Diversity and taxonomic novelty of Actinobacteria isolated from the Atacama Desert and their potential to produce antibiotics

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrechts-Universität zu Kiel

Vorgelegt von

Alvaro S. Villalobos

Kiel 2018

Referent: Prof. Dr. Johannes F. Imhoff

Korreferent: Prof. Dr. Ute Hentschel Humeida

Tag der mündlichen Prüfung:

Zum Druck genehmigt: 03.12.2018

gez. Prof. Dr. Frank Kempken, Dekan

Table of contents

Summary

Actinobacteria were isolated from selected environments of the Chilean Altiplano, from the rhizosphere of different plants near Socaire, from two hyper-saline lakes of the Atacama Desert, and from Llullaillaco Volcano Lake. The phylogenetic diversity and the potential of production of antibiotics were studied in a total of 79 isolates. Quite characteristically, each of the studied environments contained a different variety of actinobacteria. Actinobacteria isolated from the rhizosphere of plants close to Socaire revealed the presence of genera known as habitants in the rhizosphere of other plants, promoting its growth both directly and indirectly. Salar de Huasco showed a high diversity of Actinobacteria with *Nocardiopsis* as the most abundant genus, together with halophile actinobacteria, which are often found in saline environments. Isolates from Salar de Llamará belong exclusively to the *Micromonosporaceae* family, exhibiting similarity with strains obtained from mangroves and marine sediments. Actinobacteria obtained from these environments showed a high number of putative novel species.

One of the strains from Salar de Llamará, strain Llam7^{T}, was characterised as a novel genus and species of the *Micromonosporaceae* family with the name *Superstesspora tarapacensis.* Another isolate originating from Llullaillaco Volcano Lake was described as a novel species with name *Subtercola vilae* and type strain DB165^{T}. The characteristics of *S. vilae* allowing it to survive the cold environmental conditions of Llullaillaco Volcano Lake were identified through functional annotation of its genome, revealing an extensive repertoire of genes involved in membrane modulation, degradation of reactive oxygen species, and ice-binding proteins.

More than half of the isolates have the capacity to produce antibiotic substances active against Gram-positive and Gram-negative bacteria. The genomic potential of 7 of the strains affiliated with *Streptomyces*, *Kribbella* and *Superstesspora tarapcensis* was studied and revealed the potential to produce natural products. Most of the biosynthetic gene clusters for natural products revealed only low homology in their gene synteny with entries in databases, and hence might be coding for novel natural product compounds. It is concluded that the Atacama Desert and its actinobacteria constitute a promising source of taxonomic and chemical novelty, providing a cornerstone for future taxonomic studies and secondary metabolite analyses.

Zusammenfassung

Aktinobakterien wurden von verschiedenen Stellen des chilenischen Altiplano isoliert: aus der Rhizosphäre verschiedener Pflanzen in der Nähe von Socaire, aus zwei hypersalinen Seen der Atacama Wüste und aus dem Llullaillaco Vulkansee. Die phylogenetische Vielfalt und das Potential zur Antibiotikaproduktion wurden anhand von insgesamt 79 Isolaten untersucht. Für jedes der beprobten Habitate war eine bestimmte Vielfalt von Aktinobakterien charakteristisch. Aus der Rhizosphäre von Pflanzen in der Nähe von Socaire wurden unter anderem Aktinobakterien Gattungen isoliert, die bereits von anderen Pflanzen bekannt sind, und die direkt oder indirekt deren Wachstum fördern. Im Salar de Huasco wurde eine hohe Vielfalt an Aktinobakterien gefunden, mit halophilen Arten, die häufig in salzhaltigen Umgebungen vorkommen und mit *Nocardiopsis* als häufigster Gattung, zusammen. Die Isolate aus dem Salar de Llamará gehören ausschließlich zur Familie der *Micromonosporaceae* und weisen Ähnlichkeit mit Stämmen auf, die von Mangroven und aus Meeressedimenten gewonnen wurden. Unter denisolierten Aktinobakterien aus diesen Habitaten sind viele potentielle neue Arten.

Einer der Stämme aus dem Salar de Llamará, Stamm Llam7^{T}, wurde als neue Gattung und Art der *Micromonosporaceae* Familie mit dem Namen *Superstesspora tarapacensis* charakterisiert. Ein weiteres Isolat, das aus dem Llullaillaco Vulkansee stammt, wurde als die neue Spezies *Subtercola vilae* mit DB165^{T} als Typstamm beschrieben. Die funktionelle Genomannotation von *S. vilae* offenbarte ein umfangreiches Repertoire an Genen für Membranmodulation, den Abbau von reaktiven Sauerstoffradikalen und eisbindende Proteine; diese Gene ermöglichen den Aktinobakterien die kalten Umweltbedingungen des Llullaillaco Vulkansees zu überleben.

Mehr als die Hälfte der Isolate ist in der Lage, Antibiotika zu produzieren, die gegen Gram-positive und Gram-negative Bakterien wirken. Die Genome von 7 Stämmen der Gattungen *Streptomyces, Kribbella* und *Superstesspora* wurden näher untersucht und zeigten das Potenzial, Naturstoffe herzustellen. Die meisten biosynthetischen Gen-Cluster für Naturstoffe zeigten in ihrer Syntenie nur geringe Homologie mit Einträgen in Datenbanken und könnten daher für neuartige Naturstoffe kodieren. Zusammenfassend ist die Atacama-Wüste mit ihren Aktinobakterien eine vielversprechende Quelle für taxonomische und chemische Neuheit, und bildet einen Grundstein für zukünftige taxonomische Studien und sekundäre Stoffwechselanalysen.

Introduction

Geological and climatic background of Atacama Desert

Desert environments are defined as regions that receive extremely low precipitation, far less than the amount required to support the growth of most plants. Earth's deserts receive an average annual rainfall (AAR) of less than 400 mm per year (Makhalanyane et al. 2015). Deserts such as Kalahari and Mojave receive 250 and 330 mm of AAR respectively, while “True Deserts” receive less than 250 mm of AAR. Examples of true deserts are the Gobi and Sahara (194 mm and 20-100 mm, respectively). However, there is another category called “Hyper-arid” which is assigned to those deserts with an aridity index lower than 0.05 (Makhalanyane et al. 2015). This means that these environments have low AAR and high annual evapotranspiration. The Atacama Desert is included in this latest category.

The Atacama Desert is located in the north of Chile bordering Perú in the north, extending to the Copiapó river in the south. The desert extends 1000 km from north to south, approximately between latitudes 19°S and 30°S, and from the Coastal Cordillera in the west to the Andean Cordillera in the east. The hyper-arid region of Atacama Desert is in the valley bounded by the coastal mountains and the medial Cordillera de Domeyko (Houston 2006). It has been proposed that the west slope of the central Andes exhibits a pronounced rain shadow effect, causing this core zone of hyper-aridity, which extends from sea level up to 3500 m above sea level. This initial onset of hyper-aridity most likely developed progressively, starting with aridity during the Jurassic period (150 million years ago), and evolving during the Miocene period (135 million years later) into its current state as a hyper-arid desert; this was helped along by the uplifting of the Andes, which reached elevations between 1000 and 2000 m above sea level, coupled with the intensification of a cold upwelling Peruvian Current circa 10-15 million years ago.

In addition, palaeomagnetic data (Hartley et al. 2005) showed no significant latitudinal movement from the late Jurassic onwards. This, along with Atacama's location within the dry subtropic climate belt, and the presence of the cold upwelling current dating from at least the early Cenozoic (66 million years ago), resulted in climatic stability in the desert, suggesting strongly that the Atacama Desert is the oldest desert on Earth.

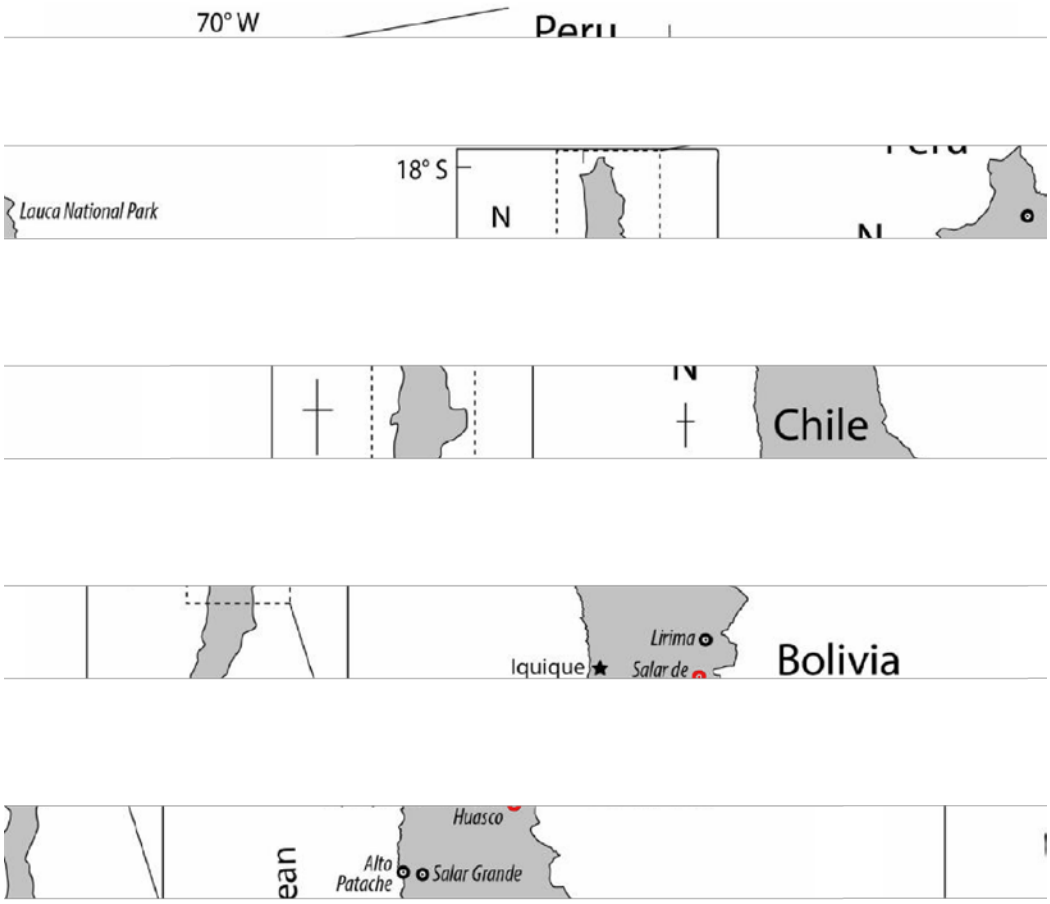


Figure 1. Map of Chile, with the zoomed region showing the Atacama Desert. The sampling sites of Socaire, Salar de Llamará and Salar de Huasco, and the isolation source of the *Subtercola vilae* DB165^{T} (Llullaillaco volcano) are shown in red dots. Map drawn by Dr. Cristina Dorador and Dr. Chris Harrod (Universidad de Antofagasta) and reproduced with the permission of the authors.

Microbiology of Atacama Desert

For a long time, the extreme aridity of the Atacama Desert and its apparent lack of flora gave the false impression that this environment could not uphold any life forms. Therefore, it became a perfect playground for the Jet Propulsion Laboratory and NASA to develop and test life detection instruments that would be used in 1975 on the Viking Mission. Cameron et al. (1966) conducted the first study of the region in Uribe train station (15 km south-east of Antofagasta), in which they characterised the soil and microflora. Aerobic bacteria were isolated using trypticase soy agar plates; anaerobic bacteria were isolated using the same medium in CO_2 chambers. In addition, microbial growth obtained from dilution tubes of thioglycolate medium indicated that a gram of soil has 10^6-10^7 microorganisms; the microbes identified in this study were affiliated with *Streptomyces* and *Mycococcus* genera (both Actinobacteria). In a further study, additional strains obtained from the first study were classified as *Bacillus subtilis*, *Bacillus brevis*, *Bacillus cereus*, and *Micrococcus casseolyticus* (Bollen et al. 1966). Later, the attention of the Atacama Desert research was renewed with a different focus. Studies of microorganisms from hypersaline lakes, in particular from Salar de Atacama, showed the diversity of halotolerant bacteria and chemotaxonomic analyses of the isolated strains (Prado et al. 1991), as well as the prevalence of cyanobacteria (Campos 1997).

Initial culture-independent studies, using denaturing gradient gel electrophoresis (DGGE), showed that the microbial communities of the hyper-arid core of Atacama Desert were dominated by Gemmatimonadetes and Planctomycetes phyla, and that Actinobacteria were present (Drees et al. 2006). In a different study cloning the 16S rRNA from environmental samples, it was shown that soils from Yungay were abundant in Actinobacteria, Proteobacteria, Firmicutes, and TM7 division bacteria; of these, 94% of the clones were affiliated with the Actinobacteria phylum (Connon et al. 2007). Twenty bacterial strains were also obtained from Atacama Desert soils, which belonged to the genera *Rhodopseudomonas*, *Sphingomonas*, *Mesorhizobium*, *Asticcacaulis*, *Bradyrhizobium*, *Bacillus*, and *Burkholderias* (Lester et al. 2007). Using next-generation sequencing technologies (NGS), the microbial diversity from different samples across the hyper-arid core of the desert revealed a unique bacterial diversity marked by high abundances of novel Actinobacteria and Chloroflexi and low levels of Acidobacteria and Proteobacteria (Neilson et al. 2012). These phyla were recurrent and dominant in many of the Atacama Desert

biomes analysed. Actinobacteria phylum has been described as being present in all cold and hot deserts (Fierer et al. 2012).

The evidence of the high prevalence of Actinobacteria in the Atacama Desert led to the first studies involving the selective isolation of this phylum. The first study showed a high diversity of strains affiliated with *Streptomyces*, *Amycolaptopsis*, and *Lechevalieria*, of which a high proportion showed taxonomic novelty (Okoro et al. 2009). Recently, NGS-based studies showed that the Actinobacteria phylum is even more abundant than previous studies have shown. From 12 samples, 67 representative families were identified, of which 16% could not be assigned to validly published taxa. The diversity observed in all of the samples was similar and dominated by members of the families *Acidimicrobiaceae*, *Geodermatophilaceae*, *Iamiaceae*, *Microbacteriaceae*, *Micrococcaceae*, *Micromonosporaceae*, *Nocardiaceae*, and *Nocardioidaceae*, as well as two unidentified taxa, FJ479147_f and HQ910322_f (Idris et al. 2017a).

In contrast to the hyper-arid core of the Atacama Desert, the different Salares in Atacama have shown astonishing bacterial diversity. For instance, in Salar de Llamará, the composition of Cyanobacteria in different microbial mats was studied using microscopy, revealing the presence of *Cyanothece*, *Synechococcus*, *Microcoleus*, *Oscillatoria*, *Gloeocapsa*, and *Gloeobacter* genera, as well as the anoxygenic phototrophic bacteria affiliated with *Chromatium* and *Thiocapsa* (Demergasso et al. 2004). The diversity of Cyanobacteria in Salar de Huasco was studied through molecular cloning of the 16S rRNA gene, revealing 78 different phylotypes affiliated with *Oscillatoriales*, *Pleurocapsales*, *Chroococcales*, and *Nostocales* orders (Dorador et al. 2008). Initially, the diversity of bacteria in Salar de Llamará, Salar de Atacama, and Salar de Ascotán indicated that Cytophaga-Flavobacteria-Bacteroidetes, Proteobacteria, and Actinobacteria phyla were frequently found in these environments (Demergasso et al. 2004). In particular, the diversity of Bacteroidetes communities from Laguna Tebenquiche, Salar de Huasco, and Salar de Ascotán revealed a high prevalence at all sites of a phylotype affiliated with *Psychroflexus* genus, while other phylotypes found were affiliated mostly with the *Flavobactericeae* family (Dorador et al. 2009). The diversity of microorganisms in unconnected wetlands from the Chilean highlands was studied, revealing that these bacterial communities were dominated mostly by Bacteroidetes and Proteobacteria (Alpha, Beta, Gamma and Delta groups). Other phyla such as Firmicutes, Actinobacteria, Planctomycetes, Verrucomicrobia, Chloroflexi, Cyanobacteria, Acidobacteria,

Deinococcus-Thermus, and the Candidate Division WS3 were present in low abundance (Dorador et al. 2013). Recently, the microbial diversity of Salar de Huasco was investigated, showing large differences between ponds: some were dominated by Proteobacteria and Bacteroidetes while others were abundant in Cyanobacteria. The lagoon, meanwhile, showed a high abundance of Gammaproteobacteria, suggesting that local environmental factors play an important role in microbial diversity within the samples (Aguilar et al. 2016). Stromatolite structures in Salar de Llamará were analysed, showing an overall higher abundance of Bacteroidetes, Proteobacteria, and Planctomycetes; these groups were more diverse during winter periods. In particular, the air-exposed part of the structures showed a predominance of Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes; in the submerged part, on the other hand, Proteobacteria (Alpha and Gamma) and Verrucomicrobia were in greater abundance (Rasuk et al. 2014).

During the last twenty years, the studies of Atacama Desert microbiology have diversified. Research with emphasis on describing the microbial diversity has proven several times that the Atacama Desert is rich in life that is diverse and unique. Formal taxonomic studies started with the description of the archaeon *Halorubrum tebenquichense*, isolated from Lake Tebenquiche. Two other strains from Lake Tebenquiche have been described: the Gammaproteobacteria *Chromohalobacter nigrandensis*, and the archaeon *Halomicrobium katesii* (Table 1). Another gammaproteobacterium of the genus *Pseudomonas* was isolated from Camarones Valley. This strain has the metabolic capacity to oxidise arsenite, a metal present in high concentration in the Atacama Desert. To date, three cryptic species of Cyanobacteria associated with a sand-rock lifestyle have been described. The phylum for which the most taxonomic strains have been described is Actinobacteria. To date, eleven validated type strains have been published. Ten of these strains have been isolated from hyper-arid soils (Table 1). In this thesis, two novel strains of Actinobacteria isolated from different sources are described. *Subtercola vilae* was isolated from water samples of Lake Llullaillaco at 6703 meters above sea level (Villalobos et al. 2018), while *Superstesspora tarapacensis* was isolated from microbial mat samples of Salar de Llamará.

Table 1. Valid type strains of Bacteria and Archaea isolated from Atacama Desert.

Species described	Isolation source	Reference
Actinobacteria		
Streptomyces leeuwenhoekii	Laguna de Chaxa, hyper-arid soil	(Busarakam et al. 2014)
Streptomyces atacamensis	Valle de la Luna, arid soil	(Santhanam et al. 2012a)
Streptomyces deserti	Salar de Atacama, soil	(Santhanam et al. 2012b)
Streptomyces bullii	Laguna de Chaxa, soil	(Santhanam et al. 2013)
Streptomyces asenjonii	Laguna de Chaxa, hyper-arid soil	(Goodfellow et al. 2017)
Lechevalieria atacamensis	Salar de Atacama, hyper-arid soil	(Okoro et al. 2010)
Lechevalieria deserti	Salar de Atacama, hyper-arid soil	(Okoro et al. 2010)
Lechevalieria roselyniae	Salar de Atacama, hyper-arid soil	(Okoro et al. 2010)
Modestobacter caceresii	Yungay, hyper-arid soil	(Busarakam et al. 2016)
Lentzea chajnantorensis	Cerro Chajnantor, gravel soil	(Idris et al. 2017b)
Subtercola vilae	Llullaillaco lagooon, water sample	This thesis, (Villalobos et al. 2018)
Superstesspora tarapacensis	Salar de Llamará, microbial mat	This thesis
Gammaproteobacteria		
Pseudomonas arsenicoxydans	Camarones Valley, sediment	(Campos et al. 2010)
Chromohalobacter nigrandesensis	Tebenquiche lake	(Prado et al. 2006)
Halobacteria (Archaea)		
Halomicrobium katesii	Tebenquiche lake, water sample	(Kharroub et al. 2008)
Halorubrum tebenquichense	Tebenquiche lake, water sample	(Lizama et al. 2002)

Natural products from Atacama Desert

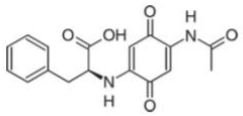
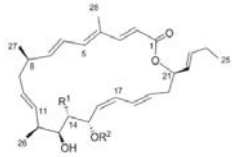
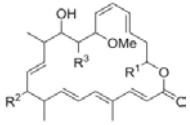
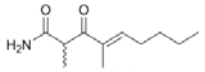
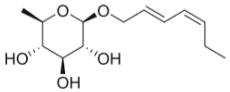
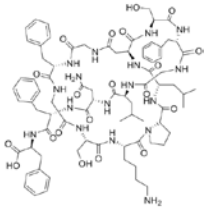
Natural products produced by microorganisms are considered a valuable resource for drug discovery due to their diverse chemical scaffolds (structures) that in many cases cannot be replicated synthetically, giving them the advantage over synthetic chemistry libraries. Among microorganisms, the Actinobacteria phylum, specifically the genus *Streptomyces*, is the richest source of natural products; these include antimicrobials, enzyme inhibitors, and anticancer compounds such as β-lactams, tetracyclines, rifamycins, aminoglycosides, macrolides, and glycopeptides (Genilloud 2017). The Actinobacteria phylum is the source of about 45% of all microbial bioactive secondary metabolites, of which 80% (7600 compounds) are produced by *Streptomyces* strains (Bérdy 2012). Interest in microbial natural products is currently renewed due to the whole genome sequencing of several representative strains of the phylum. This sequencing has revealed that different strains affiliated with several genera encoded more than 15 natural product biosynthetic gene clusters (BGCs), in contrast to the limited number of clusters found in other phyla (Doroghazi and Metcalf 2013).

Natural products discovery from Atacama Desert Actinobacteria has been prolific in the last years. Specifically, *Streptomyces* strains have proven that highly exploited genera still hide a high potential for novel natural product discovery. Different strategies have been employed to discover novel compounds. For instance, the discovery of new types of the aminobenzoquinones Abenquines A, B1, B2, C, and D (Schulz et al. 2011) followed a bioassay guide strategy, before optimising compound production using amino acids as a medium supplement. The compounds showed weak antibacterial and antifungal activity, and a moderate inhibitory effect against type 4 phosphodiesterase (PDE4b), a target enzyme for inflammatory diseases. Using a similar strategy but a different strain, the macrolactones Atacamycin A-C were discovered. These compounds also showed an inhibitory effect against PDE4b, while only Atacamycin A exhibited antiproliferative effects against adenocarcinoma and breast carcinoma cells (Nachtigall et al. 2011) (Table 2). Atacamycins are produced by a strain of *Streptomyces leeuwenhoekii* C34 isolated from hyper-arid soils of Chaxa lagoon. Different strains affiliated with *Streptomyces leeuwenhoekii* have shown promising chemical diversity. To date, seven compounds have been discovered, including the antibacterial compounds Chaxalactins A-C, isolated from *S. leeuwenhoekii* C34, and the lasso peptide Chaxapeptin, obtained from *S. leeuwenhoekii* C58; the

latter compound exhibited inhibitory activity in cell invasion assays with A549 human lung cancer cells. Three new β-diketones named asenjonamides A-C were obtained from the strain *Streptomyces asenjonii* KNN 42.f. The compounds showed antibacterial activity against Gram-positive and Gram-negative bacteria. Recently, six diene glycosides were obtained from a strain of *Lentzea chajnantorensi*. Lentzeosides A–F class of diene compounds had previously only been found in plants. This is the first time these compounds were reported from *Lentzea* genus and from a microbial source. The compounds were screened for anti-HIV activity, where lentzeoside B showed the best IC_{50} values.

To date, a total of nineteen novel metabolites have been described from the Atacama Desert Actinobacteria, where thirteen have been obtained from *Streptomyces* genus. The chemical diversity obtained to date is a clear reflection of the rich taxonomic diversity of Actinobacteria. Quite interestingly, five of the six producer strains are affiliated with novel species obtained from the Atacama Desert. All of these findings demonstrate that the microbes of the Atacama Desert are attractive for the bioprospecting of natural products.

Table 2. Novel compounds isolated from the Atacama Desert Actinobacteria.

Producer strain	Source of strain	Compounds	Structure*	Bioactivity	Reference
Streptomyces sp. strain DB634	Salar de Tara, arid soil	Abenquines A-D		Antibacterial, antifungal, and inhibition of phosphodiesterase 4b	(Schulz et al. 2011)
Streptomyces leeuwenhoekii strain C34	Laguna de Chaxa, hyper-arid soil.	Chaxalactins A-C		Antibacterial	(Rateb et al. 2011)
Streptomyces leeuwenhoekii strain C38	Laguna de Chaxa, hyper-arid soil.	Atacamycins A-C		Inhibition of phosphodiesterase 4b, antiproliferative affects against breast carcinoma and adenocarcinoma	(Nachtigall et al. 2011)
Streptomyces asenjonii strain KNN 42.f	Laguna de Chaxa, hyper-arid soil	Asenjonamides A–C		Antibacterial	(Abdelkader et al. 2018)
Lentzea chajnantorensis strain H4	Cerro Chajnantor gravel soil	Lentzeosides A–F		Anti-HIV 1	(Wichner et al. 2017)
Streptomyces leeuwenhoekii strain C58	Laguna de Chaxa, hyper-arid soil.	Chaxapeptin		Inhibitory activity in cell invasion assay with lung cancer cell lines	(Elsayed et al. 2015)

*Only the compound scaffold is shown, R indicates differences by variation of residues attached.

References

Abdelkader MSA, Philippon T, Asenjo JA, et al (2018) Asenjonamides A-C, antibacterial metabolites isolated from *Streptomyces asenjonii* strain KNN 42.f from an extreme-hyper arid Atacama Desert soil. J Antibiot (Tokyo) 71:425–431. doi: 10.1038/s41429-017-0012-0

Aguilar P, Acosta E, Dorador C, Sommaruga R (2016) Large differences in bacterial community composition among three nearby extreme waterbodies of the high Andean plateau. Front Microbiol 7:1–8. doi: 10.3389/fmicb.2016.00976

Bérdy J (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot (Tokyo) 65:385–395. doi: 10.1038/ja.2012.27

Bollen WB (1967) Identification of Chile Atacama Desert soil isolants. Progress Report Microorganisms Study JPL. Contract No 9500783.

Busarakam K, Bull AT, Girard G, et al (2014) *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 105:849–861. doi: 10.1007/s10482-014-0139-y

Busarakam K, Bull AT, Trujillo ME, et al (2016) *Modestobacter caceresii* sp. nov., novel actinobacteria with an insight into their adaptive mechanisms for survival in extreme hyper-arid Atacama Desert soils. Syst Appl Microbiol 39:243–251. doi: 10.1016/j.syapm.2016.03.007

Cameron RE, Gensel DR, Blank CB. (1966) Soil studies - desert microflora. XII. Abundance of microflora in soil samples from the Chile Atacama Desert. In Supporting Research and Advanced Developments, Space Programs Summary No. 37-38, Vol. IV, 140-147. Pasadena, CA: Jet Propulsion Lab

Campos V. (1997) Microorganismos de ambientes extremos: Salar de Atacama, Chile. In El Altiplano: Ciencia y Concieticia de los Andes, Ed. C Gonzalez, pp 143-47.

Campos VL, Valenzuela C, Yarza P, et al (2010) *Pseudomonas arsenicoxydans* sp nov., an arsenite-oxidizing strain isolated from the Atacama desert. Syst Appl Microbiol 33:193–197. doi: 10.1016/j.syapm.2010.02.007

Connon SA, Lester ED, Shafaat HS, et al (2007) Bacterial diversity in hyperarid atacama desert soils. J Geophys Res Biogeosciences. 12. doi: 10.1029/2006JG000311

Demergasso C, Casamayor EO, Chong G, et al (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. FEMS Microbiol Ecol 48:57–69. doi: 10.1016/j.femsec.2003.12.013

Dorador C, Meneses D, Urtuvia V, et al (2009) Diversity of bacteroidetes in high-altitude saline evaporitic basins in northern Chile. J Geophys Res Biogeosciences 114:1–11. doi: 10.1029/2008JG000837

Dorador C, Vila I, Imhoff JF, Witzel KP (2008) Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: An example of geographical dispersion? FEMS Microbiol Ecol 64:419–432. doi: 10.1111/j.1574-6941.2008.00483.x

Dorador C, Vila I, Witzel K-P, Imhoff JF (2013) Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. Fundam Appl Limnol 182:135–159. doi: 10.1127/1863-9135/2013/0393

Doroghazi JR, Metcalf WW (2013) Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. BMC Genomics 14:611. doi: 10.1186/1471-2164-14-611

Drees KP, Neilson JW, Betancourt JL, et al (2006) Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. Appl Environ Microbiol 72:7902–7908. doi: 10.1128/AEM.01305-06

Elsayed SS, Trusch F, Deng H, et al (2015) Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyperarid Atacama Desert. J Org Chem 80:10252–10260. doi: 10.1021/acs.joc.5b01878

Fierer N, Leff JW, Adams BJ, et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci 109:21390–21395. doi: 10.1073/pnas.1215210110

Genilloud O (2017) Actinomycetes: still a source of novel antibiotics. Nat Prod Rep 34:1203–1232. doi: 10.1039/c7np00026j

Goodfellow M, Busarakam K, Idris H, et al (2017) *Streptomyces asenjonii* sp. nov., isolated from hyper-arid Atacama Desert soils and emended description of *Streptomyces viridosporus* Pridham et al. 1958. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 110:1133–1148. doi: 10.1007/s10482-017-0886-7

Hartley A, Chong G, Houston J, Mather A (2005) 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. J Geol Soc London 162:421–424. doi: 10.1144/0016-764904-071

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. Int J Climatol. doi: 10.1002/joc.1359

Idris H, Goodfellow M, Sanderson R, et al (2017a) Actinobacterial rare biospheres and dark matter revealed in habitats of the chilean Atacama Desert. Sci Rep. 7: 8373 doi: 10.1038/s41598-017-08937-4

Idris H, Nouioui I, Asenjo JA, et al (2017b) *Lentzea chajnantorensis* sp. nov., an actinobacterium from a very high altitude Cerro Chajnantor gravel soil in northern Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 110:795–802. doi: 10.1007/s10482-017-0851-5

Kharroub K, Lizama C, Aguilera M, et al (2008) *Halomicrobium katesii* sp. nov., an extremely halophilic archaeon. Int J Syst Evol Microbiol 58:2354–2358. doi: 10.1099/ijs.0.65662-0

Lester ED, Satomi M, Ponce A (2007) Microflora of extreme arid Atacama Desert soils. Soil Biol Biochem 39:704–708. doi: 10.1016/j.soilbio.2006.09.020

Lizama C, Monteoliva-Sánchez M, Suárez-García A, et al (2002) *Halorubrum tebenquichense* sp. nov., a novel halophilic archaeon isolated from the Atacama Saltern, Chile. Int J Syst Evol Microbiol 52:149–155. doi: 10.1099/00207713-52-1-149

Makhalanyane TP, Valverde A, Gunnigle E, et al (2015) Microbial ecology of hot desert edaphic systems. FEMS Microbiol Rev 39:203–221. doi: 10.1093/femsre/fuu011

Nachtigall J, Kulik A, Helaly S, et al (2011) Atacamycins A-C, 22-membered antitumor macrolactones produced by *Streptomyces* sp. C38. J Antibiot (Tokyo) 64:775–780. doi: 10.1038/ja.2011.96

Neilson JW, Quade J, Ortiz M, et al (2012) Life at the hyperarid margin: Novel bacterial diversity in arid soils of the Atacama Desert, Chile. Extremophiles 16:553–66. doi: 10.1007/s00792-012-0454-z

Okoro CK, Brown R, Jones AL, et al (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 95:121–133. doi: 10.1007/s10482-008-9295-2

Okoro CK, Bull AT, Mutreja A, et al (2010) *Lechevalieria atacamensis* sp.nov., *Lechevalieria deserti* sp. nov. and *Lechevalieria roselyniae* sp. nov., isolated from hyperarid soils. Int J Syst Evol Microbiol 60:296–300. doi: 10.1099/ijs.0.009985-0

Prado B, Del Moral A, Quesada E, et al (1991) Numerical taxonomy of moderately halophilic Gram-negative rods isolated from the Salar de Atacama, Chile. Syst Appl Microbiol 14:275–281. doi: 10.1016/S0723-2020(11)80381-4

Prado B, Lizama C, Aguilera M, et al (2006) *Chromohalobacter nigrandesensis* sp. nov., a moderately halophilic, Gram-negative bacterium isolated from Lake Tebenquiche on the Atacama Saltern, Chile. Int J Syst Evol Microbiol 56:647–651. doi: 10.1099/ijs.0.63983-0

Rasuk MC, Kurth D, Flores MR, et al (2014) Microbial Characterization of Microbial Ecosystems Associated to Evaporites Domes of Gypsum in Salar de Llamara in Atacama Desert. Microb Ecol 68:483–494. doi: 10.1007/s00248-014-0431-4

Rateb ME, Houssen WE, Harrison WTAA, et al (2011) Diverse metabolic profiles of a *Streptomyces* strain isolated from a hyper-arid environment. J Nat Prod 74:1965–1971. doi: 10.1021/np200470u

Santhanam R, Okoro CK, Rong X, et al (2012a) *Streptomyces atacamensis* sp. nov., isolated from an extreme hyper-arid soil of the Atacama Desert, Chile. Int J Syst Evol Microbiol 62:2680–2684. doi: 10.1099/ijs.0.038463-0

Santhanam R, Okoro CK, Rong X, et al (2012b) *Streptomyces deserti* sp. nov., isolated from hyper-arid Atacama Desert soil. Antonie Van Leeuwenhoek 101:575–581. doi: 10.1007/s10482-011-9672-0

Santhanam R, Rong X, Huang Y, et al (2013) *Streptomyces bullii* sp. nov., isolated from a hyper-arid Atacama Desert soil. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 103:367–373. doi: 10.1007/s10482-012-9816-x

Schulz D, Beese P, Ohlendorf B, et al (2011) Abenquines A-D: aminoquinone derivatives produced by *Streptomyces* sp. strain DB634. J Antibiot (Tokyo) 64:763–8. doi: 10.1038/ja.2011.87

Villalobos AS, Wiese J, Aguilar P, et al (2018) *Subtercola vilae* sp. nov., a novel actinobacterium from an extremely high-altitude cold volcano lake in Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:955–963. doi: 10.1007/s10482-017-0994-4

Wichner D, Idris H, Houssen WE, et al (2017) Isolation and anti-HIV-1 integrase activity of lentzeosides A-F from extremotolerant L*entzea* sp. H45, a strain isolated from a high-altitude Atacama Desert soil. J Antibiot (Tokyo) 70:448–453. doi: 10.1038/ja.2016.78

Aim of the thesis

The aim of this thesis was to characterise the Atacama Desert Actinobacteria and elucidate their potential to produce natural products. Therefore, our study focused on four environments that had not yet been explored in Actinobacteria research: (a) Salar de Llamará, a hyper-saline basin in the Central Depression of the Atacama Desert that represents a relict wetland; (b) Salar de Huasco, a hyper-saline lake in the Chilean Altiplano; (c) Rhizosphere of different endemic plants close to Socaire; (d) Llullaillaco Volcano Lake, which represents the border of the Atacama Desert where the strain *Subtercola* DB165^{T} was isolated from.

