Amycolatopsis Camponoti Sp. Nov., New Tetracenomycin-Producing Actinomycete Isolated from Carpenter Ant *Camponotus Vagus*

Yuliya V Zakalyukina (✉ juline@soil.msu.ru)

Lomonosov Moscow State University Faculty of Soil Science: Moskovskij gosudarstvennyj universitet imeni M V Lomonosova Fakul'tet pocvovedenia <https://orcid.org/0000-0001-8943-5761>

Ilya A Osterman

Lomonosov Moscow State University Faculty of Soil Science: Moskovskij gosudarstvennyj universitet imeni M V Lomonosova Fakul'tet pocvovedenia

Jacqueline Wolf

Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures

Meina Neumann-Schaal

Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures

Imen Nouioui

Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures

Mikhail Biryukov

Sirius University of Science and Technology

Research Article

Keywords: *Amycolatopsis camponoti* sp. nov., Novel actinobacteria, Tetracenomycin X, *Camponotus vagus*, Taxonomy

Posted Date: November 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1081009/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Antonie van Leeuwenhoek on February 26th, 2022. See the published version at <https://doi.org/10.1007/s10482-022-01716-w>.

Abstract

An actinobacterial strain A_{23}^T , isolated from adult ant *Camponotus vagus* collected in Ryazan region (Russia) and established as tetracenomycin X producer, was subjected to a polyphasic taxonomic study. Morphological characteristics of this strain included well-branched substrate mycelium and aerial hyphae fragmented into rod-shaped elements. Phylogenetic analyses based on 16S rRNA gene and genome sequences showed that strain A_{23}^T was most closely related to *Amycolatopsis pretoriensis* DSM 44654^T (99.9%). Average nucleotide identity and digital DNA–DNA hybridization values between the genome sequences of isolate A_{23}^T and its closest relative, *Amycolatopsis pretoriensis* DSM 44654^T, were 39.5% and 88.6%, which were below the 70% and 95–96% cut-off point recommended for bacterial species demarcation, respectively. The genome size of the isolate A_{23}^T is 10,560,374 bp with a DNA G+C content of 71.2 mol%. The whole-organism hydrolysates contain arabinose and galactose as main diagnostic sugars as well as ribose and rhamnose. It contained MK-9(H4) as the predominant menaquinone and *iso*-C_{16:0}, *iso*-C_{15:0}, *anteiso*-C_{17:0} and C_{16:0} as the major cellular fatty acids. Based on the phenotypic, genomic and phylogenetic data, isolate A_{23}^T represents a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis camponoti* sp. nov. is proposed, and the type strain is A_{23}^T (=DSM 111725^T =VKM 2882^T).

Introduction

The genus *Amycolatopsis* (Lechevalier et al. 1986) is a member of the family *Pseudonocardiaceae* (Franco and Labeda 2014) and encompasses 85 species validly published with *Amycolatopsis orientalis* as the type species (<https://www.bacterio.net/genus/amycolatopsis> n.d.). Member of this genus are aerobic to facultatively anaerobic and characterized by branched vegetative hyphae that undergo fragmentation into rod-like and squarish elements. Whole-organism hydrolysates are rich in meso-2,6-diaminopimelic acid, arabinose and galactose as cell sugars. The peptidoglycan is of the A1g type. Muramic acid moieties are N-acetylated. Does not contain mycolic acids. The diagnostic phospholipid is phosphatidylethanolamine and/or phosphatidylmethylethanolamine while the occurrence of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides is variable (Tan and Goodfellow 2015). *Amycolatopsis* species have been described from diverse environments such as soil, vegetation, human and animal clinical sources, fresh water, rock and subterranean sites (Lee 2009). Only five *Amycolatopsis* species are known to have pathogenic properties (Huang et al. 2004; Labeda et al. 2003; Majumdar et al. 2006).

The 16S rRNA gene phylogeny provides a useful framework for inferring the relationships between genera in the *Pseudonocardiaceae* family, but did not seem to have enough taxonomic resolution to distinguish species. The use of multi-locus sequence analysis (MLSA) is able to clarify the taxonomic positions of *Amycolatopsis* species (Glaeser and Kämpfer 2015). The most reliable way to identify actinobacteria strains is the comparison of genomes from the point of view of DNA–DNA relatedness and construction whole-genome phylogenetic trees (Nouioui et al. 2018).

The studies of publicly available complete genome sequences of 117 *Amycolatopsis* strains (<https://www.ncbi.nlm.nih.gov/assembly> n.d.) have revealed that *Amycolatopsis* have comparatively large genomes from nearly 5.96 Mb (*Amycolatopsis arida* DSM 45648^T) to 10.86 Mb (*Amycolatopsis balhimycina* DSM 44591^T), GC content from 67,8% (*Amycolatopsis palatopharingis* DSM 44832^T) to 72,7% (*Amycolatopsis arida* DSM 45648^T) and circular chromosomes containing over 20 secondary metabolic gene clusters (Kumari, Singh, and Lal 2016).

