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Pseudonocardia Pini Sp. Nov., An Endophytic Actinobacterium Isolated From Roots of the Pine Tree *Callitris Preissii*

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Abstract

A Gram positive, aerobic, actinobacterial strain with rod-shaped spores, CAP47R^T, which was isolated from the surface-sterilized root of a native pine tree (*Callitris preissii*), South Australia is described. The major cellular fatty acid of this strain was *iso*-H-$C_{16:1}$ and major menaquinone was MK-8(H_4). *The diagnostic diamino acid* in the *cell-wall* peptidoglycan was *identified* as *meso*-
diaminopimelic *acid.* These chemotaxonomic data confirmed the affiliation of strain CAP47R^T to the genus *Pseudonocardia.* Phylogenetic evaluation based on 16S rRNA gene sequence analysis placed this strain in the family

Pseudonocardiaceae, being most closely related to *Pseudonocardia xishanensis* JCM 17906^T (98.8%), *Pseudonocardia oroxyli* DSM 44984^T (98.7%),

Pseudonocardia thailandensis CMU-NKS-70^T (98.7%), and *Pseudonocardia ailaonensis* DSM 44979^T (97.9%). The results of the polyphasic study which contain genome comparisons of ANIb, ANIm and digital DNA-DNA hybridization revealed the differentiation of strain CAP47R^T from the closest species with validated names. This strain represents a novel species and the name proposed for this microorganism is *Pseudonocardia pini* sp. nov., indicating the source of actinobacteria from a pine tree. The type strain is CAP47R^T (= DSM 108967^T = NRRL B-65534^T). Genome mining revealed that this strain contained a variety of genes encoding enzymes to degrade hazard chemicals.

Introduction

The genus *Pseudonocardia* belonging to family *Pseudonocardiaceae* was first proposed by Henssen (1957). It is the only family in the Order Pseudonocardiales (Labeda and Goodfellow 2012) 2014). At the time of submission of this manuscript, the genus encompasses more than 60 valid species (https://lpsn.dsmz.de/genus/Psedonocardia) (Parte et al. 2020). They were discovered from different environmental samples including soil, marine sediments, activated sludge, termite nets and gold mine core including plant tissues. Most of them were isolated from soil but there are at least 13 validly named species isolated as endophytes (Parte et al. 2020). *Pseudonocardia* strains were reported for their application in biodegradation including as bioactive compounds producers (Warwick et al. 1994; Reichert et al. 1998). The endophytic actinobacterium, strain CAP47R^T was discovered in the course of a study which focused on the biodiversity of endophytic actinobacteria from Australian native trees and screening for antimicrobial activity (Kaewkla and Franco 2013). In this report, the description of the phenotypic and genotypic properties including a genome comparative study of a *Pseudonocardia*-like strain, CAP47R^T, is presented. The data show that this strain represents a novel species, for which the name *Pseudonocardia pini* sp. nov. is proposed.

Materials And Methods

Isolation of actinobacterium

Root samples of a native pine tree (*Callitris preissii*) grown on the Bedford Park campus of Flinders University, Adelaide, South Australia (35.024636 S 138.571604 E) were used as a plant source. Sample collection and surface sterilized method were described by Kaewkla and Franco (2013). Crushed plant tissues were plated onto 10 different isolation media and incubated at 27 ^{o}C. Emergence time of each colony was recorded and whole colonies were removed from the isolation plates every week for 12 weeks. Actinobacteria were purified and maintained on half-strength potato dextrose agar (HPDA). Pure cultures were maintained on HPDA slants at 4°C and in 20% glycerol at − 80°C, for further study. Based on 16S rRNA gene sequence similarity and its phylogenetic tree position, three type strains, *Pseudonocardia xishanensis* JCM 17906^{T} (98.8%), *Pseudonocardia oroxyli* DSM 44984^{T} (98.7%) and *Pseudonocardia ailaonensis* DSM 44979^{T} (97.9%) were selected for side-by side comparison study. Reference strains; *P. oroxyli* DSM 44984^{T} and *P. ailaonensis* DSM 44979^{T} were obtained from German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) and *Pseudonocardia xishanensis* JCM 17906^{T} was obtained from Japan Collection of Microorganisms RIKEN BioResource Research Center (JCM).

