

## *Allokutzneria multivorans* sp. nov., an actinomycete isolated from soil

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An actinomycete with well-branched mycelia, designated strain YIM 120521<sup>T</sup>, was isolated from soil collected from the banks of the Nujiang River, Yunnan Province, south-west China. Both aerial and substrate mycelia were white and non-pigmented. Growth was observed at 4–40 °C (optimum 28 °C), pH 6.0–9.0 (optimum 7.0) and in 0–4 % (w/v) NaCl (optimum 0 %). Analysis of the 16S rRNA gene sequence revealed that strain YIM 120521<sup>T</sup> belongs to the genus *Allokutzneria* with the highest sequence similarity to *Allokutzneria albata* DSM 44149<sup>T</sup> (98.4 %). However, the mean DNA–DNA relatedness value between the two strains was below 70 %. Chemotaxonomic characteristics supported the inclusion of strain YIM 120521<sup>T</sup> in the genus *Allokutzneria*, with rhamnose, arabinose, glucose, galactose and mannose as the whole-cell sugars, meso-diaminopimelic acid as the cell-wall diamino acid and MK-9(H<sub>4</sub>) as the predominant menaquinone. On the basis of physiological, biochemical and chemotaxonomic characteristics, strain YIM 120521<sup>T</sup> is considered to represent a novel species of the genus *Allokutzneria*, for which the name *Allokutzneria multivorans* sp. nov. is proposed. The type strain is YIM 120521<sup>T</sup> (=JCM 17342<sup>T</sup>=DSM 45532<sup>T</sup>).

The genus *Allokutzneria* was proposed by Labeda & Kroppenstedt (2008) with the reclassification of *Kibdelosporangium albatum* (Tomita *et al.*, 1993) as *Allokutzneria albata* (Labeda & Kroppenstedt, 2008). This organism is able to produce a highly active antibiotic (cycloviracin) which exhibits antiviral activity, and is at the time of writing the only species of the genus *Allokutzneria* with a validly published name.

During the investigation on the diversity of actinobacteria associated with soil from the banks of the Nujiang River, Yunnan Province, south-west China, strain YIM 120521<sup>T</sup> was isolated on glycerol-proline agar (5 g glycerol, 1 g proline, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 1 g K<sub>2</sub>HPO<sub>4</sub>, 0.3 g CaCO<sub>3</sub>, 15 g agar, pH 7.7) after 7 days incubation at 28 °C. The strain was incubated at 28 °C in ISP 2 (International *Streptomyces* Project media, Shirling & Gottlieb, 1966) and maintained at –80 °C in a 20 % (v/v) glycerol suspension.

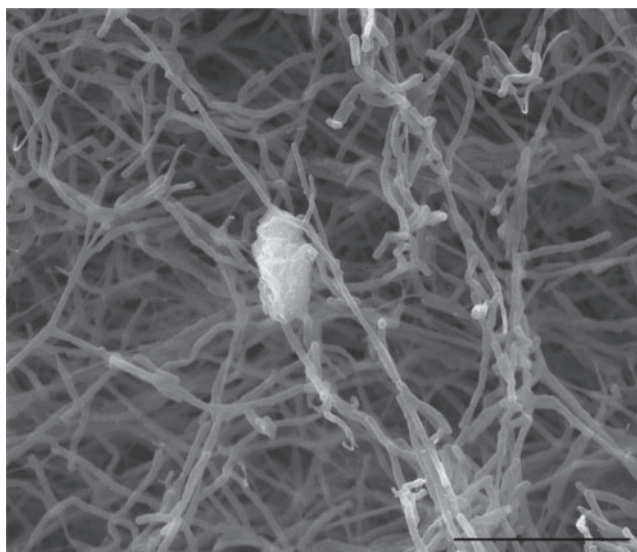
The aim of the present study was to perform a detailed taxonomic characterization of the novel strain based on a polyphasic approach. The strain is identified as a novel

member of the genus *Allokutzneria* based on the results of this study.

