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TAXONOMIC DESCRIPTION
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Description of the bacterial RNA polymerase inhibitor GE23077-producer *Actinomadura* sp. NRRL B-65521^T as *Actinomadura lepetitiana* sp. nov.

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Abstract

The filamentous actinomycete that produces the antibiotic GE23077 was isolated by the Lepetit Research Group from a soil sample collected in Thailand, and it was classified as a member of the genus *Actinomadura* on the basis of its morphology and cell-wall composition. Phylogenetic analysis based on 16S rRNA gene sequences indicated that this strain formed a distinct monophyletic line within the genus *Actinomadura,* and it was most closely related to *Actinomadura bangladeshensis* DSM 45347^T (99.31% similarity) and *Actinomadura mexicana* DSM 44485^T (98.94%). The GE23077-producing strain formed an extensively branched, non-fragmented vegetative mycelium; no pseudosporangia were formed and the arthrospores were organized in slightly twisted chains. The cell wall contained *meso*-2,6-diaminopimelic acid and the diagnostic sugar was madurose. The predominant menaquinone was MK-9(H_6), with minor amounts of MK-9(H_8) and MK-9(H_4). The diagnostic phospholipids were phosphatidylinositol and diphosphatidylglycerol. The major cellular fatty acids were $C_{16:0}$ and tuberculostearic acid (10-methyloctadecanoic acid), followed by minor amounts of $C_{18:1}\omega9c$, $C_{16:1}\omega7c$ and 10-methylheptadecanoic acid. The genomic DNA G+C content was 71.77 mol%. Significant differences in the morphological, chemotaxonomic and biochemical data, and the low DNA–DNA relatedness between the GE23077-producing strain and closely related type strains clearly demonstrate that it represents a novel species of the genus *Actinomadura*, for which the name *Actinomadura lepetitiana* sp. nov. is proposed. The type strain is NRRL B-65521^T(=LMG 31258^T=DSM 109019^T).

The genus *Actinomadura* belongs to the family *Thermomonosporaceae* of the order *Streptosporangiales* in the class *Actinobacteria* [1, 2], which for traditional reasons will be termed as actinomycetes in the present study. The genus *Actinomadura*, classified as a member of the family *Thermomonosporaceae* as emended by Stackebrandt *et al.* [3] with *Actinomadura madurae* as the type species, was first proposed by Lechevalier and Lechevalier [4] to accommodate so-called *Nocardia* species with walls containing *meso*-diaminopimelic acid but lacking arabinose and galactose in whole-cell hydrolysates. Members of the genus *Actinomadura* are aerobic, Gram stain-positive, non-acid-fast actinomycetes that can form extensively branched non-fragmenting substrate mycelium on various agar media, with aerial mycelia that differentiate into hooked, straight or irregular spiral chains of arthrospores with a surface that can be folded, irregular, smooth, spiny or warty [5]. The peptidoglycan of the cell wall contains *meso*-diaminopimelic acid (cell wall type III), and the diagnostic sugar of whole-cell hydrolysates is madurose [6]. The major phospholipids are diphosphatidylglycerol and phosphatidylinositol, corresponding to phospholipid type PI of Lechevalier *et al.* [7]. Tetra-, hexa- and octahydrogenated menaquinones with nine isoprene units are the predominant isoprenologues [8]. Complex mixtures of fatty acids with hexadecanoic ($C_{16:0}$), 14-methylpentadecanoic (iso-$C_{16:0}$) and 10-methyloctadecanoic acid (10-Me-$C_{18:0}$, tuberculostearic acid) are predominant [8].

At the time of writing (June 2020), the genus comprised 65 species with validly published names as reported in the

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Keywords: *Actinomadura lepetitiana* sp. nov; *Actinomadura* sp. NRRL B-65521; actinomycetes; antibiotics; bacterial RNA polymerase inhibitor GE23077.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; DDH, DNA-DNA hybridization.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NRRL B-65521^T is MH061375. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession JAABOU000000000. The version described in this paper is version JAABOU010000000.
Three supplementary figures are available with the online version of this article.