The work focused on (a) the characterisation of cultured diversity of Actinobacteria and their potential for antibiotic production, (b) the polyphasic identification of novel taxa of Actinobacteria, and (c) the metabolic capability to produce natural products, as encoded in the genomes of the isolates.

Chapter I: Diversity and antibiotic activity of Actinobacteria isolated from the rhizosphere of endemic plants near Socaire, Chile

Alvaro S. Villalobos, Jutta Wiese, Johannes F. Imhoff *

GEOMAR Helmholtz Centre for Ocean Research, Düsternbrooker Weg 20, 24105 Kiel, Germany

* Corresponding author at

Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Tel.: 0049-431-600 4450

Fax: 0049-431-600 4482

E-mail addresses: avillalobos@geomar.de (A.S. Villalobos), jwiese@geomar.de (J. Wiese), jimhoff@geomar.de (J.F. Imhoff).

Data in preparation for publication

Abstract

The rhizosphere of Atacama Desert plants constitutes an unexplored source of Actinobacteria with an unknown potential in the production of natural products. We investigated the diversity of Actinobacteria in rhizosphere samples of *Stipa* sp., *Adesmia* sp., *Cristaria integerrima*, *Fabiana denudata*, *Nolana* sp., and *Cumulopuntia boliviana*. Forty-seven strains were isolated and, according to analyses of the 16S rRNA gene sequence, are affiliated with nine genera of Actinobacteria. *Streptomyces* and *Nocardia* were the most abundant and diverse genera found in most of the samples. According to similarities in their 16S rRNA gene sequence, fourteen isolates affiliated with *Arthrobacter*, *Kribbella*, *Pseudarthrobacter*, *Rhodococcus*, *Nocardia*, *Pseudonocardia*, *Kocuria*, and *Streptomyces* represent putative novel species. 61.7% of the strains showed antibiotic activity against Gram-positive bacteria, while the growth inhibition of Gram-negative bacteria was only found in 17% of the isolates.

Key words: Actinobacteria; antibiotics; Atacama Desert; rhizosphere; *Streptomyces*; *Nocardia*

Introduction

The Atacama Desert, located in southern Peru and northern Chile, is classified as a hyper-arid desert, with areas that receive only 0.6 mm/year^{-1} of rainfall (Houston 2006). In the north of Chile, several priority areas for biodiversity conservation have been identified as "natural biodiversity areas" due to the high number of endemic plant species present in each (INIA, 2011).

Socaire is located between the Salar de Atacama and the Altiplano. This environment is characterised by slope vegetation divided in clear altitudinal belts (prepuna, puna and Andean steppe) up to the Andean summits, and into islands of wetland habitat in areas with surface or near-surface waters (Marquet et al. 1998). This part of the Atacama Desert is considered an area of high conservation priority (Cavieres et al. 2002), mainly due to its high level of flora endemism, which can reach up to 56% of species (Squeo et al. 1998).

In general, plants are known to have a selective pressure on soil microbial diversity, including species-specific (Marschner et al. 2001; Acosta-Martínez et al. 2008) and cultivar-specific (Germida and Siciliano 2001; Manter et al. 2010) bacterial populations. In water and nutrient-limited environments as deserts, it is suggested that bacterial diversity should be higher in the rhizosphere than in the surrounding interplant soil (Herman et al. 1995), due to the accumulation of nutrients provided by the plant at the interface of root and soil (Schlesinger et al. 1996) and its secondary metabolites (Marilley et al. 1998; Hartmann et al. 2009). On the other hand, microorganisms living in the rhizosphere influence the composition and productivity of natural plant communities (Van der Heijden et al. 1998; Van Der Heijden et al. 2006; Schnitzer et al. 2011).

Actinobacteria comprise one of the most abundant and diverse taxa in the rhizosphere and promote plant growth through nitrogen fixation, phosphate, potassium, and zinc solubilisation, production of hormones, antibiotics, lytic enzymes, and siderophores (Yadav et al. 2018). In desert plants, members of genera such as *Acidimicrobium, Rubellimicrobium,* and *Deinococcus-Thermus* are often found to promote healthy growth in plants (Köberl et al. 2011).

The presence and prevalence of Actinobacteria associated with the rhizosphere of Atacama Desert endemic plants remain unknown, making these a perfect study target for the isolation of novel antibiotic-producing strains. In this work, we explore the diversity and antibiotic activity of

Actinobacteria in rhizosphere samples obtained from *Stipa* sp., *Adesmia* sp., *Cristaria integerrima*, *Fabiana denudata*, *Nolana* sp. and *Cumulopuntia boliviana*.

Materials and methods

Samples

Rhizosphere soil samples were collected from Socaire, Chile during December 2008. Plants were identified as *Stipa* sp., *Adesmia* sp., *Cristaria integerrima*, *Fabiana denudata*, *Nolana* sp., and *Cumulopuntia boliviana.* All samples were stored at room temperature.

Isolation of Actinobacteria

For all samples, 1 g of soil was resuspended in 9 mL of Ringer 1/4 buffer (0.12 g of $CaCl_2$, 0.105 g of KCl, 0.05 g of $NaHCO_3$, and 2.25 g of NaCl in 1 L of solution) followed by the preparation of three serial dilutions (10^{-1}, 10^{-2} and 10^{-3}). All the dilutions received a heat treatment in a water bath at 56°C for 10 minutes, and the 100 µL were spread on starch-casein, Gauze, humic acid vitamin and trehalose-proline-histidine agar plates. Starch-casein medium contained 10 g of starch, 0.3 g of casein, 2 g of KNO_3, 0.05 g of $MgSO_4 \cdot 7H_20$, 2 g of K_2HPO_2, 2 g of NaCl, 0.2 $CaCO_3$, 0.01 $FeSO_4 \cdot 7H_2O$; Gauze medium was prepared using 20 g of starch, 1 g of KNO_3, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.01 g of $FeSO_4 \cdot H_2O$; humic acid vitamin composition was 1 g of humic acid, 0.5 g of Na_2HPO_4, 1.7 g of KCl, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 1 g of $CaCl_2$, B-vitamins solution (0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxin, Ca-pantothenate, inositol, p-aminobenzoic acid, and 0.25 mg of biotin); trehalose-proline-histidine (TPH) medium containing 1 g of trehalose, 0.5 g of proline, 0.5 of histidine, 0.2 $MgCl_2$, and 0.5 of KNO_3. All media were prepared at pH 7.8-8.0 and supplemented with 25 µg/mL nalidixic acid, 50 µg/mL cycloheximide, and 18 g of agar in 1 L of deionised water. Plates were incubated at 25°C until colonies were observed. The selection of the colonies was based on its shape, hardness, aerial mycelium, and presence of pigments. Pure strains were obtained and maintained on Starch-glucose-glycerol (SGG) medium which contained 10 g of starch, 10 g of glucose, 10 mL of glycerol, 5 g of soy peptone, 2 g of yeast extract, 2.5 g corn steep solids, 3 g of $CaCO_3$, 1 g of NaCl, and 18 g of agar in 1 L of deionised water. Pure cultures were cryopreserved using the Cryobank System at -20°C (MAST DIAGNOSTIC).

16S rRNA gene sequencing, identification, and phylogenetic analysis

Strains were grown in Petri dishes of SGG medium for 5 days, and total genomic DNA was extracted using DNaeasy Blood & Tissue Kit (Qiagen) DNA extraction kit, following the manufacturer's protocol and stored at -20 ºC before use. Amplification of the 16S rRNA gene was prepared using Dreamtaq using 27f (5'- AGAGTTTGATCMTGGCTCAG-3') /1492r (5'-TACGGYTACCTTGTTACGACTT-3') set of primers, the reaction mix (25 µL) contained 2 µL of genomic DNA, 1 µL (10mM) of each primer, 12.5 µL of master mix and 85 µL of DNAse-free water. The PCR reaction started with an initial denaturation at 93ºC for 2 min followed by 30 cycles of denaturation at 93 ºC for 30 sec, annealing at 55 ºC for 30 sec and extension at 72ºC for 30 sec, with a final extension at 42ºC for 1 min, and at 72ºC for 5 min. The PCR products were sequenced in IKMB sequencing facility (University of Kiel, Germany). The sequences were processed using ChromasPro software, then compared against GenBank database using BLAST, with and without type strain filter. For the phylogenetic analysis, each strain was analysed using EzBioCloud (https://www.ezbiocloud.net/), and their closest validated type strains of each phylotype were selected. Using *Rubrobacter aplysinae* DSM 27440^T used as outgroup all selected sequences were aligned using SINA Alignment Service (Pruesse et al. 2012), and then the phylogenetic analyses were performed using the MEGA6 software (Tamura et al. 2013). Tree construction was conducted using a neighbour-joining algorithm (Saitou and Nei 1987) and maximum-likelihood, using 1000 bootstrap replications.

Antibiotic activity

All strains were screened for antibacterial activity against *Staphylococcus lentus* DSM 6672^T, *Bacillus subtilis* DSM 347^T, and *Escherichia coli* DSM 498^T using a double layer assay. The isolated strains were grown in 1 mL of SGG medium for 3 days and then 10µL of the cultures were inoculated on the centre of SGG agar plates. Plates were incubated at 26 °C for 7 days. The strains of *S. lentus, B. subtilis*, and *E. coli* were grown overnight in Tryptic soy broth, containing 17 g of tryptone, 3 g of soytone, 2.5 g of glucose, 5 g of NaCl, 2.5 g of K_2HPO_4, at 28°C, subsequently 5 mL of the cultures were inoculated on 500 mL of TSA soft agar (7%) and then poured over the Petri plates that contained Actinobacteria strains. The plates were incubated at 26°C for 48 hours, and then the inhibition (clear zones around the actinobacteria colonies) was registered.

Results

In total, forty-seven Actinobacteria that affiliated with nine genera were obtained from rhizosphere samples of Socaire. Most of the strains were affiliated with *Streptomyces* (22; 46.8%) and *Nocardia* (10; 21.2%); only a few were affiliated with *Pseudarthrobacter*, *Arthrobacter*, *Micromonospora*, *Kocuria*, *Rhodococcus*, *Kribbella*, and *Pseudonocardia*. The majority of the strains were obtained from *Nolana* sp. rhizosphere with 23 strains (48.9%), followed by *Cumulopuntia boliviana* with 13 strains (27.6%) (Table 1). The majority of the strains were obtained using TPH medium (32; 68.1%) and followed by HVA medium (10; 21.3%), while culture media that contained high concentration of nutrients yielded a lower number of isolates.

Representatives of the genus *Streptomyces* were found in most of the samples except those from *Adesmia* sp., from which only *Arthrobacter* and *Pseudarthrobacter* were recovered. *Nolana* sp. samples showed the most diverse array of genera, containing *Streptomyces*, *Nocardia*, *Pseudonocardia*, *Micromonospora*, and *Rhodococcus* strains. Fourteen (29.7%) of the isolates affiliated with *Arthrobacter* (AD2), *Kribbella* (AD5), *Pseudarthrobacter* (AD8), *Rhodococcus* (AD9, Soc85), *Nocardia* (AD18, AD22, AD28), *Pseudonocardia* (AD30), *Kocuria* (Soc1), and *Streptomyces* (Soc62, T5, T11) represent putative novel species according to their 16S rRNA gene sequence similarities (<98.7%). Even though most of the strains showed a close relationship with known species (98.9-100%) (Table 1), phylogenetic analysis suggests that strains affiliated with the genus *Streptomyces* such as T4, T8, and T9 formed distant clusters from the next related type strains and might represent putative novel species (Fig 1). Seven strains affiliating to *Nocardia* showed high similarities to *Nocardia ignorata* NBRC 108230^T (98.7-99.2%) and formed 4 different phylogenetic clusters (Fig. 2). Strain T6 clustered closer to *N. ignorata* NBRC 108230^T, while strains AD16, Soc22, Soc26, and Soc28 clustered together as a group with a high bootstrap value. Strain Soc46 clustered separate from all other strains and strain AD22 represents a putative novel species separated from the *N. ignorata* NBRC 108230^T clade.

Table 1. Actinobacteria strains isolated from rhizosphere of Atacama Desert and their antibiotic activity

Strain	Length (nt)	Next related type strain	Identity (%)	Sample	Antibiotic activity*
AD2	1381	*Arthrobacter humicola* JCM 15921^T (AB279890)	98.2	*Adesmia* sp.	-
AD3	1408	*Pseudarthrobacter defluvii* 4C1-a^T (NR_042573.1)	98.8	*Adesmia* sp.	-
AD4	1439	*Pseudarthrobacter defluvii* 4C1-a^T (NR_042573.1)	99.4	*Adesmia* sp.	-
AD5	1436	*Kribbella ginsengisoli* DSM 17941^T (AB245391.1)	98.4	*Cristaria integerrima*	-
AD7	1435	*Streptomyces drozdowiczii* NRRL B-24297^T (EF654097.1)	99.2	*Fabiana denudata*	B
AD8	1443	*Pseudarthrobacter defluvii* 4C1-a^T (NR_042573.1)	98.1	*Fabiana denudata*	-
AD9	1436	*Rhodococcus marinonascens* DSM43752^T (X80617.1)	98.5	*Fabiana denudata*	-
AD12	1443	*Streptomyces drozdowiczii* NRRL B-24297^T (EF654097.1	99.1	*Nolana* sp.	-
AD16	1435	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	99.1	*Cumulopuntia boliviana*	B
AD18	1435	*Nocardia lasii* 3C-HV12^T (KP784803.1)	98.4	*Nolana* sp.	B
AD22	1411	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	98.7	*Nolana* sp.	-
AD26	1413	*Streptomyces youssoufiensis* X4^T (NR_116980.1)	99.8	*Cumulopuntia boliviana*	B, S
AD28	1417	*Nocardia anaemiae* IFM 0323^T (AB162801.1)	98.1	*Nolana* sp.	B, E, S
AD30	1446	*Pseudonocardia sichuanensis* KLBMP 1115^T (HM153789.1)	96.2	*Nolana* sp.	B, E, S
Soc1	1468	*Kocuria dechangensis* NEAU-ST5-33^T (JQ762279.3)	98.5	*Stipa* sp.	B
Soc18	1426	*Streptomyces rubiginosus* JCM 4416^T (LC034307.1)	99.5	*Nolana* sp.	B, E
Soc22	1442	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	98.9	*Nolana* sp.	E
Soc26	1434	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	99.2	*Cumulopuntia boliviana*	B
Soc28	1458	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	99.0	*Cumulopuntia boliviana*	B, S
Soc36	1333	*Micromonospora echinofusca* DSM 43913^T (LT607733.1)	99.4	*Nolana* sp.	B
Soc37	1431	*Rhodococcus ruber* DSM 43338^T (NR_026185.1)	99.9	*Nolana* sp.	-
Soc42	1329	*Micromonospora soli* NBRC 110009^T (AB981051.1)	99.4	*Nolana* sp.	B, S
Soc46	1460	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	99.0	*Nolana* sp.	E
Soc48	1340	*Kocuria himachalensis* JCM 13326^T (LC113906.1)	99.4	*Stipa* sp.	-
Soc57	1440	*Streptomyces fulvissimus* DSM 40593^T (NR_103947.1)	99.7	*Cristaria integerrima*	B, S

Table 1 continues.

Strain	Length (nt)	Next related type strain	Identity (%)	Sample	Antibiotic activity*
Soc57	1440	*Streptomyces fulvissimus* DSM 40593^T (NR_103947.1)	99.7	*Cristaria integerrima*	B, S
Soc61	1441	*Streptomyces fulvissimus* DSM 40593^T (NR_103947.1)	99.8	*Fabiana denudata*	B, E, S
Soc62	1413	*Streptomyces galilaeus* JCM 4757^T (NR_040857.1)	97.9	*Nolana* sp.	B, S
Soc63	1439	*Streptomyces galilaeus* JCM 4757^T (NR_040857.1)	99.4	*Nolana* sp.	B, S
Soc66	1418	*Streptomyces clavifer* NRRL B-2557^T (DQ026670.1)	99.5	*Nolana* sp.	-
Soc70	1441	*Streptomyces venezuelae* ATCC 10712^T (FR845719.1)	99.9	*Cumulopuntia boliviana*	B
Soc71	1435	*Nocardia lasii* 3C-HV12^T (KP784803.1)	99.1	*Cumulopuntia boliviana*	B
Soc72	1439	*Streptomyces tendae* ATCC 19812^T (NR_025871.2)	99.2	*Nolana* sp.	B
Soc75	1435	*Streptomyces griseoviridis* NBRC 12874^T (AB184210.1)	100.0	*Cumulopuntia boliviana*	B, S
Soc84	1342	*Streptomyces cyaneus* NRRL B-2296^T (AF346475.1)	99.1	*Nolana* sp.	-
Soc85	1334	*Rhodococcus marinonascens* DSM43752^T (X80617.1)	98.7	*Nolana* sp.	B
Soc89	1336	*Micromonospora soli* NBRC 110009^T (AB981051.1)	99.5	*Nolana* sp.	E, S
T1	1494	*Streptomyces sudanensis* SD504^T (EF515876.1)	98.4	*Nolana* sp.	B
T2	1469	*Streptomyces sudanensis* SD504^T (EF515876.1)	98.8	*Nolana* sp.	-
T3	1464	*Streptomyces chilikensis* RC 1830^T (JN050256.1)	99.4	*Nolana* sp.	B
T4	1347	*Streptomyces ambofaciens* ATCC 23877^T (CP012382.1)	99.5	*Nolana* sp.	-
T5	1444	*Streptomyces fragilis* NBRC 12862^T (AB184200.1)	98.6	*Cumulopuntia boliviana*	B
T6	1329	*Nocardia ignorata* IMMIB R-1434^T (AJ303008.1)	99.1	*Cumulopuntia boliviana*	E
T8	1352	*Streptomyces tendae* ATCC 19812^T (NR_025871.2)	99.1	*Cumulopuntia boliviana*	B
T9	1467	*Streptomyces chilikensis* RC 1830^T (JN050256.1)	99.1	*Cumulopuntia boliviana*	B, S
T10	1345	*Streptomyces tendae* ATCC 19812^T (NR_025871.2)	99.4	*Cumulopuntia boliviana*	B, S
T11	1435	*Streptomyces tendae* ATCC 19812^T (NR_025871.2)	98.3	*Cumulopuntia boliviana*	B, S

* B: *Bacillus subtilis* DSM 347^T; S: *Staphylococcus lentus* DSM 6672^T; E: *Escherischia coli* DSM 498^T

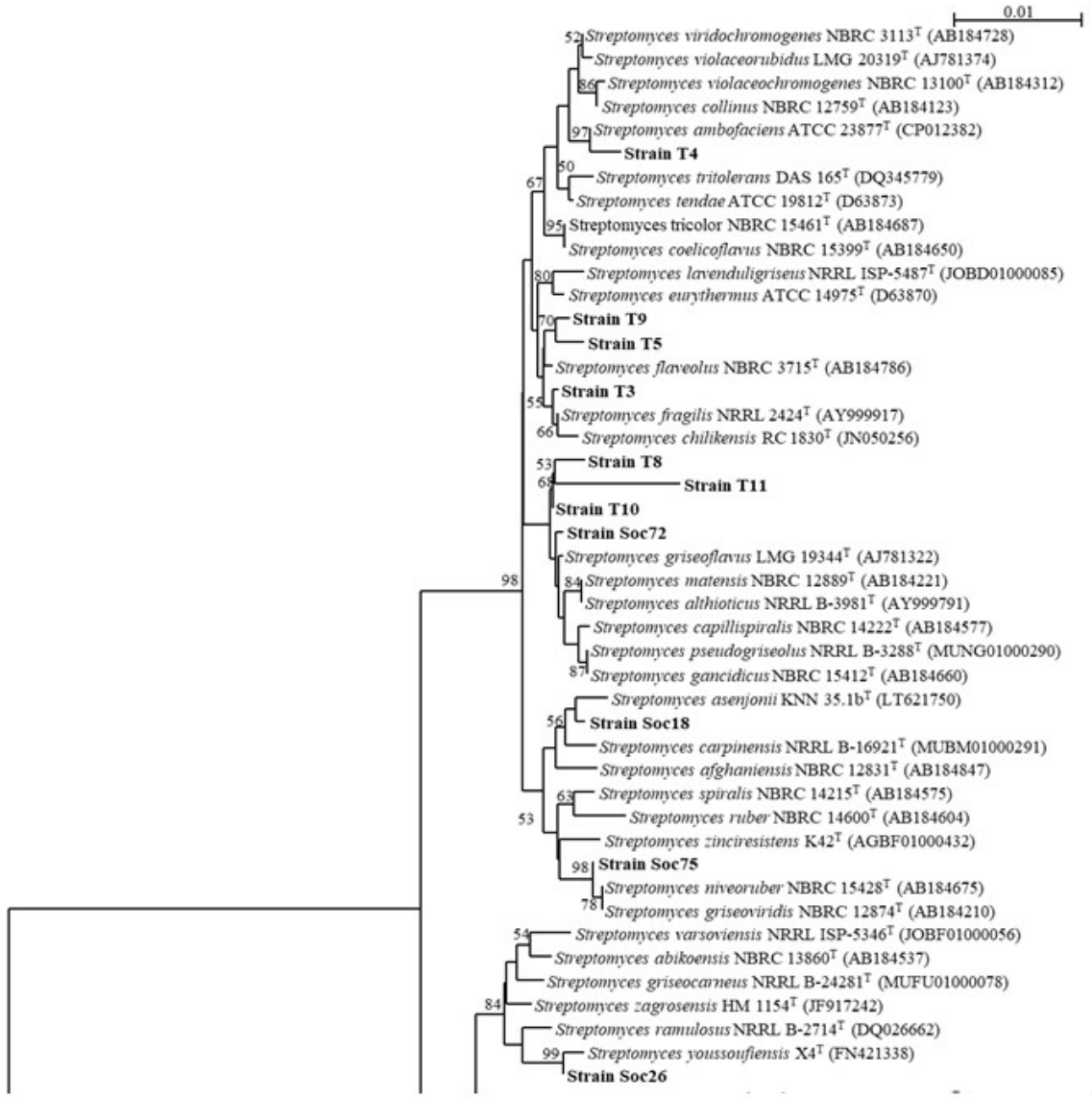


Figure 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of isolates of the genus *Streptomyces* obtained from rhizosphere samples close to Socaire, Atacama Desert with *Catenulispora rubra* DSM 44948^T as outgroup. Tree constructed with 1000 bootstrap, bar indicates 1 substitution in 100 pair bases. (Continues in the next page).

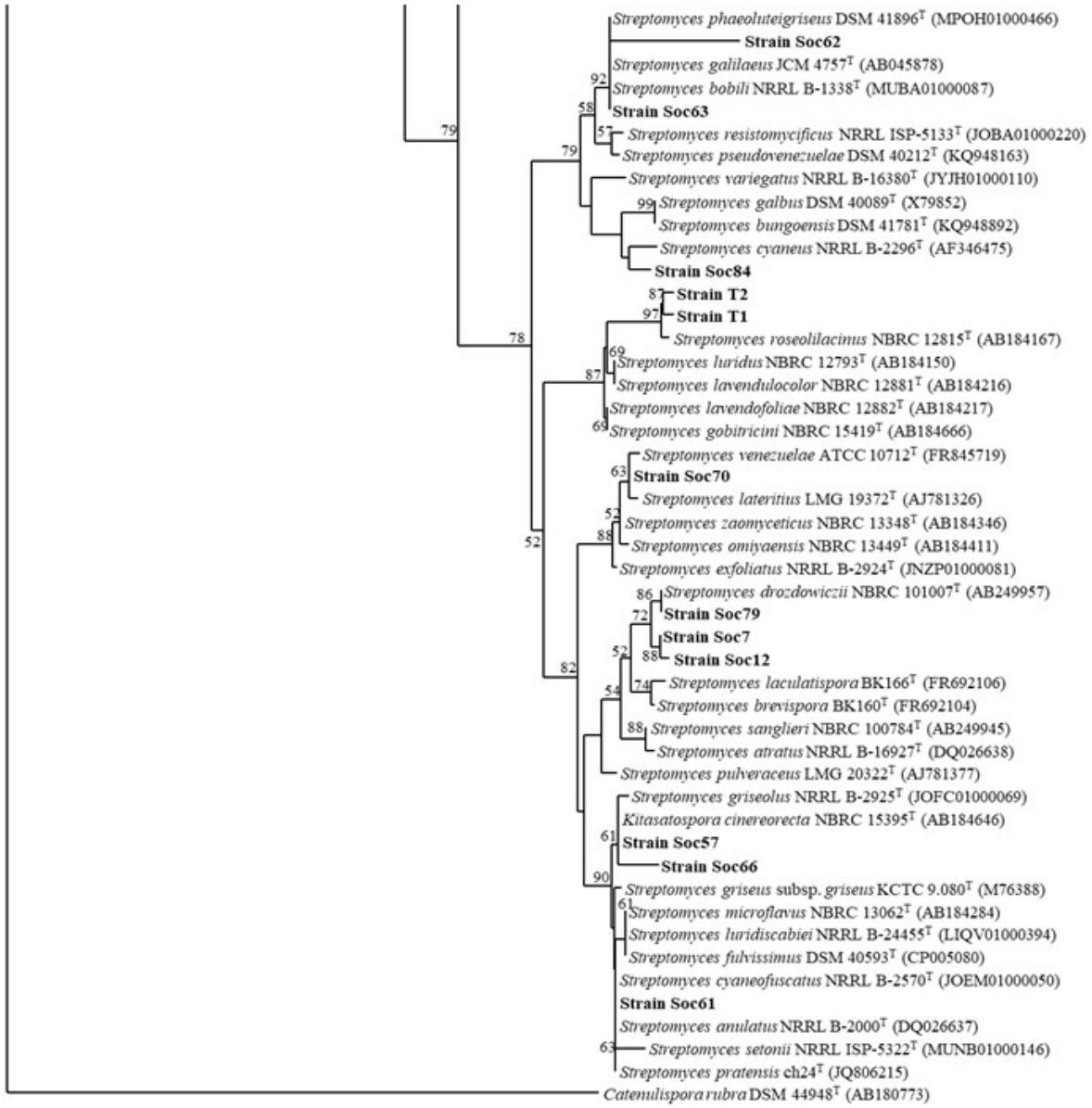


Figure 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of isolates of the genus *Streptomyces* obtained from rhizosphere samples close to Socaire, Atacama Desert with *Catenulispora rubra* DSM 44948^T as outgroup. Tree constructed with 1000 bootstrap, bar indicates 1 substitution in 100 pair bases.

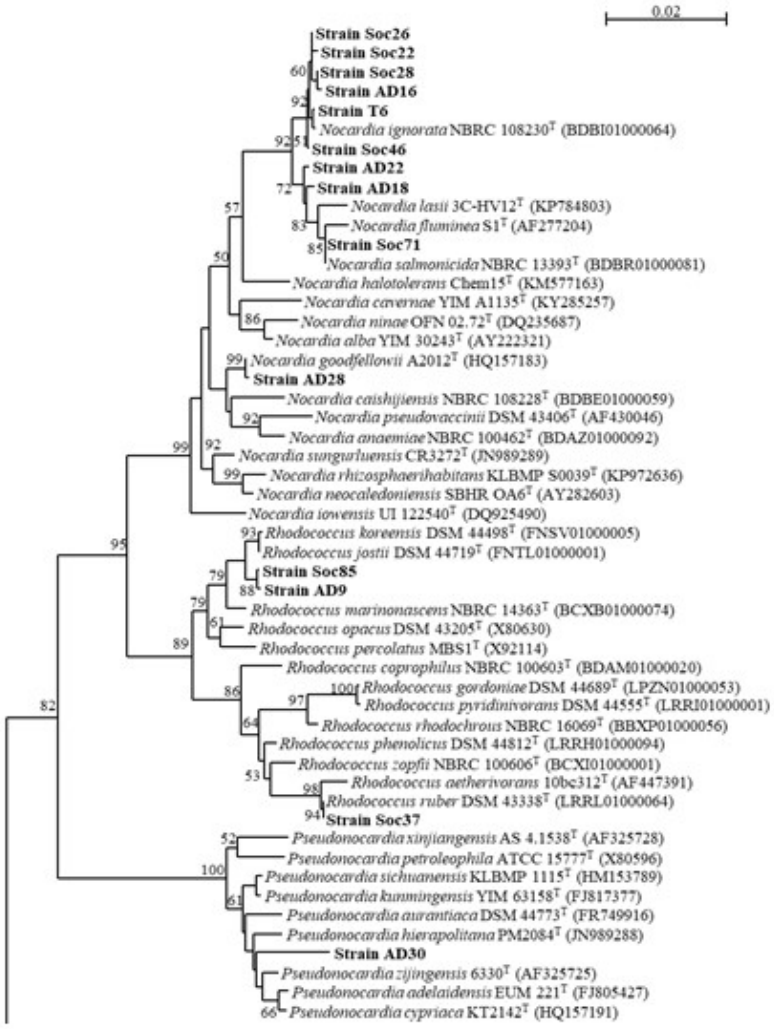


Figure 2. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of isolates from rhizosphere samples close to Socaire, Atacama Desert affiliated with *Nocardia*, *Rhodococcus*, *Pseudonocardia*, *Micromonospora*, *Kribbella*, *Kocuria*, *Arthrobacter*, and *Pseudarthrobacter* and their next related type strains with *Rubrobacter aplysinae* DSM 27440^{T} as outgroup. Tree constructed with 1000 bootstrap, bar indicates 2 substitutions in 100 pair bases. (Continues in the next page).