Cultural characteristics were tested on several media: International *Streptomyces* Project (ISP) media 2, 3, 4, 5, Czapek's agar, Nutrient agar and potato dextrose agar (PDA; Difco). (Shirling & Gottlieb, 1966). Colour of aerial and substrate mycelia and soluble pigment production were determined by comparison with chips from the ISCC-NBS Colour Charts (Kelly, 1964). The Gram reaction was determined by using the 3 % KOH method (Buck, 1982). Cell morphology was inspected under a light microscope (BH-2; Olympus) and scanning electron microscope (Quanta200; FEI). Growth at 0, 4, 10, 15, 20, 25, 28, 37, 40, 45, 50, 55 and 60 °C, pH 4.0–11.0 and NaCl tolerance ranges (0–10 %) were examined by using ISP medium 2. Catalase and oxidase tests were performed by using 3 % H<sub>2</sub>O<sub>2</sub> and an oxidase reagent (bioMérieux), respectively. Hydrolysis of gelatin, cellulose, starch and urea, milk coagulation and peptonization, H<sub>2</sub>S production and nitrate reduction were determined as described by Smibert & Kreig (1994). Acid production from carbohydrates and utilization of sole carbon and sole nitrogen sources were tested using previously described methods (Gordon *et al.*, 1974; Gordon & Mihm, 1962; Tsukamura, 1966). Strain YIM 120521<sup>T</sup> was Gram-stain-positive, produced well-branched substrate and aerial mycelia, and formed

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 120521<sup>T</sup> is JQ306329.

Two supplementary figures are available with the online version of this paper.



**Fig. 1.** Scanning electron micrograph demonstrating the morphological characteristics of strain YIM 120521<sup>T</sup> grown in ISP 2 for 21 days at 28 °C. Bar, 10 µm.

sporangium-like bodies (Fig. 1). The aerial mycelia were white and produced long straight spore chains. No pigment production was detected in any of the media tested. Optimal growth occurred in ISP medium 2. Strain YIM 120521<sup>T</sup> grew at 4–40 °C, at pH 6.0–9.0 and with 0–4 %

NaCl, with optimal growth occurring at 28 °C, pH 7.0 and with 0 % NaCl. The test for catalase activity was positive and the test for oxidase activity was negative. Milk coagulation test was positive. Tests for hydrolysis of gelatin, cellulose, starch and urea, milk peptonization, H<sub>2</sub>S production and nitrate reduction were negative. A detailed description of other physiological and biochemical characteristics of strain YIM 120521<sup>T</sup> are presented in the species description, and phenotypic characteristics compared to *Allokutzneria albata* DSM 44149<sup>T</sup>, the closest related species with a validly published name, are presented in Table 1.

Cell mass of strain YIM 120521<sup>T</sup> for chemotaxonomic analyses was cultured in ISP 2 broth at 28 °C for 14 days. Analyses of cell-wall amino acid and sugars of whole-cell hydrolysates were carried out according to the methods described by Hasegawa *et al.* (1983) and Tang *et al.* (2009). The DNA G + C content was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989). The major polar lipids were analysed according to Minnikin *et al.* (1979) and Collins & Jones (1980). Cellular menaquinones were extracted and purified using the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). The diagnostic amino acid of the cell wall of strain YIM 120521<sup>T</sup> was *meso*-diaminopimelic acid, and whole-cell sugars were rhamnose, arabinose, glucose, galactose and mannose. The DNA G + C content was 69.1 mol%. The polar lipids detected were diphosphatidylglycerol, phosphatidylglycerol,

**Table 1.** Differential characteristics of strain YIM 120521<sup>T</sup> and *Allokutzneria albata* DSM 44149<sup>T</sup>

+, Positive; –, negative; w, weakly positive. Data are from our parallel experiments.