16S rRNA gene analysis and phylogenetic trees construction

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene of strain CAP47R^{T} were described previously (Kaewkla and Franco 2013). The resultant 16S rRNA gene sequence (1441 bp) of strain CAP47R^{T} and the representatives of the closely related strains of the genus *Pseudonocardia* available from GenBank/EMBL by using CLUSTAL X (Thompson et al. 1997) were analysed using EzTaxon-e server (Yoon et al. 2017) with *Actinomyces haliotis* JCM 18848^{T} as the outgroup. The phylogenetic trees were constructed by the neighbour-joining (Saitou and Nei 1987) and maximum likelihood tree-making methods by using the software package *MEGA* version X (Kumar et al. 2018). Pairwise distances for the neighbour-joining algorithm were calculated according to the Tajima Nei model (Tajima and Nei 1984) and Tamura and Nei model (Tamura and Nei 1993) which was applied using maximum likelihood analysis with Nearest-Neighbor-Interchange (NNI). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein 1985) based on 1000 replications.

Sequencing, assembly and annotation of genome

Genomic DNA of strain CAP47R^{T} was extracted by using GenElute™ (Sigma) and the library was prepared by using a short insert size library. Illumina Hiseq X-ten platform (Illumina) was used to sequence the whole genome of this strain (2 x 150bp paired-end reads) at Beijing Genome Institute (BGI), Hongkong. The reads were *de novo* assembled by using Unicycler (0.4.8) (Wick et al. 2017). The draft assemblies of strain CAP47R^{T} genome have been submitted to GenBank. There was only a genome of the closest type strain; *P. oroxyli* DSM 44984^{T} which was available in the public database. Therefore, strain CAP47R^{T} was studied genome comparison with only *P. oroxyli* DSM 44984^{T}. The phylogenetic tree of genome of strain CAP47R^{T} and related taxa was constructed by using the Type (strain) Genome Server (TYGS) (Meier-

Kolthoff et al. 2013; Meier-Kolthoff and Göker 2019). The tree applied with FastME 2.1.6.1 (Lefort et al. 2015) from Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula *d4*. Digital DNA-DNA hybridization (dDDH) value was calculated by applying the Genome-to-Genome Distance calculator (GGDC 2.1; BLAST + method) which formula 2 (identities/HSP length) was applied for the incomplete draft genome (Meier-Kolthoff et al. 2013).

The average nucleotide identity (ANI) value between strain CAP47R^T and *P. oroxyli* DSM 44984^T was evaluated with pairwise genome alignment by using ANI-BLAST (ANIb) and ANI-MUMmer (ANIm) algorithms within the JSpeciesWS web service (Richter and Rosselló-Móra 2009; Richter et al. 2016).

Chemotaxonomic study

For the chemotaxonomic analyses, diaminopimelic acid was detected by two dimension Thin Layer Chromatography (TLC) using the method described previously (Bousfield et al. 1985) and whole-cell sugar was analysed by the TLC method of Hasegawa et al. (1983). The extraction and analysis of phospholipids were determined as described by Minnikin et al. (1984) and Komagata and Suzuki (1987) using 5% ethanolic molybdophosphoric acid, α-naphthol, Dragendorff reagent, ninhydrin and molybdenum blue reagent and periodate-Schiff spray.

Extraction and purification of isoprenoid quinones were performed using the method of Minnikin et al. (1984) with analysis of the samples by reverse phase LC-MS employing UV detection and electrospray mass spectrometry (ESI) as described by Kaewkla and Franco (2019). Strain CAP47R^T and the type strains, *P. xishanensis* JCM 17906^T and *P. oroxyli* DSM 44984^T were studied together for the analysis of whole-cell fatty acids. Cells of all strains were grown for 10 days at 25 ^oC in Trypticase soy broth (Oxoid) at 150 rpm. Wet cells (100 mg) were saponified, methylated and the fatty acid methyl esters (FAMEs) analysed using the MIDI (Microbial Identification) system (Sasser 2001). The Sherlock ACTIN6 software version 6.2B was used for analysis.

