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Kribbella antibiotica sp. nov., a Novel Nocardioform Actinomycete Strain Isolated from Soil in Yunnan, China

Wen-Jun Li[1], Dong Wang[1], Yu-Qin Zhang[1], Peter Schumann[2], Eerko Stackebrandt[2], Li-Hua Xu[1], and Cheng-Lin Jiang[1]

[1] The Key Laboratory for Microbial Resources of Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, China
[2] Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

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Summary

A novel nocardioform actinomycete strain YIM 31530^T was isolated from a soil in Yunnan, China. Based on the results of phenotypic characteristics, phylogenetic studies and DNA-DNA hybridization results, strain YIM 31530^T should be assigned to a new species of the genus *Kribbella*, for which the name *Kribbella antibiotica* sp. nov. is proposed. The type strain is YIM 31530^T (= CCTCC AA001021^T = DSM 15501^T). The GenBank accession number for the sequence reported in this paper is AY082063.

Key words: *Kribbella antibiotica* sp. nov. – Polyphasic taxonomy – 16S rDNA

Introduction

The family *Nocardioidaceae* [16, 26] contains 8 genera, namely *Nocardioides* [18], *Aeromicrobium* [14], *Friedmanniella* [21], *Kribbella* [17], *Hongia* [10], *Marmoricola* [28], *Micropruina* [22]. Recently, Sohn et al. [24] transferred *Hongia koreensis* [10] to the genus *Kribbella* [17] as *Kribbella koreensis* com.nov. based on phenotypic and phylogenetic analysis. So up to now, there are total three valid species of genus *Kribbella* [17, 24].

During our project for screening bioactive actinomycete strains from soils in Yunnan, China, one antifungal, nocardioform actinomycete strain was isolated using traditional dilution plating method. In this work, we present the polyphasic taxonomic characterization of the bioactive strain. Genotypic and phenotypic data showed that the strains YIM 31530^T should be recognized as a new species for which the name *Kribbella antibiotica* sp. nov. is proposed.

Methods

Micro-organisms and culture conditions

Strain YIM 31530^T was isolated, using the dilution plating method, from a soil sample in Yunnan Province, the west of China. The medium used for selective isolation was glycerol-asparagine agar [23] (ISP5 medium) supplemented with 50 mg/l potassium dichromate and incubated at 28 °C for about 2 weeks. The strain was maintained on ISP2 agar or ISP5 agar slants at 4 °C and as glycerol suspensions (20%,v/v) at –20 °C. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (about 150 rpm.) of ISP2 medium broth at 28 °C for 1 week.

Phenotypic characteristics

Cultural characteristics were determined after 4 weeks at 28 °C by methods used in the International *Streptomyces* Project (ISP) [23]. Morphological properties were examined by light microscopy (Olympus microscope BH-2) and scanning electron microscopy with a JEOL model JSM5600LV. Media and procedures used for determination of physiological features and carbon source utilization were those described by Shirling & Gottlieb [23] and Locci [11]. Color determination was done with color chips from the ISCC-NBS COLOR CHARTS Standard Samples No 2106 [5].

Chemotaxonomy

The amino acid and sugar analysis of whole cell hydrolysates followed procedures described by Stanek and Roberts [25]. Polar lipids were extracted, examined by two dimensional thin layer chromatograph and identified using published procedures [15]. Menaquinones were isolated using the methods of Minnikin et al. [15] and separated by HPLC [8, 9]. Cellular fatty acid composition was performed as described by Sasser [20].

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Extraction of Genomic DNA and amplification of 16S rDNA

Extraction of Genomic DNA and amplification of 16S rDNA were done as described by Xu et al. [30]. Multiple alignments with sequences of a broad selection of a*ctinobacteria* and calculations of levels of sequences similarity were carried out using CLUSTAL X [27]. A phylogenetic tree was reconstructed using neighbor-joining method of Saitou and Nei [19] from K_{nuc} values [6, 7]. The topology of the phylogenetic tree was evaluated by bootstrap resampling method of Felsenstein [2] with 1,000 replicates.

Nucleotide sequence accession numbers

The 16S rDNA sequence determined in this study has been deposited in GenBank under the accession number AY082063. The accession numbers of the reference strains, which are closely related to strain YIM 31530^T, are indicated in Fig. 2.

