

Actinoplanes aureus sp. nov., a novel protease-producing actinobacterium isolated from soil

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Abstract

A novel protease-producing actinobacterium, designated strain NEAU-A11^T, was isolated from soil collected from Aohan banner, Chifeng, Inner Mongolia Autonomous Region, China, and characterised using a polyphasic approach. On the basis of 16S rRNA gene sequence analysis, strain NEAU-A11^T was indicated to belong to the genus *Actinoplanes* and was most closely related to *Actinoplanes rectilineatus* JCM 3194^T (98.9 %). Cell walls contained *meso*-diaminopimelic acid as the diagnostic diamino acid and the whole-cell sugars were arabinose, xylose and glucose. The phospholipid profile contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides. The predominant menaquinones were MK-9(H₄), MK-9(H₆) and MK-9(H₈). The major fatty acids were C_{18:0}, C_{16:0}, C_{18:1} ω_{9c}, C_{17:0} and C_{15:0}. Genome sequencing revealed a genome size of 10,742,096 bp, a G + C content of 70.5 % and 9,514 protein-coding genes (CDSs), including 102 genes coding for protease. Genome mining analysis using antiSMASH 5.0 led to the identification of 20 putative gene clusters responsible for the production of diverse secondary metabolites. Phylogenetic analysis using the 16S rRNA gene sequences showed that the strain formed a stable clade with *A. rectilineatus* JCM 3194^T in the genus *Actinoplanes*. However, whole-genome average nucleotide identity (ANI) value and the levels of digital DNA-DNA (dDDH) hybridization between strain NEAU-A11^T and *A. rectilineatus* JCM 3194^T was 81.1 % and 24.6 % (22.2–27.0 %), respectively. The values were well below the criteria for species delineation of 70% for dDDH and 95–96% for ANI, suggesting that the isolate differed genetically from its closely related type strain. In addition, evidences from phenotypic, chemotaxonomic and genotypic studies indicate that strain NEAU-A11^T represents a novel species of the genus *Actinoplanes*, for which the name *Actinoplanes aureus* sp. nov. is proposed, with NEAU-A11^T (= CCTCC AA 2019063^T = JCM 33971^T) as the type strain.

Introduction

The genus *Actinoplanes* was first proposed by Couch (1950) as a member of the family *Micromonosporaceae*, with *Actinoplanes philippinensis* as the type species. Currently, there are 47 species with validly published and correct names (<https://lpsn.dsmz.de/genus/actinoplanes>), including the latest described species *Actinoplanes deserti* (Habib et al. 2018). *Actinoplanes* species are abundant sources of bioactive compounds (Parenti and Coronelli 1979; Simone et al. 2013; Iorio et al. 2014; Rückert et al. 2014; Huang et al. 2015), including antibiotics with therapeutic value such as teicoplanin (Sosio et al. 2004), and the alpha-glucosidase inhibitor acarbose which is a potent drug used worldwide in the treatment of type 2 diabetes (Schwientek et al. 2012). All members of the genus *Actinoplanes* are aerobic, Gram-stain positive, non-acid-fast actinomycetes that can form branched substrate mycelium on various agar media but scant aerial mycelia. Meanwhile, members of the genus have a very interesting life cycle and are characterised by motile spores and the presence of spherical, cylindrical, digitate, lobate, bottle or flask-shaped or very irregular sporangia (Zhang et al. 2012). Terminal sporangia containing flagellated spores are produced and open up to release the spores when contact with water and additional undefined substance(s) in soil extracts (Hayakawa et al. 1991). This process is known as dehiscence (Uchida et al.

2011; Higgins 1967). Goodfellow et al. (1990) provided the detailed phenotypic and chemotaxonomic analysis of the genus, and a comprehensive phylogenetic analysis of the genus was given by Tamura and Hatano (2001). The peptidoglycan of members within this genus contains *meso*-diaminopimelic acid, while, hydroxyl-diaminopimelic acid can also be present. The diagnostic sugar of whole-cell hydrolysates is xylose, moreover, small amounts of galactose and/or arabinose are also found. Phosphatidylethanolamine is the diagnostic phospholipid. The G+C contents of the genomic DNA vary from 67 to 76 % (Sazak et al. 2012).