Morphological study

Morphological characteristics of strain CAP47R^T and three type strains, *P. xishanensis* JCM 17906^T, *P. oroxyli* DSM 44984^T and *P. ailaonensis* DSM 44979^T, were compared as described by Shirling and Gottlieb (1966) on 8 different media, ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 (ISP; International Streptomyces Project), Bennett's agar, HPDA and Nutrient agar (Atlas and Parks 1993). Strain CAP47R^T was grown on HPDA for 10 days and sample preparation for the scanning electron micrograph was carried out according to Kaewkla and Franco (2019). Electron microscope (The Philips XL30 Field Emission Scanning Electron Microscope) at Adelaide Microscopy, Adelaide University, Adelaide, South Australia was used to visualize mycelial and spore morphology.

The physiological and biochemical study

The physiological characteristics of strain CAP47R^T and three closest type strains were studied together by using the following methods. Hydrolysis of skim milk, starch, utilization of four phenolic compounds as sole carbon source, catalase production, assimilation of seven organic acids were followed protocols of Kurup and Schmitt (1973). Acid production from eighteen carbohydrates, decomposition of esculin, *L*-tyrosine, and urea were studied according to the methods of Gordon et al. (1974). Growth at different pH between 4 and 10 (in 1 pH unit intervals), temperatures (4, 15, 27, 37, 45 and 55 ^oC), and NaCl concentrations (1, 3, 5, 10, 15 and 20%, w/v) were evaluated after incubation for 10–14 days on ISP 2 medium (Kurup and Schmitt 1973).

Genome mining

Secondary metabolite Analysis Shell (anti-SMASH) version 5.1.0 (Blin et al. 2009) was applied to reveal biosynthetic gene clusters (BGCs) of strain CAP47R^T. *In silico* approach was also applied to scan for genes relating to bioactive compound and enzyme production, bioremediation, and plant growth promotion. Blastp on the Uniprot database with matrix; blosum62 was used to discover the gene products and the closest similarity of microorganisms (https://www.uniprot.org/blast; The UniProt Consortium 2019).

Results And Discussion

Isolation of strain CAP47R^T

After root samples were placed on an isolation medium; Humic acid vitamin B agar (HVA) (Hayakawa and Nonomura 1987), a white small colony of CAP47R^T emerged on root tissue after incubation for 6 weeks.

16S rRNA gene analysis and phylogenetic trees construction

The results showed that strain CAP47R^T was closely embedded within the genus *Pseudonocardia*. Strain CAP47R^T showed the highest 16S rRNA gene sequence similarity with *P. xishanensis* JCM 17906^T (98.8%), *P. oroxyli* DSM 44984^T (98.7%), *P. thailandensis* CMU-NKS-70^T (98.7%), and *P. ailaonensis* DSM 44979^T (97.9%). The phylogenetic position of strain CAP47R^T was analysed and showed clearly that strain CAP47R^T was encompassed by other members of the genus *Pseudonocardia.* Strain CAP47R^T was positioned in a different clade with four closest relative type strains. The closest neighbour was *P. oroxyli* DSM 44984^T on both trees with a low bootstrap values at 56% and 41%, respectively. *P. thailandensis* CMU-NKS-70^T positioned in the farthest distance on both trees (Fig. 1 and Fig. S1).

Genome comparison

The draft genome sequence of strain CAP47R^T was 6.05 Mb with DNA G + C content determined by in *silico* genome sequence as 72.5 mol%. The genome analysis showed the ANIb, ANIm and dDDH values of

the draft genome between strain CAP47R^T and the type strain *P. oroxyli* DSM 44984^T at 81.6, 86.6 and 27.5% (c.t. 25.2–30.0%), respectively. dDDH value was lower than the threshold of 70% used to define species level (Richter and Rosselló-Móra 2009; Chun et al. 2018). In addition, according to the report of Richter and Rosselló-Móra (2009), an ANI value lower than the 95–96% cut off should delineate different species. The phylogenetic tree based on TYGS revealed the relationship between strain CAP47R^T and the related type strains. The result clearly showed that strain CAP47R^T was positioned in the same clade with the closest type strain, *P. oroxyli* DSM 44984^T. However, the phylogenetic tree of the genome obviously showed that strain CAP47R^T was grouped in the different species cluster of this type strains (Fig. 2).