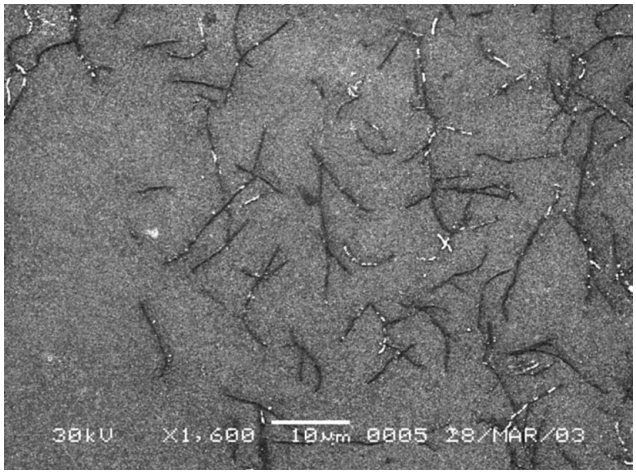

Fig. 1. Scanning electron micrographs of strain YIM31530^T grown on yeast-malt extract agar (ISP 2 medium) for 21 days at 28 °C. Bar, 10 μm.

DNA G+C content determination and DNA-DNA hybrdization

DNA for the base composition and renaturation studies was prepared following the method of Marmur [12]. The G+C content was determined using the thermal denaturation method of Marmur & Doty [13]. DNA-DNA hybridization was carried out according to described methods [1, 3, 4].

Results

Morphological observations

Morphological observation of a 28-day-old culture of strain YIM 31530^T grown on yeast-malt ext agar (ISP medium 5) revealed the strain YIM 31530^T had the typical characteristics of genus *Kribbella* (Fig. 1). Substrate mycelium were extensively branched and penetrated into agar media and they often fragmented into rod-shaped. Aerial mycelium consisting of hyphae that fragmented into short to elongated rod-like elements.

Cultural characteristics

As shown in Table 1. Strain YIM 31530^T well developed yellow white to pale yellow colonies on most media tested. It showed good growth on most media except oatmeal agar (ISP medium 3). No diffusible pigments were produced. It developed aerial hyphae on most media tested, especially Czapek's agar and glycerol-asparagine (ISP medium 5).

Physiological characteristics

The physiological properties of strain YIM 31530^T are shown in Table 2. Strain YIM 31530^T utilized almost all test carbon source. It was positive for gelation liquefaction, starch hydrolysis, melanin production, milk coagulation and peptonization, and negative for growth in cellulose, H_2S production and Nitrate reduction.

Table 1. Cultural characteristics of strain YIM 31530^T.

Medium	Aerial mycelium	Substrate mycelium	Soluble pigment
Yeast extract-malt extract (ISP* medium 2)	Light yellow	Soft yellow	–
Oatmeal agar (ISP* medium 3)	Yellow white	Yellow white	–
Inorganic salt-starch agar (ISP* medium 4)	Yellow white	Yellow white	–
Glycerol-asparagine (ISP* medium 5)	Yellow white	Yellow white	–
Czapek's agar	Yellow white	Light yellow	–
Potato agar	Gray yellow	Yellow	
Nutrient agar	Yellow white	Light yellow	–

Note: Colors taken form ISCC-NBS COLOR CHARTS (Standard Samples No 2106) [5].
* ISP, International *Streptomyces* Project [23].

