**Actinoalloteichus fjordicus** sp. nov. isolated from marine sponges: phenotypic, chemotaxonomic and genomic characterisation

Imen Nouioui · Christian Rücker · Joost Willemse · Gilles P. van Wezel · Hans-Peter Klenk · Tobias Busche · Jörn Kalinowski · Harald Bredholt · Sergey B. Zotchev

Received: 25 April 2017 / Accepted: 25 July 2017 © The Author(s) 2017. This article is an open access publication

**Abstract** Two actinobacterial strains, ADI 127-17^T^ and GBA 129-24, isolated from marine sponges *Anthodychotoma* and *Geodia barretti*, respectively, collected at the Trondheim fjord in Norway, were the subjects of a polyphasic study. According to their 16S rRNA gene sequences, the new isolates were preliminarily classified as belonging to the genus *Actinoalloteichus*. Both strains formed a distinct branch, closely related to the type strains of *Actinoalloteichus hoggarensis* and *Actinoalloteichus hymeniacidonis*, within the evolutionary radiation of the genus *Actinoalloteichus* in the 16S rRNA gene-based phylogenetic tree. Isolates ADI 127-17^T^ and GBA 129-24 exhibited morphological, chemotaxonomic and genotypic features distinguishable from their close phylogenetic neighbours. Digital DNA: DNA hybridization and ANI values between strains ADI 127-17^T^ and GBA 129-24 were 97.6 and 99.7%, respectively, whereas the corresponding values between both tested strains and type strains of their closely related phylogenetic neighbours, *A. hoggarensis* and *A. hymeniacidonis*, were well below the threshold for delineation of prokaryotic species. Therefore, strains ADI 127-17^T^ (= DSM 46855^T^) and GBA 129-24 (= DSM 46856) are concluded to represent a novel species of the genus *Actinoalloteichus* for which the name of *Actinoalloteichus fjordicus* sp. nov. (type strain ADI 127-17^T^ = DSM 46855^T^ = CECT 9355^T^) is proposed. The complete genome sequences of the new strains were obtained and compared to that of *A. hymeniacidonis* DSM 45092^T^ and *A. hoggarensis* DSM 45943^T^ to unravel unique genome features and biosynthetic potential of the new isolates.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10482-017-0920-9) contains supplementary material, which is available to authorized users.

I. Nouioui · H.-P. Klenk
School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK

C. Rücker · T. Busche · J. Kalinowski
Microbial Genomics and Biotechnology, Center for Biotechnology (CeBiTec), Bielefeld University, 33594 Bielefeld, Germany

C. Rücker
Sinskey Laboratory, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

J. Willemse · G. P. van Wezel
Molecular Biotechnology, Sylvius Laboratories, Leiden University, 2333BE Leiden, The Netherlands

H. Bredholt
Xellia Pharmaceuticals, Silurveien 2, 0380 Oslo, Norway

S. B. Zotchev (✉)
Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

e-mail: sergutzotchev@univie.ac.at

Published online: 02 August 2017 © Springer
Keywords  Marine sponges · Actinobacteria · Actinoalloteichus · Chemotaxonomy · Comparative genomics · New species · Secondary metabolite biosynthesis gene clusters

Introduction

Mycelial actinobacteria are the major sources of a variety of bioactive and potentially pharmaceutically useful compounds, some of which are being developed into anticancer agents (Feling et al. 2003; Prudhomme et al. 2008; Bhatnagar and Kim 2010) and antibiotics (Subramani and Aalbersberg 2012; Manivasagan et al. 2014). Recently, marine actinobacteria have become a focus of intensive research since they have been shown to have a remarkable potential for producing unique secondary metabolites not previously isolated from terrestrial actinobacteria (Zotchev 2012). Many such metabolites have antimicrobial activities, suggesting that they may be used as “chemical weapons” to inhibit the growth of organisms competing for nutritional sources (Davies and Ryan 2012). A role for secondary metabolites as signaling molecules has also been proposed after discovering their effect on gene expression of other bacteria exposed to sub-inhibitory concentrations of the compounds (Yim et al. 2007). Whatever their role is, it must be very important for actinobacteria, since genome sequencing typically reveals the presence of 20–40 gene clusters (most of them “silent”) dedicated to the biosynthesis of secondary metabolites in each species (Horinouchi 2007).

The genus Actinoalloteichus (Tamura et al. 2000), belonging to the family Pseudonocardiaceae (Embley et al. 1988; Stackebrandt et al. 1997) and suborder Pseudonocardineae (Labeda et al. 2011), currently encompasses five species with valid names according to LPSN classification (Euzéby 1997), with Actinoalloteichus cyanogriseus as the type species (Tamura et al. 2000). Members of the genus Actinoalloteichus form substrate and aerial mycelium with an aggregation of straight spore chains. Their cell walls contain meso-diaminopimelic acid and are rich in glutamate, glucosamine and alanine. Major cellular fatty acids are iso-C15:0, anteiso-C15:0, iso-C16:0 and C17:0. The predominant menaquinone is MK-9(H4) with the presence of MK-8(H4) and MK-9(H2) in small amounts. The diagnostic phospholipids are phosphatidylethanolamine and phosphatidylmonomethylethanolamine. The G+C content of their DNA is 72–73 mol% (Tamura et al. 2000).

