**Streptomyces pimonensis** sp. nov., isolated from the Taklimakan desert in Xinjiang, China

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**Abstract**

A novel *Streptomyces* strain, designated TRM 75549T, was isolated from a sample of sand in Pimo reclamation area, Taklimakan desert, Xinjiang, North–West China. Phylogenetic analyses of the 16S rRNA gene sequences placed strain TRM75549T within the genus *Streptomyces* with the highest similarities to *Streptomyces pilosus* NBRC 12772T (98.7%). Nonetheless, average nucleotide identity (ANI) value and the digital DNA–DNA hybridization (dDDH) value between strain TRM75549T and *S. pilosus* NBRC 12772T were, respectively, 88.2% and 44.1%, and well below 95–96% and 70% cutoff point recommended for recognizing genomospecies, respectively. A multi-locus sequence analysis of five house-keeping genes (*atpD*, *gyrB*, *recA*, *rpoB* and *trpB*) and phylogenomic analysis also illustrated that strain TRM75549T should also be assigned to the genus *Streptomyces*. Strain TRM75549T contained MK-9 (H6) and MK-9 (H8) as predominant menaquinones. The diagnostic diamino acid of cell walls were identified as LL-diaminopimelic acid and meso-diaminopimelic. The whole-cell sugar pattern of strain TRM 75549T consisted of mannose and glucose. The major fatty acids (> 5%) were iso-C14:0, iso-C15:0, anteiso-C15:0, iso-C16:1H, iso-C16:0. The polar lipids were diphosphatidylglycerol, lysophosphatidylglycerol, phosphatidylethanolamine, phospholipids, phosphatidylglycerol, phosphatidylglycerol, phosphatiylinositol mannosides and unidentified phospholipids. Strain TRM75549T could be differentiated from *S. pilosus* NBRC 12772T, based on physiological and biochemical characteristics. Thus, strain TRM75549T is representative of a novel species of the genus *Streptomyces*, for which the name *Streptomyces pimonensis* sp. nov. is proposed. The type strain is TRM75549T (= CCTCC AA 2020054T = LMG 32221T).

**Keywords** *Streptomyces pimonensis* sp. nov. · Polyphasic taxonomy · Actinomycete

**Introduction**

The genus *Streptomyces* was first proposed by Waksman and Henrici (1943). *Streptomyces* species are aerobic actinomycetes with a GC-rich genome, most of which can form branched mycelia and aerial mycelia, which usually differentiate into spore chains (Gadagkar and Kumar 2005). Many members of the genus *Streptomyces* are known to produce a variety of biologically active metabolites, including antibiotics, enzymes, enzyme inhibitors, vitamins, etc. (Liu et al. 2018). It has always been a popular germplasm resource studied by scientists, which is very important for industries such as medicine, medicine and agriculture are very important.

The strain TRM75549T in this paper was isolated from the southern edge of the Taklimakan Desert in Xinjiang, located at the junction of Pishan County and Moyu County in Hotan area, also known as Pimo Reclamation Area; the climate is highly arid, the vegetation is sparse and subject to high solar radiation, and the temperature difference between day and night is large. The environment is an ideal habitat for obtaining new species of actinomycetes bacteria. In the present study, the taxonomic status of strain TRM75549T was determined using a polyphasic approach. The results show that TRM75549T represents a novel *Streptomyces* species for which the name *Streptomyces pimonensis* is proposed.
Materials and methods

Strain isolation and culturing

In July 2020, the strain TRM75549T was isolated from a sample of sand in Pimo reclamation area, Taklimakan desert, Xinjiang, North–West China (latitude 37º22′7; longitude 79º19′19′). The strain TRM75549T was isolated by standard dilution plate method by growth on Gauze’s No. 1 medium (g/L): soluble starch 20 g, KNO₃ 1 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.5 g, NaCl 20 g, FeSO₄·7H₂O 0.01 g, Agar 16 g, pH 7.0–7.6, incubated at 30 °C for 10 days, and was preserved in 20% (V/V) glycerol at –20 °C and lyophilized in 20% skim milk powder. Biomass for chemical and molecular research was obtained by cultured the strain in trypsin soy broth for 14 days on oscillators at 180 rpm and 30 °C. The closely related type strains Streptomyces pilosus DSM 40153T (Komaki 2019; Oren and Garrity 2021) was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and was cultivated under comparable conditions as the reference strain.