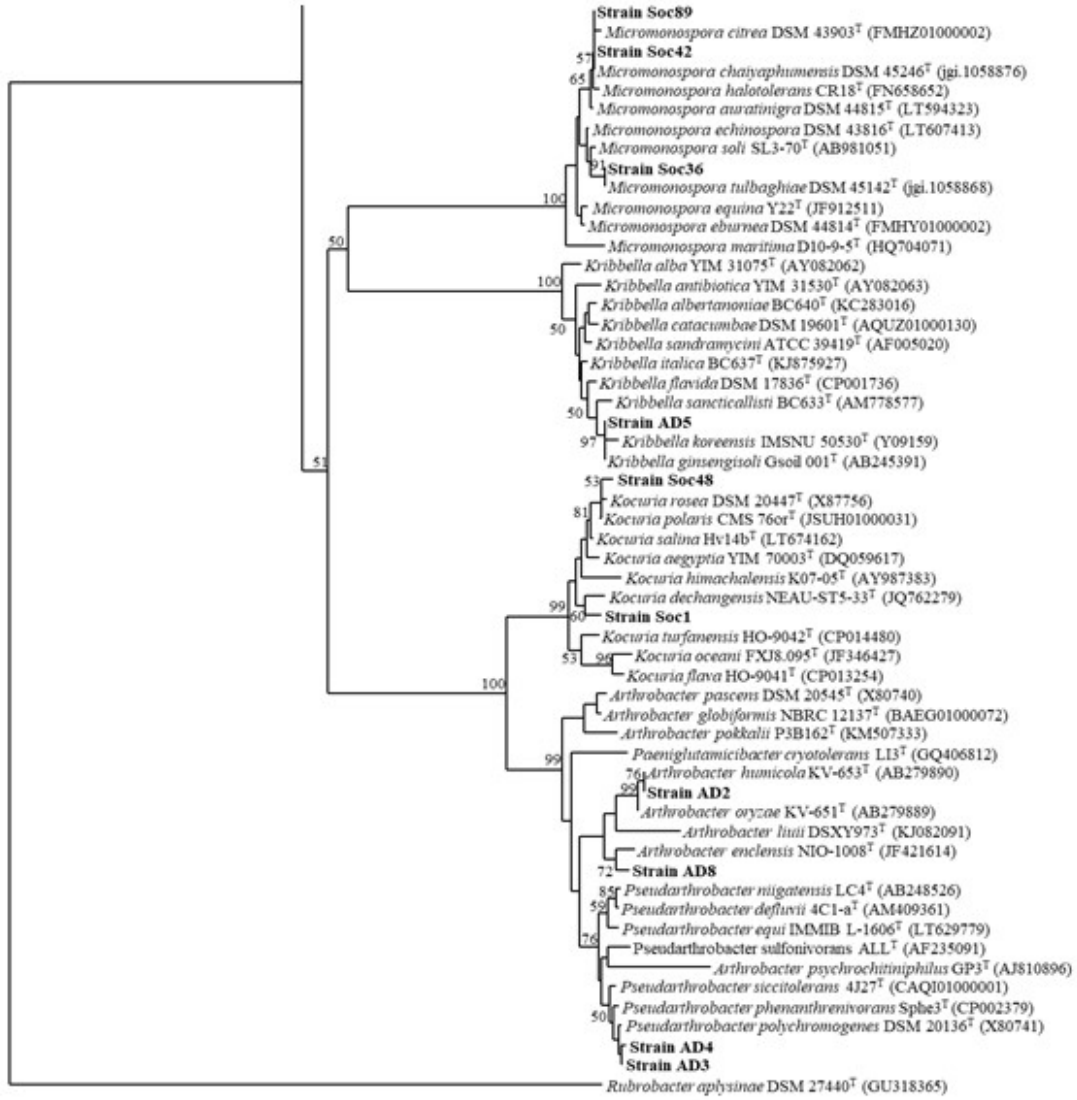


Figure 2. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of isolates from rhizosphere samples close to Socaire, Atacama Desert affiliated with *Nocardia*, *Rhodococcus*, *Pseudonocardia*, *Micromonospora*, *Kribbella*, *Kocuria*, *Arthrobacter*, and *Pseudarthrobacter* and their next related type strains with *Rubrobacter aplysinae* DSM 27440^{T} as outgroup. Tree constructed with 1000 bootstrap, bar indicates 2 substitutions in 100 pair bases.

Discussion

Actinobacteria isolated from the rhizosphere of Atacama Desert plants revealed a large number of genera first reported in the Atacama Desert such as *Arthrobacter*, *Pseudarthrobacter*, *Pseudonocardia, Nocardia*, *Kocuria*, *Kribbella*, and *Rhodococcus*. *Streptomyces* (Okoro et al. 2009) and *Micromonospora* (Carro et al. 2018), however, have been isolated previously from the Atacama Desert and most of the strains affiliated with these genera did not show similarity to previous isolates. The 16S rRNA gene sequence of strain Soc18 alone was sufficient to allow for identification as *Streptomyces asenjonii*, previously isolated from Laguna de Chaxa (Goodfellow et al. 2017). Actinobacteria obtained from rhizosphere samples of the Atacama Desert are more likely to grow in culture media with lower amount of nutrients compared with traditional Actinobacteria media.

The prevalence of the genus *Streptomyces* in most of the samples indicates their importance in the plant rhizosphere. Actinobacteria have been found to constitute an essential part of the microbial communities in the plant rhizosphere (Yadav et al. 2018). Their ecological function might be diverse, including i) protection of the plants' roots against pathogens through the production of antibiotic compounds (Adegboye and Babalola 2016); ii) a direct function involved in the promotion of plant growth such as nitrogen fixation, which has been reported for *Arthrobacter* (Verma et al. 2014), *Pseudonocardia* (Mahendra and Alvarez-Cohen 2005), *Streptomyces*, and *Micromonospora* (Sellstedt and Richau 2013) isolates; iii) solubilisation of phosphate, potassium, and zinc reported for *Arthrobacter* (Singh et al. 2016), *Kocuria* (Verma et al. 2015), and *Streptomyces* (Anwar et al. 2016).

Our study has demonstrated for the first time the diversity of Actinobacteria associated with the rhizosphere of plants from the Atacama Desert. According to our results, genera such as *Streptomyces* and *Nocardia* are predominant and diverse in the rhizosphere of Atacama Desert plants and according to their antibiotic activities constitute a promising source of novel species and antibiotic compounds.

References

Acosta-Martínez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem 40:2762–2770. doi: 10.1016/j.soilbio.2008.07.022

Adegboye MF, Babalola OO (2016) Isolation and identification of potential antibiotic producing rare actinomycetes from rhizospheric soils. J Hum Ecol 56:31–41. doi: 10.1088/0253-6102/55/5/26

Anwar S, Ali B, Sajid I (2016) Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front Microbiol 7:1334. doi: 10.3389/fmicb.2016.01334

Carro L, Razmilic V, Nouioui I, et al (2018) Hunting for cultivable *Micromonospora* strains in soils of the Atacama Desert. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:1375–1387. doi: 10.1007/s10482-018-1049-1

Cavieres LA, Arroyo MTK, Posadas P, et al (2002) Identification of priority areas for conservation in an arid zone: Application of parsimony analysis of endemicity in the vascular flora of the Antofagasta region, northern Chile. Biodivers Conserv 11:1301–1311. doi: 10.1023/A:1016001714358

Germida JJ, Siciliano SD (2001) Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol Fertil Soils 33:410–415. doi: 10.1007/s003740100343

Goodfellow M, Busarakam K, Idris H, et al (2017) *Streptomyces asenjonii* sp. nov., isolated from hyper-arid Atacama Desert soils and emended description of *Streptomyces viridosporus* Pridham et al. 1958. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 110:1133–1148. doi: 10.1007/s10482-017-0886-7

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257.

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. Int J Climatol 28: 2181–2198. doi: 10.1002/joc.1359

Köberl M, Müller H, Ramadan EM, Berg G (2011) Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. PLoS One 6: e24452. doi: 10.1371/journal.pone.0024452

Mahendra S, Alvarez-Cohen L (2005) *Pseudonocardia dioxanivorans* sp. nov., a novel actinomycete that grows on 1,4-dioxane. Int J Syst Evol Microbiol 55:593–598. doi: 10.1099/ijs.0.63085-0

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60:157–166. doi: 10.1007/s00248-010-9658-x

Marilley L, Vogt G, Blanc M, Aragno M (1998) Bacterial diversity in the bulk soil and rhizosphere fractions of *Lolium perenne* and *Trifolium repens* as revealed by PCR restriction analysis of 16S rDNA. Plant Soil 198:219–224. doi: 10.1023/A:1004309008799

Marquet PA, Bozinovic F, Bradshaw G, et al (1998) Ecosystems of the Atacama Desert and adjacent Andean area in northern Chile. Rev Chil Hist Nat 71:593–617.

Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445. doi: 10.1016/S0038-0717(01)00052-9

Okoro CK, Brown R, Jones AL, et al (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 95:121–133. doi: 10.1007/s10482-008-9295-2

Powers KC, Kalmar JM, Cinelli ME (2014) Recovery of static stability following a concussion. Gait Posture 39:611–614. doi: 10.1016/j.gaitpost.2013.05.026

Pruesse E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. doi: 10.1093/bioinformatics/bts252

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. doi: 10.1093/oxfordjournals.molbev.a040454

Schlesinger WH, Raikks JA, Hartley AE, Cross AF (1996) On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77:364–374. doi: 10.1016/j.automatica.2008.10.002

Schnitzer SA, Klironomos JN, HilleRisLambers J, et al (2011) Soil microbes drive the classic plant diversity-productivity pattern. Ecology 92:296–303. doi: 10.1890/10-0773.1

Sellstedt A, Richau KH (2013) Aspects of nitrogen-fixing actinobacteria, in particular free-living and symbiotic *Frankia*. FEMS Microbiol Lett 342:179–186. doi: 10.1111/1574-6968.12116

Singh RN, Gaba S, Yadav AN, et al (2016) First high quality draft genome sequence of a plant growth promoting and cold active enzyme producing psychrotrophic *Arthrobacter agilis* strain L77. Stand Genomic Sci. doi: 10.1186/s40793-016-0176-4

Snyder RG (1961) Vibrational spectra of crystalline n-paraffins. II. Intermolecular effects. J Mol Spectrosc 7:116–144. doi: 10.1016/0022-2852(61)90347-2

Squeo, Francisco A; Cavieres, Lohengrin; Arancio, Gina; Novoa, José; Matthei, Oscar; Marticorena, Clodomiro; Rodriguez, Roberto; Arroyo, Mary y Munoz M (1998) Biodiversidad de la flora vascular de la region de Antofagasta, Chile.pdf. Rev Chil Hist Nat 591–591.

Tamura K, Stecher G, Peterson D, et al (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi: 10.1093/molbev/mst197

Van Der Heijden MGA, Bakker R, Verwaal J, et al (2006) Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. FEMS Microbiol Ecol. 56:178–187

Verma P, Yadav A, Kazy S (2014) Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India. Int J Curr Microbiol Appl Sci 3:432–447.

Verma P, Yadav AN, Khannam KS, et al (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann Microbiol 65:1885–1899. doi: 10.1007/s13213-014-1027-4

Yadav AN, Verma P, Kumar S, et al (2018) Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In: New and future developments in microbial biotechnology and bioengineering: Actinobacteria: Diversity and Biotechnological Applications. pp 13–41

Chapter II: Phylogenetic diversity and antibiotic activity of Actinobacteria from hypersaline lakes in the Atacama Desert, Chile.

Alvaro S. Villalobos [a], Jutta Wiese [a], Johannes F. Imhoff [a,*], Ute Hentschel [a], and Cristina Dorador [b, c]

[a] GEOMAR Helmholtz Centre for Ocean Research, Düsternbrooker Weg 20, 24105 Kiel, Germany

[b] Laboratorio de Complejidad Microbiana y Ecología Funcional and Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile

[c] Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Antofagasta, Antofagasta, Chile

* Corresponding author at

Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Tel.: 0049-431-600 4450

Fax: 0049-431-600 4482

E-mail addresses: avillalobos@geomar.de (A.S. Villalobos), jwiese@geomar.de (J. Wiese), cristina.dorador@uantof.cl (C. Dorador), jimhoff@geomar.de (J.F. Imhoff).

Data in preparation for publication.

Abstract

Hypersaline lakes in the Atacama Desert are polyextremophile environments dominated by a high diversity of microorganisms. In this study, we report the isolation of Actinobacteria from Salar de Llamará (980 m asl) and Salar de Huasco (4600 m asl), and their action against Gram-positive and Gram-negative bacteria. According to phylogenetic analysis, using 16S rRNA gene sequences, thirty-two Actinobacteria isolated from the two locations were affiliated with eight families, with dominance from *Nocardiopsaceae*, *Micromonosporaceae*, and *Streptomycetaceae*; only *Micromonosporaceae* members were found in both hypersaline lakes. Eighteen strains that affiliated with *Streptomyces*, *Nocardiopsis*, *Blastococcus*, *Nocardia*, *Nonomuraea*, and *Micromonospora* were identified as potential novel species, while five strains are distantly related to *Salinispora* and *Jishengela* genera.

Most of the isolates showed close similarity to other actinobacteria isolated from environments that share similar physicochemical conditions, such as marine sediments, hypersaline/alkaline lakes, and cold deserts, among others, suggesting specific adaptation to these biomes.

More than half of the isolates produced antibiotic compounds against Gram-positive and Gram-negative bacteria. Dereplication analysis of the crude extract revealed a high degree of novelty in the compounds produced by the strains, and the incidence of nocapyrone compounds in several of the *Nocardiopsis* isolates. Results from this study demonstrate that hypersaline lakes are a rich source of microbial and chemical novelty with a high potential for antibiotic discovery.

Keywords: Salar; Actinobacteria; extreme environment; hypersaline; rare

Introduction

The Actinobacteria class is one of the richest sources of natural products (Newman and Cragg 2007). Most of the antibiotics available on the market derive from approximately 12000 described compounds produced by this group of bacteria (Bérdy 2012), including anticancer compounds, immunosuppressants, and anti-inflammatories. Most of these compounds are encoded by large biosynthetic gene clusters such as non-ribosomal peptides, polyketides, and phenazines (Fischbach and Walsh 2006), which are present in high number and diversity within actinobacteria genomes (Doroghazi and Metcalf 2013).

Actinobacteria have been explored in soils for last 60 years from soils, and lately from marine environments as well, resulting in the large number of known natural products and in an extensive collection of unpublished data of rediscovered compounds. One of the efforts made to overcome extensive rediscovery of known compounds was the exploration of actinomycetes from under-explored and extreme environments (Bull et al. 2000). Environments like deep-sea sediments, desert soils, and hypersaline environments have proven to be a rich source of novel diversity of actinobacteria, and are also associated with higher chemical diversity of natural products. Novel compounds have been identified from barophiles (Riedlinger et al. 2004; Xie et al. 2017), acidophiles (Park et al. 2014), and halophiles (Zhao et al. 2011).

Comparative studies of diversity and abundance of Actinobacteria in different environments have shown that deserts hold the highest abundance and diversity of Actinobacteria (Fierer et al. 2012), making deserts attractive spots for bioprospecting of Actinobacteria. Samples from the arid core of the Atacama Desert showed that Actinobacteria were highly abundant and represented up to 90% of the bacterial community (Drees et al. 2006); a diverse array of isolates of the genera *Streptomyces*, *Lechevalieria*, and *Amycolaptopsis* (Okoro et al. 2009), and *Micromonospora* (Carro et al. 2018) were obtained from the Atacama Desert.

The Atacama Desert not only comprises hyper-arid environments, but also contains different water bodies, locally called *Salares* (Risacher et al. 2003). These Salares are subject to extreme physicochemical conditions, including high UV radiation (600–1100W/m^{-2}), a wide range of daily temperature changes (>10-35°C), negative water balance (precipitation rates of 50 to 300 mm y^{-1} versus evaporation rates of 600 to 1200 mm y^{-1}), and variable salt concentrations (sulfate, chloride, sodium, and divalent cations) (Risacher et al. 2003). An astonishingly high diversity

with a great number of unique bacteria has been found in the microbial communities of some of these Salares (Dorador et al. 2008a, 2009, 2013).

Few Actinobacteria from the Salares have been explored as natural product producers. To date, only eleven novel compounds have been described from three *Streptomyces* isolates from the Atacama Desert. *Streptomyces* sp. strain DB634, an isolate from Salar de Tara, produces abenquines A-D (Schulz et al. 2011); *Streptomyces* sp. C38 produces atacamycin A-C (Nachtigall et al. 2011); and *Streptomyces leeuwenhoekii* $C34^T$, obtained from Laguna Chaxa, produces chaxalactins A-C (Rateb et al. 2011) and chaxapeptin (Elsayed et al. 2015).

In this study, we have explored the diversity of Actinobacteria isolates from the polyextremophilic environments of Salar de Llamará and Salar de Huasco, studying their antimicrobial activity against Gram-positive and Gram-negative bacteria. Salar de Llamará is the only hypersaline basin in the central depression of the Atacama Desert with a salinity range from 1% to 25% and pH 7,8-8 (Demergasso et al. 2004). Salar de Huasco is located in the Chilean Altiplano at 3800 m asl and has a salinity range from fresh water to saturated salt concentrations and pH 7,8-8 (Dorador et al. 2008b).

Materials and methods

Sampling

Eight samples from Salar de Huasco (Chile) were collected in June 2015. Sediments (20°15'27.1"S 68°52'18.5"W, 20°15'53.5"S 68°52'28.4"W) and salt crust samples (20°16'56.6"S 68°53'22.8"W) from the border of the lake were taken. Samples from Salar de Llamará were obtained in January 2016, where a total of eight samples were taken from sediments and silica crystals (S 21° 16.1164’ W 069° 37.101; S 21° 16.084’ W 069° 37.084’), and microbial mats (S 21° 16.087’ W 069° 37.094’). All samples were stored at 4 °C prior to the isolation of the bacteria.

Isolation of Actinobacteria

All samples were resuspended in Ringer 1/4 buffer (0.12 g of CaCl, 0.105 g of KCl, 0.05 g of $NaHCO_3$, and 2.25 g of NaCl in 1 L of solution) preparing thee dilutions,10^{-1}, 10^{-2} and 10^{-3}. The dilutions were heated in a water bath at 56°C for 10 minutes, and then 100 μL of each dilution

was spread on three different culture media. Raffinose-Histidine (RH) medium containing 1 g of raffinose and 0,1 g of histidine, Trehalose-Proline-Histidine (TPH) medium containing 1 g of trehalose, 0.5 g of proline, 0.5 of histidine, 0.2 $MgCl_2$, and 0.5 of KNO_3, and Starch-Yeast-extract-Peptone (SYP) medium containing 2 g of starch, 1 g of yeast extract, and 0.5 g of soy peptone. All media were prepared at pH 7.8-8.0 and supplemented with 25 μg/mL nalidixic acid, 50 μg/mL cycloheximide, 20 g of Tropic Marine Salt, 12 g of gellan gum, and 2 g of $CaCl_2$ in 1 L of deionised water.

Plates were incubated at 25 °C until colonies were observed. The selection of the colonies was based on its shape, hardness, aerial mycelium and presence of pigments. Pure isolates were streaked on SYP medium supplemented with 10 g of Tropic Marine salt to generate pure cultures, and then cryopreserved using the Cryobank System at -20 °C (MAST DIAGNOSTIC).

16S rRNA gene sequencing, identification, and phylogenetic analysis

Strains were grown in Petri dishes of SYP media for 5 days, and total genomic DNA was extracted using DNaeasy Blood & Tissue Kit (Qiagen) DNA extraction kit, following the manufacturer's protocol and stored at -20 ºC before use. Amplification of the 16S rRNA gene was prepared using Dreamtaq using 27f (5'- AGAGTTTGATCMTGGCTCAG-3') /1492r (5'-TACGGYTACCTTGTTACGACTT-3') set of primers, the reaction mix (25 μL) contained 2 μL of genomic DNA, 1 μL (10mM) of each primer, 12.5 μL of master mix and 85 μL of milli-Q water. The PCR reaction started with an initial denaturation at 93ºC for 2 min followed by 30 cycles of denaturation at 93 ºC for 30 seconds, annealing at 55 ºC for 30 seconds and extension at 72ºC for 30 secs, with a final extension at 42ºC for 1 min, and at 72ºC for 5 min. The PCR products were sequenced in IKMB sequencing facility (University of Kiel, Germany). The sequences were processed using ChromasPro software, then compared against GenBank database using BLAST, with and without type strain filter. Type strains present in the List of Prokaryotic Names with Standing in Nomenclature (LPSN, http://www.bacterio.net/) were used.

For the phylogenetic analysis, each strain was analysed using EzBiocloud (https://www.ezbiocloud.net/), and their closest validated type strains of each phylotype were selected. Using *Rubrobacter aplysinae* DSM 27440^T used as outgroup all selected sequences were aligned using SINA Alignment Service (Pruesse et al. 2012), and then the phylogenetic analyses were performed using the MEGA6 software (Tamura et al. 2013). Tree construction was

conducted using a neighbour-joining algorithm (Saitou and Nei 1987) and maximum-likelihood, using 1000 bootstrap replications. The sequences were deposited in the GenBank (accession numbers: MK085001-MK085032)

Antimicrobial activity test

Antimicrobial activity of the isolated strains was performed using the cross-streak method, using as target two Gram-positive bacteria, *Staphylococcus lentus* DSM 6672^T and *Bacillus subtilis* DSM 347^T and two Gram-negative bacteria, *Escherichia coli* DSM 498^T, and *Pseudomonas fluorescens* NCIMB 10586^T. Fresh culture of the isolates were grown in 1 mL of ½ strength Starch-Glucose-Glycerol (SGG) medium (Goodfellow and Fiedler 2010) (5 g Starch, 5 g glucose, 5 mL glycerol, 2.5 g soy peptone, 1 g yeast extract, 1.25 g corn steep solids, 1.5 g $CaCO_3$, and 10 g Tropic marine salts) at 26 °C for 5 days, then streaked as a line in the middle on agar plates of SGG, SYP (5 g starch, 2 g yeast extract, and 1 g soy peptone), and Starch-Peptone-Salt (10 g Starch, 5 g soy peptone, and 10 g tropic marine salt) media supplemented with 18 g of agar in 1 L of distilled water, and incubated at 26°C for 7 days (14 days for *Micromonosporaceae* family members). The target strains were cross-streaked and incubated for two days and the inhibition zones were recorded.

Results

Isolation and identification of Actinobacteria

A total of thirty-two Actinobacteria from Salar de Llamará and from Salar de Huasco were isolated. Actinobacteria obtained from Salar de Llamará (nine strains) were isolated from microbial mat samples, while strains isolated from sediment and silica crystals were not affiliated with Actinobacteria (data not shown). Actinobacteria isolated from Salar de Huasco (twenty-three strains) were obtained from sediment and arid soil close to the lake, but not from salt crusts.

The initial classification using BLAST analyses of the almost complete 16S rRNA gene sequences showed that the isolates obtained from Salar de Llamará were affiliated with the *Micromonosporaceae* family. Strains obtained from Salar de Huasco are more diverse and representatives of 8 different families: nine are affiliated with *Nocardiopsaceae*, four with *Micromonosporaceae* and *Streptomycetaceae*, two with *Geodermatophilaceae*, and single strains with *Microbacteriaceae*, *Micrococcaceae*, *Streptosporangiaceae*, and *Nonomuraea*. BLAST analysis (with and without type strain filter) also showed that most of the strains obtained from the Salares are similar to Actinobacteria obtained from biomes with similar physicochemical conditions (Table 1) such as marine sediments (12), inland saline/alkaline soils (5), rhizosphere and mangrove roots (6), soils (2), halophyte endophytes (1), stones (2), and arid soils (1).

Table 1. Actinobacteria isolated from Salar de Llamará and Salar de Huasco. Taxonomic affiliation was obtained using 16S rRNA gene sequences and BLAST. Isolates obtained from Salar de Huasco and Salar de Llamará are named with the codes Huas and Llam respectively. The habitat for the first hit in blast (no filter and type strain filter) is also indicated.

Strain	Closest Genbank entry (% similarity 16S rRNA gene)	Habitat of the closest relative	References	Closest type strain (% similarity 16S rRNA gene)	Habitat of the type strain	Reference
Huas2	*Nocardiopsis* sp. TFS73-15 (HM001280.1) (98.6%)	Marine sediments, Caribbean	(Engelhardt et al. 2010)	*Nocardiopsis aegyptia* DSM 44442 (98.9%)	Marine sediments, Alexandria, Abu Air Bay	(Sabry et al. 2004)
Huas3	*Streptomyces* sp. strain HS-NF-1046 (KX118440.1) (99.5%)	Soil sample, Jilin, China	(Gao et al. 2016)	*Streptomyces fulvissimus* DSM 40593 (99.5%)	-	(Waksman and Henrici 1948)
Huas5	*Streptomyces althioticus* isolate#C21 (LN864578.1) (99.8%)	Arid soil, Argelia	GenBank information	*Streptomyces ambofaciens* ATCC 23877 (98.7%)	-	(Pinnert-Sindico 1954)
Huas7	*Kocuria* sp. S26-8 (DQ060377.1) (99.7%)	Marine sediments, Arctic Ocean	GenBank information	*Kocuria himachalensi*s JCM 13326 (99.5%)	Cold desert of the Himalayas	(Mayilraj 2006)
Huas8	*Microbacterium* sp. YT0620 (AB376082.1) (99.3%)	Alkaline soil sample	(Ueda et al. 2008)	*Microbacterium lacus* DSM 18910 (99.3%)	Sediments, Shinji Lake, Japan	(Kageyama et al. 2007)
Huas11	*Nocardiopsis flavescens* JM-T6 (KF876899.1) (99.7%)	Endophyte in *Halocnemum strobilaceum* (Halophyte)	GenBank information	*Nocardiopsis aegyptia* DSM 44442 (99%)	Marine sediments, Alexandria, Abu Air Bay	(Sabry et al. 2004)
Huas12	*Streptomyces* sp. strain HS-NF-1046 (KX118440.1) (99.5%)	Soil sample, Jilin, China	(Gao et al. 2016)	*Streptomyces fulvissimus* DSM 40593 (99.7%)	-	(Waksman and Henrici 1948)
Huas13	*Nocardiopsis* sp. YIM 80186 (EF371474.1) (99.5%)	Saline/Alkaline environment, Qinghai Province China	GenBank information	*Nocardiopsis ganjiahuensis* DSM 45031 (99.9%)	Soil of saline, Ganjia Lake of Xinjiang Province	(Zhang et al. 2008)
Huas14	*Nocardiopsis* sp. TFS	Shallow water	(Bredholdt et al.	*Nocardiopsis tropica* DSM	Soil in the	(Evtushenko et al.

	806 (EF216368.1) (99.1%)	sediment, Trondheims fjord, Norway	2007)	44381 (99.1%)	rhizosphere of *Casuarina* sp., Seychelles	2000)
Huas15	*Nocardiopsis dassonvillei* strain HZNU (CP022434.1) (98.7%)	Pleural effusion, *Homo sapiens*	GenBank information	*Nocardiopsis dassonvillei subsp. dassonvillei* DSM 43111 (98.3%)	Soil, Japon	(Howey et al. 1990)
Huas16	*Nocardiopsis* sp. YIM 80186 (EF371474.1) (99.5%)	Saline/Alkaline environment, Qinghai Province China	GenBank information	*Nocardiopsis metallicus* DSM 44598 (99%)	Alkaline slag dump of metallurgical processing, Germany	(Schippers et al. 2002)
Huas17	*Micromonospora* sp. TFS84-03 (HM001288.1) (99.5%)	Marine sediments, Norway	(Engelhardt et al. 2010)	*Micromonospora echinofusca* DSM 43913 (99.1%)	Excrement of chukar, Beijing, zoological garden	(Kroppenstedt et al. 2005)
Huas19	*Blastococcus aggregatus* DS17 (FR865889.1) (99.5%)	Stone, Italy	(Gtari et al. 2012)	*Blastococcus aggregatus* DSM 4725 (99.2%)	Brackish water, Baltic Sea	Ahrens and Moll 1970 emend. (Urzi et al. 2004)
Huas20	*Blastococcus saxobsidens* DD2 (FO117623.1) (98%)	Sardinian wall calcarenite stone sample	(Chouaia et al. 2012b)	*Blastococcus saxobsidens* DSM 44509 (97.6%)	Calcarenite MA12, Malta	(Urzi et al. 2004)
Huas21	*Nocardiopsis* sp. TFS 806 (EF216368.1) (99.2%)	Shallow water sediment, Trondheims fjord, Norway	(Bredholdt et al. 2007)	*Nocardiopsis tropica* DSM 44381 (99.1%)	Soil in the rhizosphere of *Casuarina* sp., Seychelles	(Evtushenko et al. 2000)
Huas22	*Nocardia* sp. FMN15 (JN896621.1) (99.1%)	Zapadnaya Southwest Forest Park, Russia	Ozdemir-Kocak et al. 2016	*Nocardia rhamnosiphila* DSM 45147 (98.7%)	Suburban compost heap, Cape Town	(Everest et al. 2011)
Huas23	*Nocardiopsis* sp. SANLU-AU (JN038552.1) (98.1%)	Salt marsh rhizosphere from *Suaeta* spp.	GenBank information	*Nocardiopsis sinuspersici* DSM 45277 (98.1%)	Sandy rhizospheric soil, Sarbandar, seashore of Persian Gulf	(Hamedi et al. 2010)
Huas24	*Solwaraspora* sp. UMM479 (AY552773.1) (99.4%)	Marine sediment	(Magarvey et al. 2004)	*Micromonospora nigra* DSM 43818 (99.3%)	Salt pool in Syracuse, NY, USA.	(Kasai et al. 2000)
Huas25	*Micromonospora* sp.	Marine sediment,	(Engelhardt et al.	*Micromonospora echinofusca*	Excrement of	(Kroppenstedt et

	TFS84-03 (HM001288.1) (99.5%)	Norway	2010)	DSM 43913 (99%)	chukar, Beijing, zoological garden	al. 2005)
Huas26	*Nonomuraea* sp. TFS 1165 (EF216356.1) (99.4%)	Shallow water sediment, Trondheims fjord, Norway	(Bredholdt et al. 2007)	*Nonomuraea endophytica* DSM 45385 (97.7%)	Surface-sterilised sample of *Artemisia annua* L., Yunnan province	(Li et al. 2011)
Huas27	*Micromonospora* sp. 2602SCA9 (JQ836669.1) (99.2%)	Mangrove soil	GenBank information	*Micromonospora oryzae* DSM 102119 (98.9%)	Root internal tissues, *Oryza sativa*, Chumporn Thailand	(Kittiwongwattana et al. 2015)
Huas28	*Streptomyces* sp. NPS-554 (AB515328.1) (96.5%)	Marine sediment, Miyazaki, off the coast of Nobeoka, Japan	(Iwata et al. 2009)	*Streptomyces bohaiensis* DSM 42125 (96.8%)	Young *Scomberomorus niphonius* (pelagic fish), Eastern Liaoning Peninsula, China	(Pan et al. 2015)
Huas29	*Nocardiopsis* sp. YIM C560 (EU135693.1) (99.7%)	Haloalkaline soil, bank of Qinghai lake, China	GenBank information	*Nocardiopsis aegyptia* DSM 44442 (99.4%)	Marine sediments, Alexandria, Abu Air Bay	(Sabry et al. 2004)
Llam0	*Micromonospora* sp. CNJ878 PL04 (DQ448714.1) (99.7%)	Marine sediment, Palau	(Gontang et al. 2007)	*Micromonospora yangpuensis* DSM 45577 (98.6%)	Cup-shaped sponge, Dachan reef, China	(Zhang et al. 2012)
Llam1	*Micromonospora sp*. CNJ878 PL04 (DQ448714.1) (99.7%)	Marine sediment, Palau	(Gontang et al. 2007)	*Micromonospora narathiwatensis strain* DSM 45248 (98.6%)	Mangrove soil samples, Thailand	(Thawai et al. 2007)
Llam2	*Micromonospora sp*. CNJ878 PL04 (DQ448714.1) (99.7%)	Marine sediment, Palau	(Gontang et al. 2007)	*Micromonospora narathiwatensis strain* DSM 45248 (98.5%)	Mangrove soil samples, Thailand	(Thawai et al. 2007)
Llam7	*Micromonospora* sp. 27021/10ATCC9 (JQ836683.1) (98.4)	Mangrove soil, China	GenBank information	*Jishengella endophytica* DSM 45430 (98.4%)	Surface-sterilised roots of *Acanthus illicifolius*, mangrove reserve zone, China	(Xie et al. 2011)
Llam8	*Micromonospora* sp. 2701SIM06	Mangrove soil, China	GenBank information	*Micromonospora pattaloongensis* DSM 45245	Thai mangrove forest, Thailand	(Thawai et al. 2008)

	(JQ836686.1) (99.7%)			(98.5%)		
Llam10	*Micromonospora* sp. 27021/10ATCC9 (JQ836683.1) (98.8)	Mangrove soil, China	GenBank information	*Jishengella endophytica* DSM 45430 (98.8%)	Surface-sterilised roots of *Acanthus illicifolius*, mangrove reserve zone, China	(Xie et al. 2011)
Llam11	*Micromonospora* sp. 27021/10ATCC9 (JQ836683.1) (98.7)	Mangrove soil, China	GenBank information	*Jishengella endophytica* DSM 45430 (98.7%)	Surface-sterilised roots of *Acanthus illicifolius*, mangrove reserve zone, China	(Xie et al. 2011)
Llam13	*Micromonospora* sp. SB1-25 (FN376883.1) (98.4%)	Root nodules from *Lupinus angustifolius*, Spain.	GenBank information	*Jishengella endophytica* DSM 45430 (98.4%)	Surface-sterilised roots of *Acanthus illicifolius*, mangrove reserve zone, China	(Xie et al. 2011)
Llam15	*Micromonospora* sp. 27021/10ATCC9 (JQ836683.1) (98.8)	Mangrove soil, China	GenBank information	*Jishengella endophytica* DSM 45430 (98.8%)	Surface-sterilised roots of *Acanthus illicifolius*, mangrove reserve zone, China	(Xie et al. 2011)

The strains were assigned to 22 different phylotypes (isolates with 16S rRNA gene sequence similarity >99.5% were considered to be a single phylotype) (Table 2). Phylogenetic analyses revealed that three phylotypes (Q, T, and U) cluster closely to different genera within the *Micromonosporaceae* family (Figure 1). The strains clustered as phylotype T, using BLAST algorithm, showed high similarity to *Micromonospora yangpuensis* DSM 45577^T (98.6%) and *Micromonospora narathiwatensis* strain DSM 45248^T (98.6%), but in the phylogenetic tree they cluster close to *Plantactinospora* genus, specifically to *Plantactinospora soyae* DSM 46832^T (98.3%). The phylotype T (Huas27) has a high 16S rRNA gene sequence similarity to *Micromonospora oryzae* DSM 102119^T (98.9%), but in the tree clusters together with *Actinoplanes brasiliensis* DSM 43805^T (98.0%). In the case of phylotype U, the strains form a distinct cluster with unclear genus affiliation, related to both genera *Jishengella* and *Salinispora*.