During our investigation in the screening and diversity of actinomycetes in China, protease-producing strain NEAU-A11^T was obtained from continuous cropping soil of cucumbers collected from Aohan Banner, Chifeng, Inner Mongolia Autonomous Region, China. It produced hydrolytic enzymes, such as protease, may be involved in destroying the components of fungal cell wall, which is an important mechanism of the biocontrol of phytopathogenic fungi. In this study, we reported on polyphasic taxonomy of strain NEAU-A11^T and proposed this strain represents a new species of the genus *Actinoplanes*.

Materials And Methods

Isolation and maintenance of the organism

Strain NEAU-A11^T was isolated from soil collected from Aohan banner, Chifeng, Inner Mongolia Autonomous Region, China (42°29' N, 119°92' E). Before isolation, the soil sample was air-dried at room temperature for 14 days. For isolation, 5g of dried soil was diluted in sterile distilled water (45 ml), and then the soil suspension was incubated in a constant temperature shaker incubator at 28 °C and 250 g. for 30 min. Subsequently, the strain was isolated using the standard dilution plate method and grown on sodium succinate-asparagine agar (Piao et al. 2017) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 3 weeks of aerobic incubation at 28 °C, colonies were transferred and purified on oatmeal agar [International *Streptomyces* Project (ISP) medium 3] (Shirling and Gottlieb 1966) and maintained as glycerol suspensions (30 %, v/v) at -80 °C.

Phenotypic characterisation

The cultural characteristics of strain NEAU-A11^T were determined after growing for 14 days at 28 °C on various ISP (ISP 1-7) media (Shirling and Gottlieb 1966), Bennett's agar (Waksman 1967), Czapek's agar (Jones 1949) and nutrient agar (Jones 1949). ISCC-NBS color charts (Kelly 1964) were used to determine colours of aerial and substrate mycelia. Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi SU8010) using cultures grown on ISP 3 agar at 28 °C for 4 weeks as previously described method (Jin et al. 2019). Spore motility was identified by light microscopy (Nikon ECLIPSE E200) observation of cells suspended in 1 mM phosphate buffer (pH 7.0). Growth at different temperatures (4, 10, 15, 18, 25, 28, 32, 35, 37, 40 and 42 °C) was determined on ISP 3 agar after incubation for 14 days. The pH range for growth (pH 3-12, at intervals of 1 pH units) was

tested in Glucose-yeast extract broth (GY) (Jia et al. 2013) using the buffer system: pH 4.0-5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0-8.0, 0.1 M KH_2PO_4 /0.1 M NaOH; pH 9.0-10.0, 0.1 M NaHCO_3 /0.1 M Na_2CO_3 ; pH 11.0-12.0, 0.2 M KH_2PO_4 /0.1 M NaOH (Cao et al. 2020; Zhao et al. 2019). NaCl tolerance (0-12 %, with an interval of 1 %, w/v) for growth were tested after 14 days growth in GY broth at 28 °C. The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptization of milk, liquefaction of gelatin and production of H_2S were examined as described previously (Gordon et al. 1974; Yokota et al. 1993). Hydrolysis of Tweens (20, 40 and 80) and production of urease were tested as described by Smibert and Krieg (1994). The related type strain was also included for comparison in all tests. In addition, the protease was detected by observing whether the agar plate (5% skimmed milk powder, 2% agar, sterilized at 121 °C) produced clear rings.

Chemotaxonomic analyses

For the chemotaxonomic analysis, freeze-dried biomass was prepared from cultures grown in GY medium on a rotary shaker (250 g.) at 28 °C for 7 days. The isomer of diaminopimelic acid (DAP) in the cell wall hydrolysates was derivatized and analysed by a HPLC method (McKerrow et al. 2000). The whole-cell sugars were performed according to the procedures developed by Lechevalier and Lechevalier (1980). The phospholipids in cell were separated by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by a HPLC–UV method (Wu et al. 1989) using an Agilent Extend- C_{18} Column (150 × 4.6 mm, i.d. 5 μm), typically at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60:40, v/v) (Song et al. 2019). The presence of mycolic acids was checked by the acid methanolysis method of Minnikin et al. (1980). To determine cellular fatty acid compositions, strain NEAU-A11^T and its closely related strain were cultivated in GY medium in shake flasks at 28 °C for 7 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC–MS using the method of Xiang et al. (2011).

