***Jiangella anatolica* sp. nov. isolated from coastal lake soil**

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Introduction

The genus *Jiangella* was first proposed by Song et al. (2005) within the family *Nocardioideaceae*. However, Tang et al. (2011) established the family *Jiangellaceae* to accommodate the genera *Jiangella* (Song et al. 2005) and *Haloactinopolyspora* (Tang et al. 2011) mainly based on phylogenetic analysis of phylum *Actinobacteria*. Currently, the family *Jiangellaceae* includes the genera *Jiangella*, *Haloactinopolyspora* and the recently described *Phytoactinopolyspora* (Li et al. 2015). The type genus *Jiangella* encompasses aerobic, Gram-positive, filamentous actinomycetes with substrate mycelium fragmented into short and elongated rods. Members of the genus are characterized by the presence of LL-diaminopimelic acid in the cell-wall peptidoglycan and MK-9(H₄) as the major menaquinone. None of the species described so far contain mycolic acids. The genus accommodates *Jiangella gansuensis* YIM 002^T (Song et al. 2005), *Jiangella alba* YIM 61503^T (Qin et al. 2009), *Jiangella alkaliphila* D8-87^T (Lee 2008), *Jiangella muralis* 15-Je-017^T (Kämpfer et al. 2011) and *Jiangella mangrovi* 3SM4-07^T (Suksaard et al. 2015) isolated from desert soil, surface sterilized stem of *Maytenus austroyunnanensis*, cave soil, cellar wall and mangrove soil, respectively.

Members of the phylum *Actinobacteria* are well-known for being prolific producers of most of the bioactive secondary metabolites (Goodfellow and Fiedler 2010). Thus, selective isolation of novel actinobacteria from unexplored habitats such as marine sediments (Carro et al. 2018) or lakes (Ay et al. 2017, 2018) holds considerable importance to discovery of novel bioactive compounds. In a continuation of our investigations on actinobacterial diversity in unexplored habitats a novel strain, GTF31^T, was isolated from coastal soil sample of Gölcük Lake, a crater lake in southwest Anatolia (Turkey). Polyphasic characterization of strain GTF31^T showed that

the strain is a novel species of the genus *Jiangella*, for which the name *Jiangella anatolica* sp nov is proposed.

Materials and methods

Isolation, maintenance and cultural conditions

Strain GTF31^T was obtained from coastal soil sample of Gölcük Lake, a freshwater lake, (37° 44' 03.90'' N; 30° 29' 39.24'' E) collected in September 2013. The strain was isolated on International *Streptomyces* Project Medium 2 (ISP 2 medium) (Shirling and Gottlieb 1966) supplemented with nystatin (50 µg/ml) and rifampicin (5 µg/ml) using standard dilution-plate technique. Briefly, the soil sample was suspended in ¼ strength Ringer's solution (Oxoid) and pretreated with wet heat at 60 °C for 20 minutes. Then, the diluted (10^{-2} and 10^{-3}) suspensions were spread on ISP 2 medium. Inoculated plates were incubated at 28 °C for two weeks. The *Jiangella* isolate was purified and maintained on slopes of tryptone-yeast extract agar (DSM medium No. 680) at 4 °C and as glycerol stock solutions (25 % w/v) at – 20 °C.

Cultural properties and phenotypic tests

Cultural and morphological characteristics were observed on ISP media 1 – 7 (International *Streptomyces* Project, Shirling and Gottlieb (1966)), nutrient agar and tryptic soy agar (TSA; Difco) after incubation at 28 °C for 14 days. The ISCC-NBS Colour Charts Standard Samples No. 2106 (Kelly 1964) was used to determine the colours of aerial mycelium, substrate mycelium and diffusible pigments. Micromorphological characteristics were observed by scanning electron microscopy (JEOL JSM 6060) using cultures grown on glucose-yeast extract-malt extract (DSMZ Medium No. 65) at 28 °C for 14 days. The temperature (4, 10, 20, 28, 37, 50 °C) and pH range (7 – 12, at intervals of 1.0 pH unit) were determined on ISP 2 medium (Shirling and Gottlieb 1966). Enzyme profile of the strain was established using the commercial kit API ZYM (BioMerieux) following the manufacturer's instructions. Carbon source utilization and chemical sensitivity