The phylotypes A (Huas2 and Huas11) and S (Huas29) showed a high percentage of identity with *Nocardiopsis aegyptica* strain DSM 44442^T (98,4% and 99,8% respectively); however, only strain Huas29 (phylotype T) clusters next to it, while the strains Huas2 and Huas11 (phylotype A) form a cluster separate from their next related type strain.

According to 16S rRNA gene identities obtained from BLAST against their closed related type strains, fifteen strains can be considered potential novel species (Table 2). However, even though six phylotypes (A, D, E, G, J, and S) showed a high percentage of identity in their 16S rRNA against the type strain, their position in the phylogenetic tree in comparison with their next related type strains suggest that they might be potential candidates for novel species designation (Figure 1).

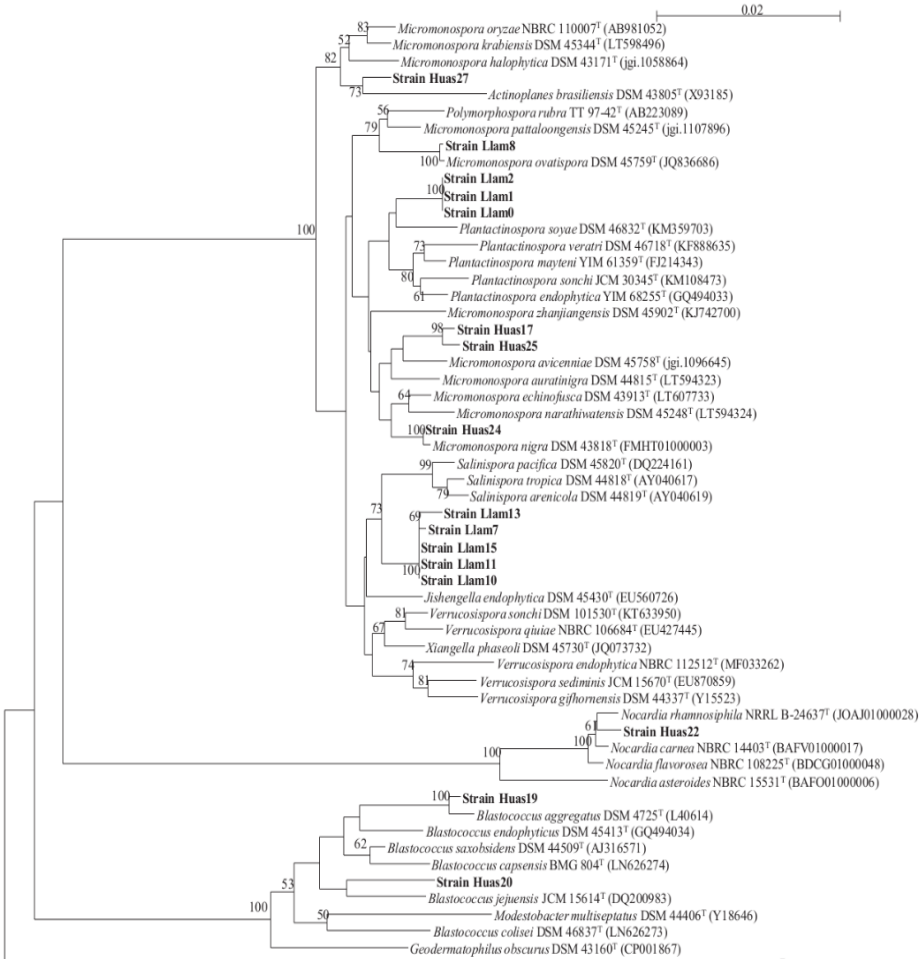


Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence comparison of the Actinobacteria isolates and the next related type strains with *Rubrobacter aplysinae* DSM 27440^T as outgroup. Numbers at the nodes represent bootstrap support (%) based on the analysis of 1000 bootstrap replications, asterisks indicate branches of the tree that were also recovered using the maximum-likelihood algorithm. Only bootstrap values ≥ 50% are indicated. Genbank accession numbers are given in parentheses. Bar indicates 0.02 substitutions per site (Continues in the next page).

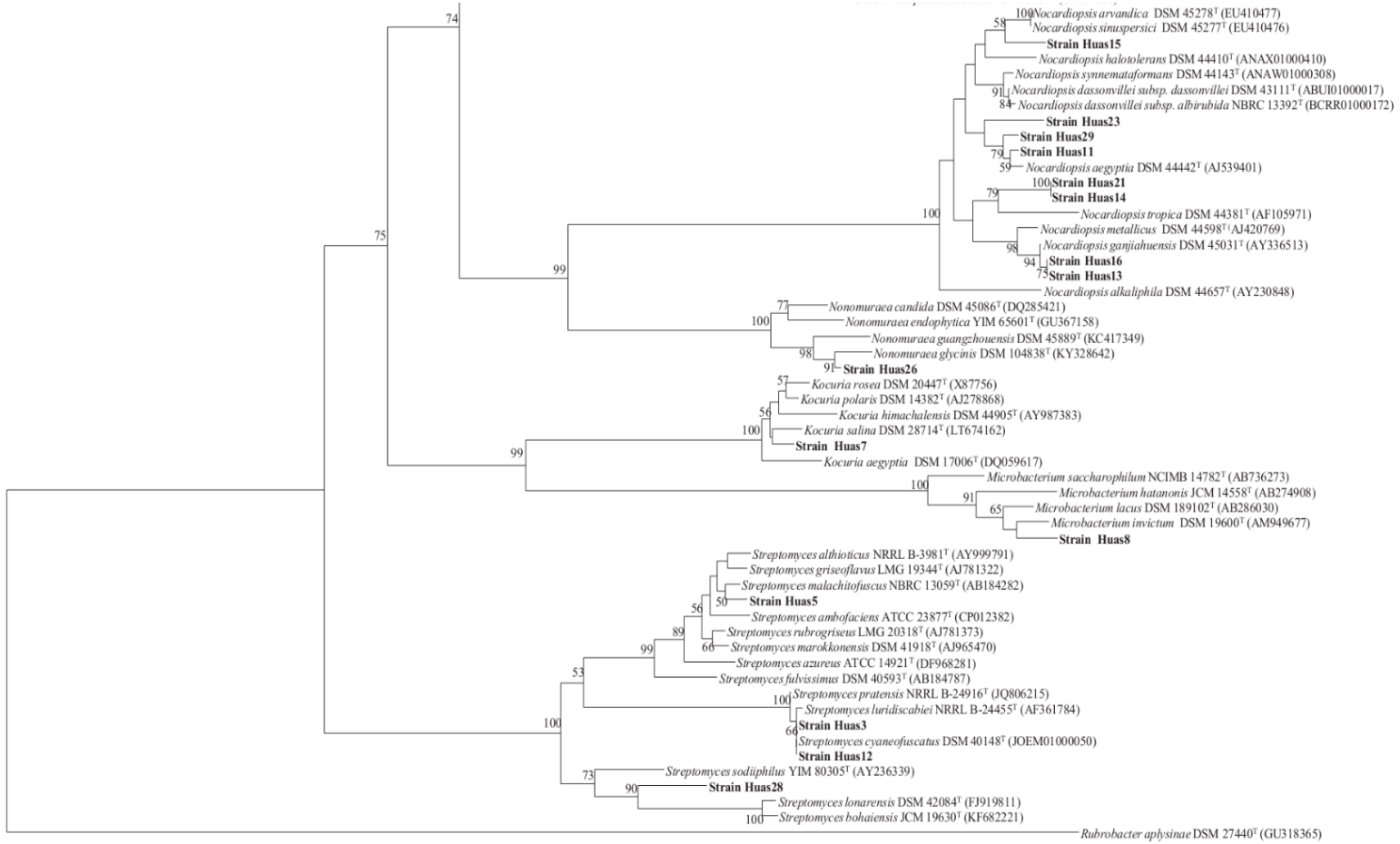


Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence comparison of the Actinobacteria isolates and the next related type strains with *Rubrobacter aplysinae* DSM 27440^T as outgroup. Numbers at the nodes represent bootstrap support (%) based on the analysis of 1000 bootstrap replications, asterisks indicate branches of the tree that were also recovered using the maximum-likelihood algorithm. Only bootstrap values ≥ 50% are indicated. Genbank accession numbers are given in parentheses. Bar indicates 0.02 substitutions per site.

Table 2. Phylotype clusters of the isolated Actinobacteria, their genus affiliation according to BLAST and to the phylogenetic analyses.

Phylotype	Strains	Genus affiliation BLAST	Phylogenetic analyses	Novel species (>98.7%)
A	Huas2, Huas11	*Nocardiopsis*	*Nocardiopsis*	No
B	Huas3, Huas12	*Streptomyces*	*Streptomyces*	No
C	Huas5	*Streptomyces*	*Streptomyces*	Yes
D	Huas7	*Kocuria*	*Kocuria*	No
E	Huas8	*Microbacterium*	*Microbacterium*	No
F	Huas13	*Nocardiopsis*	*Nocardiopsis*	No
G	Huas14, Huas21	*Nocardiopsis*	*Nocardiopsis*	No
H	Huas15	*Nocardiopsis*	*Nocardiopsis*	Yes
I	Huas16	*Nocardiopsis*	*Nocardiopsis*	No
J	Huas17, Huas25	*Micromonospora*	*Micromonospora*	No
K	Huas19	*Blastococcus*	*Blastococcus*	Yes
L	Huas20	*Blastococcus*	*Blastococcus*	No
M	Huas22	*Nocardia*	*Nocardia*	Yes
N	Huas23	*Nocardiopsis*	*Nocardiopsis*	Yes
O	Huas24	*Micromonospora*	*Micromonospora*	No
P	Huas26	*Nonomuraea*	*Nonomuraea*	Yes
Q	Huas27	*Micromonospora*	*Actinoplanes*	No
R	Huas28	*Streptomyces*	*Streptomyces*	Yes
S	Huas29	*Nocardiopsis*	*Nocardiopsis*	No
T	Llam0, Llam1, Llam2	*Micromonospora*	*Plantactinospora*	Yes
U	Llam7, Llam10, Llam13, Llam15	*Jishengella*	Unclear	Yes
V	Llam8	*Micromonospora*	Micromonospora	Yes

Antimicrobial activity

According to the performed diffusion test, 56% of the isolates showed antibiotic activity, with 30% active against the tested Gram-negative bacteria and 46% against the Gram-positive bacteria (Table 3). Among all the strains studied, 7 strains showed antibiotic activity, against *E. coli*, *Nocardiopsis* (Huas14 and Huas16), *Micromonospora* (Llam0 and Llam2), *Jishengella/Salinispora* (Llam7 and Llam11), and *Streptomyces* (Huas5); antibiotic activity against *Pseudomonas fluorescens* was shown by 6 strains, *Micromonospora* (Llam0, Llam1, and

Llam2), *Jishengella/Salinispora* (Llam7), and *Nocardiopsis* (Huas15 and Huas23). Activity against Gram-positive bacteria was detected in 12 strains, most of which share antibiotic activity against *Staphylococcus lentus* DSM 6672^T and *Bacillus subtilis* DSM 347^T. *Streptomyces* strains Huas23, Huas5, and Huas12 showed activity against both Gram-positive strains, while *Streptomyces* sp. Huas28 showed exclusively activity against *Bacillus subtilis* DSM 347^T. *Nocardiopsis* strains Huas2 and Huas15 showed activity against *Staphylococcus lentus* DSM 6672^T and *Bacillus subtilis* DSM 347^T, while *Nocardiopsis* Huas23 showed antibiotic activity only against *Staphylococcus lentus* DSM 6672^T. *Micromonospora* strains Huas24, Huas25, Llam0, Llam1, and Llam2 had activity against both Gram-positive strain tested. No correlation between phylotype and antibacterial activity was found, suggesting strain-specific genetic differences.

Table 3. Antibiotic activity of strains isolated from hypersaline samples from Salar de Huasco (Huas) and Salar de Llamará (Llam). E: *Escherichia coli* K12 DSM 498^T; P: *Pseudomonas fluorescens* NCIMB 10586^T; B: *Bacillus subtilis* DSM 347^T; S: *Staphylococcus lentus* DSM 6672^T. -: No antibiotic activity; +/- weak inhibitory activity inhibition zone < 5mm); +: inhibition zone between 5 to 10 mm; ++: inhibition zone between 10 to 20 mm; +++: inhibition zone > 20 mm. Strains that are not included in this list did not show antibiotic activity under the experimental conditions.

	SGG medium				SYP medium				SPS medium			
Strain	E	P	B	S	E	P	B	S	E	P	B	S
Huas2	-	-	-	-	-	-	-	-	-	-	+	+
Huas3	-	-	-	-	-	-	++	++	-	-	-	-
Huas5	+/-	-	+	+/-	-	-	+/-	-	-	-	+/-	-
Huas12	-	-	+/-	+	-	-	+/-	+/-	-	-	+	+/-
Huas14	-	-	-	-	-	-	-	-	++	-	-	-
Huas15	-	-	+/-	-	-	++	++	+/-	-	-	++	+
Huas16	-	-	-	-	-	-	-	-	+/-	-	-	-
Huas17	-	-	-	+/-	-	-	-	-	-	-	-	+/-
Huas23	-	-	-	-	-	+	-	+	-	-	-	-
Huas24	-	-	++	++	-	-	+++	+++	-	-	+++	-
Huas25	-	-	-	-	-	-	+++	+++	-	-	+++	-
Huas28	-	-	-	-	-	-	+/-	-	-	-	-	-
Llam0	-	++	+++	+++	-	-	++++	+++	+/-	+++	+++	++
Llam1	-	+	+++	+++	-	-	++++	+++	-	-	+++	++
Llam2	+/-	++	+++	+++	-	+/-	++++	+++	+/-	+/-	+++	+++
Llam7	+/-	+/-	-	-	-	-	-	-	-	-	-	-
Llam11	+/-	-	-	-	-	-	-	-	-	-	-	-

Discussion

Actinobacteria strains isolated from Salar de Llamará and Salar de Huasco were studied, focusing on their phylogenetic affiliation and antimicrobial properties. Salares from the Atacama Desert are under-explored habitats; despite the high salt concentration, their microbial communities are diverse and show a huge novelty of microbial taxa (Dorador et al. 2013), and play an important role in the basal structures of the ecosystem (Dorador et al. 2018). Few studies have focused on the cultivation of diverse Actinobacteria, although Okoro et al (2009) did show that soils from Salar de Atacama were diverse in *Streptomyces*, *Lechevalieria,* and *Amycolaptopsis.* *Streptomyces* obtained in this study have been described as novel species (Santhanam et al. 2012a, b, 2013; Busarakam et al. 2014). They are also a good source of new natural products (Nachtigall et al. 2011; Rateb et al. 2011; Elsayed et al. 2015). An independent study of a *Streptomyces* isolated from Salar de Tara showed that even new isolates obtained from Salares with high similarity to representative type strains can be a good source for novel compounds (Schulz et al. 2011).

In the present study, the isolates affiliated with twenty-two phylotypes and eleven different genera according to phylogenetic analyses. Compared with a previous study on soils from Atacama Desert (Okoro et al. 2009) in which only three genera were isolated (*Amycolaptopsis*, *Lechevalieria* and *Streptomyces*), the genera diversity of Actinobacteria was higher in Salar de Llamará and Salar de Huasco. Nevertheless, *Streptomyces* isolates obtained from Salar de Huasco did not show similarity in their 16S rRNA gene with strains obtained from Salar de Atacama (Okoro et al. 2009), or with the Salar de Tara isolate (Schulz et al. 2011). Interestingly, representatives of different genera were isolated from the two Salares. This demonstrates unique actinobacterial populations in each of the two Salares, whereas Salar de Huasco actinobacterial diversity was higher compared to Salar de Llamará. A recent study that focused only on the isolation of *Micromonospora* from different samples of the Atacama Desert showed that 20 representative strains showed distinct phyletic lines (Carro et al. 2018); however, none of the isolates are closely related to the strains obtained from Salar de Llamará and Salar de Huasco samples.

The differences in diversity are considered to be attributes of the nature of the studied samples originating from saline-arid soils bordering the Salares, namely from salt crusts, silica crystals,

sediment, and microbial mats. Selected culture media and conditions of isolation were identical for all samples and can therefore be excluded as responsible factors. We used uncommon carbon sources such as raffinose and trehalose, and selected amino acids to avoid the overrepresentation of the genus *Streptomyces* (Vickers et al. 1984). Also, all components of the culture media were more highly diluted in comparison with similar culture media, with the aim of mimicking environmental conditions and avoiding nutrient saturation that would favour the isolation of known microorganisms.

Due to water availability, hypersaline lakes are one of the most productive environments in the Atacama Desert, with a high diversity of microorganisms and high density of biofilms and microbial mats (Demergasso et al. 2004; Dorador et al. 2009, 2018). Culture-independent studies showed the presence of different families of Actinobacteria in Salar de Llamará and Huasco (Aguilar et al. 2016), with members affiliated with the TM146 and OM1 clades, with *Corynebacteriaceae, Cryptosporangiaceae, Demequinaceae, Mycobacteriaceae, Nitriliruptoraceae, Propionibacteriaceae, Micromonosporaceae,* and *Microbacteriaceae*. Strains affiliated with the last two families were isolated in our study (Table 1). On the other hand, members of the family *Microbacteriaceae* (*Leifsonia*) were detected by sequencing DGGE bands from Salar de Llamará (Demergasso et al. 2004), and members affiliated with *Propionibacteriaceae, Microbacteriaceae, Corynebacteriaceae, and Nocardiaceae* were detected in water samples (Pablo Aran, personal communication).

Actinobacteria are ubiquitous microorganisms; in some cases, strains affiliated with a genus can be isolated from various environmental sources. Nevertheless, strains obtained in this study showed high similarity with strains obtained from environments with similar physicochemical conditions (Table 1). Strains affiliated with *Streptomyces*, *Nonomuraea,* and *Micromonospora* have been obtained from different saline environments, including Indian coastal solar salterns (Jose and Jebakumar 2013). *Nonomuraea* strains also have been isolated from marine sediments (Bredholdt et al. 2007; Maldonado et al. 2009; Becerril-Espinosa et al. 2013). Strains affiliated with genus *Streptomyces* have been reported in different saline soils (Zvyagintsev et al. 2008; Cai et al. 2009; Akhwale et al. 2015). *Micromonospora* clones have been found in different hot spring microbial mats in Tibet (Jiang et al. 2012). Species of the genus *Nocardiopsis* include several halotolerant species that tolerate up to 20% NaCl, and which have been isolated frequently from saline environments, including from inland soils (Yang et al. 2008; Lubsanova et

al. 2014; He et al. 2015; Li et al. 2017) and marine samples (Sabry et al. 2004; Bredholdt et al. 2007; Chen et al. 2009),

Using 16S rRNA gene sequence similarity, eighteen of thirty-two strains showed an identity value below the 98.7% threshold (Yarza et al. 2014), suggesting species novelty. However, 16S rRNA in Actinobacteria has a high degree of conservation. Examples of novel species descriptions with 16S rRNA gene sequence identities higher than 99% (Meier-Kolthoff et al. 2013) have been reported for strains affiliated with the genera *Micromonospora* (Kittiwongwattana et al. 2015), *Streptomyces* (Santhanam et al. 2012a), and *Nocardiopsis* (Zhang et al. 2008), even when its DNA:DNA relatedness value is below the recommended 70% (Wayne et al. 1987). Evidence in clade formation (Figure 1), 16S rRNA gene similarities between isolates from our study, and type strains suggest that six phylotypes might be considered novel species. Specifically, the phylotype U position on the phylogenetic tree in between *Salinispora* and *Jishengella* suggests that the strains might be a novel genus (Figure 1).

Since the 1970s, the discovery of antibiotics started to fade with the high rate of rediscovery of already known compounds (Arias and Murray 2015). Nowadays, due to the huge load of information that can be obtained from compounds databases as well as the improvement in the annotation of bacterial genomes and their natural products gene clusters (Weber et al. 2015), it is easier to make an educated guess regarding the selection of potential strains that could produce novel natural products. Most of the strains isolated in this study belong to genera known as natural products producers. 7600 compounds have been described from *Streptomyces* genera,, followed by *Micromonospora* (442), *Nocardia* (316), *Nocardiopsis* (88), *Salinispora* (56), and *Nonomuraea* (32). Meanwhile for *Kocuria* and *Microbacterium* only two compounds have been reported for each, and zero for *Blastococcus* (Dictionary of Natural products 2015). (Supplementary Table 1). The availability of complete Actinobacteria genomes and the understanding of the biosynthetic gene clusters involved in the production of natural products provide valuable information about their potential as natural product producers (Doroghazi and Metcalf 2013). The complete genome sequences of type strains of representative species related to the isolates from this study showed that genes of biosynthetic pathways for more than 10 natural products are encoded in *Nocardiopsis* (Sun et al. 2010), *Salinispora* (Udwary et al. 2007), and *Micromonospora* (unpublished, Acc. Number: CP002162.1), and more than 25 in *Streptomyces* (Thibessard et al. 2015), *Nonomuraea* (D'Argenio et al. 2016), and *Nocardia*

(Vera-Cabrera et al. 2012). On the other hand, complete genomes of *Blastococcus* (Chouaia et al. 2012a), *Kocuria* (Takarada et al. 2008), and *Microbacterium* (Unpublished: Acc. Number: CP018134) show five or less natural product biosynthetic gene clusters. Strains affiliated with genera with a high number of natural product biosynthetic gene clusters but lower number of compounds described, such as *Nocardiopsis*, *Nonomuraea*, and *Micromonosporaceae* family members (*Plantactinospora*, *Actinoplanes*, etc), appear to be excellent targets for the discovery of novel compounds.

Antibiotic production in Actinobacteria has been reported extensively, with evidence in crude extracts, compounds described, and genomics endorsing it. Culture composition, culture conditions, and taxonomic affiliation of the strains is important to trigger the production of antibiotic substances (Goodfellow and Fiedler 2010). It has been reported that using media with a high carbon:nitrogen ratio enhances the antibiotic production in *Streptomyces*, *Nocardia*, and *Micromonospora* (Goodfellow and Fiedler 2010), but there are no common rules for underexploited taxa such as *Plantactinospora*, *Actinoplanes*, *Nocardiopsis*, and *Nonomuraea*. Under the condition given in our study, more than half of the strains revealed antibiotic activity (Table 2). Presumably, those strains that did not show antibiotic activities require different conditions to trigger and optimize synthesis of bioactive compounds. Also, different bioassays may reveal positive results. Antibiotic resistance is a worldwide problem (ECDC 2015); the discovery of new antibiotics to fight against Gram-Negative and Gram-Positive bacteria is urgently needed. Antibiotics against Gram-negative bacteria are difficult to find, in comparison to Gram-positive bacteria (Delcour 2009). In our study, 30% of the isolates showed activity against the Gram-negative bacteria tested.

Conclusions

Salares in the Atacama Desert represent a valuable source of novel Actinobacteria and their antibiotic compounds. A highly diverse number of Actinobacteria has been isolated from two hypersaline lakes, Salar de Llamará and Salar de Huasco by using an array of selected media. The isolated strains affiliated with a diverse set of genera, including *Streptomyces*, *Micromonospora*, *Nocardiopsis*, *Nocardia*, *Nonomuraea*, *Actinoplanes*, *Microbacterium*, *Kocuria*, *Blastococcus*, *Plantactinospora*, and a potential new genus affiliated with the *Micromonosporaceae*. Using 16S rRNA gene sequences and its phylogenetic tree analyses as criteria, 25 strains might represent novel species affiliated with each genus obtained. The ability to produce antibiotic substances is found in most of the isolates (56%), with activity against Gram-negative bacteria in 30% of the strains, against Gram-positive bacteria in 46% of the strains, and against both in 28% of the strains.

Acknowledgements

The authors gratefully acknowledge the IKMB sequencing facility (University of Kiel), Pablo Aran, Sergio Mujica, Alfonso Araya, Eduardo Acosta, and Pablo Aguilar for their help during the field work. Alvaro S. Villalobos was supported by a doctoral fellowship from Becas Chile (CONICYT) and the Deutscher Akademischer Austauschdienst (DAAD).

References

Aguilar P, Acosta E, Dorador C, Sommaruga R (2016) Large differences in bacterial community composition among three nearby extreme waterbodies of the high Andean plateau. Front Microbiol 7:1–8. doi: 10.3389/fmicb.2016.00976

Akhwale JK, Göker M, Rohde M, et al (2015) *Streptomyces alkaliphilus* sp. nov., isolated from sediments of Lake Elmenteita in the Kenyan Rift Valley. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 107:1249–1259. doi: 10.1007/s10482-015-0418-2

Arias CA, Murray BE (2015) A New Antibiotic and the Evolution of Resistance. N Engl J Med 372:1168–1170. doi: 10.1056/NEJMcibr1500292

Becerril-Espinosa A, Freel KC, Jensen PR, Soria-Mercado IE (2013) Marine Actinobacteria from the Gulf of California: Diversity, abundance and secondary metabolite biosynthetic potential. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 103:809–819. doi: 10.1007/s10482-012-9863-3

Bérdy J (2012) Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot (Tokyo) 65:385–395. doi: 10.1038/ja.2012.27

Bredholdt H, Galatenko OA, Engelhardt K, et al (2007) Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: Isolation, diversity and biological activity. Environ Microbiol 9:2756–2764. doi: 10.1111/j.1462-2920.2007.01387.x

Bull AT, Ward AC, Goodfellow M (2000) Search and Discovery Strategies for Biotechnology: the Paradigm Shift. Microbiol Mol Biol Rev 64:573–606. doi: 10.1128/MMBR.64.3.573-606.2000

Busarakam K, Bull AT, Girard G, et al (2014) *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 105:849–861. doi: 10.1007/s10482-014-0139-y

Cai Y, Chen Z, Zhang R (2009) Classification and salt-tolerance of Actinomycetes in the Qinghai lake water and lakeside saline soil. J Sustain Dev 2:107–110. doi: 10.5539/jsd.v2n1p107

Carro L, Razmilic V, Nouioui I, et al (2018) Hunting for cultivable *Micromonospora* strains in soils of the Atacama Desert. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:1375–1387. doi: 10.1007/s10482-018-1049-1

Chen YG, Wang YX, Zhang YQ, et al (2009) *Nocardiopsis litoralis* sp. nov., a halophilic marine actinomycete isolated from a sea anemone. Int J Syst Evol Microbiol 59:2708–2713. doi: 10.1099/ijs.0.009704-0

Chouaia B, Crotti E, Brusetti L, et al (2012) Genome sequence of *Blastococcus saxobsidens* DD2, a stone-inhabiting bacterium. J Bacteriol 194:2752–2753. doi: 10.1128/JB.00320-12

D'Argenio V, Petrillo M, Pasanisi D, et al (2016) The complete 12 Mb genome and transcriptome of *Nonomuraea gerenzanensis* with new insights into its duplicated "magic" RNA polymerase. Sci Rep. doi: 10.1038/s41598-016-0025-0

Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta 1794:808–16. doi: 10.1016/j.bbapap.2008.11.005

Demergasso C, Casamayor EO, Chong G, et al (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. FEMS Microbiol Ecol 48:57–69. doi: 10.1016/j.femsec.2003.12.013

Dorador C, Fink P, Hengst M, et al (2018) Microbial community composition and trophic role along a marked salinity gradient in Laguna Puilar, Salar de Atacama, Chile. Antonie Van Leeuwenhoek 111:1361–1374. doi: 10.1007/s10482-018-1091-z

Dorador C, Meneses D, Urtuvia V, et al (2009) Diversity of bacteroidetes in high-altitude saline evaporitic basins in northern Chile. J Geophys Res Biogeosciences 114:1–11. doi: 10.1029/2008JG000837

Dorador C, Vila I, Imhoff JF, Witzel KP (2008) Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: An example of geographical dispersion? FEMS Microbiol Ecol 64:419–432. doi: 10.1111/j.1574-6941.2008.00483.x

Dorador C, Vila I, Witzel K-P, Imhoff JF (2013) Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. Fundam Appl Limnol 182:135–159. doi: 10.1127/1863-9135/2013/0393

Doroghazi JR, Metcalf WW (2013) Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. BMC Genomics 14:611. doi: 10.1186/1471-2164-14-611

Drees KP, Neilson JW, Betancourt JL, et al (2006) Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. Appl Environ Microbiol 72:7902–7908. doi: 10.1128/AEM.01305-06

Elsayed SS, Trusch F, Deng H, et al (2015) Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyperarid Atacama Desert. J Org Chem 80:10252–10260. doi: 10.1021/acs.joc.5b01878

Engelhardt K, Degnes KF, Kemmler M, et al (2010) Production of a new thiopeptide antibiotic, TP-1161, by a marine *Nocardiopsis* species. Appl Environ Microbiol 76:4969–4976. doi: 10.1128/AEM.00741-10

Everest GJ, Cook AE, le Roes-Hill M, Meyers PR (2011) *Nocardia rhamnosiphila* sp. nov., isolated from soil. Syst Appl Microbiol 34:508–512. doi: 10.1016/j.syapm.2011.03.006

Evtushenko LI, Taran V V., Akimov VN, et al (2000) *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. Int J Syst Evol Microbiol 50:73–81. doi: 10.1099/00207713-50-1-73

Fierer N, Leff JW, Adams BJ, et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci 109:21390–21395. doi: 10.1073/pnas.1215210110

Fischbach MA, Walsh CT (2006) Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: Logic machinery and mechanisms. Chem Rev 106:3468–3496. doi: 10.1021/cr0503097

Gao MY, Qi H, Li JS, et al (2017) A new polysubstituted cyclopentene derivative from *Streptomyces* sp. HS-NF-1046. J Antibiot (Tokyo) 70:216–218. doi: 10.1038/ja.2016.111

Glavač NK, Djogo S, Ražić S, et al (2017) Accumulation of heavy metals from soil in medicinal plants.

