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ORIGINAL PAPER

Streptomyces xinjiangensis sp. nov., an actinomycete isolated from Lop Nur region

Cong Cheng[1] · Yu-Qian Li[1] · Mipeshwaree Devi Asem[2] · Chun-Yan Lu[1] · Xiao-Han Shi[1] · Xiao Chu[3] · Wan-Qin Zhang[4] · Deng- Di An[1] · Wen-Jun Li[2,3,5]

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Abstract A novel actinobacterial strain, designated LPA192^T, was isolated from a soil sample collected from Lop Nur, Xinjiang Uygur Autonomous Region, Northwest China. A polyphasic approach was used to investigate the taxonomic position of strain LPA192^T. The isolate showed morphological and chemotaxonomic characteristics typical of members of the genus *Streptomyces*. Peptidoglycan was found to contain $_{LL}$-diaminopimelic acid as the diagnostic diamino acid. The predominant menaquinones were MK-9(H_6) and MK-10(H_4). Polar lipids were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol. Major cellular fatty acids consist of $C_{16:0}$, *anteiso*-$C_{15:0}$ and $C_{18:1}\omega 9c$. The sugar in whole-cell hydrolysates was mannose. Phylogenetic analysis indicated that strain LPA192^T is closely related to *Streptomyces tanashiensis* LMG 20274^T (99.3 %), *Streptomyces gulbargensis* DAS131^T (99.3 %), *Streptomyces nashvillensis* NBRC 13064^T (99.3 %), *Streptomyces roseolus* NBRC 12816^T (99.2 %) and *Streptomyces filamentosus* NBRC 12767^T (99.1 %) while showing below 98.5 % sequencing similarities with other validly published *Streptomyces* species. However, DNA–DNA relatedness values between LPA192^T and the closely related type strains were below 40 %, which are much lower than 70 % threshold value for species delineation. The genomic DNA G + C content of strain LPA192^T was 69.3 mol %. Based on the differences in genotypic and phenotypic characteristics from the closely related strains, strain LPA192^T is considered to represent a novel species of the genus *Streptomyces* for which the name *Streptomyces xinjiangensis* sp. nov. is proposed. The type strain is LPA192^T (=KCTC 39601^T = CGMCC 4.7288^T).

Keywords *Streptomyces xinjiangensis* sp. nov. · 16S rRNA gene · Lop Nur · Polyphasic taxonomy

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Cong Cheng and Yu-Qian Li have contributed equally to this work.

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✉ Deng- Di An
anddi@xjnu.edu.cn

✉ Wen-Jun Li
liwenjun3@mail.sysu.edu.cn

[1] Xinjiang Key Laboratory of Special Species Conservation and Regulatory Biology, College of Life Science, Xinjiang Normal University, Urumqi 830054, People's Republic of China

[2] State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, People's Republic of China

[3] Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, People's Republic of China

[4] College of Biology and Chemistry, Xingyi Normal University for Nationalities, Xingyi 562400, Guizhou, People's Republic of China

[5] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, People's Republic of China

Introduction

Members of the phylum *Actinobacteria* are widely distributed in nature, and one important characteristic of this phylum is its role in degradation of organic matter (Lechevalier and Lechevalier 1967; Goodfellow and Williams 1983) and as source of antibiotics and other bioactive molecules (Chun et al. 1997; Labeda et al. 1997; Iwai and Takahashi 1992). Members of the genus *Streptomyces* constitute the largest actinomycete group and have been extensively studied over the past several decades for their potential agricultural, pharmaceutical or industrial applications (Watve et al. 2001; Saadoun and Gharaibeh 2002; Okoro et al. 2009; Goodfellow and Fiedler 2010; Santhanam et al. 2012; Mohammadipanah and Wink 2015; Nithya et al. 2015). Finding new actinobacterial species will presumably lead to the discovery of potentially new structural and beneficial secondary metabolites (Antony-Babu and Goodfellow 2008; Thumar et al. 2010). One way to explore new species is mining of underexplored habitats such as hyper-arid soils (Okoro et al. 2009; Santhanam et al. 2012; Mohammadipanah and Wink 2015). This paper is based on the polyphasic characterization of a novel *Streptomyces* sp. strain LPA192^T, isolated from a soil sample collected from Lop Nur, a dried salt lake in Xinjiang Uygur Autonomous Region, Northwest China. Having dried for more than 60 years, Lop Nur is now primarily a salt flats with a potential salt deposit of about 1–2 m thick (Wood 2004). Recent studies have indicated that the source is unique to several new bacterial species (Li et al. 2015a; Liu et al. 2013, 2015; Zhang et al. 2012; Zheng et al. 2014)