List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/actinomadura), including the recently described *Actinomadura harenae* [9], *Actinomadura roseirufa* [10] and *Actinomadura logoneensis* [11]. Although the primary reservoir of members of the genus *Actinomadura* remains soil (including Saharan [12] and rhizospheric soils [13]), *Actinomadura* species are widespread in nature, and have been isolated from a wide range of natural habitats such as leaves, volcanic cone and hot spring waters, honeybee hive, rocks and mud nests [14–19]. They have also been isolated from bovine manure compost [20] and from sewage ditch [21]. Additionally, strains of this genus have been reported as the agents of actinomycete mycetoma [22], whereas others have shown the potential to produce antibiotics [10, 19, 23–27].

In the course of a screening programme for discovering novel inhibitors of rifamycin-resistant RNA polymerase, the Lepetit Research Group (at that time part of Vicuron Pharmaceuticals) isolated a strain belonging to the genus *Actinomadura,* which produced the cyclic heptapeptide GE23077 that had potent activity against RNA synthesis in cell-free tests but with antibacterial activity restricted to *Moraxella catarrhalis* [26]. The product was patented and the strain was deposited as DSM 13491 [28]. Subsequent efforts to improve the GE23077 antibacterial activity and extend its antimicrobial spectrum by chemical modifications allowing its penetration into whole cells were unsuccessful; the patent was thus left aside [29]. Due to its specific and selective mode of action, we recently assisted with renewed interest in this bacterial RNA polymerase inhibitor that does not show cross-resistance with rifamycins [30, 31]. Since the GE23077-producing strain (purchased from DSMZ and coded as VA325 in our University strain collection) remained poorly characterized taxonomically, in the present study we describe its polyphasic taxonomic analysis and propose that it represents a novel species of the genus *Actinomadura*, which has been deposited as NRRL B-65521^T (=LMG 31258^T=DSM 109019^T).

The strain producing GE23077 was isolated by the Lepetit Research Group from a soil sample collected in Thailand by employing a novel method for isolating spores from uncommon soil actinomycetes [29]. One gram of soil sample dried overnight at 50 °C was resuspended in 10 ml of water containing novobiocin (10 µg ml^{-1}). After vigorous shaking the sample was centrifuged at 1500 r.p.m. for 2 min and then allowed to stand at room temperature for 2 h. Because the supernatant was mostly enriched in spores relative to vegetative forms, the supernatant was spread onto colloidal chitin agar supplemented by novobiocin (10 µg ml^{-1}) and cycloheximide (50 µg ml^{-1}) as described by Shirling and Gottlieb [32]. Plates were incubated at 28 °C for 2–3 weeks and colonies were finally transferred on ISP3 and incubated at 28 °C until growth was detectable [32]. The pure culture was then used for fermentation studies and stored as a 20% (v/v) glycerol suspension at −80 °C and as lyophilized cells for long-term preservation [26].

We extracted the genomic DNA of strain NRRL B-65521^T following the method described by Kieser *et al.* [33]. The 16S rRNA gene was amplified by PCR according to the Bact 16S Service of BMR Genomics using universal primers (Primer 1500F: AGTTTGATCCTGGCTCAG and Primer 1500R: AAGGAGGTGATCCAGCCGCA) and Platinum Taq High Fidelity (Fisher Scientific). The PCR product was sequenced by BMR Genomics using universal primers. The 16S rRNA gene sequence of strain NRRL B-65521^T was compared with those of representatives of the genus *Actinomadura* available in the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon [34]). 16S rRNA gene sequence similarity between strain NRRL B-65521^T and the type strains of related species was calculated on the basis of pairwise alignment. A multiple sequence alignment of strain NRRL B-65521^T and other related taxa was performed by using CLUSTAL W version 2.0 [35] and trimmed manually where necessary. Phylogenetic trees were reconstructed using the neighbour-joining [36] and maximum-likelihood [37] algorithms by MEGA software version X [38]. The topology of phylogenetic trees was evaluated with bootstrap analysis based on 1000 resamplings [39], to determine the confidence values of the branches in the phylogenetic tree. A distance matrix was generated using Kimura's two-parameter model [40]. All positions containing gaps and missing data were eliminated from the dataset.