Gontang EA, Fenical W, Jensen PR (2007) Phylogenetic diversity of Gram-Positive bacteria cultured from marine sediments. Appl Environ Microbiol 73:3272–3282. doi: 10.1128/AEM.02811-06

Goodfellow M, Fiedler H-PP (2010) A guide to successful bioprospecting: Informed by actinobacterial systematics. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 98:119–142

Gtari M, Essoussi I, Maaoui R, et al (2012) Contrasted resistance of stone-dwelling *Geodermatophilaceae* species to stresses known to give rise to reactive oxygen species. FEMS Microbiol Ecol 80:566–577. doi: 10.1111/j.1574-6941.2012.01320.x

Hamedi J, Mohammadipanah F, Klenk H-P, et al (2010) *Streptomyces iranensis* sp. nov., isolated from soil. Int J Syst Evol Microbiol 60:1504–1509. doi: 10.1099/ijs.0.015339-0

He S-TT, Zhi X-YY, Jiang H, et al (2015) Biogeography of *Nocardiopsis* strains from hypersaline environments of Yunnan and Xinjiang Provinces, western China. Sci Rep 5:13323. doi: 10.1038/srep13323

Howey R, Lock C, Moore L (1990) Subspecies names automatically created by Rule 46. Int. J. Syst. Bacteriol 40:317–319. doi: 10.1099/00207713-46-3-830

Iwata F, Sato S, Mukai T, et al (2009) Lorneic acids, trialkyl-substituted aromatic acids from a marine-derived actinomycete. J Nat Prod 72:2046–2048. doi: 10.1021/np900353y

Jiang H, Dong CZ, Huang Q, et al (2012) Actinobacterial diversity in microbial mats of five hot springs in central and central-eastern Tibet, China. Geomicrobiol J 29:520–527. doi: 10.1080/01490451.2011.590872

Jose PA, Jebakumar SRD (2013) Phylogenetic appraisal of antagonistic, slow growing actinomycetes isolated from hypersaline inland solar salterns at sambhar salt lake, India. Front Microbiol 4:1–9. doi: 10.3389/fmicb.2013.00190

Kageyama A, Takahashi Y, Matsuo Y, et al (2007) *Microbacterium flavum* sp. nov. and *Microbacterium lacus* sp. nov., isolated from marine environments. Actinomycetologica 21:53–58. doi: 10.3209/saj.SAJ210201

Kasai H, Tamura T, Harayama S (2000) Intrageneric relationships among *Micromonospora* species deduced from gyrB-based phylogeny and DNA relatedness. Int J Syst Evol Microbiol 50:127–134. doi: 10.1099/00207713-50-1-127

Kittiwongwattana C, Thanaboripat D, Laosinwattana C, et al (2015) *Micromonospora oryzae* sp. nov., isolated from roots of upland rice. Int J Syst Evol Microbiol 65:3818–3823. doi: 10.1099/ijsem.0.000500

Kroppenstedt RM, Mayilraj S, Wink JM, et al (2005) Eight new species of the genus *Micromonospora*, *Micromonospora citrea* sp. nov., *Micromonospora echinaurantiaca* sp. nov., *Micromonospora echinofusca* sp. nov. *Micromonospora fulviviridis* sp. nov., *Micromonospora inyonensis* sp. nov., *Micromonospora peucetia* sp. nov. Syst Appl Microbiol 28:328–339. doi: 10.1016/j.syapm.2004.12.011

Li J, Zhao G-Z, Huang H-Y, et al (2011) *Nonomuraea endophytica* sp. nov., an endophytic actinomycete isolated from *Artemisia annua* L. Int J Syst Evol Microbiol 61:757–761. doi: 10.1099/ijs.0.022558-0

Li MG, Li WJ, Xu P, et al (2003) *Nocardiopsis xinjiangensis* sp. nov., a halophilic actinomycete isolated from a saline soil sample in China. Int J Syst Evol Microbiol 53:317–321. doi: 10.1099/ijs.0.02055-0

Lubsanova DA, Zenova GM, Kozhevin PA, et al (2014) Filamentous actinobacteria of the saline soils of arid territories. Moscow Univ Soil Sci Bull 69:88–92. doi: 10.3103/S0147687414020057

Magarvey NA, Keller JM, Bernan V, et al (2004) Isolation and Characterization of Novel Marine-Derived Actinomycete Taxa Rich in Bioactive Metabolites. Appl Environ Microbiol 70:7520–7529. doi: 10.1128/AEM.70.12.7520-7529.2004

Maldonado LA, Fragoso-Yáñez D, Pérez-García A, et al (2009) Actinobacterial diversity from marine sediments collected in Mexico. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 95:111–120. doi: 10.1007/s10482-008-9294-3

Mayilraj S, Kroppenstedt RM, Suresh K, Saini HS (2006) *Kocuria himachalensis* sp. nov., an actinobacterium isolated from the Indian Himalayas. Int J Syst Evol Microbiol 56:1971–1975. doi: 10.1099/ijs.0.63915-0

Meier-Kolthoff JP, Göker M, Spröer C, Klenk H-P (2013) When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 195:413–418. doi: 10.1007/s00203-013-0888-4

Nachtigall J, Kulik A, Helaly S, et al (2011) Atacamycins A–C, 22-membered antitumor macrolactones produced by *Streptomyces* sp. C38*. J Antibiot (Tokyo) 64:775–780. doi: 10.1038/ja.2011.96

Newman DJ, Cragg GM (2007) Natural Products as Sources of New Drugs over the Last 25 Years. J Nat Prod 70:461–477. doi: 10.1021/np068054v

Okoro CK, Brown R, Jones AL, et al (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 95:121–133. doi: 10.1007/s10482-008-9295-2

Pan H-QQ, Cheng J, Zhang D-FF, et al (2015) *Streptomyces bohaiensis* sp. nov., a novel actinomycete isolated from *Scomberomorus niphonius* in the Bohai Sea. J Antibiot (Tokyo) 68:246–252. doi: 10.1038/ja.2014.137

Park HB, Lee JK, Lee KR, Kwon HC (2014) Angumycinones A and B, two new angucyclic quinones from *Streptomyces* sp. KMC004 isolated from acidic mine drainage. Tetrahedron Lett 55:63–66. doi: 10.1016/j.tetlet.2013.10.112

Pruesse E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. doi: 10.1093/bioinformatics/bts252

Rateb ME, Houssen WE, Arnold M, et al (2011) Chaxamycins A - D, bioactive ansamycins from a hyper-arid desert streptomyces sp. J Nat Prod 74:1491–1499. doi: 10.1021/np200320u

RIEDLINGER J, Reicke A, Zähner H, et al (2004) Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine *Verrucosispora* strain AB-18-032. J Antibiot (Tokyo) 57:271–279. doi: 10.7164/antibiotics.57.271

Risacher FF, Alonso H, Salazar C (2003) The origin of brines and salts in Chilean salars: A hydrochemical review. Earth-Science Rev 63:249–293. doi: 10.1016/S0012-8252(03)00037-0

Sabry SA, Ghanem NB, Abu-Ella GA, et al (2004) *Nocardiopsis aegyptia* sp. nov., isolated from marine sediment. Int J Syst Evol Microbiol 54:453–456. doi: 10.1099/ijs.0.02814-0

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. doi: 10.1093/oxfordjournals.molbev.a040454

Santhanam R, Okoro CK, Rong X, et al (2012a) *Streptomyces deserti* sp. nov., isolated from hyper-arid Atacama Desert soil. Antonie Van Leeuwenhoek 101:575–581. doi: 10.1007/s10482-011-9672-0

Santhanam R, Okoro CK, Rong X, et al (2012b) *Streptomyces atacamensis* sp. nov., isolated from an extreme hyper-arid soil of the Atacama Desert, Chile. Int J Syst Evol Microbiol 62:2680–2684. doi: 10.1099/ijs.0.038463-0

Santhanam R, Rong X, Huang Y, et al (2013) *Streptomyces bullii* sp. nov., isolated from a hyper-arid Atacama Desert soil. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 103:367–373. doi: 10.1007/s10482-012-9816-x

Schippers A, Bosecker K, Willscher S, et al (2002) *Nocardiopsis metallicus* sp. nov., a metalleaching actinomycete isolated from an alkaline slag dump. Int J Syst Evol Microbiol 52:2291–2295. doi: 10.1099/ijs.0.02279-0

Schulz D, Beese P, Ohlendorf B, et al (2011) Abenquines A-D: aminoquinone derivatives produced by *Streptomyces* sp. strain DB634. J Antibiot (Tokyo) 64:763–8. doi: 10.1038/ja.2011.87

Sun H, Lapidus A, Nolan M, et al (2010) Complete genome sequence of *Nocardiopsis dassonvillei* type strain (IMRU 509). Stand Genomic Sci 3:325–336. doi: 10.4056/sigs.1363462

Takarada H, Sekine M, Kosugi H, et al (2008) Complete genome sequence of the soil actinomycete *Kocuria rhizophila*. J Bacteriol 190:4139–4146. doi: 10.1128/JB.01853-07

Tamura K, Stecher G, Peterson D, et al (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi: 10.1093/molbev/mst197

Thawai C, Tanasupawat S, Kudo T (2008) *Micromonospora pattaloongensis* sp. nov., isolated from a Thai mangrove forest. Int J Syst Evol Microbiol 58:1516–1521. doi: 10.1099/ijs.0.65410-0

Thawai C, Tanasupawat S, Suwanborirux K, et al (2007) *Micromonospora narathiwatensis* sp . nov ., from Thai peat swamp forest soils. J Gen Appl Microbiol 293:287–293. doi: 10.2323/jgam.53.287

Thibessard A, Haas D, Gerbaud C, et al (2015) Complete genome sequence of *Streptomyces ambofaciens* ATCC 23877, the spiramycin producer. J Biotechnol 214:117–118. doi: 10.1016/j.jbiotec.2015.09.020

Udwary DW, Zeigler L, Asolkar RN, et al (2007) Genome sequencing reveals complex secondary metabolome in the marine actinomycete *Salinispora tropica*. Proc Natl Acad Sci 104:10376–10381. doi: 10.1073/pnas.0700962104

Ueda K, Tagami Y, Kamihara Y, et al (2008) Isolation of bacteria whose growth is dependent on high levels of CO_2 and implications of their potential diversity. Appl Environ Microbiol 74:4535–4538. doi: 10.1128/AEM.00491-08

Urzì C, Salamone P, Schumann P, et al (2004) *Blastococcus saxobsidens* sp. nov., and emended descriptions of the genus Blastococcus Ahrens and Moll 1970 and *Blastococcus aggregatus* Ahrens and Moll 1970. Int J Syst Evol Microbiol 54:253–259. doi: 10.1099/ijs.0.02745-0

Vera-Cabrera L, Ortiz-Lopez R, Elizondo-Gonzalez R, et al (2012) Complete Genome Sequence of *Nocardia brasiliensis* HUJEG-1. J Bacteriol 194:2761–2762. doi: 10.1128/JB.00210-12

Vickers JC, Williams ST, Ross GW (1984) A taxonomic approach to selective isolation of *Streptomycetes* from soil. Biol Biochem Biomed Asp Actinomycetes 553–561. doi: 10.1016/B978-0-12-528620-6.50045-8

Waksman S, Henrici A (1948) Family II. *Actinomycetaceae* Buchanan and family *Streptomycetaceae* Waksman and Henrici. Bergey's Man Determ Bacteriol 892–980.

Wayne LG (1988) International Committee on Systematic Bacteriology: Announcement of the report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Syst. Appl. Microbiol. 10:99–100.

Weber T, Blin K, Duddela S, et al (2015) AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. doi: 10.1093/nar/gkv437

Xie CL, Liu Q, Xia JM, et al (2017) Anti-allergic compounds from the deep-sea-derived actinomycete *Nesterenkonia flava* MCCC 1K00610. Mar Drugs 15:1–8. doi: 10.3390/md15030071

Xie QY, Wang C, Wang R, et al (2011) *Jishengella endophytica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. Int J Syst Evol Microbiol 61:1153–1159. doi: 10.1099/ijs.0.025288-0

Yang R, Zhang L-P, Guo L-G, et al (2008) *Nocardiopsis valliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. Int J Syst Evol Microbiol 58:1542–1546. doi: 10.1099/ijs.0.65090-0

Yarza P, Yilmaz P, Pruesse E, et al (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635–645. doi: 10.1038/nrmicro3330

Zhang L, Xi L, Ruan J, Huang Y (2012) *Micromonospora yangpuensis* sp. nov., isolated from a sponge. Int J Syst Evol Microbiol 62:272–278. doi: 10.1099/ijs.0.029439-0

Zhang X, Zhang L-P, Yang R, et al (2008) *Nocardiopsis ganjiahuensis* sp. nov., isolated from a soil from Ganjiahu, China. Int J Syst Evol Microbiol 58:195–199. doi: 10.1099/ijs.0.65145-0

Zhao LX, Huang SX, Tang SK, et al (2011) Actinopolysporins A-C and tubercidin as a pdcd4 stabilizer from the halophilic actinomycete *Actinopolyspora erythraea* YIM 90600. J Nat Prod 74:1990–1995. doi: 10.1021/np200603g

Zvyagintsev DG, Zenova GM, Oborotov G V. (2008) Mycelial bacteria of saline soils. Eurasian Soil Sci 41:1107–1114. doi: 10.1134/S106422930810013X

Chapter III: *Superstesspora tarapacensis* gen. nov., sp. nov., a new member of the *Micromonosporaceae* family from the hypersaline Salar de Llamará, Chile

Alvaro S. Villalobos[1], Jutta Wiese[1], Johannes F. Imhoff[1] ,Tanja Rahn[1], and Cristina Dorador[2]

[1]Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

[2]Laboratorio de Complejidad Microbiana y Ecología Funcional and Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos Universidad de Antofagasta, Antofagasta, Chile

Correspondence: Johannes F. Imhoff jimhoff@geomar.de

Keywords: *Superstesspora tarapacensis*; Actinobacteria; Atacama; polyphasic taxonomy

Running title: *Superstesspora tarapacensis* gen. nov., sp. nov.

To be submitted to International Journal of Systematic and Evolutionary Microbiology

Abstract

A new member of the *Micromonosporaceae* family was isolated from microbial mats of the hypersaline Salar de Llamará, Chile. Optimal growth was observed at 30 - 35 °C, pH 7 - 8 and 1 - 2.5 % sea salt. The 16S rRNA gene sequence revealed 98.2 - 98.9% sequence similarity with different genera of the *Micromonosporaceae*. The new isolate, strain Llam7^T, is related to *Salinispora pacifica* DSM 45820^T (98.5%) and *Jishengella endophytica* DSM 45430^T (98.6%). The cell wall of Llam7^T contains *meso*-diaminopimelic acid andLL-2,6 diaminopimelic acid, while major whole-cell sugars are glucose, mannose, xylose, and ribose. The major menaquinones are MK-9(H_4) and MK-9(H_6). As polar lipids phosphatidylglycerol, phosphatidylethonolamine, diphosphatidylglycerol and several unidentified lipids: 2 glycolipids, 1 aminolipid, 3 phospholipids, 1 aminoglycolipid, and 1 phosphoglycolipid were detected. The major fatty acids were C17:1ω8c, C15:0iso, C16:0iso, and C17:0anteiso. Based on morphological, physiological, molecular and phylogenetic data, strain Llam7^T is considered as type strain of a new species and a new genus of the family *Micromonosporaceae* for which the name *Superstesspora tarapacensisis* proposed. The type strain is Llam7^T and has a G + C content of the DNA of 71 mol%.

Introduction

The family *Micromonosporaceae* was initially proposed by by Krasil'nikov [1] with *Micromonospora* as type genus. Based on 16S rRNA gene sequences and its taxon-specific signature nucleotides the family was since repeatedly emended by Goodfellow et al.[2], Koch et al. [3], Stackebrandt et al. [4] and Zhi et al. [5]. At the time of writing, 31 genera with validly published names have been reported within this family.

In the course our research of Actinobacteria diversity from hypersaline lakes in the Atacama Desert, strain Llam7^T was isolated from a microbial mat sample collected at Salar de Llamará (Atacama Desert, Chile, S 21° 16.087' W 069° 37.094'). The sample was diluted in Ringer 1/4 buffer (0.12 g $CaCl_2$ x $2H_2O$, 0.11 g KCl, 0.05 g $NaHCO_3$, and 2.25 g of NaCl in 1 L aq. dest.) to 10^{-1}, 10^{-2} and 10^{-3}, heated in a water bath at 56°C for 10 minutes. Then 100 µL of each dilution was spread on starch-yeast-extract-peptone (SYP) medium containing in 1 L aq. dest. 2 g starch, 1 g yeast extract, and 0.5 g soy peptone supplemented with 25 µg/mL nalidixic acid, 50 µg/mL cycloheximide, 20 g Tropic Marine Salt, 12 g gellan gum, and 2 g of $CaCl_2$ x $2H_2O$ in 1 L. For genomic DNA preparation, Llam7^T cells were grown in 100 mL of SYP medium for 2 weeks at 26 °C. DNA was extracted using DNeasy®Blood&Tissue Kit (Qiagen). The quantity and quality of the extracted DNA was evaluated by 0.8 % (w/v) agarose gel electrophoresis.

Phenotypic and chemotaxonomic characterisation

Cells obtained from a liquid culture of Llam7^T in SYP under shaking conditions for 5 days at 26 °C and 120 rpm were used for Gram-stain, applying Color Gram 2 (bioMérieux Deutschland GmbH, Nürtingen, Germany) according to the manufacturer. Motility was studied by light microscopy. The cell morphology was examined using light and scanning electron microscopy. For scanning electron microscopy strain Llam7^T was grown for 21 days in SYP agar plates at 26 °C. Three colonies were cut from the agar plate, dehydrated by an ascending ethanol series (50 %, 70 %, 90 % and three times 100 % for 10 min each) [6], and critical-point dried with carbon dioxide and sputter-coated with Au/Pb. Finally, the samples were examined with scanning electron microscope.

Colonies on solid SYP medium grown for 14 d at 28 °C were elevated orange, hard, wrinkled, and dry. Old colonies grown for more than 6 weeks could penetrate into the agar, while young ones (1-2 week) could be easily removed from the agar surface. The diameter of the colonies was 2 - 4 mm. Cells obtained from these colonies were Gram-positive filaments ranging from 8 to 25 µm in length and from 0.2 0.3 µm in diameter. Cells cultured in SYP agar plates as studied with SEM were long filaments from 10 to 30 µm in length and 0.2 to 0.3 µm in width. In addition, spherical spores were formed with 0.7 to 1.4 µm in diameter (Fig. 1).

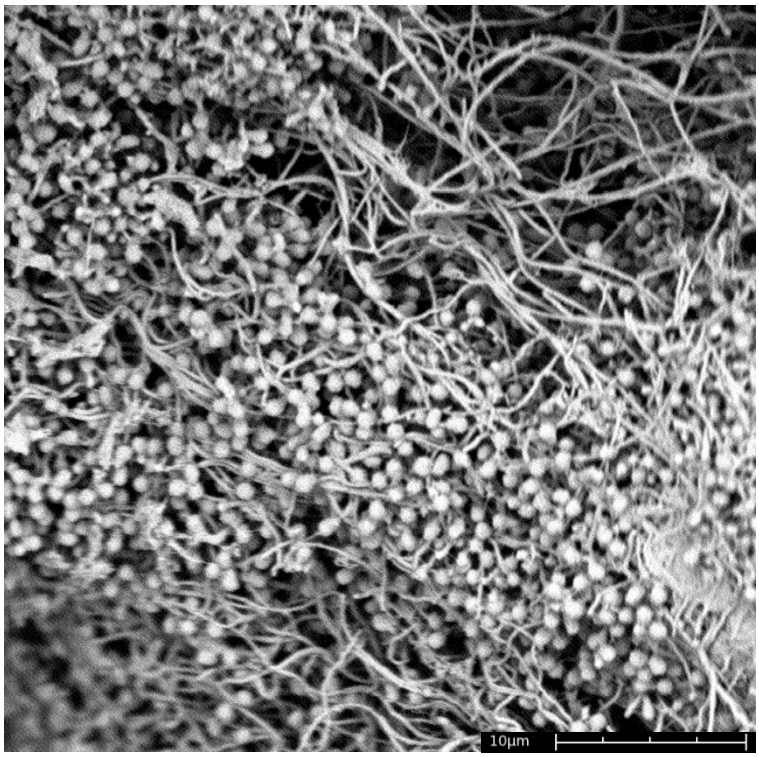


Fig. 1. Scanning electron micrograph of strain Llam7^T after cultivation grown for 21 days in SYP agar plates at 26 °C.

Growth responses to temperature and pH variation were tested on SYP medium for 30 d. The temperature for growth ranges from 15 - 35 °C with an optimum of 30 - 35 °C, similar to *Salinispora arenicola, S. pacificica and S. tropica*, while *Jishengella endophytica* could grow up to 45 °C. The pH range for growth of the strain Llam7^T was from pH 6 - pH 12 with an optimum at 7-8. Salt-depended growth was investigated after incubation at 26 °C for 30 d on SYP agar medium, supplemented with NaCl and Tropic Marine Salt® (Wartenberg, Germany), respectively, at concentrations of 0%, 1%, 2.5%, 5%, 7.5%, and 10%. Strain Llam7^T grew in a range from 0% to 5% of NaCl and Tropic Marine Salt as well with an optimum at 1% NaCl and at 1%-2.5% Tropic Marine Salt. While all representants of *Salinispora tropica, S. pacifica and S.*

arenicola did not grow in the absence of NaCl (0%) or Tropic Marine Salt, weak growth of Llam7^T was observed after long periods of incubation (up to 30 days), a characteristic which is shared with the *Jishengella endophytica*.

Physiological characteristics of Llam7^T including enzymatic activities were tested using API 20E (bioMérieux), including the oxidase assay, and API ZYM (bioMérieux) according to the manufacturer's instructions. Carbon source utilisation test were prepared using minimal medium supplemented independently with starch, trehalose, mannitol, glucose, and n-acetylglucosamin. All tests were run in duplicates. The metabolic characteristics of Llam7^T in comparison with *S. arenicola, S. pacifica, S. tropica* and *J. endophytica* are shown in Table 1. In the present study, all strains analysed showed positive activity in the tests for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, Voges–Proskauer test (production of acetoin), and gelatinase, but were negative in activities of α-galactosidase, β-glucoronidase, α-glucosidase, α-fucosidase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilisation, sulfide production, tryptophane deaminase, and indole production. The strain Llam7^T grew using starch and trehalose.

Table 1. Physiological characteristics of strain Llam7^T compared to the type strains of the genera *Jishengella* and *Salinispora*. Strain: 1, *Superstesspora tarapacensis* gen. nov., sp. nov. Llam7^T; 2, *Jishengella endophytica* DSM 45430^T; 3, *Salinispora pacifica* DSM 45820^T; 4, *Salinispora tropica* DSM 44818^T; 5, *Salinispora arenicola* DSM 44819^T

	1	2	3	4	5
Salinity					
NaCl (range)	0-5%	0-2.5%	2.5%	1-2.5%	2.5-5%
Tropic Marine Salt (range)	0-5%	0-5%	1-5%	1-5%	1-5%
pH (range)	6-12	6-13	6-13	4-12	4-13
Temperature (range)	15-35 °C	15-45 °C	20-35 °C	15-35 °C	20-35 °C
Trypsin	+	+	-	-	-
α-chymotrypsin	-	+	-	-	-
β-galactosidase	-	+	-	-	-
β-glucosidase	-	+	+	+	+
n-acetyl-β-glucosaminidase	+	-	+	+	-
α-mannosidase	+	-	+	-	-
Urease	-	+	-	-	-

The DNA base composition (G + C content) of strain Llam7^T was determined by genome sequencing using MiSeq® Reagent Kit v3 (600 cycles). The raw data were filtered using Trimmomatic [7] and then assembled using SPAdes [8]. The annotation was prepared using Prokka and barnarp [9], to obtain the complete 16S rRNA gene. The profile of the cellular fatty acids was studied using GC-analysis according to the Microbial Identification System (MIDI Inc. Del., USA) [10]. The determination of the respiratory quinones and polar lipids followed the procedure as described by Tindall [11, 12] and Tindall et al. [13], the identification of diamino acids was done according to Rhuland et al. [14], The whole-cell sugars were identified according to Staneck and Roberts [15], experiments carried out by the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany).

The DNA G + C content of strain Llam7^T is 71.0%. The major respiratory quinones were MK-9(H_6) with 52% and MK-9(H_4) with 42%. The diamino acids present in the peptidoglycan are *m*-DAP and LL-DAP. The whole cells of Llam7^T contained glucose, mannose, xylose, and ribose as major sugars. As polar lipids diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), together with unidentified lipids: 1 aminolipid (AL), 2 glycolipids (GL), 1 aminoglycolipid (GNL), 1 phosphoglycolipid (PGL) and 3 phospholipids (PL) were detected. Components of the fatty acid profile are shown in Table 2. The dominant fatty acids present in Llam7^T were C17:1ω8c (17.2%), C15:0iso (13.6%), C16:0iso (12.6%), and C17:0anteiso (11.5%) which attributed to approximately 59.5% of the total fatty acids.

Table 2 Cellular fatty acid contents (%) of strain Llam7^T and type strains of *Jishengella endophytica* DSM 45430^T, *Salinispora pacifica* DSM 45820^T, *Salinispora tropica* DSM 44818^T, *Salinispora arenicola* DSM 44819^T. Strains: 1, *Superstesspora tarapacensis* gen. nov., sp. nov. Llam7^T; 2, *Jishengella endophytica* DSM 45430^T; 3, *Salinispora pacifica* DSM 45820^T; 4, *Salinispora tropica* DSM 44818^T; 5, *Salinispora arenicola* DSM 44819^T. -, negative; tr, trace, < 0.5 %.

Fatty acid	**1**	**2**	**3**	**4**	**5**
Saturated fatty acids					
C13:0	-	-	0.6	tr	tr
C14:0	tr	tr	1.3	tr	tr
C15:0	1.3	5.0	9.8	2.2	2.7
C16:0	2.2	3.0	3.6	0.8	0.6
C16:0 10-methyl	-	1.3	-	-	-
C17:0	6.6	7.5	9.6	2.7	1.6
C17:0 10-methyl	1.1	9.0	2.4	11.2	10.1
C18:0	2.1	1.0	1.3	1.5	0.8
C19:0 10-methyl	-	-	-	tr	-
C19:0	tr	tr	0.5	tr	-
Branched-chain fatty acids					
C14:0iso	0.5	1.2	1.0	1.74	1.4
C15:1iso G	1.5	-	-	-	-
C15:1anteiso A	tr	-	-	-	-
C15:0iso	13.6	12.4	9.6	3.1	3.7
C15:0anteiso	7.8	1.9	3.1	1.3	1.5
C16:1iso H	-	tr	0.6	6.5	3.0
C16:1iso G	1.9	-	-	-	-
C16:0iso	12.6	21.4	13.6	47.5	51.1
C17:1anteiso A	2.5	-	-	-	-
C17:0iso	4.6	0.9	1.9	1.0	1.3
C17:0anteiso	11.5	1.5	2.6	1.4	2.1
C18:1iso H	-	-	-	-	tr
C18:1iso II	-	-	-	tr	-
C18:0iso	tr	1.0	-	tr	tr

Monounsaturated fatty acids					
C15:1ω8c	-	tr	-	-	-
C15:1ω6c	tr	tr	tr	0.5	0.5
C17:1isoω9c	2.6	-	1.7	1.9	2.6
C17:1anteisoω9c	-	-	-	tr	tr
C17:1ω8c	18.2	25.8	25.5	9.5	8.9
C17:1ω5c	tr	-	-	-	-
C18:1ω9c	6.1	3.5	7.5	2.4	3.8
C18:1ω7c	-	-	-	tr	tr
Others					
Summed feature 3*	2.5	1.4	0.8	tr	tr
Summed Feature 6†	tr	0.7	1.9	0.9	1.4
TBSA 10MeC18:0	tr	1	0.6	1.9	1.1

*,† Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C15:iso 2-OH and/or C16:1ω7c, while summed feature 6 consisted of C19:1ω9c and/or C19:1ω11c

Phylogeny

The 16S rRNA gene sequence similarities between strain Llam7^T and related type strains were obtained from EzBiocloud e-server (www.ezbiocloud.net; [16]). The closest relatives (31 strains) and one representative of each of the genera from the *Micromonosporaceae* family, with *Streptomyces albus* subsp. *albus* DSM 40313^T as outgroup were aligned using SINA aligner (v1.2.11) [17]. Phylogenetic trees were constructed using neighbour-joining [18] and maximum-likelihood methods [19], bootstrap method with 1000 repeats with MEGA software version 6.0 [20]. NJplot was used to draw the phylogenetic trees expressed in the Newick phylogenetic tree format [21].

The complete 16S rRNA gene sequence (1517 bp) of strain Llam7^T has high similarity values with four genera of the *Micromonosporaceae* family (98.2 - 98.9%), showing the highest 16S rRNA gene sequence similarity with *Jishengella endophytica* DSM 45430^T (98.9%), *Micromonospora olivasterospora* DSM 43868^T (98.8%), *Xiangella phaseoli* DSM 45730^T (98.54%), *Salinispora pacifica* DSM 45820^T (98.5%), and *Verrucosispora sonchi* DSM 101530^T

(98.2%). However, a neighbour-joining and maximum-likelihood phylogenetic tree, indicated that strain Llam7^T formed a distinct phylogenetic line, next to *Salinispora* species, but not to *J. endophytica* DSM 45430^T. This relationship was supported by a bootstrap value of 50% in both trees (Fig. 2).

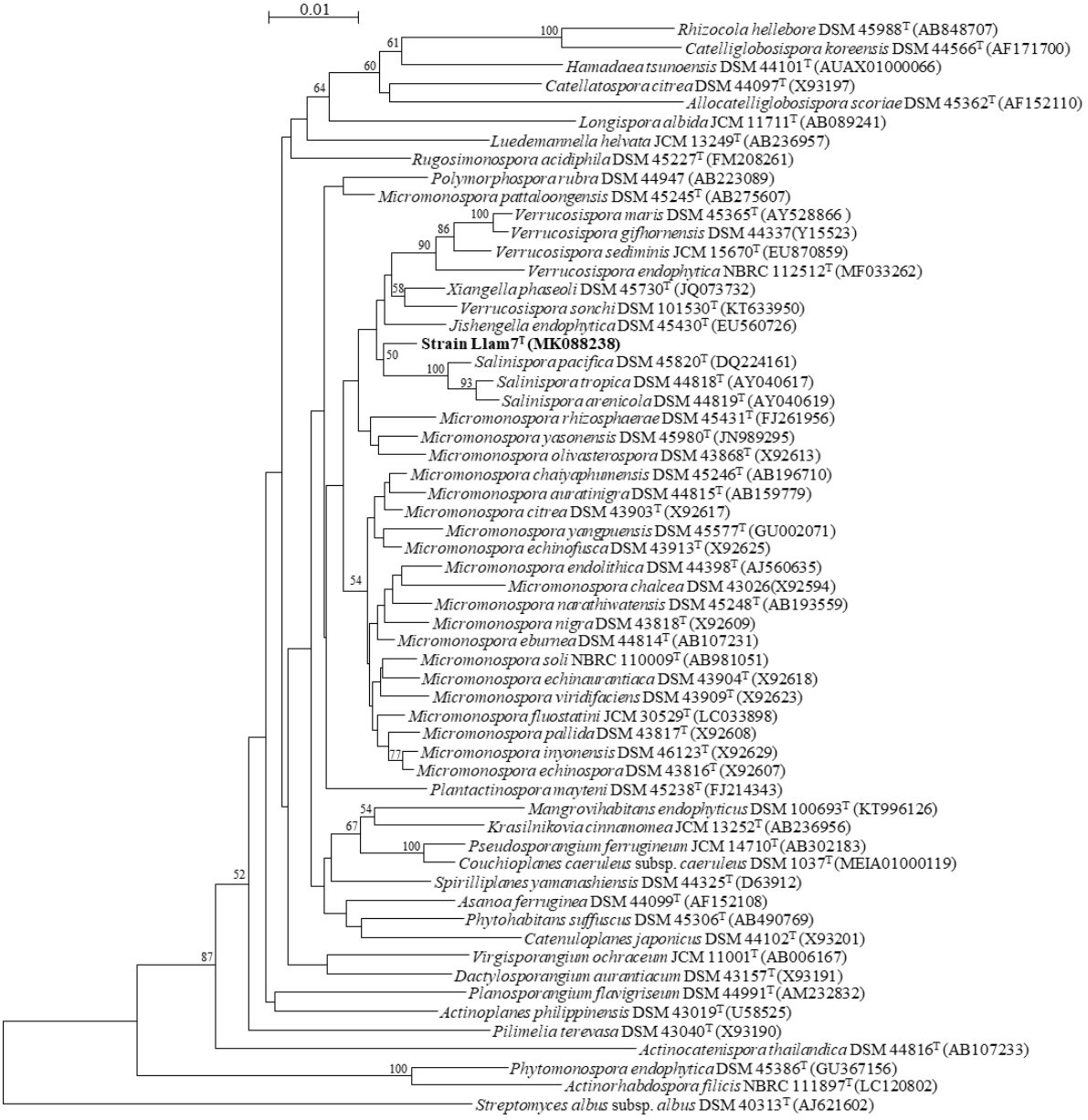


Fig. 2. Neighbour-joining tree of the *Micromonosporaceae* family based on 16S rRNA gene sequences showing the relationship of strain Llam7^T to the type species of the genera of the family. *Streptomyces albus* subsp. *albus* DSM 40313^T was used as outgroup. Bootstrap values (expressed as percentages of 1,000 replications) are given at the branches. Bar indicates evolutionary distance of 0.01.

Proposal of *Superstesspora* gen. sp. nov.

Within the family *Micromonosporaceae*, Llam7^T can be clearly distinguished from all members by contains m-DAP and LL-DAP as diamino acids in the peptidoglycan. In addition, Llam7^T differentiates from its closest *Salinispora* and *Jishengella* relatives by the presence of MK-9($H_{4.6}$) and its whole sugar content of glucose, mannose, xylose and ribose. Furthermore, Llam7^T showed the presence of a number of unidentified polar lipids not reported in members of the genera *Jishengella* and *Salinispora*, i.e. 1 aminolipid (AL), 2 glycolipid (GL), 1 aminoglycolipid (GNL), 1 phosphoglycolipid (PGL) and 3 phospholipid (PL). Even though the fatty acid composition of Llam7^T, *J. endophytica*, *S. arenicola*, *S. pacifica* and *S. tropica* is similar, specific for strain Llam7^T is the higher content of C17:0anteiso and the presence of C16:iso G and C17:1anteiso A (Table 1). Morphologically, Llam7^T can be distinguished by its cell diameter (0.2-0.3 µm) from *Jishengella endophytica* (0.6 µm). Physiological characteristics of Llam7^T regarding the salinity requirements for growing make it different to *Salinispora* members, which require it for growth.

According to the morphological, physiological (Table 1), phylogenetic (Fig. 2) and chemotaxonomic properties (Table 3), strain Llam7^T can be distinguished from all other species of *Micromonosporaceae* and is considered as a new species and a new genus for which the name *Superstesspora tarapacensis* sp. nov. and gen. nov. is proposed.

Table 3. Morphological features and chemotaxonomic characteristics of strain Llam7^T (*Salarispora* gen. nov) and validated genera of the *Micromonosporaceae* family.

Genera: 1, *Superstesspora* (This study);2, *Salinispora* [22]; 3, *Jishengella* [23]; 4, *Verrucosispora* [24]; 5, *Micromonospora*; 6, *Xiangella* [25]; 7, *Actinocatenispora* [26]; 8, *Actinoplanes* [27]; 9, *Actinorhabdospora* [28]; 10, *Allocatelliglobosispora* [29]; 11, *Asanoa* [30]; 12, *Catellatospora* [31]; 13, *Catelliglobosispora* [31]; 14, *Catenuloplanes* [32]; 15, *Couchioplanes* [33]; 16, *Dactylosporangium* [34]; 17, *Hamadaea* [35]; 18, *Krasilnikovia* [36]; 19, *Longispora* [37]; 20, *Luedemannella* [38]; 21, *Mangrovihabitans* [39]; 22, *Phytohabitans* [40]; 23, *Phytomonospora* [41]; 24, *Pilimelia* [42]; 25, *Planosporangium* [43]; 26, *Plantactinospora* [44]; 27, *Polymorphospora* [45]; 28, *Pseudosporangium* [46]; 29, *Rhizocola* [47]; 30, *Rugosimonospora* [48]; 31, *Spirilliplanes* [49]; 32, *Virgisporangium* [50].