properties were determined using GEN III Microplates in an Omnilog device (BIOLOG Inc., Hayward, CA, USA). The GEN III Microplates were inoculated with cells suspended in a viscous inoculation fluid (IF C) provided by manufacturer at 85–87 % of transmittance for strain GTF31^T and the closely related type strains. The strains were studied in two independent technical replicates. Data were exported and analysed using opm package for R (Vaas et al. 2013; Vaas et al. 2012).

Chemotaxonomy

Biomass for chemotaxonomic analyses was obtained by cultivation in shake flasks at 28 °C for 14 days on a rotary shaker (150 rpm) using glucose-yeast extract-malt extract (DSMZ Medium No. 65). Biomass was harvested by centrifugation at 9000 rpm, washed several times with sterile distilled water and then freeze-dried. Whole-cell amino acids and sugars were extracted following a standard protocol described by Lechevalier and Lechevalier (1970) and analysed by thin layer chromatography (TLC) according to Staneck and Roberts (1974). Polar lipids were extracted and analysed by two-dimensional TLC following Minnikin et al. (1984) using modifications of Kroppenstedt and Goodfellow (2006). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). The extracted menaquinones were analysed using a high performance liquid chromatography (HPLC; LC-10AS, Shimadzu 63 Co.) fitted with a reverse-phase C18 column (150 x 4.6 mm, 5 µm particle size, RP-18-Lichrosorb column, Penomenex UK) at 270 nm. Fatty acid methyl esters were extracted from fresh cultures grown on tryptic soy agar (TSA; Difco) at 28 °C for 10 days and analysed using Microbial Identification System (MIDI) Sherlock software version 6.1 and the library RTSBA6 according to the technical instructions provided by this system (Sasser 1990).

Phylogeny

Genomic DNA was extracted as described by Chun and Goodfellow (1995) and PCR amplification of 16S rRNA gene achieved using the universal primers 27F (5'–

AGAGTTTGATC(AC)TGGCTCAG–3') and 1492R (5'–ACGG(CT)TACCTTGTTACGACTT–3') (Weisburg et al. 1991). The purified PCR products were sequenced using an ABI PRISM 3730 XL automatic sequencer and an almost complete 16S rRNA gene sequence (1491 bp) was deposited in the GenBank data library and corresponding sequences of the type strains of the genus *Jiangella* were retrieved by using the EzBioCloud server (Yoon et al. 2017). A multiple sequence alignment was generated with MUSCLE program (Edgar 2004). Neighbour joining tree (Saitou and Nei 1987) was constructed in MEGA 6 (Tamura et al. 2013) using the model of Jukes and Cantor (Jukes and Cantor 1969). Phylogenetic analyses for maximum likelihood (ML) and maximum parsimony (MP) methods were conducted by the GGDC web server using the DSMZ phylogenomics pipeline (Meier-Kolthoff et al. 2014) adapted to single genes after pairwise sequence similarities were calculated according to Meier-Kolthoff et al. (2013a) via the GGDC web server (Meier-Kolthoff et al. 2013b) available at <http://ggdc.dsmz.de/>. ML tree was inferred from the alignment with RAxML (Stamatakis 2014) using rapid bootstrapping in conjunction with the autoMRE criterion (Pattengale et al. 2010) and MP tree was inferred from the alignment with TNT (Goloboff et al. 2008) using 1000 bootstraps in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence additional replicates. The sequences were checked for compositional bias using the X^2 test as implemented in PAUP* (Swofford 2002).