Actinoalloteichus strains have been isolated from different habitats: A. cyanogriseus, the type species of the genus from a soil sample collected in the Yunnan province of China (Tamura et al. 2000), the halophilic Actinoalloteichus hoggarensis from Saharan soil (Boudjelal et al. 2015), Actinoalloteichus nanshanensis from the rhizosphere of a fig tree (Xiang et al. 2011), Actinoalloteichus spitiensis from a soil in the cold desert of the Indian Himalayas (Singla et al. 2005) and Actinoalloteichus hymeniaciidonis from the sponge Hymeniacidon perleve collected at the intertidal beach of Dalian on the Chinese Yellow Sea (Zhang et al. 2006).

So far, several secondary metabolites have been isolated from Actinoalloteichus spp., including the cytotoxic macrolactam BE-14106 from soil-derived A. cyanogriseus (Fujita et al. 2016), cytotoxic cyclopentenones from A. nanshanensis sp. nov. NEAU 119 (Wang et al. 2013), antifungal nemaclafungins from marine Actinoalloteichus sp. NPS702 (Sato et al. 2012), and cytotoxic bipyridine and cyanogramide alkaloids from marine-derived A. cyanogriseus WH1-2216-6 (Fu et al. 2011, 2014).

The fact that just a few Actinoalloteichus isolates studied so far already yielded several novel compounds suggests a substantial potential of this genus for drug discovery. Here, we describe the isolation, morphological, chemotaxonomic and genome-based characterisation of two new representatives of this relatively rare genus from marine sponges collected in the Trondheim fjord (Norway). The comparison of the complete genome sequences of members of the genus Actinoalloteichus might unravel unknown gene clusters dedicated to the biosynthesis of bioactive secondary metabolites.

Materials and methods

Sampling of marine sponges

Samples of marine sponges Geodia barretti and Anthodichotoma were collected at the Tautra ridge (Trondheim fjord, Norway, 63°36’N and 10°31’E) using the MINERVA underwater remote-operated vehicle
equipped with a net and a robotic manipulator. The collected sponges did not represent endangered or protected species and the samples were collected by a national Norwegian university (Norwegian University of Science and Technology) for research purposes. Sponge samples of approximately 300 g (G. barretti, depth 62. 7 m) and 175 g (A. dichotoma, depth 60 m) were retrieved and transferred to 1–l sterile plastic containers with screw caps filled with sterile artificial seawater. Samples were kept at 10 °C during transport (about 3 h) and stored at 4 °C until processing.

Isolation, maintenance and culture conditions

Sponge pieces of approximately 2 cm³ were cut out with a sterile scalpel on a sterilised plastic cutting board, transferred to a mortar containing 18 ml sterile artificial seawater with 20% glycerol, and thoroughly ground. The obtained suspensions were transferred to 50 ml plastic tubes with 5 g sterile glass beads and vortexed at maximum speed for 2 min. Dilutions of the processed sponge samples were plated on different agar media as described in Ian et al. (2014).

The two strains, ADI 127-17T and GBA 129-24, were isolated from marine sponges A. dichotoma and G. barretti, respectively. Both isolates were obtained on ISP2 medium (International Streptomyces Project [ISP] medium 2, Shirling and Gottlieb 1966), supplemented with 50% artificial sea water, after 2 weeks of incubation at 28 °C. Strains ADI 127-17T and GBA 129-24 were maintained in 35% (v/v) glycerol at −20 and −80 °C. For most of the chemotaxonomic analyses, freeze-dried biomass for both isolates was obtained from cultures prepared in marine ISP2 broth medium (ISP2 supplemented with 50% artificial sea water) and incubated for 10 days at 28 °C with shaking at 250 revolutions per minute (rpm) while genomic DNAs were extracted from culture grown in 50 ml of 3% TSB medium (Oxoid, UK) prepared in 50% artificial sea water in 250 ml baffled flasks shacked at 250 rpm for 72 h at 28 °C.

Chemotaxonomy and morphology

Chemotaxonomic and morphological traits for strains ADI 127-17T and GBA 129-24 were determined using standards methods known to be of value in the taxonomic characterisation of the genus Actinoalloteichus. Whole cell sugar composition (Lechevalier and Lechevalier 1970), diaminopimelic acid (Staneck and Roberts 1974), menaquinone (Collins 1985) and polar lipid (Minnikin et al. 1984) profiles were detected using chromatographic methods. Fatty acid extracts (Miller 1982; Kuykendall et al. 1988) were analysed and identified by gas chromatography (Agilent 6890N instrument) using the Standard Microbial Identification (MIDI) system and the ACTIN6 database (Sasser 1990). Cryo-scanning electron microscopy was performed according to Celler et al. (2016) for description of the morphological features of both isolates after growth on ISP2 agar plates (in half-strength artificial sea water) at 28 °C for 7 days.