DNA preparation, amplification and determination of 16S rRNA sequences

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out according to the procedure developed by Kim et al. (2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene sequence of strain NEAU-A11^T, comprising 1480 bp, was obtained and compared with type strains available in the EzBioCloud server (<https://www.ezbiocloud.net/>) (Yoon et al. 2017a) and retrieved using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and then submitted to the GenBank database. The phylogenetic trees were constructed based on the 16S rRNA gene sequences of strain NEAU-A11^T and related reference species. Multiple sequences were aligned in Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 using the Clustal W algorithm and trimmed manually where was necessary. Phylogenetic trees were constructed with maximum likelihood (Felsenstein 1981) and neighbour-joining

(Saitou and Nei 1987) algorithms using MEGA 7.0 (Kumar et al. 2016). The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions (Felsenstein 1985). A distance matrix was generated using Kimura's two-parameter model (Kimura 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the EzBioCloud (Yoon et al. 2017a).

Genomic analysis, DNA-DNA hybridization and DNA G+C content

The genomic DNA of strain NEAU-A11^T was extracted by SDS method for genome sequencing and assembly. The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit. Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. A-tailed, ligated to paired-end adaptors and PCR amplified with a 350 bp insert was used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd. Illumina PCR adapter reads and low quality reads from the paired-end were filtered by the step of quality control using our own compiling pipeline. All good quality paired reads were assembled using the SOAP denovo (Li et al. 2010; Li et al. 2008) (<http://soap.genomics.org.cn/soapdenovo.html>) into a number of scaffolds. Then the filter reads were handled by the next step of the gap-closing. Whole-genome phylogeny was generated using TYGS server (<http://tygs.dsmz.de>) (Meier-Kolthoff and Göker 2019). The dDDH and ANI values were determined between the genomes of strain NEAU-A11^T and *A. rectilineatus* JCM 3194^T online at <http://ggdc.dsmz.de> using the Genome-to-Genome Distance Calculation (GGDC 2.1) (Meier-Kolthoff et al. 2013) and the ChunLab's online ANI Calculator (www.ezbiocloud.net/tools/ani) (Yoon et al. 2017b), respectively. The DNA G+C content was calculated from the genome sequence. "Antibiotics and secondary metabolite analysis shell" (antiSMASH) version 5.0 was employed to analyze the bioactive secondary metabolites (Blin et al. 2019).

Results And Discussion

Phenotypic characteristics

Morphological observation of 4-week-old culture of strain NEAU-A11^T grown on ISP 3 medium showed that it had the typical characteristics of the members of the genus *Actinoplanes*. Strain NEAU-A11^T was observed to produce branched, non-fragmenting substrate hyphae. The sporangia were 4.6-8.7 µm in size (Fig. S1) with irregular shape similar to *Actinoplanes nipponensis* (Wink et al. 2014), *Actinoplanes lichenis* (Phongsopitanun et al. 2016) and *Actinoplanes luteus* (Suriyachadkun et al. 2015). The sporangiospores were motile. Strain NEAU-A11^T was found to grow well on ISP 2, ISP 3, ISP 4, ISP 7 and nutrient agar media; grow moderately on ISP 6 medium; grow poorly on ISP 1, ISP 5 and Czapek's agar media; but no growth occurred on Bennett's agar medium. Moderate olive and strong yellow pigments were observed on ISP 6 and nutrient agar, respectively. The summary of cultural characteristics of strain NEAU-A11^T and its phylogenetic neighbors were shown in Table S1 for comparative analysis. The strain was found to grow at a temperature range of 18-37 °C (optimum temperature 28 °C), pH 6-9 (optimum

pH of 7) and NaCl tolerance of 0-4 %. The physiological and biochemical properties of strain NEAU-A11^T are shown in Table 1.