Comparison, by using the EzTaxon-e server, of the almost-complete 16S rRNA gene sequence (1492 nt) of strain NRRL B-65521^T with those of type strains of related species with validly published names indicated that strain NRRL B-65521^T is a member of the genus *Actinomadura*. The most closely related type strains were *Actinomadura bangladeshensis* DSM 45347^T [41] (99.31% similarity; 10 nucleotide differences in 1443 positions) and *Actinomadura mexicana* DSM 44485^T [42] (98.94%; 15 nucleotide differences in 1421 positions). The neighbour-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences showed that strain NRRL B-65521^T falls within the evolutionary radiation occupied by representatives of the genus *Actinomadura*, and it is included in a cluster that is also present in the maximum-likelihood tree (Fig. S1, available in the online version of this article). The two phylogenetic trees are almost completely in agreement.

We observed the morphological characteristics of strain NRRL B-65521^T by scanning electron microscopy (SEM-FEG XL-30; Philips) using cultures grown on one-tenth strength humic acid medium for 7 days at 28 °C [43]. The cultural characteristics of strain NRRL B-65521^T in parallel with those of *A. bangladeshensis* DSM 45347^T (=3-46-b3^T) and *A. mexicana* DSM 44485^T (=A290^T) were determined after incubation at 28 °C for 21 days on various ISP media [32] in addition to a few media as recommended by Waksman [44]. The agar media used for morphological and cultural studies were: yeast extract-malt agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch agar (ISP4), glycerol-asparagine agar (ISP5), peptone-yeast extract-iron agar (ISP6), tyrosine agar (ISP7), nutrient agar, Bennett's agar, Hickey–Tresner's agar and glucose-asparagine agar. Starch hydrolysis was checked on ISP4 medium and melanin production on ISP7, as previously reported [45, 46]. Colours were determined according to Maerz and Paul [47] and Tresner and Backus [48]. The ranges