Phylogeny

Genomic DNAs were extracted using the Qiagen DNeasy Blood and Tissue Kit. The 16S rRNA gene was amplified by PCR using the universal bacterial 16S rDNA primers F27 and R1492 (Lane 1991). Obtained PCR products were cloned into the Qiagen pDrive PCR cloning vector and sequenced using standard M13 vector primers at MWG Biotech (Germany). DNA sequences of almost complete 16S rRNA genes (ca 1400 nucleotides) were compared with those of the type strains of the genus Actinoalloteichus available in EzTaxon server database (http://www.ezbiocloud.net/identify) (Chun et al. 2007). Sequences were aligned using Clustal W algorithm (Thompson et al. 1997) and a phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7 (Kumar et al. 2016). The tree was computed using maximum likelihood method (ML) and the resulting tree topology was tested by bootstrap analysis performed with 5000 replicates. The 16S rRNA gene sequences for strains ADI 127-17T and GBA 129-24 have been deposited in Genbank with the accession numbers MF440323 and MF440324, respectively.

Genome sequencing, assembly and annotation

For isolation of genomic DNA, both isolates were grown aerobically in 50 ml of 3% TSB medium (Oxoid, UK) prepared in 50% artificial sea water in 250 ml baffled flasks at 28 °C, 250 rpm, for 72 h. Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega, USA) from approximately 2 g of mycelium (wet weight) using the
manufacturer’s protocol with the following modification: the clarified lysate prior to precipitation of DNA with isopropanol was extracted once with 1/2 volume of a 1:1 mixture of phenol/chloroform (pH 8.0). For sequencing and assembly, an approach that was shown to provide high quality data for actinobacterial genomes (Rückert et al. 2015). For each of the two isolates, two libraries were prepared: a WGS library using the Illumina-Compatible Nextera DNA Sample Prep Kit (Epicentre, USA) and a 5 k MatePair library using the Nextera Mate Pair Sample Preparation Kit, both according to the manufacturer’s protocol. All libraries were sequenced in 2 × 250 bp paired read runs on the MiSeq platform.

For isolate ADI 127-17T, a total of 4,487,929 reads were obtained, providing 132.3× coverage of the genome. For strain GBA 129-24, a total of 4,169,978 reads provided 124.7× coverage. Reads were assembled using the Newbler assembler v2.8 (Roche). The initial Newbler assemblies consisted of 54 contigs in two scaffolds, with a total of 76 contigs larger 100 bp for strain ADI 127-17T, and 64 contigs in two scaffolds, with a total of 90 contigs larger 100 bp for strain GBA 129-24. The Phred/Phrap/Consed software package (Ewing and Green 1998; Ewing et al. 1998; Gordon et al. 1998; Gordon 2003) was used for sequence assembly and quality assessment in the subsequent finishing process. Gaps between contigs were closed by manual editing in Consed (for repetitive elements).

Gene prediction and annotation were done using the PGAP pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Genes were identified using GeneMark (Borodovsky et al. 2003), GLIMMER (Delcher et al. 1999) and Prodigal (Hyatt et al. 2010). For annotation, BLAST searches against the NCBI Protein Clusters Database (35) were performed and the annotation was enriched by searches against the Conserved Domain Database (Klimke et al. 2009) and subsequent assignment of coding sequences to COGs. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE (Marchler-Bauer et al. 2009), Infernal (Eddy 2002), RNAmmMer (Lagesen et al. 2007), Rfam (Griffiths-Jones et al. 2005), TMHMM (Krogh et al. 2001), and SignalP (Bendtsen et al. 2004). Secondary metabolite biosynthesis gene clusters in the genomes were identified using antiSMASH 3.03 (Weber et al. 2015) and manually analysed using BLAST. The genome project was deposited in the Genomes OnLine Database (Lioliios et al. 2010). Sequencing, finishing and annotation were performed at the CeBiTec and the complete genome sequences of strain ADI127-17 and GBA 129-24 have been deposited in GenBank under the accession numbers CP016076 and CP016077-CP016078, respectively. The genome sequence of A. hoggarensis DSM 45943T has been deposited in GenBank under the accession number CP022521.

Digital DNA: DNA hybridization

Digital DNA: DNA hybridization (dDDH) analyses were performed between isolates ADI127-17T and GBA 129-24 and their close neighbours A. hoggarensis and A. hymeniacidonis using formula 2 of the Genome-to-Genome Distance Calculator server available at DSMZ website (http://ggdc.dsmz.de/distcalc2.php).