Chemotaxonomic study

The diagnostic diamino acid in the cell-wall peptidoglycan was identified as *meso*-diaminopimelic acid. Whole-cell sugars were arabinose and galactose. Lipids contained phosphatidylglycerol, phosphatidylmethylethanolamine, including three unknown glycolipids, an unknown phosphoglycolipid, an unknown phosphoglycolipid positive with ninhydrin spray and two unknown lipids positive with a ninhydrin spray (Fig. S2). Strain CAP47R^T contained MK-8(H_4) (89.7%) as the predominant menaquinone and small amounts of MK-8(H_6) (10.3%). The whole-cell fatty acid of strain CAP47R^T was of the *iso*- and *anteiso*-branched fatty acids which was the same pattern with other type strains. The major fatty acids was *iso*-H-$C_{16:1}$ (28.0%), *iso*-$C_{16:0}$ (22.3%) and *iso*-$C_{14:0}$ (13.8%) Table 1.

Morphological study

This strain showed morphology belonging to the genus *Pseudonocardia*, with well-developed white aerial mycelium on most media tested. Substrate mycelium was yellowish white on all media used. Cultural characteristics on different media are shown in Supplementary Table S1 (Kornerup and Wanscher 1978). Strain CAP47R^T did not produce melanin pigment on ISP 7 and any diffusible pigment on the media tested. Mycelia fragmented to form zig zag spores, which were straight short rods with a smooth surface (approximately 1 x 5 μm, Supplementary Fig. S3 available in online version).

The physiological and biochemical study

Other phenotypic data revealed that strain CAP47R^T was different from the closest type strains. CAP47R^T could produce acid from sorbitol but the type strain; *P. oroxyli* DSM 44984^T could not. On the other hand, the type strains; *P. oroxyli* DSM 44984^T and *P. xishanensis* JCM 17906^T could produce acid from mannose, mannitol, raffinose and could hydrolyze starch and assimilate propionate but strain CAP47R^T could not. Also, these two type strains could grow at 37°C and pH 4 but strain CAP47R^T could not.

Genome mining for BGCs and other bioactive compounds

Based on the result of the gene cluster prediction derived from the 'antiSMASH' database, gene clusters which share gene similarity with known genes at 50 % or higher may be likely to produce a variety of novel bioactive compounds. Strain CAP47R^T contained only three BCGs gene clusters including

siderophore; bacillibactin (60%), polyketide; alkylresorcinol which possess antioxidant activities (100%) and ectoine relating to drought tolerant (100%) (Supplementary Table S2).

In silico approach to scan genes revealed that strain CAP47R^T contained genes encoding enzymes which are capable of degrading recalcitrant chemicals in the environment (Supplementary Table S3). For example, 4-hydroxyacetophenone monooxygenase degrades bisphenol and aromatic ring hydroxylase degrades polycyclic aromatic hydrocarbons (PAH). Alkanesulfonate monooxygenase degrades organosulfonate and haloalkane dehalogenase degrades a variety compounds which are alkanes containing one or more halogens. 2-halobenzoate 1,2-dioxygenase small subunit degrades xenobiotic compounds in the benzoate pathway. DMSO/TMAO reductase degrades dimethyl sulfone ($DMSO_2$) (Reichert et al. 1998). Furthermore, carboxymethylenebutenolidase degrades hexachlorocyclohexane which was dimerized to produce Mirex, a banned organochlorine pesticide. Also, cyclohexanone monooxygenase and 2,4-dichlorophenol 6-monooxygenase degrade pesticides which are carcinogens. In addition, 2-nitropropane dioxygenase degrades 2-nitropropane which causes carcinomas and nitroreductase degrades polynitroaromatic explosive, 2,4,6-trinitrotoluene (TNT). The many genes relating to xenobiotic compounds degradation revealed that this strain can be applied to remove hazard chemicals in the environment. Strain CAP47R^T showed this ability which correspond to many species of genus *Pseudonocardia* (Huang and Goodfellow 2012)

Moreover, strain CAP47R^T contained genes encoding universal stress protein and enzymatic free radical scavengers in management of oxidative stresss. Biodegradation enzymes including a variety of protease and xylanase were also detected. Interestingly, strain CAP47R^T contained genes encoding to polyhydroxyalkanoate synthase and poly-beta-hydroxybutyrate polymerase which relate to polyhydroxybutyrate (PHB) production; an alternatives for production of biodegradable plastics (Getachew and Woldesenbet, 2016).