The genome of strain GTF31^T was sequenced using the Illumina HiSeq 2500 next generation sequencing platform with a 250-bp paired end protocol (MicrobesNG, Birmingham, United Kingdom) and the draft genome sequence was submitted to NCBI under the accession number POTW01000000. The genome sequence was annotated on Rapid Annotations Using Subsystems Technology (RAST) server (Aziz et al. 2008). A phylogenomic tree was built on PATRIC web server (<https://patricbrc.org/>) (Wattam et al. 2016) using FastTree algorithm (Price et al. 2010). The digital DNA-DNA hybridization (dDDH) similarities between the draft genome sequence of strain GTF31^T and publicly available genome sequence data of its close phylogenetic neighbours

were determined using formula 2 of the GGDC web server (Meier-Kolthoff et al. 2013b). In addition, the level of pairwise genome-based similarities was evaluated based on the average nucleotide identity (ANI) value determined by using online ANI calculator described by Yoon et al. (2017) available at <https://www.ezbiocloud.net/tools/ani>.

Results and discussion

Strain GTF31^T was found to grow well on ISP 1, ISP 2, ISP 3, ISP 6, nutrient agar and tryptic soy agar while moderate on ISP 5 and ISP7 media. The strain could not grow on ISP 4 medium and no diffusible pigment was observed on any media tested nor did produce melanin pigment. Substrate mycelium fragmented into elongated rods (2.1 µm x 0.3 µm) while aerial mycelium and spore formation could not be observed during 14-day incubation period on any media tested (Fig 1). The strain was able to grow at temperature and pH range of 20 – 37 °C (optimum at 28 °C) and 6 – 12 (optimum at pH 7 – 8), respectively, and in the presence of 1% NaCl. Strain GTF31^T could oxidise various compounds including acetic acid, acetoacetic acid, D-arabitol, D-cellobiose, dextrin, gelatin, β-gentiobiose, D-glucose, N-acetyl-D-glucosamine, β-methyl-D-glucoside, glycerol, L-lactic acid, D-maltose, D-mannitol, N-acetyl-β-D-mannosamine, pectin, D-salicin, sucrose, D-trehalose and turanose. The strain was able to respire in presence of aztreonam, lincomycin, nalidixic acid, potassium tellurite, rifamycin SV, 1% sodium lactate and troleandomycin whereas it could not tolerate fusidic acid, guanidine hydrochloride, lithium chloride, minocycline, niaproof, sodium bromate and tetrazolium blue (Table 1). Allantoin and urea hydrolyses and nitrate reduction tests were positive while arbutin hydrolysis was negative. In addition, strain GTF31^T produced alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase as shown by API ZYM (BioMerieux) test.

Chemotaxonomic characteristics of strain GTF31^T were consistent with those of the genus *Jiangella* such as LL-diaminopimelic acid and glucose in whole cell hydrolysate. The strain was also found to contain mannose, rhamnose and ribose. Polar lipid profile was comprised of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, four glycerophospholipids and three unidentified phospholipids (Fig S1). The predominant menaquinone was determined to be MK-9(H₄). The major cellular fatty acids were identified as anteiso-C_{15:0} (28.7%), iso-C_{16:0} (16.9%) and anteiso-C_{17:0} (16.4%) (Table S1).

An almost complete 16S rRNA gene (1491 bp) was obtained and analysed on EzBioCloud server and also on GGDC web server. Phylogenetic analyses of 16S rRNA gene sequences showed that strain GTF31^T is a member of the genus *Jiangella*. The strain was seen to be closely related to *J. gansuensis* DSM 44835^T, *J. alba* DSM 45237^T, *J. muralis* DSM 45357^T, *J. mangrovi* 3SM4-07^T and *J. alkaliphila* DSM 45079^T with 16S rRNA gene sequence similarity of 99.4%, 99.3%, 99.2%, 99.1% and 98.8%, respectively. Strain GTF31^T was found to form a distinct branch at the periphery of *Jiangella* 16S rRNA gene trees constructed by both neighbour joining (Fig 2) and maximum likelihood (Fig S2) tree-making algorithms.