The genus *Amycolatopsis* comprises a large group of commercially and medically important actinobacteria capable of producing two major types of antibiotics: glycopeptides and polyketides [12]. In this paper, we describe strain A₂₃^T, isolated from body of ant *Camponotus vagus* collecting in Ryazan region, Russia. This strain is able to produce the aromatic polyketide antibiotic tetracenomycin X (TcmX) and its new congener 6-hydroxytetracenomycin X (6-OH-Tcm X) which possess antimicrobial and cytotoxic activity (Osterman et al. 2020). As recently shown TcmX and 6-OH-Tcm X are potent inhibitors of protein synthesis due to the fact that they bind to the large subunits of prokaryotic or eukaryotic ribosomes, within the polypeptide exit tunnel (Osterman et al. 2020).

Polyphasic studies were used to clarify the taxonomic position of novel strain A₂₃^T. We propose to establish this strain as a novel species of the genus *Amycolatopsis*, with the name *Amycolatopsis camponoti* sp. nov.

Materials And Methods

Collection and microbial isolation

Strain A₂₃^T was isolated from bodies of adult workers of *Camponotus vagus* collecting in Kasimovsky District, Ryazan region, Russia (55.01138 N, 41.73078 E) (Zakalyukina, Zaytsev, and Biryukov 2021). Five individuals were washed three times in sterile distilled water and then crashed by tissue microhomogenizer with sterile saline solution. Aliquots of this mixtures were spread over M490 medium (HiMediaLab) supplemented nystatin (250 µg/mL) and nalidixic acid (10 µg/mL) and incubated for 14 days at 28°C (Zakalyukina et al. 2021). Actinobacteria isolates were purified and preserved as suspension of mycelial fragments and spores in 1:1 v/v mixture Organic medium 79 (medium DSMZ 426 (<https://www.dsmz.de/collection/catalogue/microorganisms/culture-technology/list-of-media-for-microorganisms> n.d.)) and 20% glycerol at -20°C.

16S rRNA phylogeny

Genomic DNA of strain A₂₃^T was extracted and 16S rRNA gene amplification was performed using actinomycete primers of 243F (5'-GGATGAGCCCGCGGCCTA-3') and A3R (5'-CCAGCCCCACCTTCGAC-3') (Zakalyukina et al. 2021). The 16S rRNA gene sequencing was performed with kit ABI PRISM® BigDye™ Terminator v. 3.1 and analyzed by Applied Biosystems 3730 DNA Analyzer in Center of Collective Use «Genome», Russia.

The obtained contigs were edited and assembled by GeneStudio software (Version 2.2.0.0). The 16S rRNA gene sequence of strain A₂₃^T was compared with the sequences of type strains available in the EzBioCloud server (www.ezbiocloud.net). Multiple sequences were aligned by using CLUSTAL W(Thompson, Higgins, and Gibson 1994) software and phylogenetic trees were reconstructed by using MEGA software version X(Kumar et al. 2018) (<https://www.megasoftware.net>). The neighbour-joining and maximum-likelihood algorithms(Nei, Kumar, and Takahashi 1998) were used to reconstruct phylogenetic trees with bootstrap analysis based on 1000 replicates.

Genome Features and Phylogenomic analysis

Genome of strain A₂₃^T was sequenced de novo by Novogene Co., Ltd. (<https://en.novogene.com/>), using the Illumina NovaSeq 6000 platform and fully annotated using RAST prokaryotic genome annotation service (<https://rast.nmpdr.org/>) and submitted in GenBank (assembly accession: GCA_902497555.1) (Osterman et al. 2020).

Average nucleotide identity (ANI) (Rodríguez-R and Konstantinidis 2016) and *in silico* digital DNA:DNA hybridization (DDH) were calculated using JSpecies WS (<http://jspecies.ribohost.com/jspeciesws/>), and GGDC method, with the recommended formula 2, available at the TYGS web service, respectively (Meier-Kolthoff and Göker 2019).

Phylogenomic analysis was performed using the autoMLST webserver (<https://automlst.ziemertlab.com>) with “Run ModelFinder” and “Filter inconsistent MLST genes” options (Alanjary, Steinke, and Ziemert 2019). Then aligned multigenic sequences of 98 housekeeping genes of strain A₂₃^T and its closest *Amycolatopsis* neighbors were imported into MEGA software version X, and the resulted phylogenetic tree was constructed using the Maximum Likelihood method (Felsenstein 1985) and Tamura-Nei model (Tamura, Nei, and Kumar 2004).

Phenotypic characterization

Cultural characteristics of strain A₂₃^T were observed on the range of ISP2-ISP7 media (Shirling and Gottlieb 1966) after cultivation up to 14 days at 28°C. Morphological characteristics of strain A₂₃^T on DSMZ 426 broth medium after cultivation at 28°C for 10 days were studied by scanning electron microscope (JSM-6380LA, JEOL).