Results and discussion

Actinoalloteichus isolates from marine sponges A. dichotoma and G. barretti

Two actinobacteria whose 16S rRNA genes displayed >99% identity to the type strains A. hymeniacidonis DSM 45092T and A. hoggarensis DSM 45943T were isolated from marine sponges A. dichotoma (isolate ADI127-17) and G. barretti (GBA129-24), respectively, collected at the Trondheim fjord (Norway) as described in “Materials and methods”. Both isolates have been deposited to the DSMZ culture collection with accession numbers DSM 46855T (ADI127-17) and DSM 46856 (GBA129-24), and tentatively assigned to the genus Actinoalloteichus. The 16S rRNA gene sequences of DSM 46855T and DSM 46856 were compared to the Ribosomal Database Project database (https://rdp.cme.msu.edu/), confirming the initial taxonomic classification of these isolates. Figure 1 shows the position of Actinoalloteichus spp. DSM 46855T and DSM 46856 in a 16S rRNA gene-based phylogenetic tree, which indicates that they are closely related to A. hoggarensis DSM 45943T. The inferred evolutionary history is supported by the phylogenetic tree generated using the Neighbor-Joining method (Figure S1, Supplementary materials).
While the isolates ADI 127-17 and GBA 129-24 could be cultivated on ISP2 medium supplemented with half-strength artificial sea water, they failed to growth on the same medium prepared with distilled water but with 0, 1, 2, 3, 4 and 5% NaCl. The latter results indicate that but some component(s) in the sea water is/are needed for growth of the two isolates, rather than the osmotic strength.

After 2 weeks of incubation at 28 °C on ISP2 medium prepared with half-strength sea water, strain DSM 46855\textsuperscript{T} displayed black–violet substrate and blue–violet aerial mycelium and produced no diffusible pigment. The phenotype of the strain DSM 45856 grown in the same conditions was very similar, with the only difference being aerial mycelium, which was grey–violet.

The micromorphology of *Actinoalloteichus* sp. strain DSM 46856 examined using a conventional microscope was significantly different from that of strain DSM 46855\textsuperscript{T}, as the majority of the spore chains of strain DSM 46856 were embedded within the aerial mycelium (as seen at lower magnification). The aerial hyphae appeared to stick together, indicative of the existence of an extracellular matrix, and knots were regularly observed in the aerial hyphae. The spore chains that were formed were also more homogeneous in spore length. The substrate mycelia of both strains were found to be non-fragmented.

To examine the micromorphology of the *Actinoalloteichus* isolates in more detail, cultures were grown for 7 days on ISP2 medium (in half-strength artificial sea water) and sporulating aerial hyphae were examined using cryo-scanning electron microscopy (Fig. 2). *Actinoalloteichus* sp. DSM 46855\textsuperscript{T} showed typical sporulation similar to what is observed for streptomycetes, revealing a multitude of aerial hyphae with chains of spores. However, some aerial hyphae branched and the spore sizes were relatively irregular in size, with larger and small spores in a single spore chain. Higher magnification revealed occasional aerial hyphae forming a single septum, which, up to that stage, had failed to progress to sporulation.

**Chemotaxonomy of the new *Actinoalloteichus* isolates**

Both new *Actinoalloteichus* isolates were subjected to chemotaxonomic analyses as described in Methods.
section in comparison with two type species, *A. hymeniacidonis* and *A. hoggarensis*, and the results are presented in Table 1. Analysis of phospholipid content revealed the presence of phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, two glycophostopholipids, two unidentified lipids and a glycolipid (Figs. S2–S4, Supplementary materials). None of the following polar lipids were detected: phosphatidylethanolamine, phosphatidylmethylethanolamine or phosphatidylcholine.

The same pattern of cell sugars was visualised for both *Actinoalloteichus* spp. DSM 46855<sup>T</sup> and DSM 46856: galactose, glucose, mannose, ribose, rhamnose (trace) and an unidentified sugar. The major fatty acids were found to be anteiso-15:0 (20.1, 25.6%), 17:0 (13.6, 18.3%), iso-16:0 (10.9, 10.9%), cis-9-17:1 (9.1, 10.3%), iso-15:0 (8.7, 10.6%) for strains DSM 46856 and DSM 46855<sup>T</sup>, respectively. DL-diaminopimelic acid was revealed for both isolates. The major abundant quinone was identified as MK-9(H4) for both strains.

According to the chemotaxonomic analyses, the new isolates clearly differed from *A. hymeniacidonis*, being more similar to *A. hoggarensis*. However, major fatty acid composition, menaquinone and phospholipid profiles of *Actinoalloteichus* spp. DSM 46855<sup>T</sup> and DSM 46856 were significantly different from those of *A. hoggarensis*. For example, unlike *A. hoggarensis*, the new isolates did not have phosphatidylethanolamine in their phospholipid profiles. Also, their fatty acid composition varied from that of the *A. hoggarensis*: they contained considerably less iso-C<sub>14:0</sub>, iso-C<sub>15:0</sub>, isoG-C<sub>16:1</sub>, iso-C<sub>16:0</sub>, and iso-C<sub>17:0</sub> fatty acids while having significantly more anteiso-C<sub>15:0</sub>, C<sub>16:0</sub>, anteiso-C<sub>17:0</sub>, and C<sub>17:0</sub> fatty acids.