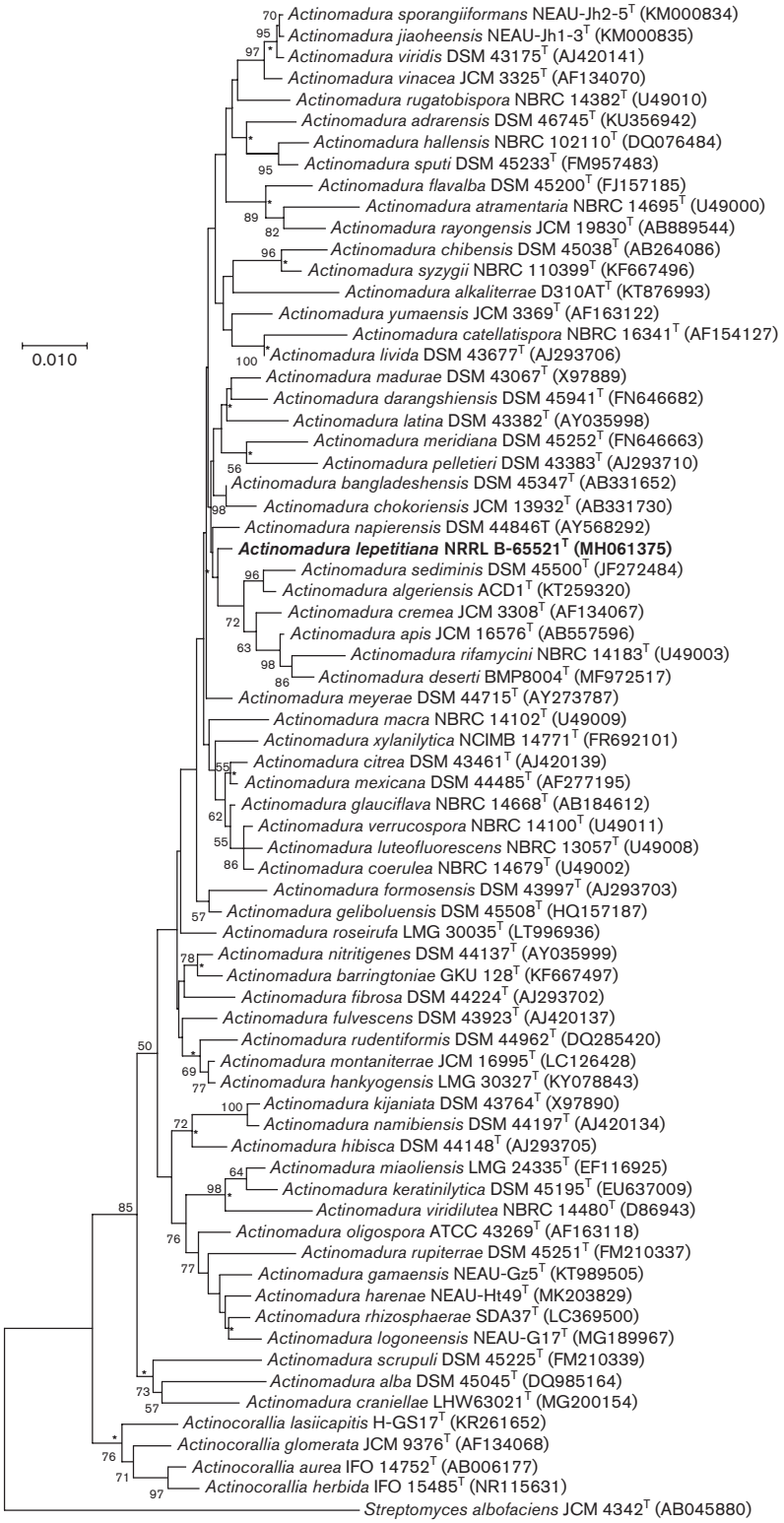

Fig. 1. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the relationship between strain NRRL B-65521^T and other species of the genus *Actinomadura*. *Streptomyces albofaciens* JCM 4342^T was used as an outgroup. The phylogenetic analysis compares 1274 sites, following the complete deletion of gaps and missing data. Asterisks denote branches that were also recovered in the maximum-likelihood tree. Numbers at branch points indicate bootstrap percentages (based on 1000 replications); only values above 50.0% are shown. Bar, 0.010 substitutions per nucleotide position.

of temperature (20–43 °C), pH (6.0–9.0) and NaCl concentration (0–2.5%, w/v) for growth of strain NRRL B-65521^T were examined on Hickey–Tresner's agar plates for 14–21 days. Carbon source (L-arabinose, cellobiose, cellulose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, ribose, salicin, starch, sucrose, D-xylose) utilization was tested using carbon source utilization medium (ISP9) [30, 49] supplemented with a final concentration of 1% (w/v) of the tested carbon source.

The morphological and cultural characteristics of strain NRRL B-65521^T were consistent with members of the genus *Actinomadura*. Strain NRRL B-65521^T showed good growth on ISP2, ISP3, ISP4, ISP6, ISP7, Hickey–Tresner's agar, Bennett's agar and nutrient agar, and moderate growth on ISP5 and glucose asparagine agar. As reported in Table 1, the colour of the substrate mycelium varied from pale yellow to pink according to the medium used [47, 48]. The strain did not produce soluble pigment with any of the media used. The aerial mycelium, when present, was white or pale pink. The aerial mycelium produced slightly twisted chains of spores (0.9–1.0 µm in diameter). Spore chains were curved to hooked and consisted of 6–8 or more non-motile spores with warty surfaces (Fig. 2). No pseudosporangia were detectable.

Strain NRRL B-65521^T tolerated temperatures of ~20–43 °C with an optimum between 28 and 37 °C. The strain grew in the pH interval from 6.0 to 9.0. Strain NRRL B-65521^T tolerated up to 2.5% (w/v) NaCl (Table 2). Starch hydrolysis and melanin production were positive. The strain grew on all carbon sources tested, but growth was more abundant on cellobiose, cellulose, D-glucose and D-xylose. The cultural and physiological characteristics of strain NRRL B-65521^T that differentiate it from the closely related *A. bangladeshensis* DSM 45347^T and *A. mexicana* DSM 44485^T [41, 42] are reported in Tables 1 and 2, and indicated in detail in the species description.