Carbon source utilization was assessed on basal media (Shirling and Gottlieb 1966) with addition of 0.04% solution of bromocresol purple at 28°C for 14 days. Nitrogen source utilization and enzyme activities were estimated using paper indicator system (MicroGene, Russia) according to the manufacturer's recommendations. The growth at different range of temperature (5°C, 10°C, 20°C, 30°C, 40°C), pH (pH 5.0, pH 6.0, pH 7.0, pH 8.0, pH 9.0) and salinity (1%, 5%, 8%) were assessed on DSMZ 426 broth media (<https://www.dsmz.de/collection/catalogue/microorganisms/culture-technology/list-of-media-for-microorganisms> n.d.) and basal media (Shirling and Gottlieb 1966) after 14 days of incubation.

Chemotaxonomy

Cell biomass of strains A_{23}^T and *A. pretoriensis* DSM 44654^T were obtained from cultures grown in Org79 and DSMZ 554 broth media and on a rotary shaker (180 r.p.m) at 28°C, respectively. After 120-hours growth cells were centrifuged, trice washed in distilled water and freeze-dried. Whole-cell sugars (Lechevalier and Lechevalier 1970), (Staneck and Roberts 1974), menaquinone (Collins et al. 1985) and diaminopimelic acids analyses (Schleifer and Kandler 1972) were carried out. Cellular fatty acids extracts were prepared using minor modifications of the protocol of Miller (Miller 1982) and Kuykendall et al. (Kuykendall et al. 1988). Gas chromatography (Agilent 6890 N instrument) was used to analyse the fatty acid methyl esters which were identified using Sherlock Microbial Identification system (MIDI, Microbial ID, Newark, DE 19711 U.S.A.) and the Actin6 database (Sasser M 1990).

Analysis of bioactive compound biosynthetic gene clusters

Secondary metabolite biosynthetic gene clusters in complete genome strain A_{23}^T (CABVGP010000000) and *A. pretoriensis* DSM 44654^T (GenBank Accessions FNUJ010000000) were identified with the bacterial version of antiSMASH 6.1.0 (<https://antismash.secondarymetabolites.org/>). Homologous regions on each genome were identified using NCBI Blastn (<https://blast.ncbi.nlm.nih.gov/>).

Results

Strain A_{23}^T showed 16S rRNA gene sequence similarity of 99.9% with *Amycolatopsis kentuckyensis* NRRL B-24129^T, *Amycolatopsis lexingtonensis* NRRL B-24131^T, *Amycolatopsis pretoriensis* DSM 44654^T, *Amycolatopsis tolypomycina* DSM 44544^T, *Amycolatopsis vancoreismycina* DSM 44592^T and *Amycolatopsis vastitatis* H5^T. The phylogenetic tree based on 16S rRNA gene sequences revealed that strain A_{23}^T formed a poorly supported distinct branch within the evolutionary radiation of the genus *Amycolatopsis* (Fig. S1).

Phylogenomic analysis based on concatenated sequences of 98 housekeeping genes showed that strain A_{23}^T formed a well-supported monophyletic clade with *A. pretoriensis* DSM 44654^T with 100% bootstrap value (Fig. 1).

The complete genome size of strain A_{23}^T was 10,560,374 bp with G+C content of 71.17 mol%, which was consistent with the G+C content of the genus *Amycolatopsis* (Nouioui et al. 2018). Similar genome features were observed for the closest neighbor *A. pretoriensis* DSM 44654^T (genome size 10,299,026 bp and G+C content 71.2 mol%). The general properties of the sequenced genomes of strain A_{23}^T are summarized in Table 1.

The ANI and *in silico* DDH values between strain A_{23}^T and strain DSM 44654^T as well as the other related species of the genus *Amycolatopsis* were 39.5% and 88.6%, below the recommended thresholds of 95-

96% and 70% for species demarcation, respectively, (Table S2) (Meier-Kolthoff and Göker 2019), (Ciufu et al. 2018).

Table 1
General features of the genome of *Amycolatopsis* strain A₂₃^T (1) and *A. pretoriensis* DSM 44654^T (2).

Genomic features	1	2
Size (bp)	10,560,374	10,299,026
Average ORF size (bp)	941.82	958.31
Total gene	10054	9886
Scaffolds	6	31
Contigs	6	31
G+C content (%)	71.17	71.17
No. of rRNA clusters	12	11
No. of tRNA clusters	58	52
Contig N50	3,441,384	828,924
Contig L50	2	5
No. proteins	9936	9807

Cells of strain A₂₃^T were aerobic, Gram-positive, non-motile. This strain showed a good growth on ISP2-ISP5 and moderately on ISP6, ISP7. The colors of substrate mycelia varied from pale yellow to reddish and brownish while aerial hyphae usually were white (Table 2). The branching mycelia (diameter 0.42 μm) fragmented into different lengths rod-shaped elements (Fig. 2), which is typically observed for *Amycolatopsis* genus (Franco and Labeda 2014).