Chemotaxonomic characteristics

All the chemotaxonomic data were consistent with the assignment of strain NEAU-A11^T to the genus *Actinoplanes*. The isolate was determined to contain *meso*-diaminopimelic acid in the cell wall. Whole-cell sugars contained arabinose, glucose and xylose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides (Fig. S2). The menaquinones were MK-9(H₈) (46.3 %), MK-9(H₆) (41.0 %) and MK-9(H₄) (12.7 %). The cellular fatty acid profile was composed of C_{18:0} (19.5 %), C_{16:0} (16.5 %), C_{18:1} ω9c (13.4 %), C_{17:0} (13.2 %), C_{15:0} (12.0 %), *iso*-C_{15:0} (9.7 %), C_{17:1} ω8c (7.0 %), *iso*-C_{17:0} (3.6 %), *anteiso*-C_{15:0} (2.0 %), C_{16:1} ω9c (1.8 %), *iso*-C_{18:0} (0.8 %), and *iso*-C_{16:0} (0.5 %). Mycolic acids were not found.

Molecular characteristics

EzBioCloud analysis based on 16S rRNA gene sequence (1480 bp; GenBank/EMBL/DDBJ accession number MW272536) indicated that strain NEAU-A11^T should be affiliated to the genus *Actinoplanes* and possessed high 16S rRNA gene sequence similarity to *A. rectilineatus* JCM 3194^T (98.9 %). Phylogenetic analysis using the 16S rRNA gene sequence showed that the strain formed a stable clade with *A. rectilineatus* JCM 3194^T in the neighbour-joining tree (Fig. 1). This relationship was also observed in the maximum-likelihood tree (Fig. S3). However, whole-genome phylogeny showed strain NEAU-A11^T formed a stable phyletic line with *Actinoplanes lutulentus* DSM 45883^T (97.6 %) (Figure 2). Based on the 16S rRNA gene sequence similarities and phylogenetic analysis, *A. rectilineatus* JCM 3194^T and *A. lutulentus* DSM 45883^T, were selected as the reference strains for comparative analysis.

The assembled genome sequence of strain NEAU-A11^T was found to be 10,742,096 bp long, composed of 109 scaffolds with an N50 of 187,467 bp and a DNA G+C content of 70.5 %. The draft genome sequence was deposited at the GenBank/EMBL/DDBJ under the accession number JADQTO000000000. NCBI Prokaryotic Genome Annotation Pipeline (PGAP) revealed four copies of the 5S rRNA genes, one copy of the 16S rRNA gene, five copies of the 23S rRNA genes, 69 tRNA genes, three copies of noncoding RNA genes and 9,514 protein-coding genes (CDSs). Detailed genomic information and other general features of genome sequences are shown in Table S2. Genome analysis showed that strain NEAU-A11^T contained 255 glycoside hydrolases (GHs), 152 glycosyl transferases (GTs), 40 carbohydrate esterases (CEs), 26 polysaccharide lyases (PLs), and 12 auxiliary activities (AAs) genes. Meanwhile, it was also showed that strain NEAU-A11^T contained 303 biosynthesis genes of antibiotics, 18 prodigiosin biosynthesis genes, 17 streptomycin biosynthesis genes, 8 biosynthesis genes of enediyne antibiotics, 6 penicillin and cephalosporin biosynthesis genes, 5 biosynthesis genes of ansamycins, 4 biosynthesis genes of vancomycin group antibiotics, 4 novobiocin biosynthesis genes, 3 acarbose and validamycin