Materials and methods

Isolation

Strain LPA192^T was isolated from a soil sample collected from Lop Nur (90°52′E, 39°58′N) during October 2010. Sample (1 g) was suspended in a flask containing 10 ml sterile water and several glass beads. The suspension was kept in an orbital shaker (28 °C, 180 rpm) for 1 h. One milliliter of the solution was serially diluted 10- and 100-folds, and 100 µl of the 10^{-3} dilution plated on Reasoner's 2A (R2A) agar medium (for composition see DSMZ 830). The plates were incubated at 28 °C for 14 days. The isolates obtained were subcultured in the same medium to obtain pure cultures. Based on the 16S rRNA gene phylogenetic profiles, strain LPA192^T was selected among other strains for further characterization using polyphasic taxonomy. Pure cultures of strain LPA192^T were maintained on R2A at 28 °C and also stored as glycerol suspensions (20 %, v/v) at −80 °C. Physiological tests were performed by cultivating the strain in R2A broth at 28 °C for 5 days as the basal growth condition, unless otherwise stated. *Streptomyces tanashiensis* CGMCC 4.1924^T, *S. nashvillensis* CGMCC 4.1741^T, *S. roseolus* CGMCC 4.2005^T and *S. filamentosus* CGMCC 4.1565^T were obtained from the China General Microbiological Culture Collection Center (CGMCC, Beijing, China), and *S. gulbargensis* CCTCC AA 206001^T from the China Center for Type Culture Collection (CCTCC, Wuhan, China). All reference strains were grown under similar culture condition for subsequent comparative analysis.

Phenotypic characteristics

Gram reaction was determined by using the standard Gram's staining method (Beveridge 2001) and confirmed by KOH lysis test (Cerny 1978). Morphology was observed using light (Philips XL 30) and scanning electron microscopy (ESEM-TMP). Cultural characteristics were examined on Gause's synthetic agar, T5 (Ming et al. 2014), International *Streptomyces* Project (ISP; Shirling and Gottlieb 1966) media, Czapek's agar (Waksman 1967), R2A agar and tryptic soy agar (TSA Difco). Color determination was carried out by using color chips from the ISCC-NBS color charts (Kelly 1964). Growth at different temperatures (4, 10, 15, 20, 28, 30, 37, 40, 45, 50, 55 and 60 °C) and NaCl tolerance (0–10.0 % w/v, with interval of 0.5 % unit) were tested on R2A medium. The pH range (4.0–13.0, at intervals of 1.0 pH unit) for growth was determined in R2A broth (28 °C, 14–21 days) using the buffer system as described by Xu et al. (2005). Catalase activity was detected by assessing production of bubbles on addition of a drop of 3 % (v/v) H_2O_2. Oxidase activity was identified by the oxidation of tetramethyl-*p*-phenylenediamine (Kovacs 1956). Antimicrobial susceptibility was tested according to Masadeh et al. (2011). Other physiological and biochemical characteristics were assessed by using the media and methods described by Gordon et al. (1974) and Williams et al. (1989). Carbon source utilization was evaluated according to the methods of Shirling and Gottlieb (1966) and Locci (1989). Nitrogen source utilization was determined as described by Williams et al. (1989).

Chemotaxonomy

Whole-cell hydrolysates were extracted, purified and analyzed by the method of Hasegawa et al. (1983) and Tang et al. (2009). Polar lipids were extracted and separated by two-dimensional thin-layer chromatography (TLC) (Minnikin et al. 1979) and analyzed as described by Collins and Jones (1980). Menaquinones were extracted according to the method of Collins et al. (1977) and identified by HPLC (Kroppenstedt 1982). Biomass for cellular fatty acid

analysis of strain LPA192^T and the reference strains was harvested from cultures grown in TSB (28 °C, 2 days). Cellular fatty acid analysis was performed by using Microbial Identification System (Sherlock version 6.1; MIDI database: TSBA6) (Sasser 1990).

Molecular analysis and DNA–DNA hybridizations

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). Almost full sequence of 16S rRNA gene of strain LPA192^T was compared with sequences of cultured species present in EzTaxon database (Kim et al. 2012). Alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using CLUSTALX program (Thompson et al. 1997). Phylogenetic analyses were performed by using three tree-making algorithms, neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) methods. Phylogenetic dendrograms were generated by using MEGA software package version 5.0 (Tamura et al. 2011). Evolutionary distance matrices of phylogenetic trees were calculated according to Kimura's two-parameter model (Kimura 1980). Bootstrap analysis was performed with 1000 replications (Felsenstein 1985). G + C content of the genomic DNA was determined by HPLC (Mesbah et al. 1989) using *Escherichia coli* JM-109 as the reference strain. DNA–DNA relatedness was carried out by the fluorometric microwell method (Ezaki et al. 1989; Li et al. 2015b) at the optimal hybridization temperature (50 °C). The experiments were set with eight replications between strain LPA192^T and its closest phylogenetic neighbors as indicated above.