Table 2

Cultural characteristics of strain A₂₃^T and its closely related phylogenetic neighbor *A. pretoriensis* DSM 44654^T

Media	1	2 ¹
<i>Yeast extract-malt extract (ISP 2)</i>		
Growth	Good	Good
Aerial spore-mass colour	White	Cream
Substrate mycelial colour	Yellow	Honey yellow
Soluble pigment	n/d ²	None
<i>Oatmeal (ISP 3)</i>		
Growth	Very good	Good
Aerial spore-mass colour	White	Cream
Substrate mycelial colour	Yellow	Yellow
Soluble pigment	Brownish	None
<i>Inorganic salts-starch (ISP 4)</i>		
Growth	Good	Good
Aerial spore-mass colour	White	Cream
Substrate mycelial colour	Pale yellow	Pale yellow
Soluble pigment	None	None
<i>Glycerol-asparagine (ISP 5)</i>		
Growth	Good	Good
Aerial spore-mass colour	White	None
Substrate mycelial colour	Reddish-yellow	Beige
Soluble pigment	Reddish	None
<i>Peptone-yeast extract iron (ISP 6)</i>		
Growth	Moderate	Sparse
Aerial spore-mass colour	White	None

¹ Data for reference species 2 are from the BacDive website (<https://bacdiver.dsmz.de/strain/13247>)

² n/d, not to distinguish.

Media	1	2 ¹
Substrate mycelial colour	Brownish-yellow	Beige
Soluble pigment	Yellow	None
<i>Tyrosine (ISP 7)</i>		
Growth	Moderate	Good
Aerial spore-mass colour	White	None
Substrate mycelial colour	Yellow	Beige
Soluble pigment	Lemon-yellow	None
¹ Data for reference species 2 are from the BacDive website (https://bacdiver.dsmz.de/strain/13247)		
² n/d, not to distinguish.		

The optimum growth temperature and pH of strain A₂₃^T were 28-30°C and pH 7, but it was able to grow between pH 6.0–9.0 and up to NaCl 5.0% (w/v). Strain A₂₃^T produced acid from glucose, fructose, sucrose, inositol, D-galactose, lactose, maltose, D-mannitol, D-xylose, L-arabinose, D-sorbitol, L-rhamnose and weakly from raffinose. No changes of pH-indicator color were observed on media with salicin, dulcitol and adonitol. Peptonization of gelatin as well as decomposition of citrate, urea and malonate were positive. Also strain A₂₃^T demonstrated noticeable urease activity, the ability to use mannitol but it was unable to hydrolyze salicin (Table 3).

Table 3

Physiological properties of strain A₂₃^T (1) and the closest-related phylogenetic neighbor *A. pretoriensis* DSM 44654^T (2)

Property	1	2
Decomposition of:		
Casein	+	+
Gelatin	+	+
Starch	-	-
Urea	+	w
Acid from:		
Adonitol	-	-
Arabinose	+	+
Fructose	+	+
Dulcitol	-	-
Galactose	+	+
Glucose	+	+
Inositol	+	+
Lactose	+	+
Maltose	+	+
Mannitol	+	-
Raffinose	w	+
Rhamnose	+	+
Salicin	-	+
Sorbitol	+	w
Sucrose	+	+
Xylose	+	+
Growth in/at:		

Data for reference species 2 are from the BacDive website (<https://bacdiver.dsmz.de/strain/13247>)

Property	1	2
1% NaCl	+	+
5% NaCl	w	+
8% NaCl	-	-
10°C	w	-
45°C	-	-
Citrate utilization	+	n/d
Malonate utilization	+	n/d
β-glucosidase	+	n/d
Data for reference species 2 are from the BacDive website (https://bacdive.dsmz.de/strain/13247)		

The whole-cell hydrolysates of strain A_{23}^T contained *meso*-diaminopimelic acid, arabinose, galactose, ribose and a trace of rhamnose as whole-cell sugars. However, strain DSM 44654^T had arabinose and galactose as the major cell sugars while ribose and rhamnose were present in traces. Both strains showed similar isoprenoid quinone profile with MK-9(H4) as the predominant one (Table S2).

The major fatty acids (>10%) for strain A_{23}^T were *iso*-C_{16:0}, *iso*-C_{15:0}, *anteiso*-C_{17:0} and C_{16:0} while the type strain of *A. pretoriensis* species had *iso*-C_{16:0} and *iso*-C_{15:0} (Table S3).

Online tool antiSMASH predicted 32 secondary metabolite gene clusters in the genome of strain A_{23}^T (Table S4) and 30 ones in *A. pretoriensis*. In total, from A_{23}^T genome it was identified 11 PKS clusters, 10 NRPS and gene NRPS-like clusters, 2 RiPPs and 4 terpenes, while from DSM 44654^T there were 10, 10, 2 and 4 accordingly.

Both A_{23}^T and *A. pretoriensis* DSM 44654^T genome contained genes responsible for the production limazepines A, C-F (Fotso et al. 2009). These limazepines' clusters were identical to that in strain *Streptomyces* sp. ICBB 8177, and showed also 82% of genes similarity with the sequence of tomaymycin in strain *Streptomyces achromogenes* (Li et al. 2009) (Fig. 3).