Morphological, cultural, physiological, and biochemical characteristic

The cultural characteristics of strain TRM75549T were determined on ISP media (ISP1, ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7) (Shirling and Gottlieb 1966), Czapek’s agar, Potato dextrose agar, Gauze’s No. 1 medium and Nutrient agar (Waksman 1967) for 14 days at 30 °C. Morphology of mycelia and spores of strain TRM75549T were observed by optical microscopy and scanning electron microscopy as described by Williams and Davies (1967) after 10 days of incubation at 30 °C on Gauze’s No. 1 medium.

Growth at different temperatures (4, 10, 15, 28, 30, 37, 40, 45, 50 and 55 °C) was determined on Gauze’s No.1 agar. The pH range of growth (pH 4.0–12.0, interval 1.0 pH units) was tested in Gauze’s No. 1 liquid medium as described in Xu et al. (2005). NaCl tolerance was determined in Gauze’s No. 1 agar supplemented with 0–10% (w/v) NaCl (interval 1%, w/v) and cultured at 30 °C for 7 days. Physiological characteristics and utilization of carbon sources were assessed following Williams et al. (1989). The uses of liquefaction of gelatin, milk peptonization and solidification, urea, starch hydrolysis, nitrate reduction, melanin production and production of H₂S were studied (Gordon 1974; Yokota et al. 1993).

Antibacterial and antifungal activity

The antimicrobial efficacy of these isolates was tested against various organisms S. aureus ATCC25923, E. faecalis ATCC29212, E. coli ATCC25922 using Kirby–Baur diffusion method (Maiti et al. 2020). In brief, lawns of test organisms were prepared on agar medium and 14-day-old colonies were placed on the lawn. The plates were kept at 4 °C for 2 h for a homogenous distribution of antimicrobial compound before the growth of test organisms followed by the incubation at 37 °C for 24 h. After incubation, the zone of inhibitions around the colony was observed and measured.

Chemotaxonomy

The cells, collected by centrifugation, were washed with distilled water, and then freeze-dried. The method proposed by Staneck and Roberts was used to determine cell wall amino acids (Staneck and Roberts 1974). The whole cell sugars were determined by the method proposed by Tang et al. (2009). Polar lipids were extracted and separated by two-dimensional TLC and identified by the method proposed by Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass according to the method proposed by Collins et al. (1977), and subjected to HPLC analysis (Wang et al. 2011). According to the method proposed by Sasser (1990), cellular fatty acids were extracted from fresh cells, and GS chromatographic analysis was performed.

Genome sequencing and phylogenetic analysis

The method of Chun and Goodfellow (1995) was used to extract genomic DNA and PCR amplification of 16S rRNA gene sequence. EzBioCloud (https://www.ezbiocloud.net/identify, Yoon et al. 2017) was used to calculate the similarity of the 16S rRNA gene sequence with other strains, and then the sequence of the strains with a close relationship were selected to construct phylogenetic trees. These sequences were aligned using MEGA 7.0 software (Kumar et al. 2015) with neighbor-joining (Saitou and Nei 1987), maximum likelihood (Felsenstein 1981), and maximum-parsimony (Fitch 1971) algorithms. The topologies of the phylogenetic trees were evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein 1985). A complete genome sequence of strain TRM77549T was obtained using an Illumina platform and assembled by Velvet 1.2.10. The G+C content of genomic DNA was
determined by whole-genome sequence sequencing. The
digital DNA–DNA hybridization (dDDH) values were
calculated on the GGDC website using formula 2 (http://
ggcd.dsmz.de/ggcd.php), originally described by Auch
et al. (2010) and updated by Meier-Kolthoff et al. (2013).
The genome sequences of strain TRM75549T (accession no.
JAHWZY000000000) and strain S. pilosus NBRC 12772T
(accession no. BMTE00000000) have been submitted to
GenBank. Housekeeping genes used for multilocus sequence
analysis (MLSA), were atpD (ATP synthase subunit D),
gyrB (DNA gyrase B subunit), recA (recombinase A), rpoB
(RNA polymerase beta subunit) and trpB (tryptophane B,
beta subunit). Each locus for each strain was concatenated
head to tail in frame as follows: atpD, gyrB, recA, rpoB and
trpB. The sequences for all loci of other related strains were
obtained from the NCBI (https://www.ncbi.nlm.nih.gov/).
AntiSMASH was used to predict the biosynthetic gene
clusters of strain TRM75549T (Kai et al. 2019).