Results and discussion

Phenotypic characteristics

Strain LPA192^T was observed to be Gram-positive, aerobic and non-motile. Strain LPA192^T grew well on R2A, yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), T5, Czapek's agar and TSA, while moderately on Gause's synthetic agar. Smooth, oval spores were borne in long, straight-to-flexuous chains (Fig. S1). Aerial mycelium was observed to be either white or gray on Gause's synthetic agar, ISP 2, ISP 3, ISP 4, ISP 5, T5, Czapek's agar, R2A and TSA. Substrate mycelium was observed to be orange yellow in majority of the tested media, but white on ISP 4, gray on TSA and vivid greenish blue on Czapek's agar media. The strain did not produce diffusible pigment (Table S1). Growth of strain LPA192^T was observed at 10–40 °C (optimum 28–37 °C), pH 5.0–8.0 (optimum pH 7.0) and 0–7 % (w/v) NaCl (optimum 0–2.5 %). The strain showed positive results for catalase, milk peptonization, milk coagulation and hydrolysis of gelatin, starch, oxidase and tweens (20, 40, 60 and 80) tests, but negative for hydrolysis of cellulose, nitrate reduction, urease and hydrogen sulfide production tests. The differential characteristics between LPA192^T and the related type strains in the genus *Streptomyces* are shown in Table 1. The detailed physiological characteristics of strain LPA192^T are given in the species description.

Chemotaxonomy

The diagnostic diamino acid of strain LPA192^T was $_{LL}$-diaminopimelic acid, while major sugar in whole-cell hydrolysates was mannose. Polar lipid profile contained phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, an unidentified aminophospholipid, an unidentified aminolipid, two unidentified phospholipids and four unidentified polar lipids (Fig. S2). The predominant respiratory menaquinones of strain LPA192^T were identified as MK-9 (H_6) (48.4 %) and MK-10 (H_4) (42.3 %). Fatty acid profile (>10 %) consisted of $C_{16:0}$ (18.9 %), *anteiso*-$C_{15:0}$ (18.7 %) and $C_{18:1}\omega 9c$ (14.0 %). Detailed fatty acid profiles (>0.5 %) of strain LPA192^T and its most closely related reference type strains are shown in Table 2.

Phylogenetic analysis and DNA–DNA relatedness

Sequence analysis of almost complete 16S rRNA gene of strain LPA192^T (1535 bp; GenBank accession number KU301049) using EzTaxon-e server showed the strain is closely related to *Streptomyces tanashiensis* LMG 20274^T (99.3 % pairwise sequence similarity), *S. gulbargensis* DAS131^T (99.3 %), *S. nashvillensis* NBRC 13064^T (99.3 %), *S. roseolus* NBRC 12816^T (99.2 %) and *S. filamentosus* NBRC 12767^T (99.1 %) while showing below 98.5 % sequencing similarities with other validly published *Streptomyces* species. Strain LPA192^T also formed a clade with these closely related type strains in all the three phylogenetic dendrograms (Fig. 1, Figs. S3 and S4). DNA–DNA relatedness values between strain LPA192^T and type strains *S. tanashiensis* CGMCC 4.1924^T, *S. gulbargensis* CCTCC AA206001^T, *S. nashvillensis* CGMCC 4.1741^T, *S. roseolus* CGMCC 4.2005^T and *S. filamentosus* CGMCC 4.1565^T were 21.8 ± 3.5, 11.7 ± 0.7, 33.3 ± 1.7, 15.5 ± 2.2 and 33.9 ± 2.2 %, respectively, which is significantly lower than 70 % threshold value used for recognition of genomic species (Stackebrandt and Goebel 1994). The genomic DNA G + C content of strain LPA192^T was 69.3 mol %.