For comparative analysis of antibiotic production, we cultivated strain NRRL B-65521^T and the closest related strains *A. bangladeshensis* DSM 45347^T and *A. mexicana* DSM 44485^T in a medium previously optimized for GE23077 production, consisting of: glucose 2% (w/v), yeast extract 0.2% (w/v), soybean meal 0.8% (w/v), NaCl 0.1% (w/v) and $CaCO_3$ 0.4% (w/v) [26]. The cultures were incubated for 192 h on a rotary shaker at 200 r.p.m. and at 28 °C, and sampled every 24 h. Biomass was measured as dry weight after harvesting the mycelium by centrifugation for 10 min at 4000 *g* and then dehydrating the pellet for 24 h in a 50 °C oven. To follow GE23077 production, 20 ml of the harvested broth was filtered to remove the mycelium and the supernatant was loaded on 2 ml pre-activated Diaion HP-20 resin and then eluted with 5 ml methanol. Samples were concentrated to 1.5 ml in a rotavapor before HPLC injection. HPLC analyses were conducted on a 5-µm particle-size Ultrasphere ODS (Beckman) column (4.6 by 250 mm) eluting at a flow rate of 1 ml min^{-1} in isocratic mode for 25 min of 82% Phase A and 18% Phase B. Phase A and B were solutions of methanol/40 mM ammonium formate buffer 1:99 (v/v) and methanol/40 mM ammonium formate 70:30 (v/v) [26], respectively.

As shown in Fig. S2a, in the antibiotic production medium, *A. bangladeshensis* DSM 45347^T and *A. mexicana* DSM 44485^T grew well with a biomass production comparable or even higher than for strain NRRL B-65521^T, but neither of them produced any GE23077. In contrast, GE23077 production was detectable in cultures of strain NRRL B-65521^T (Fig. S2b).

Biomass for chemotaxonomic study was prepared by growing strain NRRL B-65521^T in ISP2 broth [32] at 200 r.p.m. for 5 days at 30 °C; cells were harvested by centrifugation, washed twice in distilled water, re-centrifuged and freeze-dried. The steroisomeric form of diaminopimelic acid was determined by TLC [50]. The whole-cell sugar pattern was determined according to Saddler *et al.* [51]. Isoprenoid quinones were extracted and purified according to the procedure of Minnikin *et al.* [52] and analysed by HPLC [53]. Polar lipids and fatty acids were extracted and analysed by the Identification Service of the Leibniz-Institut DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). Briefly, polar lipids were separated by two-dimensional silica gel TLC (Macherey-Nagel Art. No. 818 135). The first direction was developed in chloroform/methanol/water (65:25:4, by vol.), and the second in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipid material was detected using molybdatophosphoric acid and specific functional groups were detected using spray reagents specific for defined functional groups according to Tindall *et al.* [54]. For the extraction of whole-cell fatty acids, the wet biomass was extracted using minor modifications [55] of the method of Miller [56]. Analyses were carried out as described by Kroppenstedt *et al.* [8] and fatty acid methyl ester peaks were separated by the Microbial Identification System (MIDI; Microbial ID) and analysed using the TSBA 40 database.

Strain NRRL B-65521^T contained *meso*-diaminopimelic acid as the cell-wall diamino acid and the whole-cell sugar was madurose (type B). As shown in Fig. S3, the major phospholipids were phosphatidylinositol and diphosphatidylglycerol. The predominant menaquinone of the strain was MK-9(H_6) (58.94%), while minor amounts of MK-9(H_8) (12.61%) and MK-9(H_4) (5.83%) were also present. The major cellular fatty acids were $C_{16:0}$ (32.47%) and tuberculostearic acid (10-methyloctadecanoic acid) (25.29%), followed by moderate amounts of $C_{18:1}\omega9c$ (8.88%), $C_{16:1}\omega7c$ (6.86%), and 10-methylheptadecanoic acid (5.25%).