Results and discussion

After the strain TRM75549T was cultured at 30 °C for
7 days, it was grown on eight kinds of culture medium,
and the colors of aerial mycelia and substrate mycelia were
recorded. The strain was observed to grow well on ISP 1,
ISP 4, ISP 5, ISP 7, Gauze’s No. 1 agar, moderately well on
ISP 2, Czapek’s agar, Potato dextrose agar, with slow growth
on ISP 3 and ISP 6, and none on nutrient agar. The results
of the antibacterial experiment showed that TRM75549T
inhibits E. coli with an average diameter of 2 cm, inhibits
E. faecalis with an average diameter of 1.5 cm, and inhibits
S. aureus with an average diameter of 1.3 cm; Strepto-
mycetes pilosus DSM 40153T inhibits E. coli with an average
diameter of 1.1 cm, and inhibits E. faecalis, with an average
diameter of 1.3 cm, but S. aureus has no obvious transparent
circle (Supplementary Fig. S2). Strain TRM75549T could
be distinguished from S. pilosus DSM 40153T by some phe-
notypic characteristics, in particular cultural characteristics
(Supplementary Table S1). Morphological characteristics of
strain TRM75549T were observed using SEM (Supplemen-
tary Fig. S1). The strain was observed to form an abundant
white aerial mycelium, occasionally twisted, which differ-
entiates into spore chains. Each spore was observed to be
olivary with a hairy surface. Strains grow at 15–45 °C, pH
6.0–9.0 and 0–9% NaCl, and are best grown at 30 °C and pH
7.0 at 1% (w/v) NaCl.

The cell wall of strain TRM75549T contained LL-diami-
nopimelic acid and meso-diaminopimelic (Supplementary
Fig. S6), and the whole cell hydrolyzed sugars were man-
nose and glucose (Supplementary Fig. S7). The polar lipids
were diphosphatidylglycerol (DPG), lysophosphatidylglyc-
erol (LPG), phosphatidylethanolamine (PE), phospholipids
(PLS), phosphatidylglycerol (PG), phosphatidylinositol (PI),
phosphatidylinositol mannosides (PIM) and an unidenti-
fied phospholipid (Supplementary Fig. S3). The predomi-
nant menaquinones were MK-9 (H6) and MK-9 (H8). The
major fatty acids (> 5%) were iso-C14:0 (10.64%), iso-C 15:0
(5.01%), anteiso-C15:0 (11.98%), iso-C16:1 H (12.87%), iso-
C16:0 (33.46%).

The G + C content in the draft genome sequence of strain
TRM75549T was identified as 72.14 mol%. The GenBank
login number of the 16S rRNA gene sequence of strain
TRM75549T is MW479154, and the closest phylogenetic
neighbor was S. pilosus NBRC 12772T (GenBank accession
no. AB184842). The average nucleotide identity value and
the digital DNA–DNA hybridization value between strain
TRM75549T and S. pilosus NBRC 12772T were 88.20 and