Macrotermycin biosynthetic gene clusters with 96% similarity to that of *Amycolatopsis* strain sp. M39 (Beemelmans et al. 2017) were found in the genome of strains A_{23}^T and DSM 44654^T (Fig. S4).

Previously described tetracenomycin biosynthesis gene cluster and its paralogous Tcm2 cluster (Osterman et al. 2020) were detected in 1.7 and 2.5 regions of genome A_{23}^T accordingly. Despite the relatively closeness, no Tcm clusters were detected in the genome sequence of strain DSM 44654^T.

Discussion

Amycolatopsis strains are well known as producers for the commercially used vancomycin and other glycopeptide antibiotics as well as for the production of the ansamycin rifamycin. Other compounds with antibacterial, antifungal or antiviral properties that have been derived from *Amycolatopsis* strains are quartromycin, octacosamicin, chelocardin, kigamicin and the macrotermycins A-D (Kumari et al. 2016), (Beemelmans et al. 2017), (Chen et al. 2016).

It has been shown that there is a relationship between the distribution of biosynthetic gene clusters (BGCs) and phylogenetic lineages in *Amycolatopsis* genomes (Adamek et al. 2018). *Amycolatopsis* type strains that produce, or have the potential to produce, a particular class of antibiotic are phylogenetically related. Owing to this phylogenetic clustering, it is possible to predict the antibiotic-production ability of a novel *Amycolatopsis* strain by its association in the tree, constructed with the antibiotic biosynthetic gene sequences. It should be noted that the presence of these genes does not necessarily mean that the strains will produce the antibiotic. The genes may not be expressed at all in the strain (silent genes) or may only be expressed under specific conditions (Kisil, Efimenko, and Efremenkova 2021).

Thus, a phylogenetic characterization, together with a genome analysis will allow to evaluate the prospects of new *Amycolatopsis* strains from the point of view of the synthesis of biologically active substances.

In an ongoing high-throughput screen of synthetic and natural products it was detected the presence of a translation inhibitor in the culture media of the unclassified *Amycolatopsis* sp. A₂₃ (Osterman et al. 2020). The active compound was purified, analyzed and identified as aromatic polyketide antibiotic tetracenomycin X (TcmX). Furthermore, bioinformatic analysis validated the presence of the gene cluster for TcmX biosynthesis in complete genome of *Amycolatopsis* sp. A₂₃ (Osterman et al. 2020). TcmX binds to the large subunit, within the polypeptide exit tunnel thereby inhibiting protein synthesis. Possession antimicrobial and cytotoxic activity as well as distinct mode of action make this relatively narrow class of aromatic polyketides promising for drug development.

Thus, the revealed ability of strain A₂₃^T to produce bioactive compounds calls to clarify its taxonomic position.

Paiwise 16S rRNA gene sequence similarity was 99% between strain A₂₃^T and a wide range of *Amycolatopsis* type strains (Figure 1). Genome based phylogeny showed that strain A₂₃^T is closely related to *Amycolatopsis pretoriensis* DSM 44654^T forming together a well-supported subclade. (Figure 2). The dDDH and ANI values between the studied strain and its relatives were below the recommended threshold for prokaryotic species demarcation. In addition, morphological, physiological and chemotaxonomic properties distinguished strain A₂₃^T from its close neighbour strain DSM 44654^T (Tables 2, 3, S2, S3). Therefore, isolate A₂₃^T merits to be assigned as a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis camponoti* sp. nov. is proposed.

The secondary metabolite genes of actinobacteria are mainly organized into biosynthetic gene clusters (BGCs), which encoding for closely related biosynthetic pathways that produce highly similar chemical compounds (Adamek et al. 2018). All of the *Amycolatopsis* strains possess a small core-genome (a common set of genes for all strains of this taxon), but have a huge pan-genome indicating a great potential for the production of secondary metabolites. Polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) gene clusters are main types of BGCs responsible for the synthesis the majority of antibiotics. The most part of these clusters are unique to *Amycolatopsis* genus, located mainly in the non-core region (Adamek et al. 2018). The genome of strains A₂₃^T encompasses the tetracenomycin clusters and is rich in BGCs associated with PKS, NRPS, NRPS-like clusters, RiPPs and terpenes (Table S4).

The genome mining of strain A₂₃^T revealed that this strain has the potential to produce wide range of secondary metabolites including limazepines A, C-F and macrotermycins A-D (Table S4). The limazepines belong to the growing group of the pyrrolo[1,4]benzodiazepine antitumor antibiotics. Their inherent antitumor and antibacterial activities are due to their ability to regulate gene expression by recognizing and binding with DNA GC base pairs (Fotso et al. 2009). Macrotermycins A-D are 20-membered glycosylated polyketide macrolactams's had antibacterial and also selective antifungal activity and were isolated from a termite-associated actinomycete, *Amycolatopsis* sp. M39 (Beemelmans et al. 2017). The DSM 44654^T genome contained the same clusters encoded limazepines and macrotermycins, supporting the view that phylogenetic similarity implies the presence of closely related biosynthetic pathways (Adamek et al. 2018).