Genomics of *Actinoalloteichus* spp. DSM 46855<sup>T</sup> and DSM 46856

Due to the increasing interest in identifying new actinobacterial species that can be potential sources of novel bioactive secondary metabolites, the new *Actinoalloteichus* isolates were subjected to genome analyses. Sequencing, finishing and annotation were performed, and the complete genome sequences deposited in GenBank with accession numbers CP016076 (DSM 46855<sup>T</sup>), CP016077 (DSM 46856 chromosome) and CP016078 (DSM 46856 plasmid). A summary of the project information is shown in Table 2.

The genome of strain DSM 46855<sup>T</sup> consists of one circular chromosome of 7,120,854 bp (70.09% G+C content), while the genome of strain DSM 46856 consists of one circular chromosome of 7,215,977 bp (70.11% G+C content) and a plasmid of 59,408 bp (69.21% G+C content). Among a total of 6064 predicted genes in the DSM 46855<sup>T</sup> genome, 5986 appear to be protein coding, while these numbers for DSM 46856 are 6271 and 5986, respectively. In total, 4360 protein-coding genes of DSM 46855<sup>T</sup> (71.90%) and 4412 protein-coding genes of strain DSM 46856 (70.36%) were assigned a putative function, the remaining were annotated as hypothetical proteins. The properties and the statistics of the genomes are briefly summarised in Tables 2 and 3, and the circular
In order to resolve the ambiguity regarding relat-edness of new isolates and *A. hoggarensis* DSM 45943T stemming from phylogenetic analysis of their 16S rRNA genes (Fig. 1), the genome of the latter bacterium was sequenced (Genbank accession number CP022521). Like the genome of *A. hymeniacidonis* DSM 45092T (Schaffert et al. 2016) with 6306 kbp (68.08% G+C content), the genome of *A. hoggarensis* DSM 45943T is significantly smaller than those of the two *Actinoalloteichus* strains DSM 46855T and DSM 46856, consisting of a single, circular chromosome of 6607 kbp with a G+C content of 71.15%.

The significantly different genome sizes and G+C contents provided further evidence that the *Actinoalloteichus* strains DSM 46855T and DSM 46856 represent a distinct novel species. Based on the

---

**Table 1** Chemotaxonomic characteristics of the strains DSM 46855T, DSM 46856 and the type strains of the closely related species *A. hymeniacidonis* DSM 45092T and *A. hoggarensis* DSM 45943T

|                  | DSM 45092T | DSM 45943T | DSM 46856 | DSM 46855T |
|------------------|------------|------------|-----------|------------|
| iso-14:0         | 4          | 3.7        | 2.0       | 2.1        |
| iso-6:15:0       | 6          | —          | —         | —          |
| iso-15:0         | —          | 21.6       | 8.7       | 10.6       |
| anteiso-15:0     | 20         | 11.7       | 25.6      | 20.1       |
| 15:0             | 6          | 4.1        | 4.7       | 5.6        |
| iso-16:1         | —          | —          | —         | —          |
| 16:1             | —          | —          | —         | —          |
| isoG-16:1        | 6          | 5.8        | 2.5       | 2.2        |
| iso-16:0:0       | 16         | —          | —         | —          |
| iso-16:0         | —          | 18.2       | 10.9      | 10.9       |
| 16:0             | —          | —          | 1.8       | 1.6        |
| iso-17:0         | —          | 4.0        | 1.7       | 2.0        |
| anteiso-17:0     | 4          | 4.2        | 10.8      | 9.6        |
| W8C-17:1 = cis9-17:1 | 19 | —         | 9.1       | 10.3       |
| 17:0             | 11         | 8.8        | 13.6      | 18.3       |
| MK-8(H4)         | —          | —          | —         | 3.6        |
| MK-9(H2)         | —          | —          | —         | 0.4        |
| MK-9(H4)         | 64         | +(54%)a    | 99.7      | 58.1       |
| MK-9(H6)         | 23         | +(3.2%)a   | —         | 4.1        |
| MK-9(H8)         | 12         | —          | —         | —          |
| MK-10(H4)        | —          | +(34.2%)a  | 0.4       | 9.4        |
| Phosphatidylethanolamine | +     | +          | —         | —          |
| Phosphatidylglycerol  | +      | +          | +         | +          |
| Phosphatidylinositol | +      | +          | +         | +          |
| Phosphatidylinositol mannoside | +   | +          | —         | —          |
| Glycophospholipids | —        | —          | +         | +          |
| Diphosphatidylglycerol | —     | +          | +         | +          |
| Phosphatidylmonomethyl-ethanolamine | —    | —          | —         | —          |
| Phosphatidyl choline | —      | —          | —         | —          |
| meso-A2 pm       | +         | +          | +         | +          |
| Galactose, glucose, mannose, ribose, rhamnose (trace) unidentified sugar | +   | +          | +         | +          |

* Absolute values for *A. hoggarensis* DSM 45943T not reported.
available genome sequences for *Actinoalloteichus* spp. DSM 46855^T^, DSM 46856, DSM 45092^T^ and DSM 45943^T^, which appear most related based on the 16S rRNA gene sequences, we performed genome-to-genome distance calculations (Table 4). The 16S rRNA gene sequences of *Actinoalloteichus* spp. DSM 46855^T^, DSM 46856 were 99.9% identical to that of the most closely related species, *A. hoggarensis* DSM 45943^T^.