For draft genome sequencing and assembly, the genomic DNA of strain NRRL B-65521^T was checked by agarose gel electrophoresis to evaluate DNA integrity and purity and via a NanoDrop UV-Vis spectrophotometer (ThermoFisher Scientific) and Qubit assay (ThermoFisher Scientific) to estimate DNA concentration. Whole-genome sequencing was performed by BMR Genomics on the Illumina MiSeq platform. A-tailed, ligated to paired-end adaptors and PCR-amplified library with a 300 bp insert were prepared at BMR Genomics. Illumina PCR adapter reads and low-quality reads from the paired-end were filtered by a quality

Table 1. Comparison of the cultural characteristics of strain NRRL B-65521^T and the type strains of the most closely related species of the genus *Actinomadura* with validly published names

Strains: 1, NRRL B-65521^T; 2, *A. bangladeshensis* DSM 45347^T; 3, *A. mexicana* DSM 44485^T. G, growth (+++, good; ++, moderate; +, poor; –, no growth); AM, aerial mycelium (–, not produced; +, poorly produced); SM, substrate mycelium; SP, soluble pigment (–, not produced). Colour chart from [47, 48].

Medium		1	2	3
ISP2	G	+++	++	+++
	AM	–	–	+
	SM	Pale pink	Bamboo	Light yellow
	SP	–	–	Yellow
ISP3	G	+++	+++	+++
	AM	+	+	+
	SM	Pink	Bamboo	Light brown
	SP	–	–	–
ISP4	G	+++	+++	+++
	AM	–	+	–
	SM	Deep pink	Light beige	Pale pink
	SP	–	–	–
ISP5	G	++	+++	++
	AM	+	–	–
	SM	Pale pink	Light beige	Light brown
	SP	–	–	–
ISP6	G	+++	+++	++
	AM	+	–	–
	SM	Pale yellow to brownish	Deep orange-yellow	Light brown
	SP	–	–	–
ISP7	G	+++	+++	+++
	AM	+	+	+
	SM	Pink	Red wood	Red wood
	SP	–	–	–
Nutrient agar	G	+++	++	+++
	AM	+	–	–
	SM	Pale yellow	Bamboo	Light ivory
	SP	–	–	–
Hickey–Tresner's agar	G	+++	+++	+++
	AM	+	+	+
	SM	Pale yellow	Light brown	Light ivory
	SP	–	–	–
Glucose asparagine agar	G	++	++	+++
	AM	–	+	+
	SM	Light pink	Light ivory	Light ivory
	SP	–	–	–

Continued

Table 1. Continued

Medium		1	2	3
Bennet's agar	G	+++	++	+++
	AM	–	–	–
	SM	Pink	Light brown	Pink to pale red
	SP	–	–	–