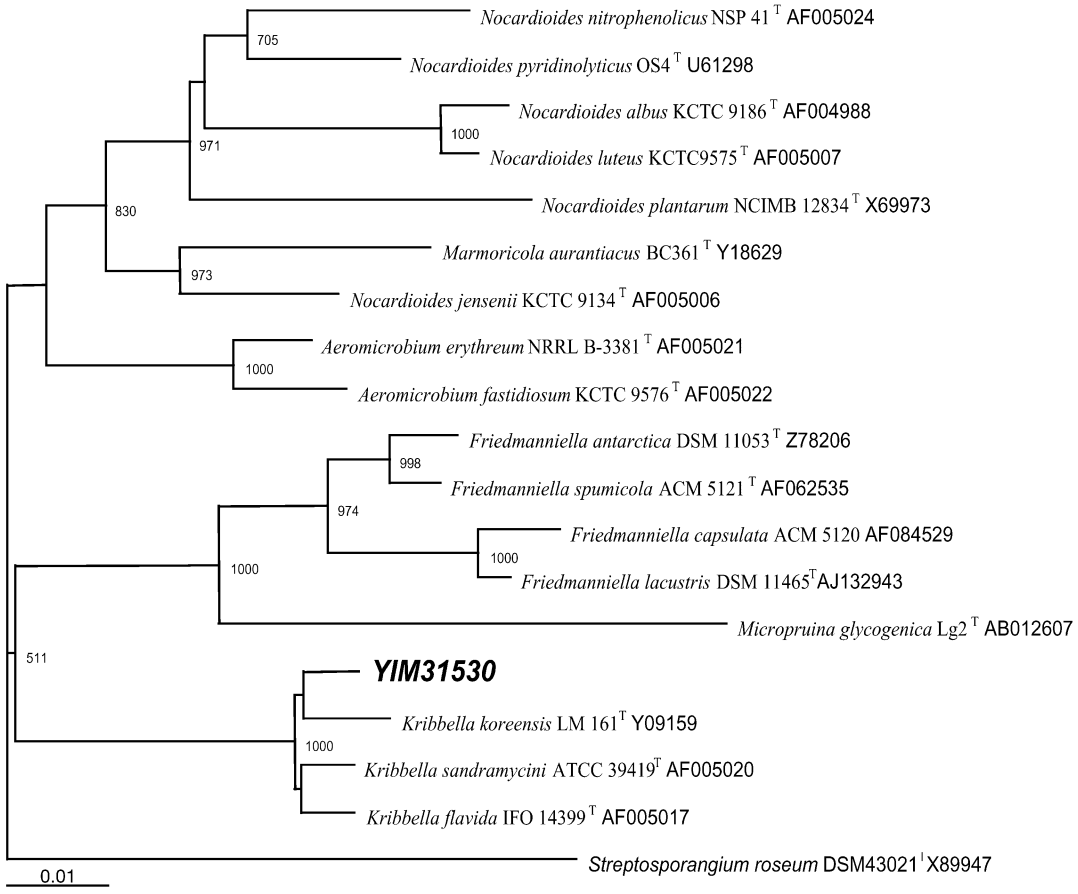

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM31530^T among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Streptosporangium roseum* DSM43021^T (X89947) was used as root. Bar, 1% sequence divergence.

Chemotaxonomic characteristics

The purified cell walls of strain YIM 31530^T contained LL-diaminopimelic acid and glycine. Whole-cell hydrolysates contained ribose, xylose and glucose. The polar lipids mainly contained phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant menaquinone and fatty acids were MK-9 (H_4) and anteiso-$C_{15:0}$, iso-$C_{15:0}$, respectively.

Phylogenetic analysis

Almost complete 16S rDNA sequence data of strain YIM 31530^T (1481bp) was determined. A phylogenetic tree was constructed by the neighbor-joining and maximum-likelihood methods based on the 16S rDNA sequences of strain YIM 31530^T and those of its closest relatives, using *Streptosporangium roseum* (DSM 43021^T) as an outgroup (Fig. 2). Phylogenetic analysis has shown that strain YIM 31530^T groups with the other three members (*Kribbella koreensis*, *Kribbella sandramycini*, *Kribbella flavida*) of genus *Kribbella*, and also form a separate branch with them. It had sequence similarity 98.6%, 98.8%, 98.4% with the latter three type strains, respectively, while with other actinomycete type strains, which contains LL-diaminopimelic acid in the cell wall having no more than 93% sequence similarity. This result clearly indicate that the isolate YIM 31530^T be member of the genus *Kribbella*.

The DNA base composition and DNA-DNA hybridizations

The DNA G+C content of strain YIM 31530^T was 67 mol%. DNA-DNA hybridization rates determined with the type strains *Kribbella koreensis* (LM 161^T), *Kribbella sandramycini* (KCTC 9609^T), *Kribbella flavida* (IFO 14399^T) of genus *Kribbella* were all below the threshold value of 70% (Wayne et al., 1987) (53.8%, 35.4%, 18%, respectively) indicating that strain represents a distinct species of genus *Kribbella*.

Taxonomic conclusions

The novel isolate YIM 31530^T is a typical nocardioform actinomycete strain and this organism exhibited characteristics similar to those validly described type strains of genus *Kribbella*. for example, it has morphological and chemotaxonomic characteristics of genus *Kribbella*; it has high 16S rDNA similarity to these organisms. However, the isolate YIM 31530^T had many phenotypic characteristic difference with the other three type strains of genus *Kribbella* (Table 2). Additionally, DNA-DNA relatedness provided important data for determining the taxonomic position of the new isolate. DNA-DNA hybridization among the new isolate YIM 31530^T and the other three type strains *Kribbella koreensis* (LM 161^T), *Kribbella sandramycini* (KCTC 9609^T), *Kribbella flavida* (IFO 14399^T) of genus *Kribbella* revealed significantly lower than 70% similarity values, indicating that strain YIM 31530^T represents a new species in accordance with the recommendations of the committee on reconciliation of the committee on reconciliation of approaches to bacterial systematics [29]. As the genomic