| Cluster | Region | Length (bp) | Most similar known cluster | Type | Similarity (%) |
|---------|--------|-------------|-----------------------------|------|----------------|
| 1–2     | 2.1    | 143,516–164,601 | Albaflavenone | Terpene | 100 |
| 2–6     | 6.1    | 48,389–71,157 | SapB | RlPP:Lanthip | 100 |
| 3–8     | 8.2    | 133,780–155,939 | Geosmin | Pidade Terpene | 100 |
| 4–17    | 17.1   | 64,941–75,339 | Ectoine | Other | 100 |
| 5–20    | 20.1   | 42,010–64,619 | Citrulasin D | RlPP | 100 |
| 6–4     | 4.1    | 80,325–92,097 | Desferrioxamin B/desferrioxamine E | Other | 83 |
| 7–11    | 11.2   | 96,541–140,395 | Paenibactin | NRP | 83 |
| 8–11    | 11.1   | 43,228–94,339 | Coelbactin | NRP | 81 |
| 9–10    | 10.1   | 247–45,109 | Deimino-antipain | NRP | 77 |
| 10–11   | 11.1   | 143,713–216,222 | Spore pigment | Polyketide | 66 |
| 11–31   | 31.1   | 1–32,456 | Diisonitrile antibiotic SF2768 | NRP | 66 |
| 12–18   | 18.1   | 20,424–45,677 | Carotenoid | Terpene | 63 |
| 13–73   | 73.1   | 1–9,114 | Hopene | Terpene | 53 |
| 14–72   | 72.1   | 1–9,353 | Cosmomyces B | Polyketide | 50 |
44.10%, respectively, well below 95–96 and 70% cutoff point recommended for delineating species. The phylogenetic tree constructed from the 16S rRNA gene sequence through the neighbor-joining method showed that the strain TRM75549\textsuperscript{T} formed a unique clade (Fig. 1), which was also restored in the maximum-likelihood trees and maximum-parsimony
trees (Supplementary Figs. S4, S5). The MLSA with the concatenated \textit{atpD}, \textit{gyrB}, \textit{recA}, \textit{rpoB} and \textit{trpB} genes showed that the strain did not cluster with \textit{S. pilosus} NBRC 12772\textsuperscript{T}, forming two separate branches and the MLSA distances were much greater than the generally accepted threshold value of 0.007 for species delineation (Fig. 2). A total of 39 secondary metabolites synthetic gene clusters were compared by antiSMASH. The antiSMASH biosynthetic gene clusters of TRM75549\textsuperscript{T} with a similarity greater than 50\% are shown in Table 1. All the data suggested that strain TRM75549\textsuperscript{T} was a member of the genus \textit{Streptomyces}. However, on the basis of a combining compare of phylogenetic distinctness and differences in chemotaxonomic and physiological characteristics (Table 2), it is considered that strain TRM75549\textsuperscript{T} is the representative of a new species of \textit{Streptomyces}, named \textit{Streptomyces pimonensis} sp. nov. is proposed.

**Table 2** Biochemical characteristics of strain TRM75549\textsuperscript{T} compared to its phylogenetic relatives

| Characteristic                        | 1                  | 2                  |
|---------------------------------------|--------------------|--------------------|
| Spore-chain morphology                | Straight           | Spirale            |
| Spore surface                         | Hairy              | Spiny              |
| Optimum temperature \(^{\circ}\text{C}\) | 30                  | 28                 |
| pH tolerance                          | 6.0–9.0            | 7.0–9.0            |
| NaCl tolerance (% w/v)                | 0–9                | 0–5                |
| Glucose                               | +                  | +                  |
| L(+)-Arabinose                        | +                  | +                  |
| Celloibiose                           | +                  | +                  |
| D(+)-Fructose                         | +                  | +                  |
| D(+)-Galactose                        | +                  | +                  |
| D(+)-Maltose                          | +                  | +                  |
| D(+)-Mannitol                         | +                  | +                  |
| Lactose                               | +                  | –                  |
| Xylose                                | +                  | +                  |
| Raffinose                             | –                  | –                  |
| myo-Inositol                          | w                  | +                  |
| Sucrose                               | w                  | –                  |
| L-Rhamnose                            | –                  | +                  |
| Gelatin liquefaction                  | +                  | w                  |
| Starch hydrolysis                     | +                  | +                  |
| Nitrate reductase                     | +                  | +                  |
| Urea                                  | –                  | –                  |
| \(\text{H}_2\text{S}\) production    | –                  | +                  |
| Melanin production                    | –                  | –                  |
| Milk peptonization                    | –                  | +                  |
| Major whole-cell sugars               | Glucose            | Glucose            |
|                                       | mannose            | mannose            |
| Major cell-wall diamino acid          | LL-DAP             | LL-DAP             |
|                                       | meso-DAP           | meso-DAP           |
| Phospholipids                         | DPG, PE, PI, PG, PIM, PLS, LPG | DPG, PE, PC, PG, NPG, PLS, LPG |
| Predominant menaquinones              | MK-9(H\(_8\)), MK-9(H\(_4\)) | MK-8,MK-8(H\(_2\)) MK-8(H\(_4\)), MK-9(H\(_6\)) |
| Major cellular fatty acids (>10%)     | iso-C\(_{16:0}\), iso-C\(_{16:1}\), anteiso-C\(_{15:0}\) | iso-C\(_{16:0}\), anteiso-C\(_{15:0}\), iso-C\(_{15:0}\) |