Genus	**Single spore**	**Sporangia**	**Spore motility**	**Diamino acid(s)**	**Whole-cell sugars**	**Fatty-acid type**	**Major MK(s)**	**Phospho-lipid type**
1	+	–	–	*m*-DAP and LL-DAP	Glu, Man, Xyl, Rib	3a	9($H_{4,6}$)	II
2	+	–	–	*m*-DAP	Ara, Gal, Xyl	3a	9(H_4)	II
3	+	–	–	*m*-DAP	Xyl, Man, Ara, Rib, Glc	3a	9($H_{4,6,8}$)	II
4	+	–	–	*m*-DAP	Man, Xyl, Rib	2d	9(H_4)	II

5	+	–	–	*m*-DAP	Ara, Xyl	3b	10($H_{4,6}$), 9($H_{4,6}$)	II
6	–	–	–	*m*-DAP	Man, Glc, Gal	3a	9($H_{4,6}$)	III
7	–	–	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	3b	9($H_{4,6}$)	II
8	–	+	+	*m*-DAP	Ara, Xyl	2d	9(H_4), 10(H_4)	II
9	–	–	–	*m*-DAP	Gal, Glu, Man, Rib	3b	10($H_{4,6}$)	II
10	–	–	–	3-OH-DAP	Glc, Rha, Rib, Xyl, Ara, Gal, Man	3b	10($H_{4,6}$), 9(H_4)	II
11	–	–	–	*m*-DAP	Ara, Rha, Rib, Xyl, Gal, Man, Glc	2d	10($H_{6,8}$)	II

12	–	–	–	*m*- and 3-OH-DAP	Ara, Xyl, Gal	3b	9($H_{4,6}$), 10(H_4)	II
13	–	–	–	*m*-DAP	Rha, Rib, Gal, Xyl, Man, Glc	3b	10(H_4)	II
14	–	–	+	l-Lys	Xyl	2c	9(H_8), 10(H_8)	III
15	–	Pseudo-sporangia	+	l-Lys	Ara, Gal, Xyl	2c	9(H_4)	II
16	–	+	+	*m*-DAP	Ara, Xyl	3b	9($H_{4,6,8}$)	II
17	–	–	–	*m*- and 3-OH-DAP	Xyl, Gal, Man, Rib, Ara, Rha	3b	9(H_6)	II
18	–	Pseudo-sporangia	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	2d	9($H_{6,4,8}$)	II

19	–	–	–	*m*-DAP	Ara, Gal, Xyl	2d	10($H_{4,6}$)	II
20	–	+	–	*m*-DAP	Gal, Glc, Man, Rha, Rib, Xyl, Ara	2d	9($H_{6,4}$)	II
21	+	–	–	*m*-DAP	Gal, Glc, Man, Ara, Xyl, Rib	2d	9($H_{6,8}$)	II
22	–	–	–	*m*-DAP, l-Lys	Gal, Glc, Man, Rib, Xyl	2d	9(H_6), 10($H_{4,6}$)	II
23	+	–	–	*m*-DAP	Gal, Glc, Rib, Man	2d	8(H_2), 9(H_2), 10($H_{2,4,6}$)	III
24	–	+	+	*m*-DAP	Ara, Xyl	2d	9($H_{2,4}$)	II
25	–	+	+	*m*-DAP	Ara, Xyl	3b	9(H_4), 10(H_4)	II

26	+	–	–	*m*-DAP	Ara, Xyl, Gal, Glc	2d	10($H_{6,8,4}$)	II
27	–	–	–	*m*-DAP	Xyl	2a	9($H_{4,6}$), 10($H_{4,6}$)	II
28	–	Pseudo-sporangia	–	*m*- and 3-OH-DAP	Ara, Gal, Glc, Man, Xyl, Rib	2d	9(H_6)	II
29	–	–	–	3,4-OH-DAP	Gal, Xyl, Man, Rib	2d	9($H_{4,6}$)	II
30	+	–	–	3-OH-DAP	Ara, Gal, Xyl	2c	9($H_{8,6}$)	II
31	–	–	+	*m*-DAP	Man, Glc, Xyl, Gal	2d	10(H_4)	II
32	–	+	+	3-OH-DAP	Gal, Glc, Man, Rha, Xyl	2d	10($H_{4,6,8}$)	II

Description of *Superstesspora* gen. nov.

Superstesspora [Su.per´stes L. noun *Superstes*, survivor; spo´ra a seed, in bacteriology a spore; Su.per´stes.spo´ra N. L. fem. n. a spore-forming bacterium surviving harsh conditions].

Bacteria of this genus are aerobic, Gram-positive, cells forming long filaments, and produce single non-motile spherical spores. The peptidoglycan contains meso-diaminopimelic acid and LL-2,6 diaminopimelic acid. Glucose, mannose, xylose, and ribose are major whole-cell sugars. The major menaquinones are MK-9(H_4) and MK-9(H_6). Polar lipids present are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethonolamine as well as a number of unidentified Lipids (2 glycolipids, 3 phospholipids and 1 aminolipid, 1 aminoglycolipid, and 1 phosphoglycolipid). The major fatty acids are C17:1 ω8c, C15:0 iso, C16:0 iso, and C17:0 anteiso. The DNA G+C content is 71.0 mol%. The type species of the genus is *Superstesspora tarapacensis*.

Description of *Superstesspora tarapacensis* sp. nov.

Superstesspora tarapacensis [ta.ra.pa.cen'sis. N.L. fem. adj. pertaining to Tarapacá, a region in Chile where Salar de Llamará is located].

Cells grown in liquid medium were long filaments from 10 to 30 µm in length and 0.2 to 0.3 µm in width. Single non-motile spherical spores with a diameter between 0.7 to 1.4 µm were formed. Growth optima are at 30 - 35 °C and at pH 7-8. Growth range is from 15 - 35 °C and from pH 6 - pH 12 and from 0 to 5% of NaCl and Tropic Marine Salt with growth optima at 1% and 1 to 2.5% respectively.

Cells grow aerobically, using starch and trehalose, and produce trypsin, n-acetyl-β-glucosaminidase, α-mannosidase, alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, gelatinase, and acetoin. The peptidoglycan contains *meso*-diaminopimelic acid and LL-2,6 diaminopimelic acid, while the whole-cell sugar present are glucose, mannose, xylose, and ribose. The polar lipids are phosphatidylglycerol, phosphatidylethonolamine, diphosphatidylglycerol and unidentified glycolipids (2), aminolipid (1), phospholipids (2),

aminoglycolipid (1), and phosphoglycolipid (1). The predominant menaquinone is MK-9($H_{4,6}$). Major cellular fatty acids are C17:1 w8c, C15:0 iso, C16:0 iso, and C17:0 anteiso respectively.

The type strain Llam7^T was isolated from the hypersaline Salar de Llamará, Chile. The G + C content of the DNA of the type strain is 71.0 mol%.

Protologue

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the type strain Llam7^T is MK088238.

Author Statements

Funding information. There was no external funding source.

Acknowledgements. Alvaro S. Villalobos thanks the ISOS supervisors Ute Hentschel Humeida (GEOMAR, Kiel, Germany) and Frank Sönnichsen (University Kiel, Germany) for fruitful discussions. He also is grateful for support by a doctoral fellowship from Becas Chile (CONICYT) and the Deutscher Akademischer Austausch Dienst (DAAD).

Conflicts of interest. The authors declare that there is no conflict of interest.

Abbreviations

SYP, Starch-yeast extract-soy peptone medium; DSMZ, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen and Zellkulturen; BCCM, Belgium Co-ordinated Collection of Microorganisms.

References

1. **Krasil'nikov NA**. Ray Fungi and Related Organisms-Actinomycetales. *Moscow Akad Nauk SSSR (in Russ)* 1938;272.
2. **Goodfellow M, Stanton LJ, Simpson KE, Minnikin DE**. Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* 1990;136:19–36.
3. **Koch C, Kroppenstedt RM, Rainey FA, Stackebrandt E**. 16S ribosomal DNA analysis of the genera *Micromonospora, Actinoplanes, Catellatospora, Catenuloplanes, Couchioplanes, Dactylosporangium,* and *Pilimelia* and emendation of the family *Micromonosporaceae*. *Int J Syst Bacteriol* 1996;46:765–768.
4. **Stackebrandt E, Rainey FA, Ward-Rainey NL**. Proposal for a new hierarchic classification system, Actinobacteria classis nov. *Int J Syst Bacteriol* 1997;47:479–491.
5. **Zhi XY, Li WJ, Stackebrandt E**. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* 2009;59:589–608.
6. **Boyde A, Wood C**. Preparation of animal tissues for surface-scanning electron microscopy. *J Microsc* 1969;90:221–249.
7. **Bolger AM, Lohse M, Usadel B**. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
8. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al.*** SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
9. **Seemann T**. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
10. **Sasser M**. Identification of bacteria by gas chromatography of cellular fatty acids. 1990. Epub ahead of print 1990. DOI: 10.1017/S0022215114003314.
11. **Tindall BJ**. A Comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
12. **Tindall BJ**. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
13. **Tindall BJ, Sikorski J, Smibert RA, Krieg NR**. Phenotypic characterization and the principles of comparative systematics: Chapter 15. *Methods Gen Mol Microbiol Third Ed*. Epub ahead of print 2007. DOI: 10.1128/9781555817497.

14. **Rhuland LE, Work E, Denmanb RF, Hoare DS**. The Behavior of the Isomers of α,∈-Diaminopimelic Acid on Paper Chromatograms. *J Am Chem Soc* 1955;77:4844–4846.

15. **Staneck JL, Roberts GD**. Simplified Approach to Identification of Aerobic Actinomycetes by Thin-Layer Chromatography. *Appl Microbiol*. Epub ahead of print 1974. DOI: 10.1016/0011-684X(82)90109-5.

16. **Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, *et al.*** Introducing EzTaxon-e: A prokaryotic 16s rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.

17. **Pruesse E, Peplies J, Glöckner FO**. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.

18. **Saitou N, Nei M**. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.

19. **Felsenstein J**. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J Mol Evol* 1981;17:368–376.

20. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S**. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.

21. **Perrière G, Gouy M**. WWW-Query: An on-line retrieval system for biological sequence banks. *Biochimie* 1996;78:364–369.

22. **Maldonado LA, Fenical W, Jensen PR, Kauffman CA, Mincer TJ, *et al.*** *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 2005;55:1759–1766.

23. **Xie QY, Wang C, Wang R, Qu Z, Lin HP, *et al.*** *Jishengella endophytica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 2011;61:1153–1159.

24. **Rheims H, Schumann P, Rohde M, Stackebrandt E**. *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol* 1998;48:1119–1127.

25. **Wang X, Jia F, Liu C, Zhao J, Wang L, *et al.*** *Xiangella phaseoli* gen. nov., sp. nov., a member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 2013;63:2138–45.

26. **Seo SH, Lee SD**. *Actinocatenispora rupis* sp. nov., isolated from cliff soil, and emended description of the genus *Actinocatenispora*. *Int J Syst Evol Microbiol* 2009;59:3078–3082.

27. **Stackebrandt E, Kroppenstedt RM**. Union of the genera *Actinoplanes* couch, *Ampullariella* couch, and *Amorphosporangium* couch in a redefined genus *Actinoplanes*. *Syst Appl Microbiol* 1987;9:110–114.

28. **Mingma R, Tanaka K, Ōmura S, Takahashi Y, Matsumoto A**. *Actinorhabdospora filicis* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 2016;66:3071–3077.

29. **Lee DW, Lee SD**. *Allocatelliglobosispora scoria*e gen. nov., sp. nov., isolated from volcanic ash. *Int J Syst Evol Microbiol* 2011;61:264–270.

30. **Xu XX, Qu Z, Wang H, Lin HP, Wang C, *et al.*** *Asanoa hainanensis* sp. nov., isolated from rhizosphere soil of *Acrostichum speciosum* in a mangrove, and emended description of the genus *Asanoa*. *Int J Syst Evol Microbiol* 2011;61:2384–2388.

31. **Ara I, Bakir MA, Kudo T**. Transfer of *Catellatospora koreensis* Lee et al. 2000 as *Catelliglobosispora koreensis* gen. nov., comb. nov. and *Catellatospora tsunoense* Asano et al. 1989 as *Hamadaea tsunoensis* gen. nov., comb. nov., and emended description of the genus *Catellatospora* As. *Int J Syst Evol Microbiol* 2008;58:1950–1960.

32. **Kudo T, Nakajima Y, Suzuki K**. *Catenuloplanes crispus* (Petrolini et al. 1993) comb. nov.: incorporation of the genus *Planopolyspora* Petrolini 1993 into the genus *Catenuloplanes* Yokota et al. 1993 with an amended description of the genus *Catenuloplanes*. *Int J Syst Bacteriol* 1999;49:1853–1860.

33. **Tamura T, Nakagaito Y, Nishii T, Hasegawa T, Stackebrandt E, *et al.*** A new genus of the order Actinomycetales, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* 1994;44:193–203.

34. **Thiemann JE, Pagani H, Beretta G**. A new genus of the *Actinoplanaceae*: *Dactylosporangium*, gen. nov. *Arch Mikrobiol* 1967;58:42–52.

35. **Chu X, Li S, Chen W, Asem MD, Duan YQ, *et al.*** *Hamadaea flava* sp. nov., isolated from a soil sample and emended description of the genus *Hamadaea*. *Int J Syst Evol Microbiol* 2016;66:1818–1822.

36. **Ara I, Kudo T**. *Krasilnikovia* gen. nov., a new member of the family *Micromonosporaceae* and description of *Krasilnikovia cinnamonea* sp. nov. *Actinomycetologica* 2007;21:1–10.

37. **Shiratori-Takano H, Yamada K, Beppu T, Ueda K**. *Longispora fulva* sp. nov., isolated from a forest soil, and emended description of the genus *Longispora*. *Int J Syst Evol Microbiol* 2011;61:804–809.

38. **Ara I, Kudo T**. *Luedemannella* gen. nov., a new member of the family *Micromonosporaceae* and description of *Luedemannella helvata* sp. nov. and *Luedemannella flava* sp. nov. *J Gen Appl Microbiol* 2007;53:39–51.

39. **Liu S-W, Tuo L, Li X-J, Li F-N, Li J, *et al.*** *Mangrovihabitans endophyticus* gen. nov., sp. nov., a new member of the family *Micromonosporaceae* isolated from *Bruguiera sexangula*. *Int J Syst Evol Microbiol* 2017;67:1629–1636.

40. **Inahashi Y, Matsumoto A, Omura S, Takahashi Y**. *Phytohabitans flavus* sp. nov., *Phytohabitans rumicis* sp. nov. and *Phytohabitans houttuyneae* sp. nov., isolated from plant roots, and emended description of the genus *Phytohabitans*. *Int J Syst Evol Microbiol* 2012;62:2717–23.

41. **Li J, Zhao GZ, Zhu WY, Huang HY, Xu LH, *et al.*** *Phytomonospora endophytica* gen. nov., sp. nov., isolated from the roots of *Artemisia annua* l. *Int J Syst Evol Microbiol* 2011;61:2967–2973.

42. **Kane WD**. A new genus of *Actinoplanaceae, Pilimelia*, with a description of two species, *Pilimelia terevasa* and *Pilimelia anulata*. *J. Elisha Mitchell Sci. Soc.* 1966;82:220–230.

43. **Wiese J, Jiang Y, Tang SK, Thiel V, Schmaljohann R, *et al.*** A new member of the family *Micromonosporaceae*, *Planosporangium flavigriseum* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 2008;58:1324–1331.

44. **Zhu WY, Zhao LX, Zhao GZ, Duan XW, Qin S, *et al.*** *Plantactinospora endophytica* sp. nov., an actinomycete isolated from *Camptotheca acuminata* Decne., reclassification of *Actinaurispora siamensis* as *Plantactinospora siamensis* comb. nov. and emended descriptions of the genus *Plantactinospora* and *Plantactino*. *Int J Syst Evol Microbiol* 2012;62:2435–2442.

45. **Tamura T, Hatano K, Suzuki K-II**. A new genus of the family *Micromonosporaceae*., *Polymorphospora* gen. nov., with description of *Polymorphospora rubra* sp. nov. *Int J Syst Evol Microbiol* 2006;56:1959–1964.

46. **Ara I, Matsumoto A, Bakir MA, Kudo T, Omura S, *et al.*** *Pseudosporangium ferrugineum* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol* 2008;58:1644–1652.

47. **Matsumoto A, Kawaguchi Y, Nakashima T, Iwatsuki M, Omura S, *et al.*** *Rhizocola hellebori* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* containing 3, 4-dihydroxydiaminopimelic acid in the cell-wall peptidoglycan. *Int J Syst Evol Microbiol* 2014;64:2706–2711.

48. **Monciardini P, Cavaletti L, Ranghetti A, Schumann P, Rohde M, *et al.*** Novel members of the family *Micromonosporaceae*, *Rugosimonospora acidiphila* gen. nov., sp. nov. and *Rugosimonospora africana* sp. nov. *Int J Syst Evol Microbiol* 2009;59:2752–2758.

49. **Tamura T, Hayakawa M, Hatano K**. A new genus of the order Actinomycetales, *Spirilliplanes* gen. nov., with description of *Spirilliplanes yamanashiensis* sp. nov. *Int J Syst Bacteriol* 1997;47:97–102.

50. **Otoguro M, Ishida Y, Tamura T, Yamamura H, Suzuki K, *et al.*** *Virgisporangium aliadipatigenens* sp . nov ., Isolated from Soil in Iriomote Island and Emended Description of the Genus *Virgisporangium*. *Society* 2010;24:39–44.

Chapter IV: *Subtercola vilae* sp. nov., a new Actinobacterium from an extremely high-altitude cold volcano lake in Chile

Alvaro S. Villalobos[a], Jutta Wiese[a], Pablo Aguilar[b, c], Cristina Dorador[b, d], Johannes F. Imhoff[a,*]

[a] Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

[b] Laboratorio de Complejidad Microbiana y Ecología Funcional and Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile

[c] Lake and Glacier Ecology Research Group, Institute of Ecology, University of Innsbruck, Techniker Str. 25, 6020 Innsbruck, Austria

[d] Centre for Biotechnology and Bioengineering (CeBiB), Universidad de Antofagasta, Antofagasta, Chile.

The GenBank/EMBL/DBBJ accession number for the 16S rRNA of strain DB165^T (= DSM 105013^T = JCM 32044^T) is MF276890.

* Corresponding author at

Marine Microbiology, GEOMAR Helmholtz Center for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Tel.: 0049-431-600 4450

Fax: 0049-431-600 4482

E-mail addresses: avillalobos@geomar.de (A.S. Villalobos), jwiese@geomar.de (J. Wiese), pablo.aguilar-espinosa@uibk.ac.at (P. Aguilar), cristina.dorador@uantof.cl (C. Dorador), jimhoff@geomar.de (J.F. Imhoff).

Published in Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2018;111(6):955–63

Abstract

A new Actinobacterium, strain DB165^{T}, was isolated from cold waters of Llullaillaco Volcano Lake (6170 m asl) in Chile. Phylogenetic analysis based on 16S rRNA gene identifies strain DB165^{T} as belonging to the genus *Subtercola* in the family Microbacteriaceae, sharing 97.4% of sequence similarity with *Subtercola frigoramans* DSM 13057^{T}, 96.7% with *Subtercola lobariae* DSM 103962^{T}, and 96.1% with *Subtercola boreus* DSM 13056^{T}. The cells were observed to be Gram-positive, form rods with irregular morphology, and grow best at 10-15 °C, pH 7 and in the absence of NaCl. The cross-linkage between the amino acids in its peptidoglycan is type B2γ, 2,4-diaminobutyric acid is the diagnostic diamino acid, the major respiratory quinones are MK-9 and MK-10, the polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, 5 glycolipids, 2 phospholipids, and 5 additional polar lipids. The fatty acid composition of DB165^{T} (5%>) contains i-14:0, i-16:0, a-15:0, a-17:0, and the dimethylacetal i-16:0 DMA. The genomic DNA G+C content of the strain DB165^{T} is 65 mol%. Based on the phylogenetic, phenotypic, and chemotaxonomic analyses presented in this study, the strain DB165^{T} (= DSM 105013^{T} = JCM 32044^{T}) represents a new species in the genus *Subtercola*, for which the name *Subtercola vilae* sp. nov. is proposed.

Keywords: Cold environments; Llullaillaco volcano; *Microbacteriaceae*; New species; *Subtercola vilae*

Introduction

Members of the family *Microbacteriaceae* are widely distributed in terrestrial and aquatic environments or associated with macroorganisms (Evtushenko 2012). Some representatives, including species of the genus *Subtercola* were found in cold environments such as glacial ice (Christner et al. 2007), boreal groundwater (Männistö et al. 2000), and Antarctic sediments (Li et al. 2010). At present the genus *Subtercola* comprised three validly described species, *Subtercola boreus, Subtercola frigoramans, and Subtercola lobariae,* the first two were isolated from Finnish groundwater (Männistö et al. 2000) and the third one from the lichen *Lobaria retigera* (Si et al. 2017). Based on the high similarity of 16S rRNA gene sequences (>96%) other isolates from cold habitats, such as Antarctic and Artic waters as well as glaciers were found to be affiliated with *Subtercola* (Singh et al. 2014; Zhang et al. 2013; Peeters et al. 2011).

In this study, we characterise strain DB165^T, isolated from a water sample of Llullaillaco Volcano Lake (6170 m) in Chile, one of the highest-elevation lakes on Earth. According to its distinct properties, strain DB165^T is proposed as the type strain of the new species *Subtercola vilae*.

Materials and methods

Isolation and cell morphology

Strain DB165^T was obtained from a water sample collected at the Llullaillaco Volcano Lake (S24º 42.878', W68º33.310') on 18 January 2013, using R2A medium (DIFCO) supplemented with 18 g agar l^{-1}. Pure cultures were obtained after three successive transfers of single colonies to R2A medium plates. Stock cultures were maintained in SGG medium containing 10 g starch, 10 g glucose, 10 ml glycerol (99.7% v/v), 5 g soy peptone, 2.5 g corn steep solids, 2 g yeast extract, 3 g $CaCO_3$, 1 g NaCl, and 18 g agar in 1 l deionised water (Goodfellow and Fiedler 2010). DB165^T was cryopreserved using CRYOBANK (Mast Diagnostica GmbH, Germany) for long term storage at -80 ºC.

Gram-staining was prepared using the Color Gram 2 kit (BioMérieux, France), following the manufacturer's protocol. Endospore staining was performed using the green malaquite method and light microscopy (Schaeffer and Fulton, 1933). Cell morphology, shape and size were

determined using scanning electron microscopy (SEM) according to Gärtner et al. (2008), after cultivation of trypticase soy medium (Trypticase Soy Broth (Becton, Dickinson and company, France) supplemented with 18 g agar l^{-1}.

Physiological characteristics

Enzyme activities and utilisation of carbon sources for strain DB165^T, *S. boreus* DSM 13057^T, and *S. frigoramans* DSM 13057^T were examined using API ZYM, API 20E, and API 50CH (BioMérieux, France), following manufacturer's recommendations. The effect of sodium chloride (0, 0.1, 0.3, 0.6, 0.9, 1, 2.5, 5, 7.5, and 10% w/v) and pH (2, 3, 4, 5, 6, 7, 8, 9, and 10) on the growth was tested according to Kutzner (1981), using the ISP2 medium containing 4 g yeast extract, 10 g malt extract, 4 g dextrose, and 18 g agar in 1 L of distilled water. The optimal range of temperature was tested at 5 °C, 10 °C, 15 °C, 20 °C, 28 °C, and 30 °C using SGG medium.

Chemotaxonomic analyses

Polar lipids were extracted according to a modified protocol of Bligh and Dyer (1959), and the total lipid material was detected using molybdatophosphoric acid and specific functional groups were detected using spray reagents specific for defined functional groups (Tindal et al. 2007). The lipoquinones were extracted and identified using the two-stage method described by Tindall (1990a, b). After cultivation at 25 °C, fatty acid methyl esters were obtained by saponification, methylation and extraction using minor modifications of the method of Miller (1982) and Kuykendall et al. (1988) The fatty acid methyl esters mixtures were separated using Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711 U.S.A.)

The peptidoglycan was obtained from 4 g wet weight pellet according to the method of Schleifer (1985). The peptidoglycan analyses were performed according to Schumann (2011).

Analyses of polar lipids, respiratory quinones, whole-cell fatty acids, and peptidoglycan analyses were carried out by the Identification Service of the DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

DNA base composition

DNA was extracted using DNeasy Blood & Tissue kit (QIAGEN). The G+C content was calculated from the genome sequence, which was determined with Nextseq 500 (Illumina). The

quality of the sequences was checked and filtrated using Trimmomatic (adapters, >Q30,>1000 bp) (Bolger et al. 2014). The genome was assembled using SPAdes (Kmer=121) (Bankevich et al. 2012).

Phylogenetic analyses

DNA was extracted using DNeasy Blood & Tissue kit (QIAGEN) with modifications. The 16S rRNA gene sequence was amplified by PCR using PureTaq Ready-To-Go PCR beads (GE Healthcare) and sequencing according to Gärtner et al. (2008).

The 16S rRNA gene sequence of strain DB165^T was aligned with sequences of 22 selected type strains of the family *Microbacteriaceae*, including species of *Subtercola*, *Frondihabitans*, and *Agreia*, and in addition *Cellulomonas carbonis* KCTC 19824^T as outgroup using SINA (Pruesse et al. 2012). Phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood algorithms using MEGA version 6.0 (Tamura et al. 2013). The tree topologies were evaluated with bootstrap analyses based on 1000 replicates.

Results

Morphological and physiological characteristics

Colonies of strain DB165T are sticky, golden yellow after growth at the optimal growth temperature of 10 °C to 15 ºC for 6-7 days (also after 7-10 days at 28 ºC), but are pale yellow after growth at 5 ºC for 2-3 weeks. Optimum growth is at 10-15 °C (range from 5-28 °C). No growth occurs at 30 °C. Strain DB165T tolerates only low concentrations (up to 0.9%) of NaCl and grows best in the absence of NaCl. The pH-range is from 5 to 8 with an optimum at pH 7. Cells show no motility and form no spores, they are irregular short rods of 0.5 µm width and 1.0-1.2 µm length, Gram-positive and have an irregular shape as seen under SEM. Some of the cells are thicker at the ends. Occasionally, coccoid cells were observed (Fig. 1). Variable cell shapes were also reported for *S. boreus* and *S. frigoramans* (Männistö et al. 2000).

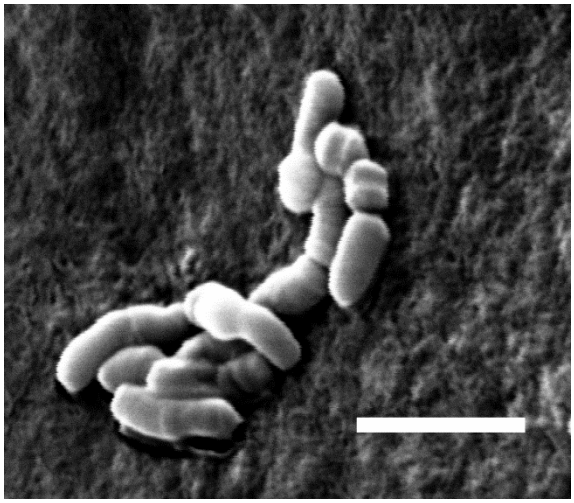


Figure 1. Scanning electron micrograph of *Subtercola vilae* DB165^T grown on trypticase soy medium for 7 d at 28 ºC. Scale bar indicates 2 µm.

The metabolic properties of strain DB165^T, in comparison with the type strains of *S. boreus* and *S. frigoramans* are shown in Table 1. Strain DB165^T metabolised inositol, $_D$-sorbitol, $_D$-sucrose, $_D$-melibiose, glycerol, $_L$-arabinose, $_D$-xylose, methyl-β$_D$-xylopyranoside, $_D$-galactose, $_D$-glucose, $_D$-fructose, $_D$-mannose, $_L$-rhamnose, $_D$-mannitol, amygdalin, arbutin, esculin, salicin, $_D$-cellobiose, $_D$-maltose, $_D$-trehalose, $_D$-melezitose, gentiobiose, and $_D$-turanose.

Table 1. Physiological characteristics of *Subtercola vilae* DB165^T compared to the type strains of *Subtercola frigoramans* and *Subtercola boreus*. In the present study, all *Subtercola* strains showed positive activity in the test for esterase lipase (C8), leucine arylamidase, acid phosphatase, Naphthol-AS-Bl-phosphohydrolase, α-glucosidase, β-glucosidase, β-galactosidase (weak in API ZYM test), $_D$-glucose, $_D$-mannitol, inositol, $_D$-sorbitol, $_L$-rhamnose, $_D$-sucrose, $_D$-melibiose, amygdalin, $_L$-arabinose, glycerol, $_D$-xylose, $_D$-galactose, $_D$-fructose, esculin, $_D$-cellobiose, $_D$-maltose, $_D$-trehalose, and $_D$-turanose.; and negative activity for lipase (C14), α-chymotrypsin, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, sulfide production, urease, gelatinase, erythritol, $_L$-xylose, $_L$-sorbose, dulcitol, methyl-α_D-mannopyranoside, methyl-α_D-glucopyranoside, n-acetylglucosamine, inulin, $_D$-raffinose, starch, glycogen, xylitol, D-lyxose, $_D$-tagatose, $_D$-fucose, $_D$-arabitol, $_L$-arabitol, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate.

	S. vilae DB165^T	*S. frigoramans* DSM 13057^T	*S. boreus* DSM 13056^T
APIZYM			
Alkaline phosphatase	-	(+)	+
Esterase (C 4)	+	(+)	+
Valine arylamidase	(+)	(+)	+
Cysteine arylamidase	(+)	-	+
Trypsin	(+)	-	-
α-galactosidase	(+)	-	-
β-glucoronidase	-	-	+
n-acetyl-β-glucosaminidase	-	(+)	-
α-mannosidase	-	(+)	(+)
API20E			
Arginine dihydrolase	-	(+)	-
Citrate as unique carbon source	-	(+)	(+)
Tryptophane deaminase	+	-	-
Tryptophanase	+	-	-
Voges-Proskauer test	+	-	-

API 50CH			
$_D$-arabinose	-	-	+
$_D$-ribose	-	-	+
methyl-β_D-xylopyranoside	+	-	-
$_D$-mannose	+	-	+
arbutin	+	-	-
salicin	+	-	-
$_D$-lactose (bovine origin)	-	-	+
$_D$-melezitose	+	-	-
gentiobiose	+	-	-

+, positive activity; (+), weak activity; -, no activity.

Chemotaxonomic characteristics

The polar lipids of the strain DB165^T consisted of phosphatidylglycerol, diphosphatidylglycerol, 5 unidentified glycolipids, 2 unidentified phospholipids, and unidentified 5 lipids. The diamino acid in the peptidoglycan was identified as DAB (2,4-diaminobutyric acid). The molar ratio of alanine:glycine:glutamic acid:DAB was 1.1:1.0:0.04:1.7. Instead of glutamic acid high amounts of 3-hydroxyl-glutamic acid were found. Assuming that much of the glutamic acid is replaced by 3-hydroxyl-glutamic acid and despite of this replacement, the amino acid composition is consistent with peptidoglycan type B2γ. The major isoprenoid quinones of strain DB165^T were MK-9 (47%) and MK-10 (39%). Minor amounts of MK-11 (6%) and MK-8 (4%) were also present. The G+C content of the genomic DNA of the strain DB165^T was 65.0 mol%.

Major fatty acids of the strain DB165^T were iso- and anteiso-saturated C-15 and C16 fatty acids with a-15:0 (50%), i-16:0 (17 %), and i-16:0 DMA (17%) as major components (Table 2).

Table 2. Fatty acid profiles of *Subtercola vilae* DB165^T, *Subtercola frigoramans* DSM 13057^T, *Subtercola boreus* DSM 13056^T, and *Subtercola lobariae* DSM 103962^T. Percent of total peak area of ion chromatograms is indicated. *

	S. vilae DB165^T	*S. frigoramans* DSM 13057^T	*S. boreus* DSM 13056^T	*S. lobariae* DSM 103962^T
14:00	tr	-	-	-
14:0 2-OH	-	-	-	10.3
16:00	tr	-	-	tr
i-14:0	5.5	6.7	tr	2.3
i-15:0	tr	tr	4.3	1.4
i-16:0	17.2	10.2	4.2	6.7
i-17:0	-	-	-	-
a-15:0	50.0	46.1	51.6	68.8
a-17:0	6.7	6.8	3.5	4.2
a-15:1	-	-	tr	-
16:0 DMA	tr	-	tr	-
i-15:0	tr	-	1.7	1.5
i-16:0	17.0	13.3	11.9	6.9
a-15:0	3.2	10.3	11.0	9.7
a-17:0	1.9	2.9	4.0	2.6
References	This study	Männistö et al.	Männistö et al.	Si et al. 2017

tr, traces (< 1%); -, not detected; a-, anteiso-branched fatty acid; i-, iso-branched fatty acid; DMA, 1,2 dimethyl acetals.

*Cells for fatty acid and dimethyl acetals were grown at 25 °C, except for *S. lobariae* where DMAs were obtained from cells cultivated at 20 °C.