Table 2. Comparison of physiological and chemical characteristics between strain YIM 31530^T and three species of genus *Kribbella.*

Characteristics	*K. antibiotica* YIM 31530^T	*K. koreensis* LM 161^T	*K. sandramycini* KCTC 9609^T	*K. flavida* IFO 14399^T
Gelation liquefaction	+	+	+	–
Milk peptonization	+	+	+	+
Milk coagulation	+	+	+	+
Starch hydrolysis	+	–	–	–
Nitrate reduction	–	+	–	+
H_2S Production	–	–	–	–
Growth in cellulose	–	–	–	–
Malanin production	+	+	–	+
Carbon utilization				
Glucose	+	+	+	+
Lactose	+	+	–	+
Galactose	+	+	+	W
Fructose	+	+	+	+
Sucrose	+	+	+	+
Maltose	+	+	+	+
Mannose	+	+	–	+
Arabinose	+	+	+	–
Xylose	+	+	+	–
Raffinose	+	+	+	–
Rhamnose	+	+	+	+
Glycerol	+	+	+	+
Inositiol	+	+	+	+
Mannitol	+	+	+	+
Sorbitol	+	+	+	+
Trisodium citrate	+	+	–	+
Sodium oxalate	+	+	–	+
Sodium acetate	+	+	+	+
Chemical characteristics				
Wall peptidoglycan	LL-DAP	LL-DAP	LL-DAP	LL-DAP
Cell sugars	glu, xyl, rib	man, glu, gal, rib	man, glu, gal	man, glu, gal
Polar lipids	DPG, PC, PG, PI	DPG, PC PG, PI	PC	PC
Major menaquinone(s)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)
Predominant fatty acids	ai-$C_{15:0}$; ai-$C_{15:0}$;	i-$C_{15:0}$ i-$C_{15:0}$; i-$C_{16:0}$	ai-$C_{15:0}$; ai-$C_{15:0}$; i-$C_{16:0}$	i-$C_{15:0}$; i-$C_{16:0}$
G+C mol%	67	71.3	70	68.3

Note: some data taken from Park et al. [17], Lee et al.[10] and Sohn et al. [24].
Abbreviations: +, positive reaction; –, negative reaction; w, weakly positive reaction.
DAP, diaminopimelic acid; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine. Abbreviations for MK-9(H_4), partially saturated menaquinone with four of nine isoprene units hydrogenated. Abbreviations for fatty acids exemplified by ai-$C_{15:0}$, 12-methyltetradecanoic acid; i-$C_{15:0}$, 13-methyltetradecanoic acid.

distinctness is also expressed by differences in some metabolic properties (Table 2), we propose the name *Kribbella antibiotica* sp. nov. for strain YIM 31530^T.

Description of *Kribbella antibiotica* sp. nov.

Kribbella antibiotica

(an.ti.bio.ti.ca, Gr. pref. *anti* – against; gr. n. *bios* – life, M. L. fem. adj.; against lfe, antibiotic)

The cell of the organism are non-motile, aerobic. It developed aerial hyphae on most media tested, especially Czapek's agar and glycerol-asparagine (ISP medium 5). The substrate mycelium are extensively branched and they often fragmented into rod-shaped. Aerial mycelium consisting of hyphae that fragment into short to elongated rod-like elements. No diffusible pigment is produced. It could utilize almost all test carbon sources, such as glucose, lactose, galactose, fructose, sucrose, maltose, mannose, arabinose, xylose, ribose, raffinose, rhamnose, glycerol, mannitol, inositol, sorbitol, trisodium citrate, sodium oxalate, sodium acetate. It is positive for gelation liquefaction, starch hydrolysis, milk coagulation, milk peptonization, malanin production and negative for growth in cellulose, H_2S production and nitrate reduction. The cell wall of strain YIM31530^T contained LL-diaminopimelic acid. Whole-cell sugars were glucose, xylose and ribose. The principal menaquinone is MK-9 (H_4). The phospholipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidyl- inositol. The predominant fatty acids are anteiso-$C_{15:0}$; iso-$C_{15:0.}$ The DNA G+C content was 67 mol%. Optimum growth at 28 °C and pH 7.0. Isolated from soil in Yunnan Province, the west of China. The type strain is YIM 31530^T (= CCTCC AA001021^T = DSM 15501^T).

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Corresponding author:
Cheng-Lin Jiang, The Key Laboratory for Microbial Resources of Ministry of Education, P.R. China, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, P.R.China
Tel.: ++86-871 5034 139; Fax: ++86-871 5173 878;
e-mail: lihxu@ynu.edu.cn or liact@hotmail.com