Conclusion

In conclusion, strain CAP47R^T can be distinguished from other members of the genus *Pseudonocardia; P. xishanensis* JCM 17906^T, *P. oroxyli* DSM 44984^T and *P. ailaonensis* DSM 44979^T by dDDH, ANIb and ANIm values, phylogenetic tree of genome, and its phenotypic characters. The name *Pseudonocardia pini* sp. nov. is proposed for this novel species. Genome mining predicted that this strain contained a variety of genes encoding enzymes which could be used to degrade hazard chemicals in the environment. Also, strain CAP47R^T contained genes relating to PHB production which can be applied to produce bioplastic.

Description of Pseudonocardia pini sp. nov.

Pseudonocardia pini (pi'ni. L. gen. n. *pini* of pine, isolated from root of pine tree)

Aerobic, Gram-stain positive, non-acid/alcohol-fast. Catalase positive. Growth occurs between 25 and 32 ^oC but grows well at 27 ^oC. Growth occurs between pH 5.0 and 10.0 but grows well at pH 7.0. Growth

occurs in the presence of NaCl 5% (w/v). Colonies are small and tough and aerial mycelium develop well. Aerial mycelium fragments into long rod-shaped spores with a smooth surface (approximately 1 x 5 μm). Colony colour is yellowish white to pale white. Diffusible pigments are not produced on any medium used. Cells can produce acid from arabinose, fructose, fucose, galactose, glucose, maltose, *myo*-inositol, ribose, sorbitol, sucrose and trehalose but not from cellulose, *meso*-erythritol. Strain CAP47R^T hydrolyzes esculin and urea but not *L*-tyrosine, skim milk and starch. Strain CAP47R^T can assimilate acetate, citrate, *L*-lactate, malate but not benzoate, propionate and tartrate. Polar lipids are phosphatidylglycerol (PG), phosphatidylmethylethanolamine (PME), including three unknown glycolipids, an unknown phosphoglycolipid, an unknown phosphoglycolipid positive with ninhydrin spray and two unknown lipid positive with ninhydrin spray. The major menaquinones of strain CAP47R^T are MK-8(H_4) (89.7%). The major cellular fatty acids of strain CAP47R^T are *iso*-H-$C_{16:1}$ (28.0%), *iso*-$C_{16:0}$ (22.3%) and *iso*-$C_{14:0}$ (13.8%). The DNA G + C content of the type strain is 72.5%.

The type strain, CAP47R^T (= DSM 108967^T = NRRL B-65534^T) is an endophytic actinobacterium isolated from the surface sterilized root of a native pine tree (*Callitris preissii*) which grows on the Flinders University campus, Adelaide, South Australia.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and accession number for the draft genome of strain CAP47R^T are MT792901 and JACBXB000000000, respectively.

Declarations

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Author contributions

Dr. Onuma Kaewkla and Prof. Chris Franco planned the experiments. Dr. Kaewkla carried out the experimental work and prepared the draft manuscript. Prof. Franco contributed to improve the manuscript and provided the facilities.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have 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.

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Tables

Table 1. Whole-cell fatty acid composition (%) of *Pseudonocardia pini* CAP47R^T and related species of *Pseudonocardia.*

Strain: 1, *Pseudonocardia pini* CAP47R^T; 2, *Pseudonocardia xishanensis* JCM 17906^T; and 3, *Pseudonocardia oroxyli* DSM 44984^T. Only fatty acids detected at more than 0.1% of the total are presented. -, not detected. All the data are from this study.