biosynthesis genes, 2 carbapenem biosynthesis genes, 2 isoquinoline alkaloid biosynthesis genes, 2 neomycin, kanamycin and gentamicin biosynthesis genes and 1 flavonoid biosynthesis gene. Genome mining analysis using antiSMASH 5.0 led to the identification of 20 putative gene clusters responsible for the production of diverse secondary metabolites. There were 17 gene clusters displayed very low similarity to the known gene clusters of xantholipin, azinomycin B, dynemicin A, oligomycin, diazepinomicin, himastatin, azalomycin F3a, mediomycin A and so on. Therefore, strain NEAU-A11^T has great potential to produce novel bioactive compounds. The other three biosynthetic gene clusters with more than 50 % similarity to the known clusters were related to the production of alkyl-*O*-dihydrogeranyl-methoxyhydroquinones, citrulassin E and desferrioxamin B. Especially, one putative gene cluster shared 100 % similarity to the reported type III polyketide biosynthetic gene cluster of alkyl-*O*-dihydrogeranyl-methoxyhydroquinones in *Actinoplanes missouriensis* (Awakawa et al. 2011). Hence, it is speculated that strain NEAU-A11^T had significant potential to be a rich source for producing various bioactive compounds. In addition, 102 protease (such as YP_003344293, YP_008738346, YP_003836524, YP_007213461, YP_003300042) genes were identified in the genome of NEAU-A11^T. Moreover, the results of agar plates bioassay showed that strain NEAU-A11^T had the ability to produce protease (Fig. S4). Proteases may be involved in the biological control of plant-pathogenic fungi because they may play an important role in fungal cell wall lysis (Liu et al. 2019). Therefore, strain NEAU-A11^T is a potential biological control agent.

Digital DNA-DNA hybridization and ANI values were employed to further clarify the relatedness between strain NEAU-A11^T and *A. rectilineatus* JCM 3194^T and *A. lutulentus* DSM 45883^T. The levels of digital DNA-DNA hybridization between them was 24.6 % (22.2-27.0 %) and 24.8 % (22.5-27.3 %), respectively, which was below the threshold value of 70 % recommended by Wayne et al. (Wayne et al. 1987) for assigning strains to the same genomic species. Similarly, the low ANI values between strain NEAU-A11^T and its reference strains *A. rectilineatus* JCM 3194^T and *A. lutulentus* DSM 45883^T were found to be 81.1 % and 81.6 %, respectively, a result below the threshold used to delineate prokaryote species (Richter and Rossello-Mora 2009; Chun and Rainey 2014).

Beside of the genotypic evidence above, strain NEAU-A11^T also can be distinguished from its closely related strains by phenotypic characteristics (Table 1). Differential cultural characteristics contain: NaCl tolerance of strain NEAU-A11^T is up to 4 % (w/v), which is higher than that of *A. rectilineatus* JCM 3194^T (3 %); and strain NEAU-A11^T could grow at the maximum temperature of 37 °C, which is lower than that of *A. rectilineatus* JCM 3194^T (42 °C) and higher than that of *A. lutulentus* DSM 45883^T (32 °C). Other phenotypic differences include: coagulation and peptization of milk, hydrolysis of Tweens (40 and 80) and aesculin, production of H₂S and utilization of d-mannitol, d-mannose, d-galactose, d-sorbitol, inositol, lactose, l-alanine, l-glutamine, l-proline, l-serine, l-threonine and maltose. Therefore, based on a combination of chemotaxonomic, morphological, molecular and physiological data, strain NEAU-A11^T represents a novel species of the genus *Actinoplanes*, for which the name *Actinoplanes aureus* sp. nov. is proposed.

Description of *Actinoplanes aureus* sp. nov.

Actinoplanes aureus (au're.us. L. masc. adj. *aureus* golden)

An aerobic, Gram-stain-positive, protease-producing actinobacterium that forms well-developed substrate mycelia carrying irregular sporangia (4.6-8.7 μm). The sporangiospores are motile. Grows well on ISP 2, ISP 3, ISP 4, ISP 7 and nutrient agar media; grows moderately on ISP 6 medium; grows poorly on ISP 1, ISP 5 and Czapek's agar media; but no growth occurs on Bennett's agar medium. Moderate olive and strong yellow pigments are observed on ISP 6 and nutrient agar, respectively. Growth occurs at pH 6-9 (optimum pH 7), temperature range of 18-37 °C (optimum 28 °C) and in the maximum presence of 4 % NaCl (w/v). Positive for hydrolysis of aesculin and starch and production of H₂S, but negative for hydrolysis of Tweens (20, 40 and 80), liquefaction of gelatin, production of urease, coagulation and peptization of milk, decomposition of cellulose and reduction of nitrate. Utilize d-glucose, lactose and raffinose as the sole carbon source for growth, while the following substrates are not used as carbon sources: d-fructose, d-galactose, d-mannitol, d-mannose, d-ribose, d-sorbitol, d-xylose, dulcitol, inositol, l-arabinose, l-rhamnose, maltose and sucrose. l-Asparagine, l-aspartic acid, l-glutamic acid and l-tyrosine are utilized as the sole nitrogen source, but not creatine, glycine l-alanine, l-arginine, l-glutamine, l-proline, l-serine or l-threonine. Cell walls contain *meso*-diaminopimelic acid as the diagnostic diamino acid and the whole-cell sugars are arabinose, xylose and glucose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides. The menaquinones are MK-9(H₈), MK-9(H₆) and MK-9(H₄). The predominant cellular fatty acids (> 10 %) are C_{18:0}, C_{16:0}, C_{18:1} ω 9c, C_{17:0} and C_{15:0}. The DNA G+C content of the type strain is 70.5 %.