However, both DDH and ANI estimates obtained clearly suggest that strains DSM 46855^T^ and DSM 46856 belong to the same species (DHH = 97.6%, ANI = 99.68%), which is distinct from *A. hoggarensis* (DHH = 30.9%, ANI = 85.96% compared with strain DSM 46855^T^). Based on the analyses described above, we propose that the isolates DSM 48655^T^ and DSM 48656 represent a new species of the genus *Actinoalloteichus*, with the proposed name *Actinoalloteichus fjordicus*. It is notable that *A. fjordicus* is the first representative of the genus *Actinoalloteichus* which appears to be an obligate marine species. The Digital Protologue database (Rossello-Móra et al. 2017) Taxon Number for strain DSM 46855^T^ is TA00145.

### Description of *A. fjordicus* sp. nov.

*Actinoalloteichus fjordicus* sp. nov. (fjor'di,cus. N.L. masc. adj. fjordicus, referring to a Norwegian fjord, the site of isolation).

Aerobic, Gram-stain positive, catalase positive actinobacterium that forms an extensively branched substrate mycelium that bears aerial hyphae that differentiate into straight chains of smooth surfaced spores of unusual bluish on ISP2 medium. Grows from 20 to 35 °C, optimally at ~28 °C, optimally at ~pH 7.0, and requires at least 50% sea water in the media for growth. Additional cultural and phenotypic features are cited in the text and in Table 1. Chemotaxonomic characteristics are typical of the genus *Actinoalloteichus*.

The type strain, AD1127-7^T^ (= DSM 46855^T^ = CECT 9355^T^), was isolated from a sponge sample of *Antho dichotoma* collected at a depth of 60 m below sea level at the Tautra ridge in the Trondheim fjord in Norway (63°36’N and 10°31’E). The GenBank accession numbers for the 16S rRNA gene sequences of DSM 46855^T^ and the related strain DSM 46856 are MF440323 and MF440324, respectively.

Gene clusters for biosynthesis of secondary metabolites in the genomes of *A. fjordicus*

All sequenced actinobacterial genomes contain gene clusters for biosynthesis of secondary metabolites, but the information regarding the types of compounds that representatives of the genus *Actinoalloteichus* can produce is scarce. Moreover, to our knowledge, only one biosynthetic gene cluster from *Actinoalloteichus*
has been characterised so far (Zhu et al. 2012). Recently, we sequenced the genome of sponge-derived *A. hymeniacedonis*, and described analysis of its secondary metabolite biosynthesis gene clusters, SMBGCs (Schaffert et al. 2016). Considering the above, it was of interest to compare the biosynthetic potential of the latter, as well as terrestrial *Actinoalloteichus* spp. genomes (albeit draft quality) with those of the new isolates.

The genomes of DSM 46855\textsuperscript{T}, DSM 46856, *A. hymeniacedonis*, *A. hoggarensis*, *A. cyanogriseus* and *A. spitinensis* were analysed for the presence of SMBGCs using the online version of software antiSMASH 4.0 (Weber et al. 2015). The results of the analysis were manually curated to confirm or edit borders of the clusters, identify closest homologues in the databases based on BLAST searches, and to gain more detailed insight into the biosynthesis of the corresponding compounds. The results of the analysis are presented in Table 5.

The new *A. fjordicus* strains were found to harbour 27 identical SMBGCs, the genome of DSM 46855\textsuperscript{T} containing one additional cluster compared to that of DSM 46856, presumptively governing biosynthesis of a peptide-nucleoside compound. Five gene clusters that are likely to be responsible for the biosynthesis of known secondary metabolites, ectoine (two gene clusters, Sadeghi et al. 2014), nocardicin (Gunsiin...
et al. 2004), maduropeptin (Van Lanen et al. 2007) and dihydromaltophilin (Yu et al. 2007) were identified in both DSM 46855T and DSM 46856 genomes. One ectoine biosynthesis gene cluster was found in all the analysed genomes, but the second one was only identified in A. hoggarensis and A. hymeniacedonis. While the nocardicin gene cluster was present in the genomes of A. hoggarensis, A. cyanogriseus and A. spitiensis, it could not be identified in the A. hymeniacedonis genome. The maduropeptin gene cluster was identified in A. cyanogriseus, but not in the genomes of other Actinoalloteichus species investigated in this study. The dihydromaltophilin gene cluster was identified in all analysed genomes. Compared to other analysed Actinoalloteichus spp., seven SMBGCs appeared to be unique for the A. fjordicus strains (Table 5). Of those, 4 (clusters 2, 7, 14 and 24) could not be matched to any cluster available in the public databases as of March 2017, suggesting that DSM 46855T and DSM 46856 may be interesting sources of