Table 1 Differential characteristics between strain LPA192^T and its closely related type strains in the genus *Streptomyces*

Characteristics	1	2	3	4	5	6
Milk coagulation	+	+[a]	+	+	−	−
Starch hydrolysis	+	+	−[a]	w	w	+
Oxidase activity	+	+	−	+	−	−
Growth at 45 °C	−	−	+	−	−	−
Growth at 7 % NaCl, w/v	+	−	−[a]	−	+	+
pH range	5–8	5–8[a]	5–8	5–8	6–8	6–8
Utilization of sole carbon source						
$_D$(+)-galactose	+	+	−[a]	+	+	+
Inositol	+	−	−[a]	−	−	−
Lactose	+	+	−	+	−	−
maltose	+	+	−	+	+	+
$_D$-mannose	+	+	−	+	−	+
raffinose	−	−	−[a]	+	−	−
$_L$-rhamnose	+	+[a]	−[a]	+	+	−
$_D$(+)-trehalose	−	−	−	+	−	−
$_D$(+)-xylose	+	+	+	+	−[a]	+
Nitrogen source utilization						
$_L$-tryptophan	−	+	−	+	−	−
Antimicrobial susceptibility						
Erythromycin	w	+	w	w	w	+
Chloramphenicol	+	+	+	+	w	w
Ciprofloxacin	−	−	+	w	+	w
Penicillin G	w	−	−	−	−	+
Tetracycline	+	w	+	+	w	+

Strains: 1. LPA192^T ; 2. *Streptomyces tanashiensis* CGMCC 4.1924^T; 3. *Streptomyces gulbargensis* CCTCC AA 206001^T; 4. *Streptomyces nashvillensis* CGMCC 4.1741^T; 5. *Streptomyces roseolus* CGMCC 4.2005^T; 6. *Streptomyces filamentosus* CGMCC 4.1565^T

All data were generated in this study under similar culture conditions

+ (positive), utilization; − (negative), no utilization; w (weakly positive), utilization

[a] Data differ from published results (Dastager et al. 2007; Shirling and Gottlieb 1968a, b)

On the basis of low DNA–DNA relatedness values, phylogenetic analysis, morphological, physiological and chemotaxonomic data, the strain LPA192^T is considered to represent a new species of the genus *Streptomyces*, for which the name *Streptomyces xinjiangensis* sp. nov. is proposed.

Description of *Streptomyces xinjiangensis* sp. nov.

Streptomyces xinjiangensis (xin.ji.ang.en'sis. N.L. adj. *xinjiangensis* pertaining to Xinjiang, a province of China from where the sample was collected).

Aerobic, non-motile, Gram-staining positive. Long, smooth, oval-shaped spores are arranged in straight chains. Aerial mycelium is white or gray without fragmentation. Substrate mycelium is orange yellow. Diffusible or melanoid pigments are not produced. Growth occurs at 10–40 °C, pH 5.0–8.0 and with 0–7 % (w/v) NaCl. Positive for catalase, milk peptonization, milk coagulation, gelatin, starch, oxidase and tweens (20, 40, 60 and 80) hydrolysis tests, but negative for cellulose hydrolysis, nitrate reduction, urease and hydrogen sulfide production tests. Utilizes $_D$-arabinose, $_D$-fructose, $_D$(+)-galactose, $_D$-mannose, $_D$(+)-xylose, inositol, lactose, L-rhamnose, maltose as sole carbon sources, but not $_D$(+)-trehalose or raffinose. Utilizes $_L$-aspartic acid, $_L$-arginine, $_L$-asparagine, $_L$-cystine, $_L$-glutamic acid, $_L$-histidine, $_L$-isoleucine, $_L$-lysine, $_L$-methionine, $_L$-phenylalanine, $_L$-serine, $_L$-threonine, $_L$-tyrosine and $_L$-valine as sole nitrogen sources, but not $_L$-tryptophan. Cell-wall diamino acid is $_{LL}$-diaminopimelic acid. Whole-cell hydrolysates contain mannose. Major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol. The predominant menaquinones are MK-9 (H_6) and MK-10(H_4). Major fatty acids (>10 %) are $C_{16:0}$, *anteiso*-$C_{15:0}$ and $C_{18:1}\omega 9c$. The DNA G + C content of strain LPA192^T is 69.3 mol %. The type strain LPA192^T (=KCTC 39601^T = CGMCC 4.7288^T) was isolated from a soil sample collected from Lop Nur in Xinjiang province in Northwest China. The

Table 2 Fatty acid profiles (>0.5 %) of strain LPA192^T and its closely related type strains in the genus *Streptomyces*