The type strain is NEAU-A11^T (=CCTCC AA 2019063^T=JCM 33971^T), isolated from soil collected from Aohan banner, Chifeng, Inner Mongolia Autonomous Region, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-A11^T is MW272536. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JADQTO000000000. The version described in this paper is version JADQTO000000000.1.

Abbreviations

ANI, average nucleotide identity; BA, Bennett's agar; CA, Czapek's agar; CCTCC, China Center for type Culture Collection; dDDH, digital DNA:DNA hybridization; DPG, Diphosphatidylglycerol; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; GC-MS, Gas Chromatography-Mass Spectrometer; GY, Glucose-yeast extract medium; ISCC-NBS, Inter-society color council-national bureau of standards; ISP, International *Streptomyces* Project; JCM, Japan Collection of Microorganisms; MEGA, Molecular Evolutionary Genetics Analysis; NA, Nutrient agar; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; TLC, Thin-Layer Chromatography.

Declarations

Consent to participate and/or Consent to publish

This research doesn't involve human subjects, so the informed consent to participate and consent to publish are not obtained.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics 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.

Consent

All authors have seen a copy of the manuscript and have approved its submission.

Data availability

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-A11^T is MW272536. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JADQTO000000000. The version described in this paper is version JADQTO000000000.1.

Authors' contributions

Xiujun Sun performed the laboratory experiments, analysed the data, and drafted the manuscript. Xianxian Luo contributed to the biochemical characterisation. Chuan He and Zhenzhen Huang contributed to the polyphasic taxonomy. Junwei Zhao contributed to the fatty acids determination. Beiru He and Xiaowen Du contributed to the morphological analyses. Wensheng Xiang participated to the discussions of each section of experiments. Jia Song and Xiangjing Wang designed the experiments and revised the manuscript.

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Table

Table 1. Differential characteristics of strain NEAU-A11^T and its closely related species of the genus *Actinoplanes*. All the data from this study were obtained under the same conditions except where marked.

Strains: 1, NEAU-A11^T; 2, *A. rectilineatus* JCM 3194^T; 3. *A. lutulentus* DSM 45883^T

+, positive; –, negative; ND, no data available.

^a Data from Gao et al. (Gao et al. 2014)

Characteristics	1	2	3^a
Tolerance of NaCl (% w/v)	0-4	0-3	0-4
Temperature range (°C)	18-37	10-42	15-32
Hydrolysis of aesculin	+	-	+
Degradation of Tween 40	-	+	ND
Degradation of Tween 80	-	+	ND
Production of H ₂ S	+	-	-
Coagulation and peptization of milk	-	+	-
Liquefaction of gelatin	-	+	+
Carbon utilization			
d-galactose	-	+	+
d-mannitol	-	+	+
d-mannose	-	+	+
d-sorbitol	-	+	+
Inositol	-	+	+
Lactose	+	-	+
Maltose	-	+	+
Nitrogen utilization			
l-alanine	-	+	-
l-glutamine	-	+	+
l-proline	-	+	ND
l-serine	-	+	+
l-threonine	-	+	+