Many studies of the actinobacterial symbionts of insects have repeatedly led to discovery of biologically active natural products (Beemelmans et al. 2017), as well as new taxa in Actinobacteria. The complex system of relationships between prokaryotes with their fast and flexible metabolism and insect hosts still promises a very wide scope for new discoveries.

Conclusions

Based on phenotypic, phylogenetic and genomics analyses, strain A₂₃^T, producer of a valuable substance tetracenomycin X, is considered as a type strain of a novel species with the proposed name, *Amycolatopsis camponoti*.

Description of *Amycolatopsis camponoti* sp. nov.

Amycolatopsis camponotii (cam.po.no'ti. N.L. gen. n. camponoti of *Camponotus*, referring to the insect *Camponotus vagus* Scopoli, from which the type strain was isolated).

Aerobic, Gram-stain-positive, non-motile and filamentous actinobacteria. The aerial mycelia fragments into rod-shaped fragments (0.42 µm in diameter). Well-developed substrate mycelium varies from pale yellow to brownish or reddish yellow, and the color of aerial mycelium usually is white on most media.

When growing for three weeks in some liquid media, it produces soluble pigments that ranges from faintly brown to red.

The optimum growth temperature and pH of strain A_{23}^T are 28–30°C and pH 7, but it is able to grow between pH 6.0–9.0 and up to NaCl 5.0% (w/v). The strain metabolizes L-arabinose, fructose, D-galactose, glucose, inositol, lactose, maltose, D-mannitol, L-rhamnose, D-sorbitol, suchrose, and D-xylose and able to degrade gelatin and produce urease. Additional phenotypic features are listed in Table 3. The whole-cell hydrolysates are rich in *meso*-diaminopimelic acid, arabinose, galactose, ribose and a trace of rhamnose as cell sugars. Major cellular fatty acids (>10%) are *iso*-C_{16:0} (21,87%), *iso*-C_{15:0} (18,41%), *anteiso*-C_{17:0} (11,48%) and C_{16:0} (10,38%). The predominant menaquinone is MK-9(H4), while MK-9(H2) and MK-8(H4) are present as minor components.

The type strain is A_{23}^T (=DSM 111725^T=VKM 2882^T), isolated from bodies of ants *Camponotus vagus* in Ryazan region, Russia. The genome size of the isolate A_{23}^T is 10,560,374 bp with a DNA G+C content of 71.2 mol%. The GenBank accession number for the 16S rRNA gene sequence and the genome assembly of strain A_{23}^T are KY952635 and GCA_902497555, respectively.

Declarations

Acknowledgments The authors are indebted to Marlen Jando and Gabriele Pötter (Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) for support with the growth cultures.

Authors' Contributions YVZ carried out the experiments, visualization, analysis the data, and writing draft manuscript, IAO conceptualized the project, JW, MN-S carried out chemotaxonomical assays, IN corrected and reviewed the draft, MVB supervised the project. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by Ministry of Science and Higher Education of the Russian Federation (Agreement № 075-15-2021-1085) and in part by Sirius University.

Data Availability Statement The GenBank accession number for the 16S rRNA gene sequence and the genome assembly of strain A_{23}^T are KY952635 and GCA_902497555, respectively. Detailed data on genome analysis A_{23}^T are presented IMG/M DataBase (<https://gold.jgi.doe.gov/>).