![Fig. 3](Image)

**Fig. 3** Graphical maps of the chromosomes of a A. fjordicus DSM 46855T and b A. fjordicus DSM 46856. From the outside to the center: Genes on forward strand (color by COG categories), genes on reverse strand (color by COG categories—see Table 3), RNA genes (tRNAs green, rRNAs red, other RNAs black), G+C content, G+C skew

| Strain                  | DDH        | A. fjordicus DSM 46855T | A. fjordicus DSM 46856 | A. hoggarensis DSM 45943T | A. hymeniacedonis DSM 45092T |
|------------------------|------------|------------------------|------------------------|---------------------------|-----------------------------|
| A. fjordicus DSM 46855T| 100.0%     | 97.6%                  | 30.9%                  | 24.5%                     |
| A. fjordicus DSM 46856 | 100.0%     |                        | 30.9%                  | 24.5%                     |
| A. hoggarensis DSM 45943T | 100.0%     |                        | 100.0%                 | 23.9%                     |
| A. hymeniacedonis DSM 45092T | 100.0%     |                        |                        |                           |

| Strain                  | ANI        | A. fjordicus DSM 46855 | A. fjordicus DSM 46856 | A. hoggarensis DSM 45943T | A. hymeniacedonis DSM 45092T |
|------------------------|------------|------------------------|------------------------|---------------------------|-----------------------------|
| A. fjordicus DSM 46855 | 100.00%    | 99.68%                 | 85.96%                 | 80.05%                    |
| A. fjordicus DSM 46856 | 100.00%    |                        | 85.98%                 | 79.98%                    |
| A. hoggarensis DSM 45943T | 100.00%   |                        | 100.00%                | 79.51%                    |
| A. hymeniacedonis DSM 45092T | 100.00%    |                        |                        |                           |
previously unknown natural products. The knowledge on the respective SMBGCs and their putative products will help to identify corresponding compounds, and may assist in their testing and development as potential drug candidates.

Acknowledgements Open access funding provided by University of Vienna. SBZ acknowledges support from the Norwegian University of Science and Technology (Norway) and University of Vienna (Austria). Anika Winkler and Katharina Hanuschka (CeBiTec, Bielefeld University) are acknowledged for help with genome sequencing.

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
References

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: signalP 3.0. J Mol Biol 340:783–795

Bhatnagar I, Kim S (2010) Immense essence of excellence: marine microbial bioactive compounds. Mar Drugs 8:2673–2701

Borodovsky M, Mills R, Besemer J, Lomsadze A (2003) Prokaryotic gene prediction using GeneMark and GeneMark.hmm. Curr Protoc Bioinformatics 1:4–5

Boudjelal F, Zitouni A, Bouras N, Schumann P, Sproer C, Borodovsky M, Mills R, Besemer J, Lomsadze A (2003) Immense essence of excellence: marine microbial bioactive compounds. Mar Drugs 8:2673–2701

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: signalP 3.0. J Mol Biol 340:783–795

Bhatnagar I, Kim S (2010) Immense essence of excellence: marine microbial bioactive compounds. Mar Drugs 8:2673–2701

Borodovsky M, Mills R, Besemer J, Lomsadze A (2003) Prokaryotic gene prediction using GeneMark and GeneMark.hmm. Curr Protoc Bioinformatics 1:4–5

Boudjelal F, Zitouni A, Bouras N, Schumann P, Sproer C, Sabauo N, Klenk HP (2015) Actinoalloteichus hoggarensis sp. nov., an actinomycete isolated from Saharan soil. Int J Syst Evol Microbiol 65:2006–2010

Celler K, Koning RI, Willemse J, Koster AJ, van Wezel GP (2016) Cross-membranes orchestrate compartmentalization and morphogenesis in Streptomyces. Nat Commun 7:ncomms11836

Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol 57:2259–2261

Collins MD (1985) Analysis of isoprenoid quinone. In: Gottschalk G (ed) Methods in Microbiology. Academic Press, London, pp 329–366

Davies J, Ryan KS (2012) Introducing the parvome: bioactive compounds in the microbial world. ACS Chem Biol 7:252–259

Delcher AL, Harmon D, Kasif S, White O, Salzberg SL (1999) Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641

Ewing B, Green P (1998) Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8:186–194

Fujita K, Sugiyama R, Nishimura S, Ishikawa N, Arai MA, Ishibashi M, Kakeya H (2016) Stereochemical assignment and biological evaluation of BE-14106 unveils the importance of one acetate unit for the antifungal activity of polyene macrolactams. J Nat Prod 79:1877–1880

Gordon D (2003) Viewing and editing assembled sequences using Consed. Curr Protoc Bioinform 2(11.2.1):43