Figures

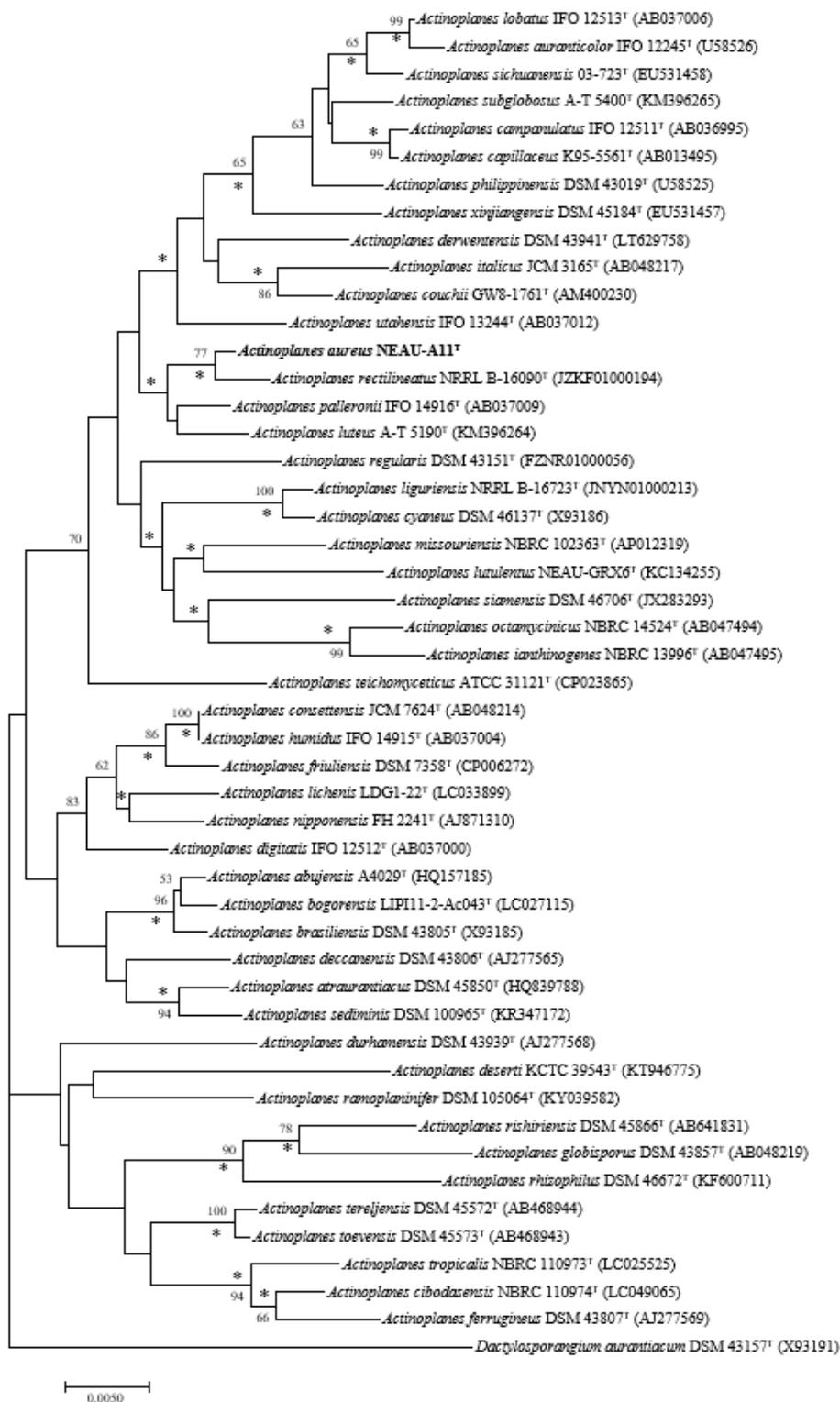


Figure 1

Neighbour-joining tree based on 16S rRNA gene sequences showing relationships between strain NEAU-A11T (1480 bp) and the 47 phylogenetically closely related representative species with validly-published names in the genus *Actinoplanes*. The out-group used was *Dactylosporangium aurantiacum* DSM 43157T. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. Asterisks