Characteristic	YIM 120521 <sup>T</sup>	<i>A. albata</i> DSM 44149 <sup>T</sup>
Temperature range for growth (°C)	4–40	17–45
Melanoid pigments	–	+
Hydrolysis of:		
Potato starch	–	+
Gelatin	–	+
Acid production from:		
L-Arabinose	–	w
Dulcitol	w	–
D-Galactose	–	+
Lactose	–	w
Raffinose	+	–
D-Ribose	–	+
D-Sorbitol	w	–
Sucrose	–	w
Decomposition of:		
Hypoxanthine	+	–
Xanthine	+	–
Menaquinones		
Predominance	MK-9(H <sub>4</sub> )	MK-9(H <sub>4</sub> )
Traces	MK-9(H <sub>2</sub> ), MK-9	MK-9(H <sub>2</sub> ), MK-9(H <sub>6</sub> ), MK-10(H <sub>4</sub> )
DNA G + C content (mol%)	69.1	71.6

**Table 2.** Cellular fatty acid contents of strain YIM 120521<sup>T</sup> and *Allokutzneria albata* DSM 44149<sup>T</sup>

Data (>0.5 %) were taken from this study after culture on trypticase soy agar (Difco) at 28 °C for 3 days. —, Not detected.

Fatty acid (%)	YIM 120521 <sup>T</sup>	<i>A. albata</i> DSM 44149 <sup>T</sup>
iso-C <sub>14:0</sub>	1.9	4.0
iso-C <sub>15:0</sub>	21.9	16.0
anteiso-C <sub>15:0</sub>	2.0	2.9
C <sub>15:1</sub> ω6c	1.7	1.7
iso-C <sub>16:0</sub> H	1.0	7.9
iso-C <sub>16:0</sub>	17.0	29.1
Summed feature 3*	1.6	1.6
C <sub>16:0</sub>	4.5	4.3
iso-C <sub>17:0</sub>	3.5	2.8
anteiso-C <sub>17:0</sub>	1.4	2.5
C <sub>17:1</sub> ω8c	14.8	4.8
C <sub>17:0</sub>	7.0	6.6
iso-C <sub>16:0</sub> 3-OH	0.7	1.5
iso-C <sub>17:0</sub> 3-OH	16.1	10.2
C <sub>17:0</sub> 3-OH	—	0.7
Summed feature 9*	1.4	—
C <sub>18:0</sub>	0.6	—

\*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 comprised C<sub>16:1</sub>ω7c/C<sub>16:1</sub>ω6c; summed feature 9 comprised iso-C<sub>17:1</sub>ω9c and/or 10-methyl C<sub>16:0</sub>.

phosphatidylethanolamine, OH-phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol and two unknown lipids (PL1 and PL2) (Fig. S1, available in IJSEM Online). The predominant isoprenoid quinone was MK-9(H<sub>4</sub>) (83.8 %); MK-9(H<sub>2</sub>) (8.3 %) and MK-9 (7.9 %) were present as minor components.

Cell mass for analysis of cellular fatty acids was obtained from cultures grown on trypticase soy agar (Difco) at 28 °C for 3 days. The fatty acids were extracted as described by Sasser (1990), analysed according to the standard protocol of the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6). The major fatty acids (≥5.0 %) of strain YIM 120521<sup>T</sup> were iso-C<sub>15:0</sub> (21.9 %), iso-C<sub>16:0</sub> (17.0 %), iso-C<sub>17:0</sub> 3-OH (16.1 %), C<sub>17:1</sub>ω8c (14.8 %) and C<sub>17:0</sub> (7.0 %). The detailed components are listed in Table 2.

The phylogenetic position of strain YIM 120521<sup>T</sup> was determined by 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the protocol of Li *et al.* (2007) and the 16S rRNA gene was amplified from the chromosomal DNA by using primers 27F and 1492R (Lane, 1991). Sequences were initially analysed by using BLAST search against the GenBank database and the EzTaxon-e database (<http://eztaxon-e.ezbiocloud.net/>; Kim *et al.*, 2012). After multiple alignments using CLUSTAL X (Thompson *et al.*, 1997), the phylogenetic and molecular evolutionary analyses were conducted using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony

(Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The MEGA 4 software (Tamura *et al.*, 2007) and PHYLIP package (Felsenstein, 1993) were used to reconstruct phylogenetic trees. Evolutionary distance matrices (distance options according to Kimura's two-parameter model) were calculated as described by Kimura (1980). Bootstrap values were determined based on 1000 replications (Felsenstein, 1985). For DNA hybridization analysis, genomic DNA of the tested strains was prepared according to the method of Marmur (1961). The DNA–DNA relatedness value was determined according to the fluorometric microwell method (Ezaki *et al.*, 1989; He *et al.*, 2005) and the experiment was repeated five times. 16S rRNA gene sequence analysis revealed that strain YIM 120521<sup>T</sup> shared the highest 16S rRNA gene sequence similarity with *Allokutzneria albata* DSM 44149<sup>T</sup> (98.4 %), which illustrated that strain YIM 120521<sup>T</sup> belonged to the genus *Allokutzneria*. Phylogenetic analysis revealed that the strain YIM 120521<sup>T</sup> clustered with *Allokutzneria albata* DSM 44149<sup>T</sup> forming two branches with a high bootstrap value (90 %) in a distinct clade (Fig. 2), and showed the affiliation of strain YIM 120521<sup>T</sup> to the genus *Allokutzneria*. The corresponding consequences were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms (Fig. S2). This result was supported by the chemotaxonomic characteristics (such as consistent diagnostic amino acid, sugars and predominant isoprenoid quinone) and morphological traits (well-branched substrate and aerial mycelia and forming sporangium-like bodies). All these morphological and chemical characteristics strongly supported the affiliation of the novel strain to the genus *Allokutzneria*.

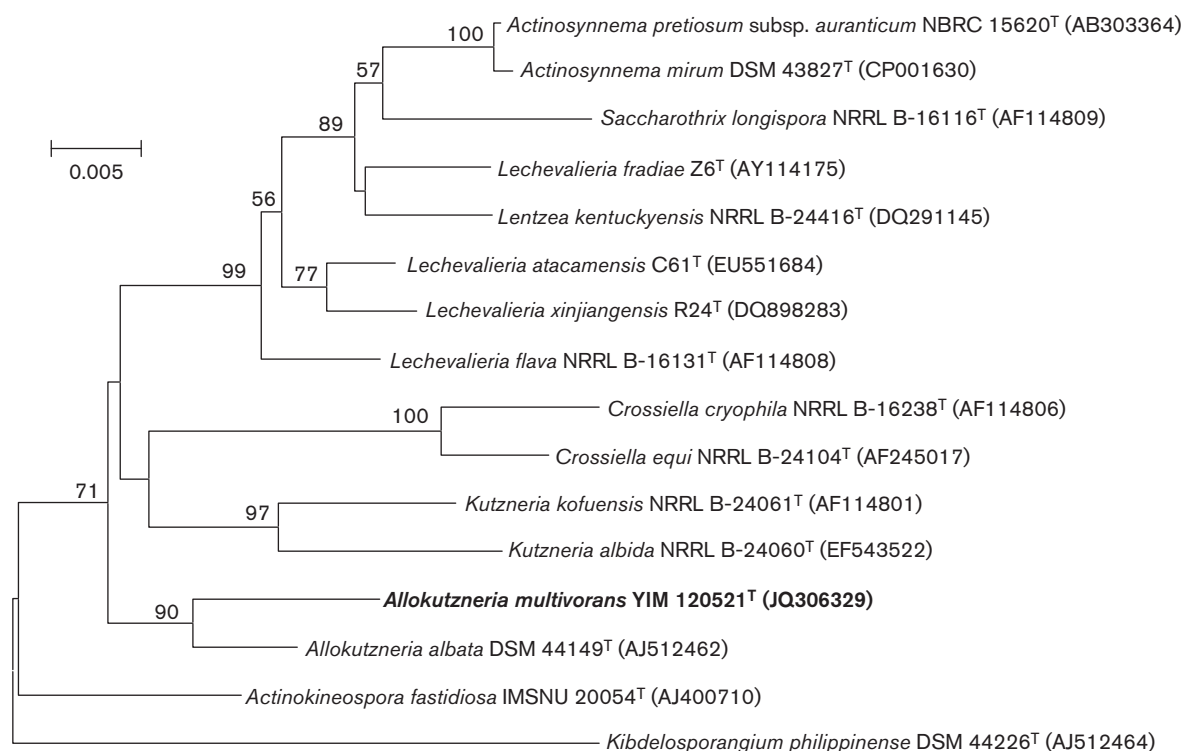
The mean DNA–DNA relatedness value between strain YIM 120521<sup>T</sup> and *Allokutzneria albata* DSM 44149<sup>T</sup> was only 34.9 % (maximum standard deviation, 9 %), and the reciprocal value was 55.5 % (maximum standard deviation, 2 %), which are below the cut-off point recommended by Wayne *et al.* (1987) for confirming separation of strain YIM 120521<sup>T</sup> from its nearest phylogenetic neighbour. Furthermore, many physiological and biochemical features of strain YIM 120521<sup>T</sup> and *Allokutzneria albata* DSM 44149<sup>T</sup> were different from each other, such as temperature range of growth, hydrolysis of potato starch and gelatin, acid production from various compounds, decomposition of nitrogen sources, traces of menaquinones, DNA G + C content (Table 1) and fatty acid compositions (Table 2).