The draft genome sequence of strain GTF31^T was deposited on GenBank with accession number of POTW00000000 and annotated with RAST server. The genome is composed by 256 contigs with N50 value of 54,053. The numbers of coding sequences and RNAs are 6444 and 51, respectively. Total genome size is 7 Mb and G+C content is 72.5%. In phylogenomic tree constructed on PATRIC web server, strain GTF31^T formed a distinct cluster with other members of the genus *Jiangella* (Fig 3). The calculated digital DDH values between strain GTF31^T and type species of *J. gansuensis*, *J. alba*, *J. muralis* and *J. alkaliphila* ranged from 24.4 to 34.4%, values which are well below the proposed threshold of 70% for species delineation (Wayne et al. 1987). In addition to low DDH values, ANI values calculated on EzBioCloud web server (Table S2), which ranged between 81.0 – 87.9 % (value below the threshold of 95-96 %) (Richter and

Rosselló-Móra 2009), confirmed differentiation of strain GTF31^T from its phylogenetic neighbours.

On the basis of phylogenetic, chemotaxonomic and morphological analyses, strain GTF31^T showed characteristics of the genus *Jiangella* but differs from previously described species. Therefore, it is clear that strain GTF31^T represents a novel member of the genus *Jiangella* for which *Jiangella anatolica* sp. nov. is proposed.

Description of *Jiangella anatolica* sp. nov.

Jiangella anatolica (a.na.to'li.ca. N. L. fem. adj. *anatolica*, referring the place from which the type strain was isolated).

Aerobic, Gram-positive actinomycete that forms mycelia fragmenting into elongated rods. Substrate mycelium is cream or pebble grey on ISP 1, ISP 2, ISP 3, ISP 5, ISP 6, ISP 7, nutrient agar and tryptic soy agar media. Shows good growth on ISP 1 – 3, ISP 6, nutrient agar and tryptic soy agar media while moderate on ISP 5 and ISP 7 media. Optimal growth occurred at 28 °C and pH 7 – 8. Tolerates aztreonam, lincomycin, nalidixic acid, potassium tellurite, rifamycin SV, 1% sodium chloride, 1% sodium lactate and troleandomycin. Positive for allantoin and urea hydrolyses, nitrate reduction tests and production of acid phosphatase, alkaline phosphatase, α -chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, lipase (C14), α -mannosidase, trypsin, naphthol-AS-BI-phosphohydrolase. Whole cell hydrolysate contains LL-DAP, glucose, mannose, rhamnose and ribose. The predominant menaquinone is MK-9(H₄). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycerophospholipids and unidentified phospholipids. Major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. The G+C content of the type strain is 72.5 % and the size of the genome is 7.0 Mb.

The type strain GTF31^T (= DSM 100984^T = CECT 9378^T) was isolated from coastal soil sample of Gölcük Lake, Isparta, Turkey. The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequence and whole genome sequence of strain GTF31^T are KU255546 and POTW01000000, respectively.

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Author contributions

HA, NS and KI designed the study. HA, IN and LC conducted physiological experiments. HPK, HA, IN, JMI designed chemotaxonomic analyses. DC carried out scanning electron microscopy analysis.

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.

Supplementary material

Table S1 Fatty acids composition of strain GTF31^T, *Jiangella gansuensis* JCM 13367^T, *J. alba* JCM 16901^T, *J. muralis* JCM 17970^T

Table S2 Genome sequence comparison between strain GTF31^T and closely related type strains of the genus *Jiangella*. Genome accession numbers are given in brackets

Fig. S1 Polar lipid profile of strain GTF31^T. Key: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; GPL1-4, unidentified glycerophospholipids; PL1-3, unidentified phospholipid

Fig. S2 Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain GTF31^T and closely related type strains. The tree was inferred under the GTR+GAMMA model. The numbers above the branches are support values when larger than 60% from maximum likelihood (left) and maximum parsimony (right) bootstrapping. The branches are scaled in terms of the expected number of substitutions per site

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