indicate branches also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

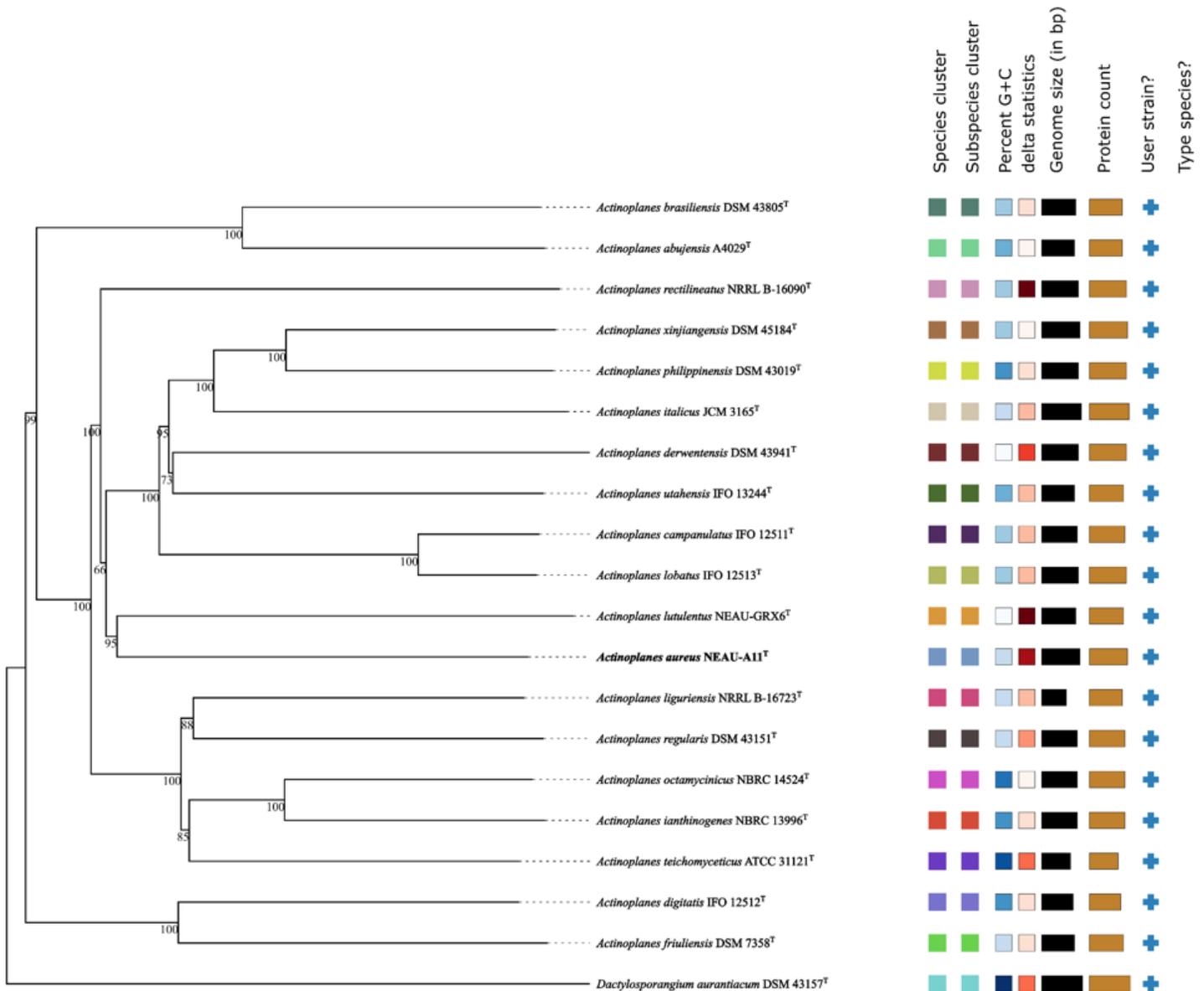


Figure 2

Whole-genome sequence tree generated with TYGS for strain NEAU-A11T and closely related species of the genus *Actinoplanes*. The out-group used was *Dactylosporangium aurantiacum* DSM 43157T. Tree inferred with FastME from GBDP distances calculated from genome sequences. Branch lengths are scaled in terms of GBDP distance formula d5; numbers above branches are GBDP pseudo-bootstrap support values from 100 replications.

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