Fatty acid	1	2	3
iso-$C_{12:0}$	0.3	-	-
$C_{13:0}$	0.3	-	-
$C_{12:0}$ 3OH	0.2	-	-
iso-$C_{14:0}$	13.8	3.7	3.7
$C_{14:0}$	0.76	1.1	0.7
$C_{14:1}$ w5c	0.4	0.3	-
iso-H-$C_{15:1}$	-	0.5	-
iso-$C_{15:0}$	5.0	3.1	6.3
anteiso-$C_{15:0}$	1.3	0.4	0.5
$C_{15:1}$ w6c	3.6	0.4	0.2
iso-H-$C_{16:1}$	28.0	21.8	15.9
iso-$C_{16:0}$	22.3	28.9	44.2
$C_{16:1}$ w7c	1.3	-	-
$C_{16:0}$	1.6	4.2	2.1
$C_{15:0}$ 3OH	0.5	-	-
anteiso-$C_{17:1}$ w9c	-	-	0.2
iso-$C_{17:0}$	0.3	0.3	1.2
anteiso-$C_{17:0}$	0.6	0.6	1.2
$C_{17:1}$ w8c	0.8	0.3	0.2
$C_{17:1}$ w6c	0.6	1.5	1.5
$C_{17:0}$	0.7	0.4	-
$C_{16:1}$ 2OH	-	-	0.6
$C_{17:0}$ 10-methyl	4.8	1.4	1.0
iso-H-$C_{15:1}$	0.7	-	0.4

iso-$C_{18:0}$	-	1.2	2.1
$C_{16:1}$ w7c	-	5.1	2.8
iso-$C_{17:1}$ w9c	12.2	24.9	15.2

Table 2. Differential characteristics between *Pseudonocardia pini* CAP47R^T and related species of *Pseudonocardia.*

Strain: 1, *Pseudonocardia pini* CAP47R^T; 2, *Pseudonocardia oroxyli* DSM 44984^T; 3, *Pseudonocardia xishanensis* JCM 17906^T and 4, *Pseudonocardia ailaonensis* DSM 44979^T. +, positive or present; w, weakly positive; -, negative or absent. Catalase was positive for all strains. All strains could produce acid from arabinose, fructose, fucose, galactose, glucose, maltose, *myo*-inositol, ribose, sucrose and trehalose but not from cellulose and *meso*-erythritol. They could hydrolyze esculin and urea. All strains could assimilate acetate, citrate, *L*-Lactate, malate but not benzoate and tartrate. They could not use toluene, phenol, and benzene as sole carbon source. All strains could grow at 1% and 3% NaCl (w/v), pH between 5 and 10 and at 27 ^{o}C. They could not grow at 15% and 20% NaCl (w/v), at 4 ^{o}C, 45 ^{o}C and 55 ^{o}C.

Characteristics	1	2	3	4	Characteristics	1	2	3	4
Acid production from:					**Hydrolysis of :**				
mannose	-	+	+	+	L-tyrosine	-	-	-	w
mannitol	-	+	+	+	Skim milk	-	+	-	-
methyl-D-glucopyranoside	-	-	+	-	Starch	-	+	+	+
raffinose	-	+	+	+	**Use of organic acid:**				
sorbitol	+	-	-	-	propionate	-	+	+	+
xylose	-	-	-	+					
Growth at:					**Use of phenolic compounds:**				
NaCl 5% (w/v)	w	+	w	-	Pyridine	-	-	-	+
NaCl 10% (w/v)	-	w	-	-					
15 ^{o}C	-	w	w	w					
27 ^{o}C	+	+	+	+					
37 ^{o}C	-	+	+	-					
pH 4	-	+	w	-					

Supplementary Information

Supplementary Figure S1. Maximum likelihood tree showing the relationship between *Pseudonocardia pini* CAP47R^T and members of the genus *Pseudonocardia* with *Actinomyces haliotis* JCM 18848^T as the outgroup. Bootstrap percentage based on 1000 resamplings are listed at the nodes. The scale bar represents 0.02 changes per nucleotide.

Supplementary Figure S2. Two-dimensional thin-layer chromatography of polar lipids of *Pseudonocardia pini* CAP47R^T. Chloroform-methanol-water (65:25:4) was used in the first direction, followed by chloroform-acetic acid-methanol-water (40:7.5:6:2) in the second direction. Abbreviations: PG; Phosphatidylglycerol, PME; phosphatidylmethylethanolamine, UnGly; unknown glycolipid, UnPGly; unknow phosphoglycolipid, UnPGlyA; unknow phosphoglycolipid positive with ninhydrin spray, UnA; unknown lipid with ninhydrin spray, B: brown color, X; starting point.

Supplementary Figure S3. Scanning electron micrograph of strain CAP47R^T grown on HPDA for 10 days at 27 ^oC. Bar = 5μm.