Gordon D, Abajian C, Green P (1998) Consed: a graphical tool for sequence finishing. Genome Res 8:195–202

Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A (2005) Pfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res 33(Database issue):D121–D124

Gunior M, Breazeale SD, Lind AJ, Ravel J, Jane JW, Townsend CA (2004) The biosynthetic gene cluster for a monocyclic beta-lactam antibiotic, nocardicin A. Chem Biol 11:927–938

Horinouchi S (2007) Mining and polishing of the treasure trove in the bacterial genus Streptomyces. Biosci Biotechnol Biochem 71:283–299

Hyatt D, Chen G, Locascio PF, Land ML, Larimer FW, Hauser LJ (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119

Ian E, Malko DB, Sekurova ON, Bredholt H, Rückert C, Borisova ME, Albersmeier A, Kalinowski J, Gelfand MS, Zotchev SB (2014) Genomics of sponge-associated Streptomyces spp. closely related to Streptomyces albus J1074: insights into marine adaptation and secondary metabolism biosynthesis potential. PLoS ONE 9:e96719

Klimek W, Agarwala R, Badredtin A, Chetvernin S, Ciufo S, Fedorov B, Kyrятин B, O’Neill K, Resch W, Resenichak S, Schaf S, Tolstoy I, Tatusova T (2009) The National Center for Biotechnology Information’s Protein Clusters Database. Nucleic Acids Res 37(Database issue):D216–D223

Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

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 38:358–361

Lane DJ (1991) 16S/23S rRNA sequencing. Nucleic acid techniques. In: Stackebrandt E, Goodfellow M (eds) Bacterial Systematics. Wiley, Chichester, pp 115–175

Springer
Lechevalier MP, Lechevalier HA (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443

Litiöös K, Chen IA, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, Kyrpides NC (2010) The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Res 38(Database issue):D346–D354

Manivasagan P, Kang K, Sivakumar K, Li-Chan Eunice C Y, Oh H, Kim S (2014) Marine actinobacteria: an important source of bioactive natural products. Environ Toxicol Pharmacol 38:172–188

Marchler-Bauer A, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, He S, Hurwitzi DI, Jackson JD, Ke Z, Lanzycicki CJ, Liebert CA, Liu C, Lu F, Lu S, Marchler GH, Mulkovand M, Song JS, Tasneem A, Tharsi N, Yamashita RA, Zhang D, Zhang N, Bryant SH (2009) CDD: specific functional annotation with the Conserved Domain Database. Nucleic Acids Res 37(Database issue):D205–D210

Minnikin DE, O’Donnell AG, Goodfellow M, Alderson G, Athalaye M, Schaal K, Parleth JJ (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 16:584–586

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

Prudhomme J, McDaniel E, Ponts N, Bertani S, Fenical W, Jensen P, Le Roch K (2008) Marine actinomycetes: a new strategy for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Van Lanen SG, Oh TJ, Liu W, Wendt-Pienkowski E, Shen B (2007) Characterization of the maduropeptin biosynthetic gene cluster from Actinomadura madurae ATCC 39144 supporting a unifying paradigm for enediyne biosynthesis. J Am Chem Soc 129:13082–13094

Wang XJ, Zhang J, Wang JD, Qian PT, Liu CX, Xiang WS (2013) Novel cyclopentenone derivatives produced by a rare actinobacterial strain Actinoalloteichus nanseanensis sp. nov. NAU 119. Nat Prod Res 27:1863–1869

Weber T, Blin K, Duddela S, Krug D, Kim HU, Brucclori R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH (2014) antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 42:W237–W243

Xiang W, Liu C, Wang X, Du J, Xi L, Huang Y (2011) Actinoalloteichus nanseanensis sp. nov., isolated from the rhizosphere of a fig tree (Ficus religiosa). Int J Syst Evol Microbiol 61:1165–1169

Yim G, Wang HH, Davies J (2007) Antibiotics as signalling molecules. Philos Trans R Soc Lond B Biol Sci 362:1195–1200

Yu F, Zaleta-Rivera K, Zhu X, Huffman J, Millet JC, Harris SD, Yuen G, Li XC, Du L (2007) Structure and biosynthesis of heat-stable antifungal factor (HSAF), a broad-spectrum antymycotic with a novel mode of action. Antimicrob Agents Chemother 51:64–72

Zhang H, Zheng W, Huang J, Luo H, Jin Y, Zhang W, Liu Z, Huang Y (2006) Actinoalloteichus hymenioides sp. nov., an actinomycete isolated from the marine sponge Hymenioides perleve. Int J Syst Evol Microbiol 56:2309–2312

Zhu Y, Fu P, Lin Q, Zhang G, Zhang H, Li S, Ju J, Zhu W, Zhang C (2012) Identification of caerulomycin A gene cluster implicates a tailoring amidohydrolase. Org Lett 14: 2666–2669

Zotchev SB (2012) Marine actinomycetes as an emerging resource for the drug development pipelines. J Biotechnol 158:168–175