Strains: 1. TRM75549\textsuperscript{T}; 2. \textit{Streptomyces pilosus} DSM 40153\textsuperscript{T}; All data are from this study

+ Positive, w weakly positive, – negative

**Description of \textit{Streptomyces pimonensis} sp. nov.**

\textit{Streptomyces pimonensis} (pimonensis (pi.mo.nen’sis. N.L. masc. adj. pimonensis, pertaining to Pimo reclamation area, Taklimakan desert, Xinjiang, North–West China, from where the type strain was isolated).
Aerobic, gram-positive actinomycetes. The aerial mycelium is tortuous, and each spore was observed to be olivary with a hairy surface. Grow well on ISP 1, ISP 4, ISP 5, ISP 7, Gauze’s No. 1 agar, grow moderately on ISP 2, Czapek’s agar, Potato dextrose agar. Grows at 0–9% (w/v) NaCl, pH 6.0–9.0 and 15–45 °C, with optimum growth at 1% (w/v) NaCl, pH 7.0–8.0, and 30 °C, respectively. Glucose, arabinose, D-sorbitol, D-xylene, fructose, maltose, mannitol, lactose, D-galactose, inositol, ribose, cellobiose are utilized. Sucrose, rhamnose, raffinose, trehalose are not utilized. Starch hydrolysis, nitrate reduction, cellulose decomposition, degradations of Tweens 20, 40, 60 and 80 are positive, whereas urease, gelatin liquefaction, milk degradations, degradations of Tweens 20, 40, 60 and 80 are positive, whereas urease, gelatin liquefaction, milk degradations, peptonization and solidification, oxidase, melanin production are negative. The cell wall contains LL-diaminopimelic acid and meso-diaminopimelic. Whole cell hydrolysates contain mannose and glucose. The polar lipids were diphosphatidylglycerol (DPG), lysophosphatidylglycero (LPG), phosphatidyl ethanolamine (PE), phospholipids (PLS), phosphatidylglycerol (PG), phosphatidyl inositol (PI), phosphatidylinositol mannosides (PIM) and an unidentified phospholipid. The menaquinone system contains MK-9 (H6) and MK-9 (H8). The major fatty acids are iso-C14:0 (10.64%), iso-C15:0 (5.01%), anteiso-C15:0 (11.98%), iso-C16:1 (12.87%), iso-C16:0 (33.46%). The G+C content in the draft genome sequence of strain TRM75549T is 72.14 mol%.

The type strain is TRM75549T (= CCTCC AA 2020054T = LMG 32221T), isolated from the Pimo, Taklimakan desert, Xinjiang, North–West China.

The GenBank/EMBL/DBJ accession number for the genome and 16S rRNA gene sequence of strain TRM75549T is JAHWZY000000000 and MW479154.

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Author contributions PZ participated in the experiment and preparation of the first draft. XL, XL, ZL, ZX, and CW gave guidance during the experiment. LZ contributed to reagents, instrumentation and the financial support for this work.

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Declarations

Conflict of interest The authors state that there is no conflict of interest.

Ethical approval This article does not contain any research conducted by any author on human participants or animals.

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