Based on these analyses, strain YIM 120521<sup>T</sup> is considered to represent a novel species of the genus *Allokutzneria*, for which the name *Allokutzneria multivorans* sp. nov. is proposed.

### Description of *Allokutzneria multivorans* sp. nov.

*Allokutzneria multivorans* (mul.ti.vo'rans. L. adj. *multus* many, numerous; L. v. *voro* to devour, swallow; N.L. part. adj. *multivorans* devouring numerous aerial mycelia).

Gram-stain-positive, well-branched substrate and aerial mycelia actinomycete that forms sporangium-like bodies



**Fig. 2.** Phylogenetic tree based on 16S rRNA gene sequences, showing the nearest neighbours of strain YIM 120521<sup>T</sup>. The tree was generated by the neighbour-joining method. All the branches were also recovered by maximum-likelihood and maximum-parsimony tree-making algorithms (Fig. S2). The sequence of *Kibdelosporangium philippinense* DSM 44226<sup>T</sup> was used as an outgroup. Bootstrap percentages  $\geq 50\%$ , generated from 1000 resamplings, are indicated at branch points. Bar, 0.005 substitutions per nucleotide position.

(Fig. 1). The aerial mycelium is white and produces long straight spore chains. Soluble pigments are not found in any of the tested media. The temperature range for growth is 4–40 °C, with optimal growth at 28 °C. The pH range for growth is pH 6.0–9.0, with optimal growth at pH 7.0. Tolerates up to 4% NaCl. Positive for catalase activity and milk coagulation, but negative for oxidase activity, milk peptonization, H<sub>2</sub>S production, nitrate reduction, hydrolysis of gelatin, cellulose, starch and urea. Utilizes L-arabinose, cellobiose, dextrin, dulcitol, D-fructose, fucose, D-glucose, D-galactose, glycerol, inositol, lactose, maltose, D-mannose, mannitol, raffinose, L-rhamnose, sorbitol, succinic acid, sodium DL-malate, L-sorbose and D-xylose as sole carbon sources, but does not utilize xylitol. Amresco, hypoxanthine, L-serine and xanthine can be used as sole nitrogen sources. Acid is produced from D-arabinose, adonitol, cellobiose, dulcitol, dextrinose, fructose, fucose, glycerol, D-glucose, glycogen, arab-inose, D-lyxose, mannose, mannitol, maltose, rhamnose, raffinose, salicin, D-sorbitol, synanthrin and D-xylose. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Whole-cell hydrolysates contain rhamnose, arabinose, glucose, galactose and mannose. MK-9(H<sub>4</sub>) is the predominant respiratory quinone; MK-9(H<sub>2</sub>) and MK-9 are present as minor components.

Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, OH-phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol and two unknown lipids (PL1 and PL2) are present as polar lipids. The main cellular fatty acids ( $>0.5\%$ ) are listed in Table 2.

The type strain is YIM 120521<sup>T</sup> (=JCM 17342<sup>T</sup>=DSM 45532<sup>T</sup>), isolated from soil collected from the banks of the Nuijiang River in Yunnan province, south-west China. The DNA G+C content of the type strain is 69.1 mol%.

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