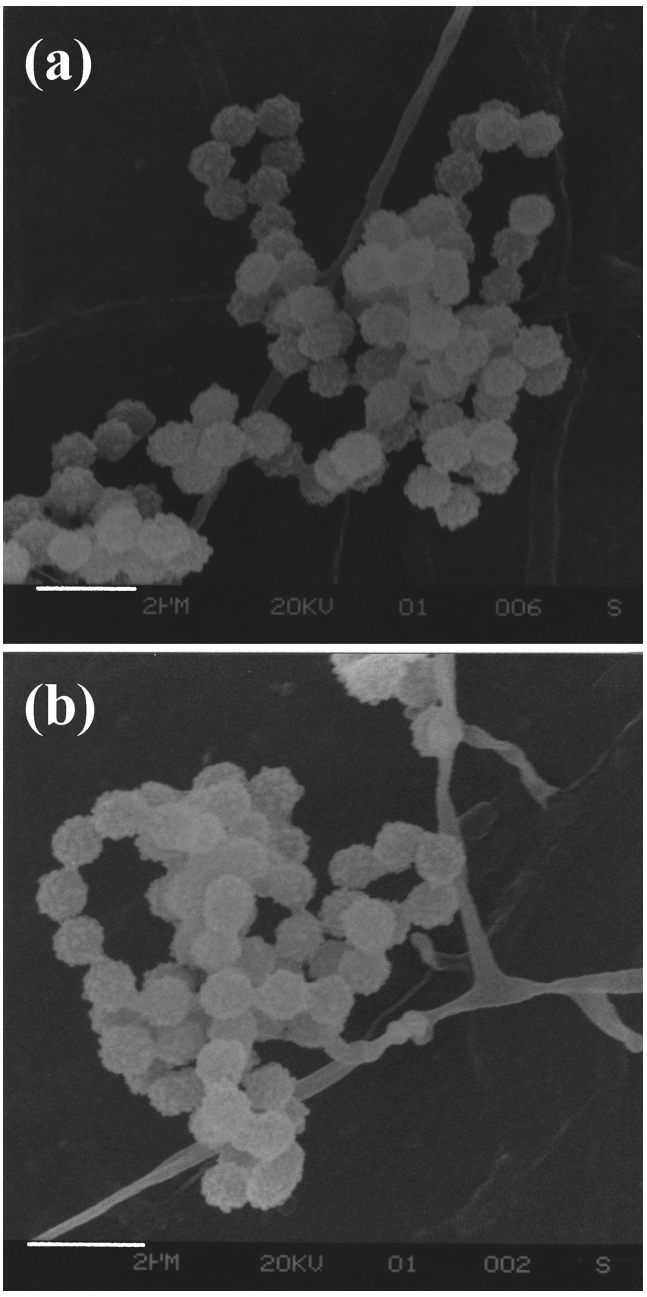

Fig. 2. Scanning electron micrographs showing spore morphology. The strain was grown on humic acid at 28 °C for 14 days. Bars, 2.0 µm.

control step using the BMR Genomics compilation pipeline. Adapter trimming was conducted with Cutadapt (https://cutadapt.readthedocs.io [57]), we used an in-house compilation pipeline to remove residual PCR primer sequences and filtered low-quality bases (Q<30) and short reads (length <150 bp). All good-quality paired reads were assembled by using SPAdes v3.12.0 (http://cab.spbu.ru/software/spades/ [58]) into a number of scaffolds. The filter reads were then handled by the next step of the gap-closing. Evaluation of assembly output was performed with QUAST v4.6.3 (http://quast.sourceforge.net/quast [59]).

The assembled genome sequence of strain NRRL B-65521^T was 8592328 bp long and composed of 2016 contigs with an

Table 2. Physiological properties that differentiate strain NRRL B-65521^T from the type strains of the most closely related species of the genus *Actinomadura*

Strains: 1, NRRL B-65521^T; 2, *A. bangladeshensis* DSM 45347^T; 3, *A. mexicana* DSM 44485^T. Only results that differentiate among the strains are reported. +, growth/degradation; –, absence of growth/degradation; ND, not determined.

Characteristic	1	2	3
Temperature for growth (°C)	20–43	20–45	25–37
pH range	6–9	5–9	7–9
NaCl tolerance (%, w/v)	2.5	4	2.5
Starch hydrolysis	+	–	–
Melanin production	+	–	+
Growth on sole carbon source			
L-Arabinose	+	+	++
Cellobiose	++	++	+
D-Fructose	+	+	–
D-Galactose	+	–	–
myo-Inositol	+	–	+++
Lactose	+	–	–
D-Mannitol	+	+	–
D-Mannose	+	–	–
Raffinose	+	–	ND
Rhamnose	+	–	+
Xylose	++	+	ND

N50 value of 35526 bp, a DNA G+C content of 71.77 mol% and a coverage of 75×. It was deposited at DDBJ/ENA/GenBank under accession JAABOU000000000. The version described in this paper is version JAABOU010000000. Genome analysis by Prokka v1.14.5 revealed eight copies of the rRNA gene, 100 tRNA genes and one copy of tmRNA. The 16S rRNA gene sequence from the whole genome sequence shared 100% similarity to that from PCR sequencing, proving that the genome sequence was not contaminated.

