumoe* DSM 44121^T; 7, *P. citrea* DSM 43110^T; 8, *P. flava* CC 0387^T. Data are from this study, Takahashi *et al.* (1987), Kalakoutskii *et al.* (1989), Busse *et al.* (2003), Jiang *et al.* (2009) and Martin *et al.* (2010).

Characteristic	1	2	3	4	5	6	7	8
Menaquinones (%)								
MK-9(H ₈)	3	—	—	—	—	—	+	—
MK-9(H ₆)	46	4	—	15	48	+	+	—
MK-9(H ₄)	29	92	93	64	36	+	+	86
MK-9(H ₂)	3	4	7	2	2	+	+	4.8
MK-9(H ₀)	—	—	—	—	—	—	+	2.64
MK-8(H ₄)	19	—	—	10	6	—	—	6.65
Polar lipids†	DPG, PG, PI, PL, GL	DPG, PG, GL, PGL, PL	DPG, PG, two GLs, PGL, PL	DPG, PG, PI, GL, PGL	DPG, PG, PI, PL, three GLs	DPG, PI	PG, PI, a glucosamine phospholipid	DPG, PG, PL, GL
Cell-wall amino acids and type‡	Ala/Glu/Lys (1.90:1.64:1.0), A4α	Glu/Gly/Ala/Lys (0.98:0.69:2.88:1.0), A3α	Glu/Gly/Ala/Lys (1.05:0.43:3.4:1.0), A3α	Ala/Glu/Lys (2.3:2.0:1.0), A4α	Lys (Glu, Ala)	Lys, A3α	Lys/Ala, A3α	Lys (Ala)
Cell-wall sugars§	Gal, Glc	Rha, Gal, Glc	Rha, Gal, Glc	Rha, Gal	Rha, Gal, Glc	Rha, Glc, Rib	Gal	Rha, Gal, Glc, Rib

*MK-9 (hydrogenation was not specified).

†DPG, Diphosphatidylglycerol; GL, unknown glycolipid; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PL, unknown phospholipid.

‡Amino acids in parentheses are minor components.

§Gal, Galactose; Glc, glucose; Rha, rhamnose; Rib, ribose.

||Whole-cell sugars.

alanine, glutamic acid and lysine (1.90:1.64:1.0). The predominant menaquinones were MK-9(H₆) (46 %) and MK-9(H₄) (29 %). Minor amounts of MK-8(H₄) (19 %), MK-9(H₂) (3 %) and MK-9(H₈) (3 %) were also detected. Menaquinone MK-8(H₄) has also been found in *P. umidemergens*, *P. kroppenstedtii* and *P. flava* but not in the other four species of the genus *Promicromonospora*. The phospholipid profile comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unidentified phospholipid and an unknown glycolipid (Supplementary Fig. S2, available in IJSEM Online). The major fatty acids were anteiso-C_{15:0} (64.80 %) and iso-C_{15:0} (13.86 %) (Supplementary Table S1, available in IJSEM Online), which were similar to those obtained by Martin *et al.* (2010) for the type strains of other members of the genus *Promicromonospora*. The DNA G + C content of strain YIM 61515^T was 70.4 mol%. Strain YIM 61515^T could be differentiated from the type strains of other members of the genus *Promicromonospora* by a range of chemotaxonomic characteristics (Table 2).

For 16S rRNA gene sequence analysis, extraction and amplification of genomic DNA was carried out as described previously (Li *et al.*, 2007). An almost-complete 16S rRNA gene sequence (1437 bp) was aligned with selected sequences obtained from public databases using CLUSTAL X version 1.8 (Thompson *et al.*, 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) trees were constructed using MEGA version 3.1 (Kumar *et al.*, 2004). To evaluate the stability of the trees, bootstrap analysis was performed using a consensus tree based on 1000 randomly generated trees (Felsenstein, 1985). DNA–DNA hybridization was performed according to the fluorometric micro-well method (Ezaki *et al.*, 1989; He *et al.*, 2005).

Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 61515^T was affiliated with the genus *Promicromonospora*. Strain YIM 61515^T was most closely related to *P. aerolata* V54A^T and *P. vindobonensis* V45^T (99.4 and 99.1 % 16S rRNA gene sequence similarity, respectively). Lower levels of 16S rRNA gene sequence similarity were observed between strain YIM 61515^T and the other type strains of recognized species of the genus *Promicromonospora*: 98.4 % with *P. kroppenstedtii* RS16^T, 98.3 % with *P. umidemergens* 09-Be-007^T, 98.1 % with *P. sukumoe* DSM 44121^T and *P. citrea* DSM 43110^T, and 96.3 % with *P. flava* CC 0387^T. In an earlier study, Busse *et al.* (2003) proposed that isolates of the genus *Promicromonospora* that show ≤98.6 % 16S rRNA gene sequence similarity with recognized members of the genus probably represent novel species; the 16S rRNA gene sequence similarities between strain YIM 61515^T and the less closely related members of the genus *Promicromonospora* (96.3–98.4 %) were slightly lower than this value. In the neighbour-joining phylogenetic tree (Fig. 1), strain YIM 61515^T formed an independent lineage in a cluster containing *P. aerolata* V54A^T, *P. vindobonensis* V45^T and *P. umidemergens* 09-Be-007^T, which was supported by a high bootstrap value. This relationship was also evident in the 16S rRNA gene sequence dendrogram generated with the maximum-parsimony algorithm. In view of this, DNA–DNA hybridization was performed only between strain YIM 61515^T and members of the genus *Promicromonospora* with which the isolate exhibited ≥98.4 % 16S rRNA gene sequence similarity. DNA–DNA relatedness values between strain YIM 61515^T and *P. vindobonensis* V45^T, *P. aerolata* V54A^T and *P. kroppenstedtii* RS16^T were 45.1 ± 3.3, 43 ± 2.1 and 35.1 ± 1.7 %, respectively. These values were well below the 70 % cut-off value recommended for the circumscription of bacterial genomic species (Wayne *et al.*, 1987),

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1437 nt), showing the relationships between strain YIM 61515^T and members of the genus *Promicromonospora* and the family *Promicromonosporaceae*. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony method. Bar, 0.005 substitutions per nucleotide position.

indicating that strain YIM 61515^T represents a novel species of the genus *Promicromonospora*.

On the basis of the morphological, chemotaxonomic, phenotypic and genetic characteristics, it is evident that strain YIM 61515^T should be assigned to a novel species in the genus *Promicromonospora*, for which the name *Promicromonospora xylanilytica* sp. nov. is proposed.

Description of *Promicromonospora xylanilytica* sp. nov.

Promicromonospora xylanilytica [xy.la.ni.ly'ti.ca. N.L. n. *xylanum* xylan, a plant polymer; N.L. adj. *lyticus* -a -um (from Gr. adj. *lutikos* -ê -on) able to loose, able to dissolve; N.L. fem. adj. *xylanilytica* xylan-dissolving].

Gram-positive and aerobic. Forms white to yellowish white colonies with characteristic wrinkly surfaces. No aerial mycelium is produced. Substrate mycelia develop well and fragment into non-motile, coccoid, Y-shaped, V-shaped or curved bacillary elements. Grows well on ISP 2, ISP 3, ISP 4, ISP 5 and nutrient agar. Grows at 10–37 °C (optimum 28 °C), at pH 6.0–9.0 and with 0–9 % (w/v) NaCl. Positive for catalase and nitrate reduction, but negative for milk coagulation and peptonization, gelatin liquefaction and H₂S production. The cell-wall peptidoglycan contains alanine, glutamic acid and lysine. Galactose and glucose are present as cell-wall sugars. The predominant menaquinones are MK-9(H₆) and MK-9(H₄). The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, an unidentified phospholipid and an unknown glycolipid. The major fatty acids (>5 %) are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}.

The type strain, YIM 61515^T (=DSM 21603^T=CCTCC AA 208046^T), was isolated from surface-sterilized leaves of *M. austroyunnanensis* collected from the tropical rainforest in Xishuangbanna, Yunnan Province, south-west China. The DNA G+C content of the type strain is 70.4 mol%.

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