Fatty acids	1	2	3	4	5	6
$C_{12:0}$	2.9	–	–	–	–	–
2OH–$C_{12:0}$	1.0	–	–	–	–	–
3OH–$C_{12:0}$	2.4	–	–	–	–	–
iso-$C_{14:0}$	2.1	2.5	1.5	1.6	4.0	2.4
$C_{14:0}$	1.2	0.5	0.5	–	0.6	0.8
iso-$C_{15:0}$	5.7	11.3	8.6	8.1	7.5	10.2
anteiso-$C_{15:0}$	18.7	29.5	29.9	35.3	32.0	34.0
iso-$C_{16:0}$	5.9	12.8	10.2	8.9	15.6	11.3
$C_{16:0}$	18.9	8.5	10.9	8.9	11.0	7.7
anteiso-$C_{17:1}$ $\omega 9c$	–	0.5	1.2	0.6	1.2	0.8
iso-$C_{17:0}$	4.2	12.2	9.7	11.3	7.5	10.3
anteiso-$C_{17:0}$	5.4	17.9	20.0	20.6	12.8	16.7
cyclo-$C_{17:0}$	0.9	0.8	0.9	1.3	–	0.5
$C_{17:0}$	0.8	0.6	0.6	0.6	0.8	0.9
$C_{18:3}$ $\omega 6c$(6, 9, 12)	0.5	–	–	–	–	–
$C_{18:1}$ $\omega 9c$	14.0	–	–	–	–	–
$C_{18:0}$	1.8	0.6	2.3	0.6	1.4	1.0
Summed feature 3	9.9	–	0.8	–	1.7	0.8
Summed feature 4	1.0	0.8	1.6	0.5	1.9	1.2
Summed feature 9	0.5	0.5	1.4	0.5	1.0	0.9

Strains: 1. LPA192^T ; 2. *Streptomyces tanashiensis* CGMCC 4.1924^T; 3. *Streptomyces gulbargensis* CCTCC AA 206001^T; 4. *Streptomyces nashvillensis* CGMCC 4.1741T; 5. Streptomyces roseolus CGMCC 4.2005^T; 6. *Streptomyces filamentosus* CGMCC 4.1565^T

All data were generated in this study. Biomass was harvested from TSB at 28 °C. Values are percentage of total fatty acids. –, not detected; Fatty acids that represent <0.5 % were omitted. Summed feature 3: $C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c$; Summed feature 4: *anteiso*-$C_{17:1}$ B and/or iso I; Summed feature 9: *methyl*-$C_{16:0}$ or iso-$C_{17:1}\omega 9$

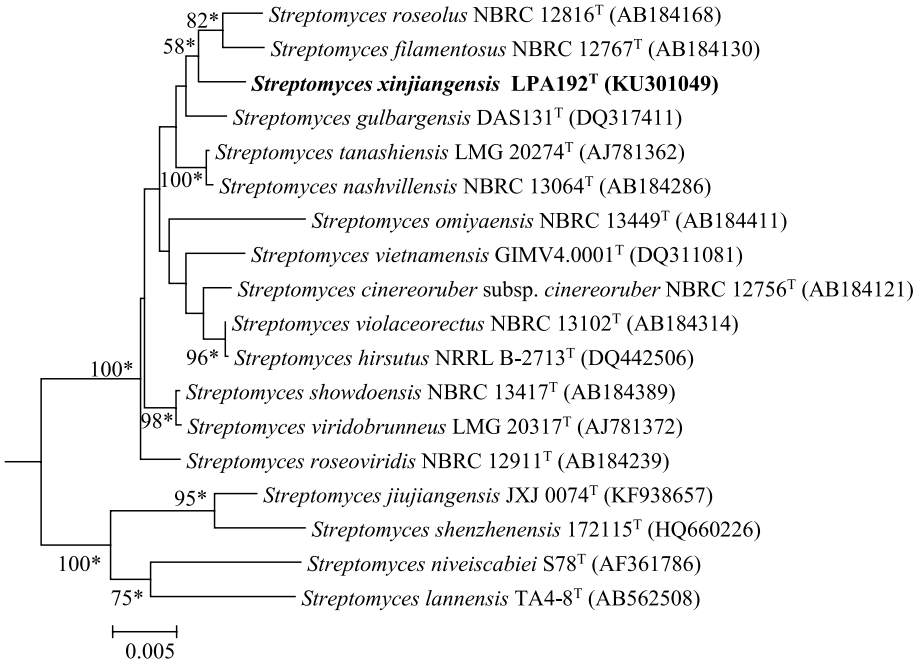

Fig. 1 Unrooted neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain LPA192^T and its closely related strains. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at the branch points. *Asterisks* indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods. *Bar*, 0.005, nucleotide substitutions per site

GenBank accession number for the 16S rRNA gene sequence of strain LPA192^T is KU301049.

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