According to the phenotypic and genotypic results, strain NRRL B-65521^T is different from the type strains of closely related species. To confirm this, the level of DNA–DNA hybridization between strain NRRL B-65521^T and the closest strains *A. bangladeshensis* DSM 45347^T and *A. mexicana* DSM 44485^T was determined by the Identification Service of the Leibniz-Institut DSMZ. Cells were disrupted by using a Constant Systems TS 0.75 KW device (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* [60]. DNA–DNA hybridization (DDH) was carried out as previously described [61, 62], under consideration of the modifications reported, using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostated 6×6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian) [63]. DNA–DNA relatedness values were examined from two independent determinations. Additionally, average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were determined by comparing the genomes of strain NRRL B-65521^T with those of *A. bangladeshensis* DSM 45347^T (SMJW01000100.1) and *A. mexicana* DSM 44485^T (FZNP01000053.1) using the ortho-ANIu algorithm from Ezbiocloud (https://www.ezbiocloud.net/tools/orthoaniu [64]) and the Genome-to-Genome Distance Calculator (GGDC 2.1) at http://ggdc.dsmz.de [65].

Based on DDH, strain NRRL B-65521^T showed DNA–DNA relatedness of 19.8±4.66% to *A. bangladeshensis* DSM 45847^T and 32.85±0.63% to *A. mexicana* DSM 44485^T; both of these values are clearly below the 70% threshold for the definition of bacterial species [62]. Consistently, the level of dDDH between NRRL B-65521^T and *A. bangladeshensis* DSM 45847^T was 29.60% and 44.00% between NRRL B-65521^T and *A. mexicana* DSM 44485^T, again significantly below the 70% threshold [62]. Similarly, the ANI values of 85.09 and 91.18% found between strain NRRL B-65521^T and *A. bangladeshensis* DSM 45847^T and *A. mexicana* DSM 44485^T, respectively, are below the threshold used to delineate prokaryote species by genome comparison [66, 67].

On the basis of the data from our polyphasic study, the GE23077-producing strain NRRL B-65521^T differs from closely related species of the genus *Actinomadura*; therefore, strain NRRL B-65521^T represents a novel species, for which the name *Actinomadura lepetitiana* sp. nov. is proposed.

DESCRIPTION OF *ACTINOMADURA LEPETITIANA* SP. NOV.

Actinomadura lepetitiana (le.pe.ti.tia'na. N.L. fem. adj. *lepetitiana* from the Lepetit Research Group, which isolated and studied the strain).

Aerobic, Gram-stain-positive actinomycete that forms extensively branched, non-fragmented pale yellow to pink vegetative mycelium on ISP2, ISP3, ISP4, ISP6, ISP7, nutrient agar, Hickey–Tresner's agar and Bennett's agar. Aerial mycelium, when present, is white to pale pink. Globose spores with verrucose surface are produced on humic acid medium. Pigments are not produced. No growth occurs above 43 °C or below 20 °C; optimal growth occurs in the range from 28 to 37 °C. Grows in the range of pH from 6.0 to 9.0 and with up to 2.5% (w/v) NaCl. Starch hydrolysis and melanin production are positive. Cellulose, cellobiose, D-glucose and D-xylose are utilized as sole carbon sources; in addition, arabinose, D-fructose, D-galactose, *myo*-inositol, lactose, D-mannitol, maltose, D-mannose, raffinose, L-rhamnose, ribose, salicin, sucrose and starch are used but the growth on them is less abundant. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The whole-cell diagnostic sugar is madurose. The predominant menaquinone is MK-9(H_6), while minor amounts of MK-9(H_4) and MK-9(H_8) are also detected. The diagnostic phospholipids are phosphatidylinositol and diphosphatidylglycerol. The major fatty acids (>10.0%) are $C_{16:0}$ and tuberculostearic acid (10 methyloctadecanoic acid).

The type strain, NRRL B-65521^T (=LMG 31258^T=DSM 109019^T), was isolated by the Lepetit Research Group from a soil sample collected in Thailand and identified as a producer of an inhibitor of bacterial RNA polymerase during a screening programme for discovering novel antibiotics. The G+C content of the genomic DNA of the type strain is 71.77 mol%. The NCBI accession number for the 16S rRNA gene sequence of strain NRRL B-65521^T is MH061375. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession JAABOU000000000. The version described in this paper is version JAABOU010000000.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This article does not contain any studies with human participants or animals performed by any of the authors.

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