Figures

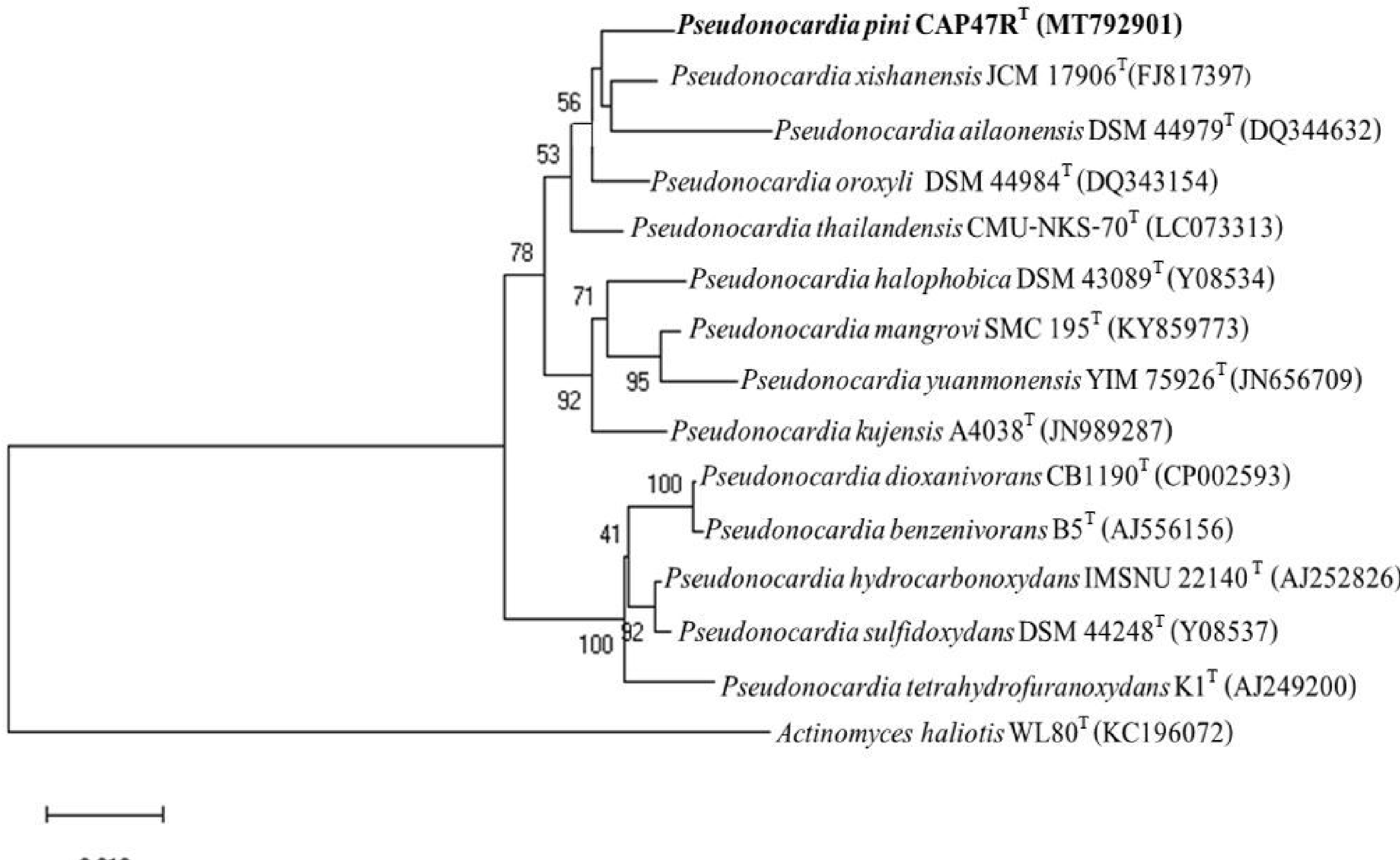

Figure 1

Neighbour-joining tree showing the relationship between Pseudonocardia pini CAP47RT and members of the genus Pseudonocardia with Actinomyces haliotis JCM 18848T as the outgroup. Bootstrap percentage based on 1000 resamplings are listed at the nodes. The scale bar represents 0.01 changes per nucleotide.