Conflicts of Interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

References

1. Adamek M, Alanjary M, Sales-Ortells H, Goodfellow M, Bull AT Anika Winkler, Daniel Wibberg, Jörn Kalinowski, and Nadine Ziemert. 2018. "Comparative Genomics Reveals Phylogenetic Distribution Patterns of Secondary Metabolites in Amycolatopsis Species." *BMC Genomics* 19(1). doi: 10.1186/s12864-018-4809-4
2. Alanjary M, Steinke K, Ziemert N (2019). "AutoMLST: An Automated Web Server for Generating Multi-Locus Species Trees Highlighting Natural Product Potential.. " *Nucleic Acids Res* 47(W1):W276–W282. doi: 10.1093/nar/gkz282
3. Beemelmans C, Ramadhar TR, Kim KH, Klassen JL, Cao S, Wyche TP, Hou Y, Poulsen M, Bugni TS, Currie CR, and Jon Clardy (2017). "Macrotermycins A–D, Glycosylated Macrolactams from a Termite-Associated *Amycolatopsis* Sp. M39.. " *Org Lett* 19(5). doi: 10.1021/acs.orglett.6b03831
4. Bergey's Manual® of Systematic Bacteriology (2012) *Bergey's Manual® of Systematic Bacteriology*. edited by M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K. Suzuki, W. Ludwig, and W. B. Whitman. New York, NY: Springer New York
5. Chen Su, Wu Q, Shen Q, Wang H (2016). "Progress in Understanding the Genetic Information and Biosynthetic Pathways behind Amycolatopsis Antibiotics, with Implications for the Continued Discovery of Novel Drugs.. " *ChemBioChem* 17(2):119–128. doi: <https://doi.org/10.1002/cbic.201500542>
6. Ciufu S, Kannan S, Sharma S, Badretdin A, Clark K, Turner S, Brover S, Schoch CL, Kimchi A, DiCuccio M (2018). "Using Average Nucleotide Identity to Improve Taxonomic Assignments in Prokaryotic Genomes at the NCBI.. " *Int J Syst Evol MicroBiol* 68(7):2386–2392. doi: 10.1099/ijsem.0.002809
7. Collins MD, Goodfellow M, Minnikin DE, Alderson G (1985). "Menaquinone Composition of Mycolic Acid-Containing Actinomycetes and Some Sporoactinomycetes.. " *J Appl Bacteriol* 58(1). doi: 10.1111/j.1365-2672.1985.tb01431.x
8. Felsenstein J (1985). "Confidence Limits on Phylogenies: An Approach Using The Bootstrap.. " *Evolution* 39(4):783–791. doi: <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
9. Fotso, Serge TM, Zabriskie PJ, Proteau PM, Flatt DA, Santosa, Sulastri, and Taifo Mahmud (2009). "Limazepines A–F, Pyrrolo[1,4]Benzodiazepine Antibiotics from an Indonesian *Micrococcus* Sp.. " *J Nat Prod* 72(4). doi: 10.1021/np800827w
10. Franco CMM, and David P. Labeda (2014) *The Order Pseudonocardiales*. The Prokaryotes. Springer Berlin Heidelberg, Berlin, Heidelberg
11. Glaeser SP, Peter, Kämpfer (2015). "Multilocus Sequence Analysis (MLSA) in Prokaryotic Taxonomy.. " *Syst Appl Microbiol* 38(4):237–245. doi: 10.1016/J.SYAPM.2015.03.007
12. <https://.> n.d. "<https://Www.Bacterio.Net/Genus/Amycolatopsis.”>
13. <https://www.dsmz.de/collection/catalogue/microorganisms/culture-technology/list-of-media-for-microorganisms>. n.d. "<https://Www.Dsmz.de/Collection/Catalogue/Microorganisms/Culture-Technology/List-of-Media-for-Microorganisms.”>
14. <https://.> n.d. "<https://Www.Ncbi.Nlm.Nih.Gov/Assembly.”>

15. Huang Y, Paściak M, Liu Z, Xie Q, and Andrzej Gamian (2004). "Amycolatopsis Palatopharyngis Sp. Nov., a Potentially Pathogenic Actinomycete Isolated from a Human Clinical Source.. " *Int J Syst Evol MicroBiol* 54(2):359–363. doi: 10.1099/ijs.0.02685-0
16. Kisil O, Tatiana A, Efimenko, and Olga v. Efremenkova (2021). "Looking Back to Amycolatopsis: History of the Antibiotic Discovery and Future Prospects.. " *Antibiotics* 10(10). doi: 10.3390/antibiotics10101254
17. Kumari R, Singh P, and Rup Lal (2016). "Genetics and Genomics of the Genus Amycolatopsis.. " *Indian Journal of Microbiology* 56(3):233–246. doi: 10.1007/s12088-016-0590-8
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). "MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms.. " *Mol Biol Evol* 35(6):1547–1549. doi: 10.1093/molbev/msy096
19. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE (1988). "Fatty Acids, Antibiotic Resistance, and Deoxyribonucleic Acid Homology Groups of Bradyrhizobium Japonicum.. " *Int J Syst Bacteriol* 38(4):358–361. doi: 10.1099/00207713-38-4-358
20. Labeda DP, Donahue JM, Williams NM, Sells SF, Henton MM (2003). "Amycolatopsis Kentuckyensis Sp. Nov., Amycolatopsis Lexingtonensis Sp. Nov. and Amycolatopsis Pretoriensis Sp. Nov., Isolated from Equine Placentas.. " *Int J Syst Evol MicroBiol* 53(5):1601–1605. doi: 10.1099/ijs.0.02691-0
21. Lechevalier MP, Lechevalier HA (1970). "Chemical Composition as a Criterion in the Classification of Aerobic Actinomycetes.. " *Int J Syst Evol MicroBiol* 20:435–443
22. Lechevalier MP, Prauser H, Labeda DP, Ruan JS (1986). "Two New Genera of Nocardioform Actinomycetes: Amycolata Gen. Nov. and Amycolatopsis Gen. Nov.. " *Int J Syst Bacteriol* 36(1):29–37. doi: 10.1099/00207713-36-1-29
23. Lee SD (2009). "Amycolatopsis Ultiminotia Sp. Nov., Isolated from Rhizosphere Soil, and Emended Description of the Genus Amycolatopsis.. " *Int J Syst Evol MicroBiol* 59(6):1401–1404. doi: 10.1099/ijs.0.006577-0
24. Li W, Chou ShenChieh, Khullar A, Gerratana B (2009). "Cloning and Characterization of the Biosynthetic Gene Cluster for Tomaymycin, an SJG-136 Monomeric Analog.. " *Appl Environ Microbiol* 75(9). doi: 10.1128/AEM.02325-08
25. Majumdar S, Prabhakaran SR, Shivaji S, and Rup Lal (2006). "Reclassification of Amycolatopsis Orientalis DSM 43387 as Amycolatopsis Benzoatilytica Sp. Nov.. " *Int J Syst Evol MicroBiol* 56(1):199–204. doi: 10.1099/ijs.0.63766-0
26. Meier-Kolthoff JP, Markus, Göker (2019). "TYGS Is an Automated High-Throughput Platform for State-of-the-Art Genome-Based Taxonomy.. " *Nat Commun* 10(1):2182. doi: 10.1038/s41467-019-10210-3
27. Miller LT (1982). "Single Derivatization Method for Routine Analysis of Bacterial Whole-Cell Fatty Acid Methyl Esters, Including Hydroxy Acids.. " *J Clin Microbiol* 16:584–586
28. Nei M, Kumar S, Takahashi K (1998). "The Optimization Principle in Phylogenetic Analysis Tends to Give Incorrect Topologies When the Number of Nucleotides or Amino Acids Used Is Small.. " *Proc*