16S rRNA gene sequence analyses

The 16S rRNA gene sequence (1432 bp) of the strain DB165^{T} (deposited under the accession number MF276890) matches to different *Subtercola* species in a range of 96.1% to 97.4% of similarity, *S. frigoramans* DSM 13057^{T} (97.4% similarity), *S. lobariae* DSM 103962^{T} (96.7% similarity), and *S. boreus* DSM 13056^{T} (96.1% similarity). However, it also showed high similarity to to *Frondihabitans* species, *Frondihabitans peucedani* DSM 22180^{T} (96.8% similarity) and *Frondihabitans australicus* DSM 17894^{T} (96.6% similarity) The phylogenetic analysis (Fig. 2) based on the consensus 16S rRNA gene sequences (1416 bp) showed that strain DB165^{T} forms a cluster with *Subtercola* species and *Agreia* with a strong bootstrap support, while *Frondihabitans* species cluster together, but the separation with the *Subtercola*/*Agreia* cluster is not well supported by bootstraps. In the *Subtercola* clade, strain DB165^{T} is found in a distinct cluster with *S. frigoramans* DSM 13057^{T} with strong bootstrap support.

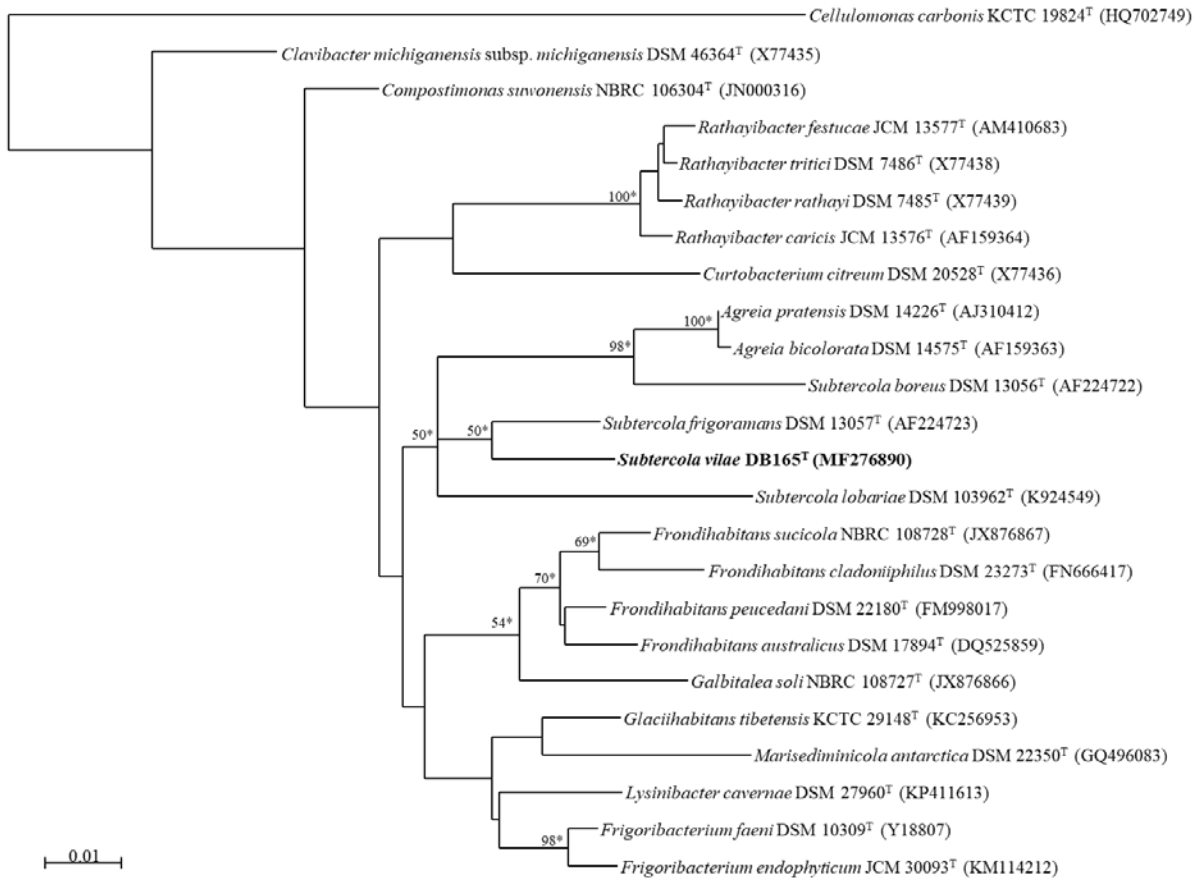


Figure 2. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence comparison of strain DB165^T and species of the family *Microbacteriaceae* with *Cellulomonas carbonis* KCTC 19824^T as outgroup. Numbers at the nodes represent bootstrap support (%) based on the analysis of 1000 bootstrap replications, asterisks indicate branches of the tree that were also recovered using neighbour-joining algorithm. Only bootstrap values ≥ 50% are indicated. Genbank accession numbers are given in parentheses. Bar indicates 0.01 substitutions per site.

Discussion

Subtercola species are characterised by a peptidoglycan type B2γ with DAB as diamino acid, MK-9 and MK-10 as major respiratory quinones, and a similar polar lipids profile (Table 3). Their relative content of fatty acids is quite similar, whereby the compositions of *S. vilae* is most similar to that of *S. frigoramans* (Table 2).

Subtercola vilae strain DB165^T has a peptidoglycan type B2γ in which the glutamic acid is almost completely replaced by 3-hydroxyl-glutamic acid, as is found in all other *Subtercola* species (Männistö et al., 2000, Si et al., 2017). The metabolic characteristics that differentiate strain DB165^T from *S. boreus* and *S. frigoramans* are utilisation of methyl-β_D-xylopyranoside,

arbutin salicin, $_{D}$-melezitose and gentobiose as carbon sources and the enzymatic activities of trypsin, α-galactosidase, tryptophanase deaminase, and tryptophanase as well as the production of acetoin. In contrast to DB165^{T}, *S. frigoramans* exhibited as unique features arginine dihydrolase and n-acetyl-β-glucosaminidase. *S. frigoramans* and *S. boreus* can use citrate as unique carbon source and showed the enzymatic activities of alkaline phosphatase and α-mannosidase. *S. boreus* showed β-glucoronidase enzymatic activity and can use $_{D}$-arabinose, $_{D}$-ribose, and $_{D}$-lactose, carbon sources that strain DB165^{T} and *S. frigoramans* cannot use. The fatty acid profile of strain DB165^{T} is similar to the type strains of other *Subtercola* species. Major differences are in the content of i-16:0, with 17% in strain DB165^{T} compared to 4.2-10.2% in the other *Subtercola* species and a low content of 3% a-15:0 DMA compared to 9.7-11.0% in the other *Subtercola* species (Table 2).

The phylogenetic analysis of the 16S rDNA gene sequences clearly shows the close relationship of strain DB165^{T} to *Subtercola* species rather than to *Agreia* and *Frondihabitans* species. The genus *Agreia* forms a sub-cluster within *S. boreus* and different chemotaxonomic traits have been proposed to distinguish the two genera (Schumann et al. 2003). Though both genera have a cross-linkage between the amino acids in the peptidoglycan of type B2γ, in the case of *Subtercola* species, the cross-linkages have DAB, while *Agreia* species have $_{L}$-DAB connected to $_{D}$-Orn. *Frondihabitans* species can be clearly by the peptidoglycan, which is of the B2β type (Zhang et al., 2007). Fatty acids play an important role in the differentiation of the genera. While the presence 1,2 dimethyl acetals i-16:0 DMA and a-17:0 DMA is observed in all *Subtercola* species, while *Agreia* only contains i-15:0 DMA in low proportions (>4.2%) (Schumann et al. 2003; Behrendt et al. 2002). *Frondihabitans* species have a fatty acid profile totally different from *Subtercola* and *Agreia* species, having 18:1 and 14:0 2-OH as major fatty acids but lacking1,2 dimethyl acetals. The major menaquinones of *Subtercola* species, including the strain DB165^{T}, comprise MK-9 and MK-10, while in *A. bicolorata* DSM 14575^{T} MK-10 and in *A. pratensis* DSM 4246^{T} MK-10 and MK-11 are dominant, and in *Frondihabitans* MK-8 and MK-7. The presence of MK-9 as major component can be used as a marker to differentiate these three genera (Table 2). It should be mentioned that *A. pratensis* which was originally classified as *S. pratensis* contains as major menaquinones MK-10 (51%) and MK-11 (21%), but in addition 13% of MK-9 (Behrendt et al., 2002, Evtushenko et al., 2001). Irrespective of the problematic taxonomic position of *Agreia* species and the similarity of 16S rRNA gene sequences with *Frondihabitans*,

the phylogenetic relationship (Fig. 2) and chemotaxonomic criteria clearly support the classification of strain DB165^T as species of the genus *Subtercola* (Table 3). Based on the phenotypic and genetic analyses presented in this work, strain DB165^T is considered to represent a new species of the genus *Subtercola*, for which the name *Subtercola vilae* sp. nov. is proposed.

Table 3. Diagnostic key characteristics of the genera *Subtercola*, *Agreia*, and *Frondihabitans* in comparison to *Subtercola vilae* DB165^T

	Subtercola vilae DB165^T	*Subtercola*[a]	*Agreia*[b]	*Frondihabitans*[c]
Peptidoglycan type	B2γ	B2γ	B2γ	B2β
Cell wall diamino acid	DAB	DAB	$_L$-DAB $_D$-Orn	$_D$-Orn
Respiratory quinones	MK-9, MK-10	MK-9, MK-10	MK-10 MK-11	MK-7, MK-8, MK-9
Polar lipids	PG, DPG, GL, PL, L	PG, DPG, GL, PL	PG, DPG	PG, DPG, GL, AL, PL
Major Cellular Fatty acids (>10%)	a-15:0, i-16:0	a-15:0, i-16:0	a-15:0, i:16:0, a-17:0	18:1, 14:0 2-OH, a-15:0
Major 1,1-dimethyl acetals (>5%)	i-16:0 DMA	i-16:0 DMA, a-15:0 DMA	ND	ND
G+C content [mol %]	65	64-68	65-67	65-71
Isolation source	Volcano lake at 6170 m asl	Boreal groundwater, lichen	Leaf gall, phyllosphere of grasses	Associated to plants and lichen
References	This work	Männistö et al. 2000, Si et al. 2017	Evtushenko et al 2001, Behrendt et al. 2002	Kim et al. 2014

PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; GL, glycolipids; PL, phospholipids; AL, aminolipid; L, lipids; ND, not detected.

Description of *Subtercola vilae* sp. nov.

Subtercola vilae (vi'lae, of Vila, named in honour to Irma Vila, a Chilean limnologist with outstanding contributions to the microbiology and ecology of lakes in the Chilean Altiplano and Atacama Desert).

Cells are short, irregular rods 0.5 µm wide and 1.0-1.2 µm long. Colonies are golden yellow, circular convex. Growth occurrs chemoheterotrophically under oxic conditions. Optimum growth is at 10-15 °C (range from 5-28 °C), at pH 7 (range from pH 5-8) and in the absence of NaCl. Cells produce esterase C4, esterase lipase C8, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidase, tryptophane deaminase, and tryptophanase and acetoin. Weak activity is observed for valine arylamidase, cysteine arylamidase, trypsin, and α- and β-galactosidase. Carbon sources used under oxic conditions are, inositol, $_{D}$-sorbitol, $_{D}$-sucrose, $_{D}$-melibiose, glycerol, $_{L}$-arabinose, $_{D}$-xylose, methyl-β_{D}-xylopyranoside, $_{D}$-galactose, $_{D}$-glucose, $_{D}$-fructose, $_{D}$-mannose, $_{L}$-rhamnose, $_{D}$-mannitol, amygdalin, arbutin, esculin, salicin, $_{D}$-cellobiose, $_{D}$-maltose, $_{D}$-sucrose, $_{D}$-trehalose, $_{D}$-melezitose, gentiobiose, and $_{D}$-turanose. The cell-wall peptidoglycan is a B2γ type with DAB as the diagnostic amino acid and 3-hydroxyl-glutamic acid instead of glutamic acid.

Major menaquinones are MK-9 and MK-10. Polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, 5 unidentified glycolipids, 2 unidentified phospholipids, and 5 unidentified lipids. The major cellular fatty acids are a-15:0, i-16:0, a-17:0, and i-14:0, while 14:0 and 16:0 are found only in traces. Major dimethylacetals are i-16:0 DMA, a-15:0 DMA and a-17:0 DMA, while 16:0 DMA and i-15:0 DMA are present in trace amounts. The G+C content of the DNA of the type strain is 65.0 mol%.

The type strain DB165^{T} (= DSM 105013^{T} = JCM 32044^{T}) was isolated from Llullaillaco Volcano Lake in Chile.

Acknowledgements

The authors gratefully acknowledge sequence analysis by the IKMB sequencing facility (University of Kiel, Germany), SEM sample preparation by Rolf Schmaljohann (GEOMAR, Kiel, Germany), providing of samples from Llullaillaco volcano by Thomas Bowen and Matt Farson (California State University, USA). Alvaro S. Villalobos thanks the ISOS supervisors Ute Hentschel Humeida (GEOMAR, Kiel, Germany) and Frank Sönnichsen (University Kiel, Germany) for fruitful discussions. He also is grateful for support by a doctoral fellowship from Becas Chile (CONICYT) and the Deutscher Akademischer Austausch Dienst (DAAD).

References

Bankevich A, Nurk S, Antipov D et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455-477

Behrendt U, Ulrich A, Schumann P, Naumann D, Suzuki K (2002) Diversity of grass-associated *Microbacteriaceae* isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of *Subtercola pratensis* sp. nov., *Curtobacterium herbarum* sp. nov. and *Plantibacter flavus* gen. nov., sp. nov. Int J Syst Evol Microbiol 52:1441-1454

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120

Christner BC, Mosley-Thompson E, Thompson LG, Zagorodnov V, Reeve JN (2007) Isolation and identification of bacteria from ancient and modern ice core archives. In: Casassa G, Sepúlveda FV, Sinclair RM (eds) The Patagonian icefields: A unique natural laboratory for environmental and climate change studies. Springer, New York, pp 9-15

Evtushenko LI (2012) Family XI. *Microbacteriaceae*. In: Whitman W, Goodfellow M, Kämpfer P, Busse HJ, Trujillo M, Ludwig W, Suzuki KI, Parte, A. (eds) Bergey's manual of systematic bacteriology, Volume 5: The Actinobacteria, Springer, Berlin, pp 807-994

Evtushenko LI, Dorofeeva LV, Dobrovolskaya TG et al (2001) *Agreia bicolorata* gen. nov., sp. nov., to accommodate actinobacteria isolated from narrow reed grass infected by the nematode *Heteroanguina graminophila*. Int J Syst Evol Microbiol 51:2073-2079

Gärtner A, Wiese J, Imhoff JF (2008) *Amphritea atlantica* gen. nov., sp. nov., a gammaproteobacterium from the Logatchev hydrothermal vent field. Int J Syst Evol Microbiol 58:34-39

Goodfellow M, Fiedler H-P (2010) A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie van Leeuwenhoek 98:119-142

Kim SJ, Lim JM, Ahn JH et al (2014) Description of *Galbitalea soli* gen. nov., sp. nov., and *Frondihabitans sucicola* sp. nov. Int J Syst Evol Microbiol 64:572-578

Kutzner HJ (1981) The family *Streptomycetaceae*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes – a handbook on habitats, isolation and identification of bacteria, Springer, Berlin, pp 2028-2090

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

Li HR, Yu Y, Luo W, Zeng YX (2010) *Marisediminicola antarctica* gen. nov., sp. nov., an actinobacterium isolated from the Antarctic. Int J Syst Evol Microbiol 60:2535-2539

Männistö MK, Schumann P, Rainey FA et al (2000) *Subtercola boreus* gen. nov., sp. nov. and *Subtercola frigoramans* sp. nov., two new psychrophilic actinobacteria isolated from boreal groundwater. Int J Syst Evol Microbiol 50:1731-1739

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

Peeters K, Ertz D, Willems A (2011) Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. Syst Appl Microbiol 34:360-367

Pruesse E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823-1829

Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evo 4:406-425

Schaeffer AB, Fulton MD (1933) A simplified method of staining endospores. Science 77:194

Schleifer KH (1985) Analysis of the chemical composition and primary structure of murein. Methods Microbiol 18:123-156

Schumann P (2011) Peptidoglycan structure. Methods Microbiol 38:101-129

Schumann P, Behrendt U, Ulrich A, Suzuki KI (2003) Reclassification of *Subtercola pratensis* Behrendt et al. 2002 as *Agreia pratensis* comb. nov. Int J Syst Evol Microbiol 53:2041-2044

Si H, Shi F, Zhang L et al (2017) *Subtercola lobariae* sp. nov., an actinobacterium of the family Microbacteriaceae isolated from the lichen *Lobaria retigera*. Int J Syst Evol Microbiol 67:1516-1521

Singh P, Hanada Y, Singh SM, Tsuda S (2014) Antifreeze protein activity in Arctic cryoconite bacteria. FEMS Microbiol Lett 351:14-22

Tamura K, Stecher G, Peterson D et al (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725-2729

Tindall BJ (1990a) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66:199-202

Tindall BJ (1990b) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. Syst Appl Microbiol 13:128-130

Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Chapter 15: Phenotypic characterization and the principles of comparative systematics. In: Reddy C, Beveridge T, Breznak J, Marzluf G, Schmidt T, Snyder L (eds) Methods for general and molecular microbiology, 3rd edn. ASM Press, Washington DC, pp 330-393

Zhang DC, Brouchkov A, Griva G, Schinner F, Margesin R (2013) Isolation and characterization of bacteria from ancient Siberian permafrost sediment. Biology (Basel) 2:85-106

Zhang L, Xu Z, Patel BKC (2007) *Frondicola australicus* gen. nov., sp. nov., isolated from decaying leaf litter from a pine forest. Int J Syst Evol Microbiol 57:1177-1182

Chapter V: Cold-adaptation of *Subtercola vilae* DB165^T an isolate from a high-altitude cold volcano lake as revealed by its genome analysis

Alvaro S. Villalobos [1], Jutta Wiese [1], Johannes F. Imhoff [1,*], Cristina Dorador [2], Alexander Keller [3], Ute Hentschel [1]

[1] Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany; avillalobos@geomar.de (A.S.V); jwiese@geomar.de (J.W); uhentschel@geomar.de (U.H)

[2] Laboratorio de Complejidad Microbiana y Ecología Funcional and Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Universidad de Antofagasta, Antofagasta, Chile; cristina.dorador@uantof.cl

[3] Department of Bioinformatics, Biocenter, University of Würzburg, Am Hubland, Würzburg, Germany; a.keller@biozentrum.uni-wuerzburg.de

* Corresponding author at jimhoff@geomar.de

Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Tel.: 0049-431-600 4450

Fax: 0049-431-600 4482

Submitted to Microorganisms (in revision).

Abstract

Subtercola vilae DB165^T was isolated from Llullaillaco Volcano Lake in Chile (6170 m above sea level), which is characterised as an oligotrophic cold environment and covered by an ice layer. In order to gain insight into its ability to adapt to life in this extreme environment, we analysed the genome with a particular focus on properties related to possible adaptation to cold environments. General properties of the genome are presented, including carbon and energy metabolism as well as secondary metabolite production. The genome properties of *Subtercola vilae* DB165^T and the related species *Subtercola boreus* DSM 13056^T, *Agreia bicolorata* DSM 14575^T, and *Agreia pratensis* DSM 14246^T are compared. The repertoire of genes in the genome of *S. vilae* DB165^T that are linked to adaptation to the harsh conditions found in Llullaillaco Volcano Lake include several mechanisms to transcribe proteins at low temperatures, such as a high number of tRNAs and cold shock proteins. In addition, *S. vilae* DB165^T is capable of producing a number of proteins to cope with oxidative stress, which is of particular relevance in low temperature environments in which reactive oxygen species are more abundant. Furthermore, it gains the capacity to produce cryo-protectants and to control membrane fluidity at low temperatures. To combat against ice crystal formation, two new ice-binding proteins were identified which are unique to *S. vilae* DB165^T. These results indicate that *Subtercola vilae* can employ different strategies to survive the extreme and cold conditions prevalent in Llullaillaco Volcano Lake.

Keywords: Cold adaptation; *Subtercola vilae*; genome analysis; systematic affiliation; Llullaillaco Volcano

Introduction

The genus *Subtercola* was initially described with two species isolated from boreal water in Finland, *Subtercola frigoramans* and *Subtercola boreus* [1]. Now two more species have been described: *Subtercola lobariae*, which was isolated from the lichen *Lobaria retigera* in China [2], and *Subtercola vilae*, isolated from Llullaillaco volcano in Chile [3]. 16S rRNA gene sequences of different isolates assigned to Subtercola genus have been found only in cold environments such as glaciers, cryoconite holes, permafrost soil, digestive tracts of Antarctic krill, cloudy water samples [4], Arctic lichens, and Antarctic soils [5] (Figure 1), suggesting that these species are well adapted to cold environments.

Subtercola vilae DB165^{T} was isolated from Llullaillaco Volcano Lake at 6170 m above sea level (Llullaillaco volcano, Chile) [3]. The environment has been characterised as a cold oligotrophic environment. During different expeditions in summer time, the lake was covered by an ice layer, with the temperature of lower water masses was at 3-4 °C (6.8 m depth). The temperature at the soil surface on a summer day may vary from -10 °C to > 50 °C due to the high radiation present [6,7]. A recent study demonstrated that the microbial communities of Llullaillaco soils are dominated by Actinobacteria, with more than 90% represented by members of the genus *Pseudonocardia* [7]. Bacteria affiliated with the class Actinobacteria are not only abundant in Llullaillaco soils, but also along the Andean mountains [7,8].

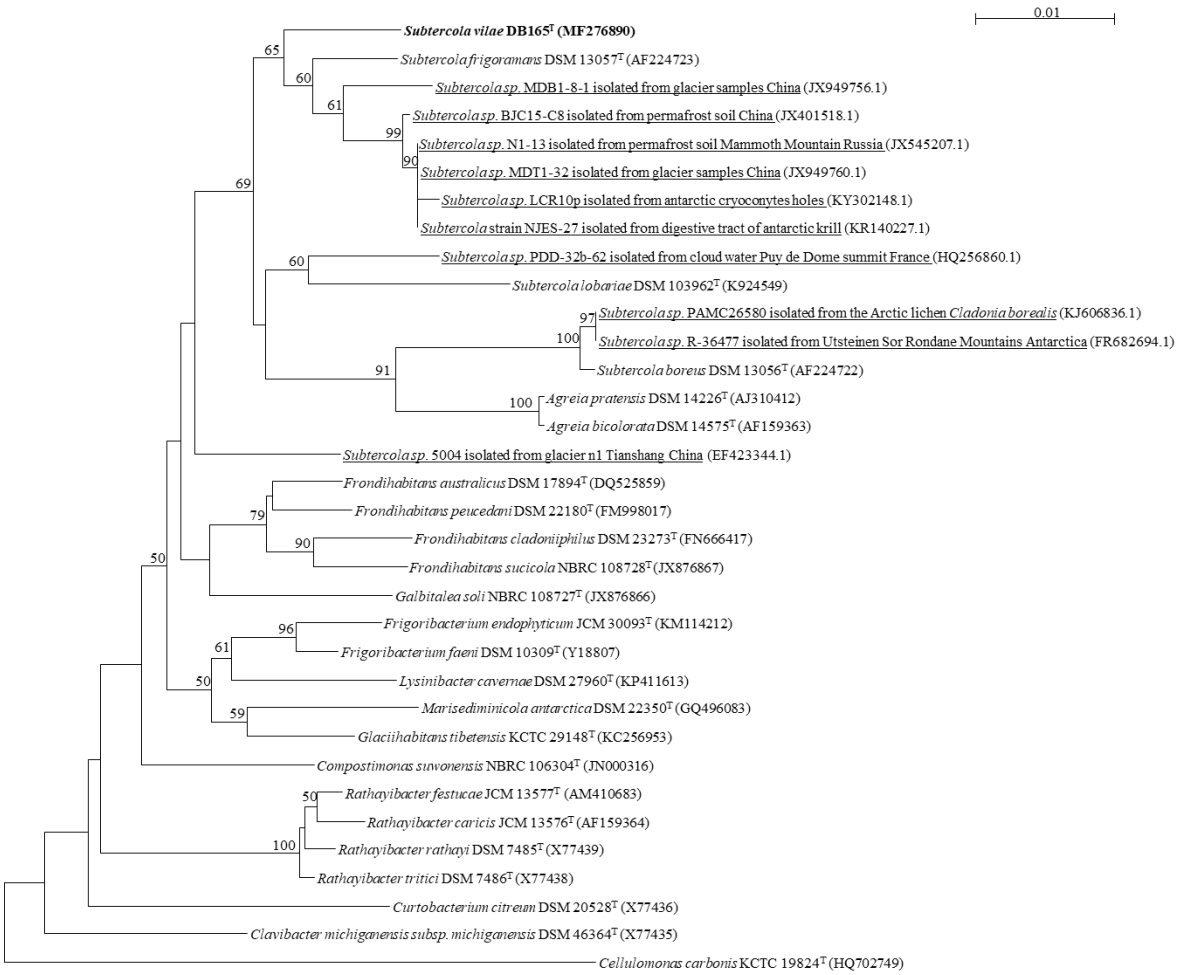


Figure 1. Phylogenetic tree based on the 16S rRNA gene sequences highlighting the position of *Subtercola vilae* DB165^T and other sequences annotated as *Subtercola* strains deposited in NCBI, relative to phylogenetically closely related type strain within the family *Microbacteriaceae*. The sequences were aligned on the SINA online service.

Taking into consideration the environmental distribution of the genus *Subtercola* in cold environments, along with the relevance of Actinobacteria in Llullaillaco volcano, the genome of *Subtercola vilae* DB165^T was sequenced as the first genome of a *Subtercola* species and from a cultured species of Actinobacteria obtained from Llullaillaco volcano.

In order to gain insight into its ability to live in this extreme environment, we here present a classification alongside chemotaxonomic characteristics of *Subtercola vilae* DB165^T, in addition to the genome sequence, assembly, annotation, and in particular properties related to the possible adaptations to cold environments.

Materials and methods

S. vilae DB165^T (DSM 105013^T = JCM 32044^T) cells were grown in SGG medium containing 10 g starch, 10 g glucose x H2O, 10 ml glycerol (99.7% v/v), 5 g soy peptone, 2.5 g corn steep solids, 2 g yeast extract, 3 g CaCO3, 1 g NaCl, and 18 g agar in 1 L deionised water [9] for 5 days at 23 °C. DNA was extracted using DNeasy®Blood&Tissue Kit (Qiagen). The quality and quantity of the extracted DNA was evaluated by 0.8 % (w/v) agarose gel electrophoresis. The genomic DNA library was generated using Nextera XT (Illumina Inc.) according to the manufacturer's instructions. After fragmentation, size-selection was performed using NucleoMag NGS Clean-up and Size Select (Macherey-Nagel) to obtain a library with median insert-size around 400 bp. After PCR enrichment, the library was validated with a high-sensitivity DNA chip and Bioanalyzer 2100 (both Agilent Technologies, Inc.) and additionally quantified using the Qubit dsDNA HS assay (Life Technologies). Four sequencing runs were performed on a NextSeq device using v2 2 × 150 bp chemistry, and the genome was multiplexed together with thirteen other bacterial genomes from other sources. In total, 1,304,036,262 bp raw paired-end sequences were subjected to the Trimmomatic software for adapter and quality trimming (mean Phred quality score ≥ 30) [10] filtering of sequences containing ambiguous bases and a minimum length of 200 bp. The remaining 1,206,508,976 bp were assembled with SPAdes assembler using enabled error pre-correction and k-mer sizes ranging from 15 to 127 (step size of 10) [11]. The assemblies obtained were analysed using QUAST [12], whereas 127-kmers showed the bests quality.

Open reading frames were identified using Prodigal in Prokka and barrnap for rRNA genes [13] An additional gene prediction and functional annotation was performed with the Rapid Annotation using Subsystem Technology webserver [14,15], and for natural product biosynthetic gene clusters antiSMASH 3.0 was used [16]. The genome completeness was analysed with CheckM [17]. A maximum likelihood phylogenetic tree was calculated using 107 essential single-copy genes on bcgTree [18] with 1000 bootstrap. For the genome comparison between *Subtercola vilae* DB165^T, *Subtercola boreus* DSM 13056^T (NZ_NBWZ01000001.1), *Agreia bicolorata* DSM 14575^T [19], and *Agreia pratensis* DSM 14246^T [20], the genomes of *Agreia* type strains were obtained from Genbank and annotated using the same Prokka pipeline used for *S. vilae* DB165^T. Comparison analysed were performed in Anvi'o 5 [21] using Prokka

annotation, Clusters of Orthologous Groups (COGs) functional annotation, Average Nucleotide Identity (ANI) by PyANI [22], and Anvi'o pangenome pipeline using DIAMOND [23].

Putative ice-binding motif in *S. vilae* DB165^{T} open reading frames were identified using blastp against an in-house Actinobacteria anti-freeze protein database, which comprise amino acid sequences of Anti-freeze protein obtained from UniProt [24]. The hits were further analysed by protein homology modeling using Phyre2 server [25] and corroborated according the position of functional threonine residues on the surface of the proteins using Visual Molecular Dynamic (VMD 1.9.3) software [26].

The sequencing project was completed in January 2017 and sequence data were deposited as a Whole Genome Shotgun (WGS) project in Genbank under the Bioproject PRJNA491396 and the accession number QYRT00000000 consisting of 103 contigs ≥1000 bp. The version described on this paper is QYRT00000000. The annotated genome is available in RAST under the ID number 2056433.4.

Results

Genome properties

The draft genome sequence of *S. vilae* DB165^T was assembled into 103 contigs (≥ 1000 bp) containing a total of 4,043,135 bp with an average G+C content of 65.1% (Table 1). From a total of 3879 predicted genes, 3797 (97.8 %) codify for proteins and 2434 (62.7%) were annotated with a putative function. Genes not linked to a function were annotated as hypothetical or unknown function. We annotated a total of eighty-two rRNA genes (2.11%) divided into five rRNA genes (three 5S rRNA, one 16S rRNA, and one 23S rRNA) and fifty-nine tRNA genes. Furthermore, a total of 1416 (36.5%) of the coding sequences were assigned using COGs to twenty-four different classes. Distribution of these genes and their percentage representation are listed in Table 2. Annotation obtained via RAST assigned a total 2089 sequences to twenty-seven subsystem categories. The highest ranking among the subsystem categories are those concerned with metabolism of carbohydrates (20.1 %), amino acids and derivatives (15.4 %), cofactors, vitamins, prosthetic groups, pigments (10.7 %), proteins (9.5 %), as well as fatty acids, lipids, and isoprenoids (5.1 %) (Figure 2). Completeness of the genome was calculated by CheckM, using the lineage marker set for Actinomycetales (UID1593), from which 99.5% of the proteins were present in *S. vilae* DB165^T draft genome.

Table 1. Genome statistics according to Prokka annotation

Attribute	Value	% of total
Genomes Size (bp)	4043135	100
Contigs	103	
DNA G+C content	65.1	
Total of genes	3879	100
Coding sequences	3797	97.8
Genes with function prediction	2434	62.7
Genes assigned to COGs	1416	36.5
RNA genes	82	2.11
rRNA genes	5	0.1
Pseudo genes	0	0
5S rRNA	3	0.07
16S rRNA	1	0.02
23S rRNA	1	0.02
tRNA	59	1.5
Other RNA	18	0.46

Table 2. Number of protein coding genes of S. vilae DB165^T associated with the general COG functional categories

Code	Value	%age	Description
J	163	5.92	Translation, ribosomal structure and biogenesis
A	1	0.04	RNA processing and modification
K	263	9.55	Transcription
L	115	4.18	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	31	1.13	Cell cycle control, Cell division, chromosome partitioning
V	60	2.18	Defence mechanisms
T	94	3.41	Signal transduction mechanisms
M	125	4.54	Cell wall/membrane biogenesis
N	12	0.44	Cell motility
U	17	0.65	Intracellular trafficking and secretion
O	95	3.45	Posttranslational modification, protein turnover, chaperones
C	152	5.52	Energy production and conversion
G	342	12.42	Carbohydrate transport and metabolism
E	242	8.79	Amino acid transport and metabolism
F	91	3.3	Nucleotide transport and metabolism
H	182	6.61	Coenzyme transport and metabolism
I	118	4.28	Lipid transport and metabolism
P	165	5.99	Inorganic ion transport and metabolism
Q	66	2.4	Secondary metabolites biosynthesis, transport and catabolism
R	295	10.71	General function prediction only
S	99	3.59	Function unknown
-	1467	37.52	Not in COGs

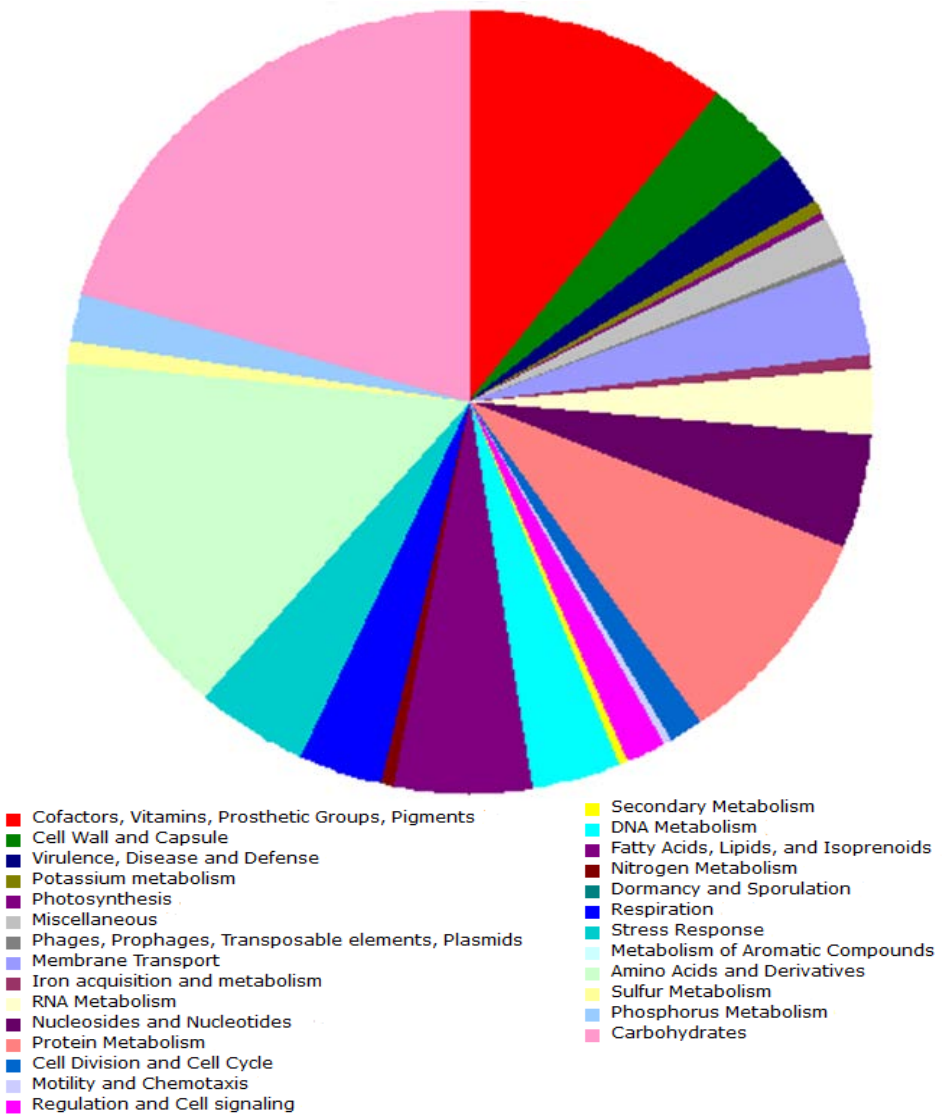


Figure 2. Metabolic subsystems of Subtercola vilae DB165^{T} annotated through the RAST webserver.