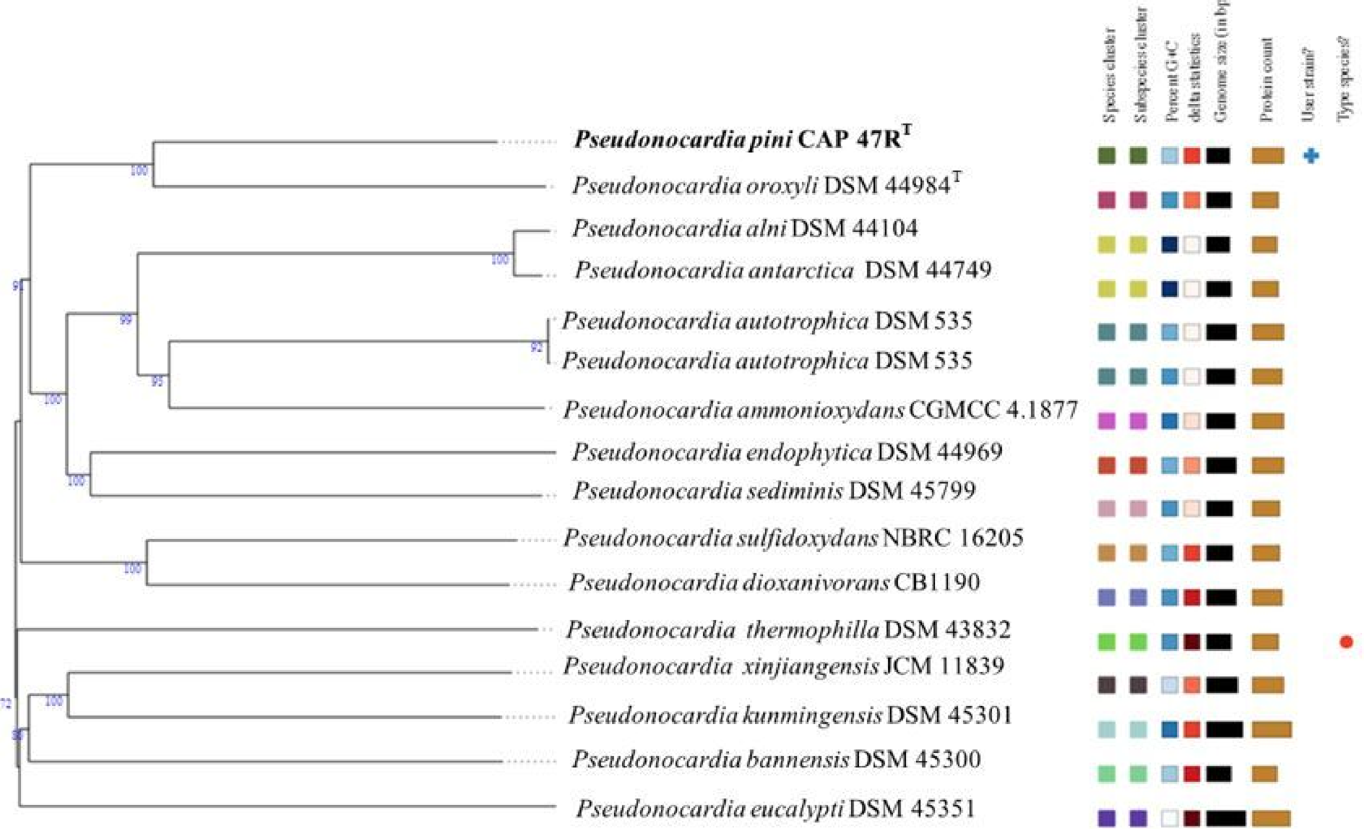

Figure 2

Phylogenomic tree based on TYGS results showing the relationship between strain CAP47RT with related type strains. The numbers above branches are GBDP pseudo-bootstrap support values >60 % from 100 replications, with an average branch support of 89.2%. The tree was rooted at the midpoint (Farris 1972). Leaf labels are annotated by affiliation to species and subspecies clusters, genomic G+C content, δ values and overall genome sequence length, number of proteins, and the kind of strain (Meier-Kolthoff and Göker 2019).

Supplementary Files

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

- FigSMLR2.pptx
- figs3spore.jpg
- TableS1.docx
- TableS2BCG.docx
- TableS3genomeCAP47R.docx