- Natl Acad Sci USA 95(21):12390–12397. doi: 10.1073/pnas.95.21.12390
29. Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, Kyrpides NC, Pukall H-P, Klenk M (2018) Goodfellow, and Markus Göker. "Genome-Based Taxonomic Classification of the Phylum Actinobacteria." *Frontiers in Microbiology* 9:2007. doi: 10.3389/fmicb.2018.02007
30. Osterman IA, Wieland M, Maviza TP, Lashkevich KA, Lukianov DA, Ekaterina S, Komarova DI, Shiriaev SA, Leyn JE, Zlamal A, Skvortsov, Vadim N, Tashlitsky, Vladimir I, Polshakov J, Cheng YS, Polikanov AA, Bogdanov AL, Osterman, Sergey E, Dmitriev Roland Beckmann, Olga A. Dontsova, Daniel N. Wilson, and Petr v. Sergiev. 2020. "Tetracenomycin X Inhibits Translation by Binding within the Ribosomal Exit Tunnel." *Nature Chemical Biology* 16(10). doi: 10.1038/s41589-020-0578-x
31. Luis Rodríguez-R (2016) and Konstantinos Konstantinidis. "The Enveomics Collection: A Toolbox for Specialized Analyses of Microbial Genomes and Metagenomes."
32. Sasser M (1990) "Bacterial Identification by Gas Chromatographic Analysis of Fatty Acids Methyl Esters (GC-FAME)." *Newark, DE: MIDI Labs, Inc.*
33. Schleifer KH, and O. Kandler (1972). "Peptidoglycan Types of Bacterial Cell Walls and Their Taxonomic Implications.. " *Bacteriological Reviews* 36(4). doi: 10.1128/br.36.4.407-477.1972
34. Shirling EB, and D. Gottlieb (1966). "Methods for Characterization of Streptomyces Species.. " *Int J Syst Bacteriol* 16(3):313–340. doi: 10.1099/00207713-16-3-313
35. Stanek JL, and Glenn D. Roberts (1974). "Simplified Approach to Identification of Aerobic Actinomycetes by Thin-Layer Chromatography.. " *Appl Microbiol* 28(2). doi: 10.1128/am.28.2.226-231.1974
36. Tamura K, Nei M (2004) and Sudhir Kumar. "Prospects for Inferring Very Large Phylogenies by Using the Neighbor-Joining Method." *Proceedings of the National Academy of Sciences* 101(30):11030–35. doi: 10.1073/PNAS.0404206101
37. Thompson JD, Desmond G, Higgins, Gibson TJ (1994). "CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice.. " *Nucleic Acids Res* 22(22):4673–4680. doi: 10.1093/nar/22.22.4673
38. Zakalyukina Y, Zaytsev AR, Biryukov Mv (2021). "Study of Cellulose-Destroying Activity of Actinobacteria Associated with Ants.. " *Moscow University Biological Sciences Bulletin* 76(1). doi: 10.3103/S0096392521010065

Figures

Figure 1

Maximum-likelihood evolutionary tree based on concatenated multilocus gene sequences (a total of 91571 positions in the final dataset) of 30 *Amycolatopsis* species and strain A23T. *Nocardia farcinica*

NBRC 15532T was used as the outgroup. Numbers on branches indicate the percentage bootstrap values of 1000 replicates, only values >50% are indicated. Bar, 0.05 substitutions per nucleotide position

Figure 2

Scanning electron micrograph of strain A23T, showing aerial mycelium that fragmented into rod-shaped elements after incubation on DSMZ 426 broth medium at 28°C for 10 days

Figure 3

The BGCs in genome of *Amycolatopsis* A23T A, the structure of region 1.7 (location: 2,798,054 - 2,907,649 nt, total: 109,596 nt); B, limazepines cluster of *Streptomyces* sp. ICBB 8177; C, tetracenomycin X biosynthesis cluster

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AmycolatopsiscamponotiSupplZakalyukinaetal.docx](#)