Carbon and energy metabolism

The genome of *S. vilae* DB165^{T} reveals its potential to metabolise a wide range of sugars, ranging from monosaccharides such as mannose, D-ribose, xylose, D-gluconate ketogluconates, L-arabinose, D-galacturonate, and D-glucoronate; di- and oligosaccharides such as trehalose, sucrose, fructooligosaccharides (FOS), raffinose, maltose, maltodextrin, lactose, and galactose; the polysaccharides glycogen; sugar alcohols such as glycerol, glycerol-3-phosphate, mannitol, and inositol. *S. vilae* DB165^{T} has the potential to utilise the amino sugar chitin and its monomers to convert them to fructose-6-phospate for the glycolysis pathway.

S. vilae DB165^T has encoded in its genome the enzymes 3-hydroxybutyryl-CoA dehydrogenase (hbd), 3-hydroxybutyryl-CoA epimerase (hbe), 3-ketoacyl-CoA thiolase (atoB), and enoyl-CoA hydratase (eh). These are needed for the production of poly-hydroxy-butyrates (PHB), which serve as an intracellular carbon and energy reserve [27], helpful against desiccation and osmotic stress, and increases UV resistance [28]. The genome of *S. vilae* DB165^T also codifies for polyphosphate (PolyP) formation (exopolyphosphatase, polyphosphate kinase and polyphosphate glucokinase) molecules that serve in phosphate storage and protect against desiccation, UV radiation and temperature stress [29].

Three different rhodopsin genes are encoded in *S. vilae* DB165^T. Two of them (fig|2056433.4.peg.750; fig|2056433.4.peg.1678) showed high identity with genes annotated as rhodopsin in *Subtercola boreus* (WP_116415267.1; WP_116281575.1), while the third one resembles a xanthorhodopsin [30] from *Clavibacter michiganensis* (WP_079533889.1). The presence of rhodopsin in *S. vilae* DB165^T suggests that it might be able to transform energy from sunlight [31].

Secondary metabolite production

The antiSMASH analysis revealed the presence of three different secondary metabolite gene clusters for the biosynthesis of a type 3 polyketide, a terpene, and a cluster that contained core biosynthetic genes for the non-ribosomal peptide synthesis pathway but was not categorised in this compound family.

The detected polyketide type 3 synthetase cluster has a total of 36 genes, where 3 genes showed high identity and were in the same order as the alkylresorcinol synthetic cluster of *Agreia* species, while other annotated genes affiliated with aminotransferase class V amidase, short chain dehydroganases/reductase, aldo/ketoreductase family oxidoreductase, pullulanase type I, and alpha-glucosidase, suggesting modifications in the alkylresorcinol scaffold. The genes found in this cluster showed a 63% resemblance to a polyketide type-3 cluster found in *Agreia* sp. Leaf335 and 55% of genes showed similarity with *A. bicolorata* VKM Ac-1804T. Alkylresorcinol compounds can be easily incorporated into cell membranes, causing considerable changes to their structure and properties [32]. Some also show antibiotic activity [33].

The terpene biosynthetic cluster consists of twenty-four genes with high homology to carotenoid biosynthetic clusters. Genes annotated as core biosynthetic genes had high identity with phytoene

synthase, lycopene beta elongase BC, while additional biosynthetic genes include polyprenyl synthetase, dehydrogenase, and a short-chain dehydrogenase/reductase. The carotenoids in *S. vilae* DB165^T might play an important role as membrane modulators at low temperatures [34], and also as antioxidants [35].

The third uncategorised gene cluster has a total of thirteen genes, in which the core biosynthetic genes are an amino acid adenylation protein similar to the one found in the saframycin A biosynthetic gene cluster, and a zinc metalloprotease. An additional biosynthetic gene 4-aminobutyrate aminotransferase that show identity with the present in saframycin A biosynthetic gene cluster. The gene structure of the cluster seems to be conserved in *Microbacteriaceae* species of the genera *Agreia, Clavibacter*, and *Cellulomonas*.

Cold stress adaptation of *Subtercola vilae* DB165^T

In order to identify adaptation to the harsh conditions of Llullaillaco volcano environment, annotated genes sorted in the subsystem categories of RAST and an ice-binding motif in-house database were used.

Comparative analysis of tRNA species, predicted using barrnap, revealed that *Subtercola* strains isolated from cold waters show a higher number of tRNA genes: *S. vilae* DB165^T encodes fifty-nine and *S. boreus* DSM 13056^T fifty-three, in contrast to *Agreia* species found in nematodes and plants (fifty-one in *A. bicolorata* DSM 14575^T and fifty in *A. pratensis* DSM 14246^T). A higher number and diversity of tRNAs might help to counteract their slow mobilisation to the translation sites [36,37].

Membrane fluidity

An important challenge to life at cold temperatures is the ability to maintain the cell membrane in a liquid-crystalline state. One of the strategies suitable for this purpose involves the production of unsaturated fatty acids. The *S. vilae* DB165^T genome encodes thirty-two proteins involved in the production of fatty acids. We found a total of fourteen copies of FabG gene that codify for 3-oxoacyl-[acyl-carrier-protein] reductase, the enzyme that catalyses the onset of reduction and condensation of fatty acids, as well as the synthesis of branched fatty acids [38,39]. The coding sequence of 3-oxoacyl-acyl-carrier-protein synthase II (FabF/KAS-II) is involved in the elongation of fatty acids and has been reported in the productions of antesio-15:0 in *Listeria*

monocytogenes, helping in its membrane fluidity and survival at low temperatures [40]. Anteiso-15:0 was found as a major fatty acid in *S. vilae* DB165^T [3] as well as in other Subtercola species, *S. frigoramans*, *S. boreus*, and *S. lobariae* [1,2]. The genome also encodes acyl-CoA thioesterase II (TesB), an enzyme that has been proposed as catalysing short to medium length 3-hydroxy acyl chains and PlsC gene for 1-acyl-sn-glycerol-3-phosphate acyltransferase, which catalyses the conversion of intermediates in phospholipid synthesis and 3-ketoacyl-(acyl-carrier-protein) reductases, enhancing the production of polyunsaturated lipids [38,39].

Cryoprotectants

Genes involved in the production and uptake of choline, glycine, and betaine were found in *S. vilae* DB165^T genome. These compounds maintain the membrane fluidity at low temperature and also prevent cold-induced aggregation of cellular proteins [41]. Intracellular proteins can also be protected by the production of the sugar trehalose [42]. S. vilae encodes the complete pathway for the biosynthesis of trehalose and utilisation of trehalose. In addition, *S. vilae* DB165^T contains eleven copies of a trehalose permease transport system (SugB).

Temperature shifts

The fast production of cold-inducible proteins is an important adaptation to low temperatures [43]. In *S. vilae* DB165^T we found three different cold-shock (Csp) proteins with high identity to CspA and CspC. These proteins act as RNA chaperones, destabilising mRNA secondary structures formed at low temperatures, and enhancing translation efficiency [44]. On the other hand, we found eleven heat shock proteins that prevent denaturation of cellular proteins at high and low temperatures [45].

Oxidative stress

Metabolic reactive oxygen species (ROS) generate intracellular damage in proteins, membranes, and DNA. More dissolved oxygen can be found in the water at low temperatures and may increase the potential of possible damage [46]. Psychrophilic microorganisms are adapted to this harsh condition; one of their adaptations against damage caused by ROS is the production of different enzymes involved in detoxification of the superoxide radical (O_2-). *S. vilae* DB165^T encodes several enzymes to fight against the ROS stress. The enzymes found are deferrochelatase/peroxidase (EfeN), thioredoxin/glutathione peroxidase (BtuE),

deferrochelatase/peroxidase (EfeB), putative heme-dependent peroxidase, catalase-peroxidase (katG: one copy), putative non-heme bromoperoxidase (BpoC), catalases (katA), and superoxide dismutases [Mn;Fe; Cu-Zn] (sodABC).

Ice-binding proteins

In order to understand possible mechanisms to cope with ice crystal formation inside the cell, we screened the amino acid sequences of all *S. vilae* DB165^T genes against an in-house ice-binding motif database. We obtained two hits of hypothetical proteins with an ice-binding motif in the *S. vilae* DB165^T genome: Svil_00062, which consists of 389 amino acids, and Svil_00202, with 380 amino acids. Comparison of both proteins with the Genbank database using blastp showed that the proteins share only 50-52% of the amino acid sequence with proteins annotated as hypothetical and DUF3494 domain-containing, a domain related to ice-binding properties (Table 3a). This suggests that the conserved domain may have the function of binding with ice, whereas the other part of the proteins is not similar to anything yet reported in the database. As the ice-binding domain revealed a low identity with DUF3494 domain-containing proteins (40-45%) in the database, the sequences of both proteins were modelled by Phyre2 server. The result showed that the proteins were structurally similar to the antifreeze protein observed in *Colwellia psychrerythraea* 34H, which was used as backbone. The pattern in the β-strands and α-helices of the protein, as well as in the threonine residues, are displayed in parallel and face out the β-strands (Figure 3). As the placement of these residues is considered essential for the ice binding function, it can be concluded that both proteins may have the predicted function [47]. As the other part of the proteins did not show hits with high coverage and identity (Table 3b), the two proteins quite likely represent a new type of ice-binding protein. Interestingly, we could not find either of the two proteins in the genome of the *S. boreus* DSM 13056^T, *A. bicolorata* DSM 14575^T, and *A. pratensis* DSM 14246^T.

Table 3. Identification of CDS that contain ice-binding motifs in *S. vilae* DB165^T genome. a) BLAST results of predicted and best-scored proteins with ice-binding motifs in *S. vilae* DB165^T genome of Genbank. b) BLAST of the two different regions of *S. vilae* DB165^T predicted ice-binding proteins, in which is indicated the low coverage and identity of the first segment of the protein and the last segment of the protein which has the ice-binding motif.

a) Locus tag	Blast hit	Query coverage	Identity	Accession number
Svilae_00062	DUF3494 domain-containing protein [*Streptomyces fradiae*]	50%	40%	WP_050363635.1
1 to 389 aa	DUF3494 domain-containing protein [*Arthrobacter alpinus*]	50%	45%	WP_074712914.1
Svilae_00202	hypothetical protein UT69_C0002G0034 [Candidatus *Yanofskybacteria* bacterium]	52%	43%	KKR37772.1
1 to 380 aa	hypothetical protein A2207_02115 [Candidatus *Yanofskybacteria* bacterium]	52%	43%	OGN35513.1
b) Locus tag	Blast hit	Query coverage	Identity	Accession number
Svilae_00062				
1 to 186 aa	Alpha-tubulin suppressor [*Microbacterium* sp. ru370.1]	39%	39%	SDO93348.1
Svilae_00062				
187 to 389 aa	DUF3494 domain-containing protein [*Streptomyces xinghaiensis*]	97%	41%	WP_039820269.1
Svilae_00202				
1 to 179 aa	hypothetical protein [*Rhodococcus* sp. 06-1477-1B]	39%	45%	WP_094735903.1
Svilae_00202				
180 to 380 aa	hypothetical protein UT69_C0002G0034 [Candidatus *Yanofskybacteria* bacterium]	99%	43%	KKR37772.1

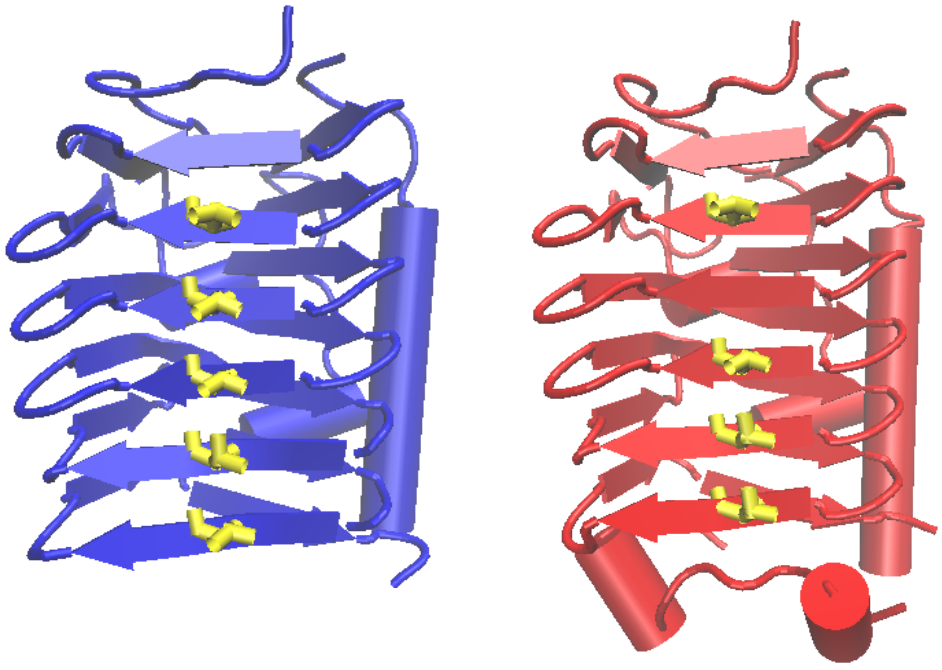


Figure 3. Cartoon representations of ice binding motif models of Svilae_00062 (blue) and Svilae_00202 (red). The putative ice-binding surface with ordered threonine residues (yellow) is shown. Arrows and ribbons represent β-strands and α-helices, respectively.

Genome comparison of *Subtercola vilae* DB165^T, *Subtercola boreus* DSM 13056^T, *Agreia bicolorata* DSM 14575^T, and *Agreia pratensis* DSM 14246^T

The differences between *Subtercola* and *Agreia* are small, causing problems in initial species description followed by species emendation [48]. 16S rRNA gene comparison between *S. vilae* DB165^T and *Agreia* type strains showed similarities of around 96% and a branch separation in their phylogenetic tree [3]. Chemotaxonomic markers such as cell wall diamino acid and 1,1-dimethyl acetals are essential to differentiate strains affiliated with either *Subtercola* or *Agreia* [3,48]. However, genomic evidence of chemotaxonomic markers used for taxa description are scarce [49]. In order to use genomic data to see differences between *Subtercola* and *Agreia* strains, we prepared a maximum-likelihood phylogenetic tree of essential single-copy genes, using different type strains of *Microbacteriaceae* and a pangenome approach to see differences in detail between *S. vilae* DB165^T, *S. boreus* DSM 13056^T, *A. bicolorata* DSM 14575^T, and *A. pratensis* DSM 14246^T. The maximum-likelihood tree generated supports the separation of

Subtercola and *Agreia* species, with a high bootstrap value in each separation node and with a separation distance comparable to type species of other genera (e.g: *Herbiconiux*/ *Cnuibacter*) (Figure 4).

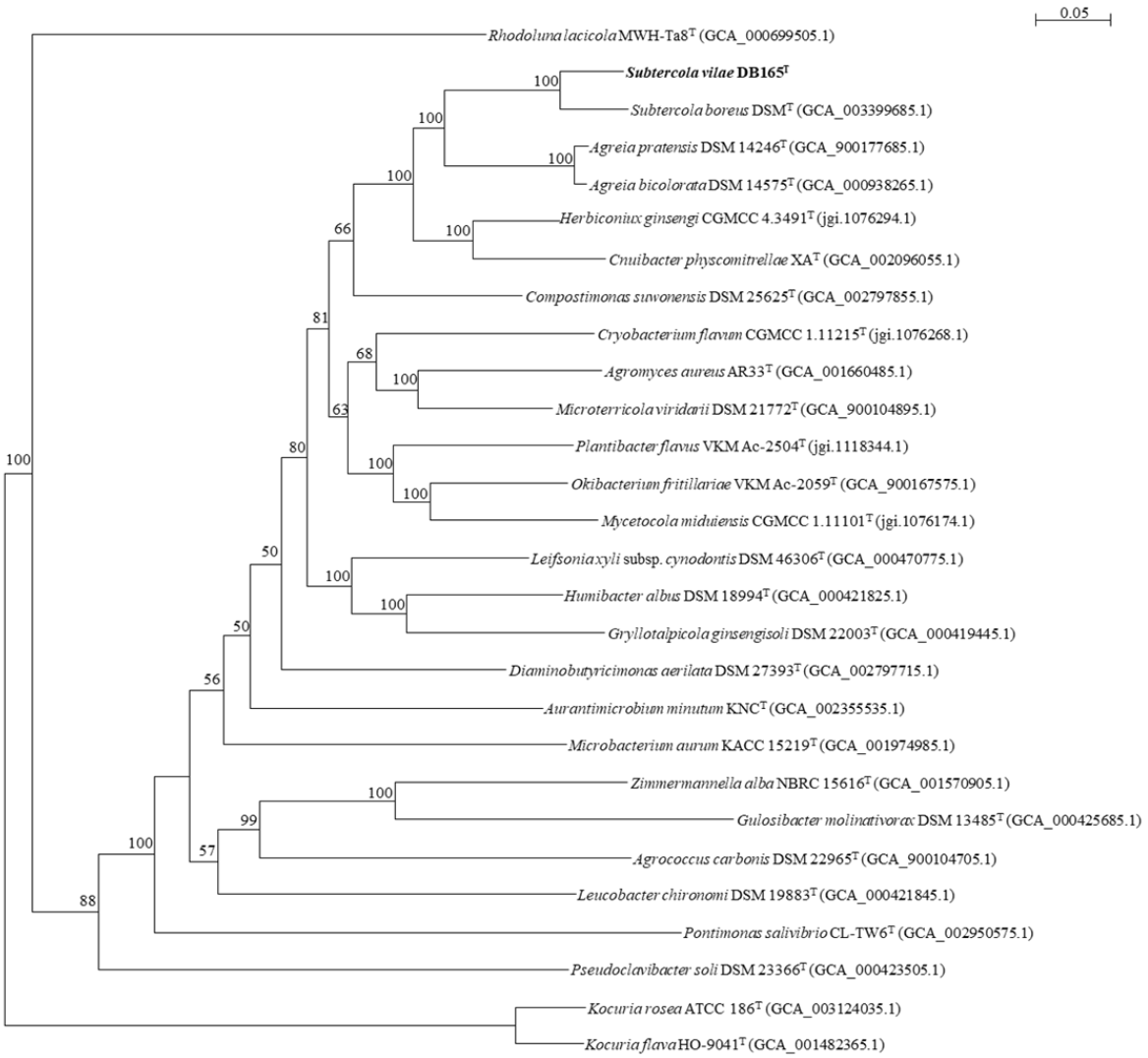


Figure 4. Maximum-likelihood tree based on 107 essential single-copy genes (amino acids) of *Subtercola vilae* DB165^T and *Microbacteriaceae* type strains and two *Kocuria* type strains as outgroup. Project accession is indicated in brackets. Bootstrap values >50% are indicated. Bars indicates 0.05 amino acids substitutions per site.

A detailed analysis using the pangenome approach showed that the average nucleotide identity (ANI) of *S. vilae* DB165^T is 78% against *S. boreus* DSM 13056^T, and 75% against *A. bicolorata* DSM 14575^T and *A. pratensis* DSM 14246^T, while in between the two *Agreia* species it is 87% (Figure 5). A total of 121 of 14781 genes were shared between *S. vilae* DB165^T, *S. boreus* DSM 13056^T, *A. bicolorata* DSM 14575^T, and *A. pratensis* DSM 14246^T. A total of 2568 genes present

in *S. vilae* DB165^{T} are unique, and 2770 are only present in *S. boreus* DSM 13056^{T}; meanwhile, 748 genes are shared between the two strains. *S. vilae* DB165^{T} shares 141 genes with *A. bicolorata* DSM 14575^{T} and 363 with *A. pratensis* DSM 14246^{T}. On the other hand, 1801 genes were shared between both *Agreia* species, a higher number than in the *Subtercola* species (Figure 5). In terms of functional annotation, 87.6% of the genes shared between the four strains were annotated in COG. Interestingly, the percentage of genes annotated in COG is lower in the unique genes of each strain, 22% for *S. vilae* DB165^{T}, 30% for *S. boreus* DSM 13056^{T}, 31% for *A. bicolorata* DSM 14575^{T}, and 23% for *A. pratensis* DSM 14246^{T}. Genes only present in *S. vilae* DB165^{T} are involved in the flux of different ions like Na, Cl, Mg, Cu, Fe, and Pb, in sugar transport like ABC transport permeases, bacteriorhodopsins, peptidoglycan/xylase/chitin deacetylase, and trehalose synthetase, among others. Most of the functions possibly involved in the survival at low temperature are shared between *S. vilae* DB165^{T} and *S. boreus* DSM 13056^{T}. These include peroxiredoxin, peroxidases, ferrodoxin, 2 cold shock proteins (cspA), and ferrodoxins. In terms of transport proteins, two genes of divalent metal cation transporters for Fe, Co, Zn, and Cd are present in both *Subtercola* type strains and high-affinity Fe^{+2}/Pb^{+2} permeases, transporters that might help the cells in the detoxification of free radicals caused by the metals. On the other hand, three different cold shock proteins were found in *Subtercola*/*Agreia* shared genes, three catalases and three copies of the fatty acid desaturase (desA). These findings suggest that most of the cold adaptation mechanisms described might yet play an important role in the analysed strains, which are encoded deeply in the *Subtercola*/*Agreia* cluster.

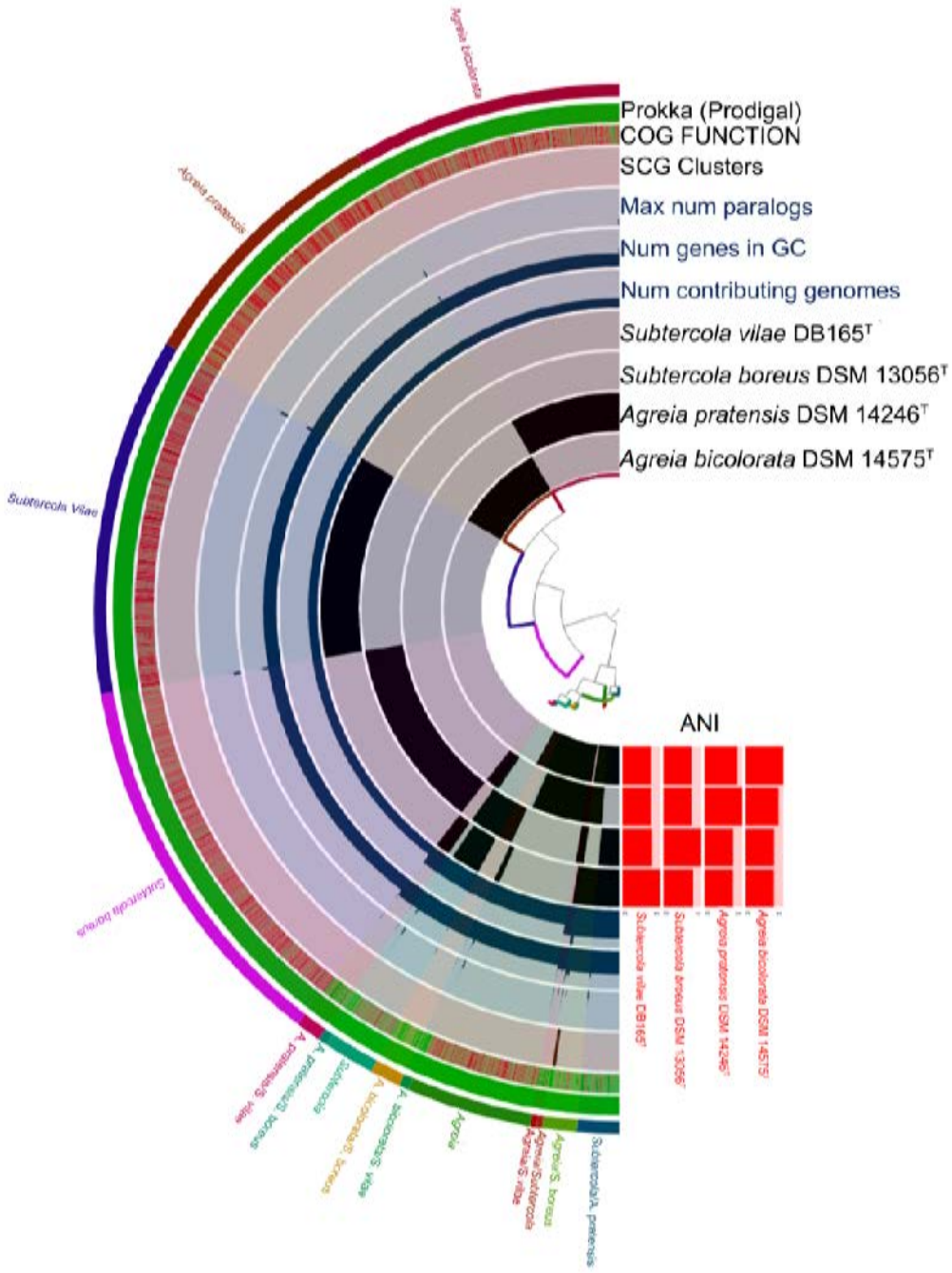


Figure 5. Pangenome analysis of *Subtercola vilae* DB165^{T} and their next relative strains *Subtercola boreus* DSM 13056^{T}, *Agreia bicolorata* DSM 14575^{T}, and *Agreia pratensis* DSM 14246^{T}. ANI analysis is indicated in red boxes, a 100% of similarity is shown in a total red box. Genes annotated with a COG function are annotated with green colour, whereas red indicated unknown function. In the outside layer of it is indicated the proportion of genes present in *Agreia* spp. and *Subtercola* spp. strains, genes present only in each strain, shared between *Subtercola* spp. strains, shared between *Agreia* spp. strains, and genes present in between specific strains of the genera *Subtercola* and *Agreia*.

Conclusions

S. vilae DB165^T is the first available genome reported from the genus *Subtercola*, which was isolated from a poly-extremophilic environment of the Llullaillaco Volcano Lake. The genome of *S. vilae* DB165^T encodes a repertoire of genes linked to the harsh condition of Llullaillaco (Figure 6). These features include several mechanisms for transcribing proteins at low temperatures, such as a high number of tRNAs, and cold shock proteins. *S. vilae* DB165^T is capable of producing several proteins to deal with oxidative stress, which is of higher relevance in low temperature environments, in which reactive oxygen species are more abundant. We propose that these two new ice-binding proteins, which are present uniquely in *S. vilae* DB165^T, combat ice crystal formation. Genome comparison of *S. vilae* DB165^T against closely related type strains of the species *S. boreus*, *A. bicolorata*, and *A. pratensis* showed distinct differences between these species. While all of them exhibited a large part of strain-specific genes in their genomes, several genes involved in cold adaptation were shared between the strains, suggesting that the features might be more common among the *Microbacteriaceae* family. However, among all strains analysed, only *S. vilae* DB165^T showed the presence of genes that might interact with ice crystals. The data presented in this work showed that *S. vilae* DB165^T can employ different strategies to live at cold temperatures such as those prevalent in Llullaillaco Volcano Lake.

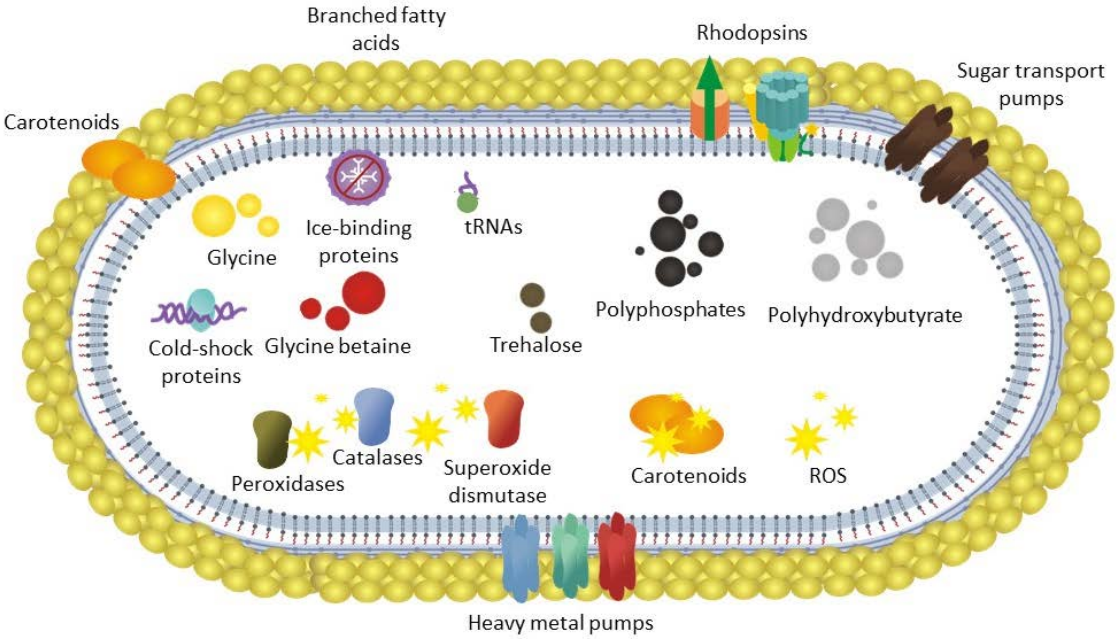


Figure 6. Traits annotated in *Subtercola vilae* DB165^{T} genome considered to be involved in its adaptation to Llullaillaco Volcano Lake.

Acknowledgments

The authors gratefully acknowledge Tanja Rahn for her technical assistance in the DNA extraction, Martin Jhan and Beate Slaby for their support on the bioinformatic softwares, Wiebke Sickel for the genome sequencing, and Alexa Garin-Fernandez for her contribution in the Figure 6 preparation. Alvaro S. Villalobos was supported by a doctoral fellowship from Becas Chile (CONICYT) and the Deutscher Akademischer Austausch Dienst (DAAD).

Conflicts of interest

The authors declare no conflict of interests.

References

1. Mannisto, M. K.; Schumann, P.; Rainey, F. A.; Kampfer, P.; Tsitko, I.; Tiirola, M. A.; Salkinoja-Salonen, M. S. *Subtercola boreus* gen. nov., sp. nov. and *Subtercola frigoramans* sp. nov., two new psychrophilic actinobacteria isolated from boreal groundwater. Int. J. Syst. Evol. Microbiol. 2000, 50, 1731–1739, doi:10.1099/00207713-50-5-1731.
2. Si, H.; Shi, F.; Zhang, L.; Yue, H.; Wang, H.; Zhao, Z. *Subtercola lobariae* sp. nov., an actinobacterium of the family *Microbacteriaceae* isolated from the lichen *Lobaria retigera*. Int. J. Syst. Evol. Microbiol. 2017, 67, 1516–1521, doi:10.1099/ijsem.0.001753.
3. Villalobos, A. S.; Wiese, J.; Aguilar, P.; Dorador, C.; Imhoff, J. F. *Subtercola vilae* sp. nov., a novel actinobacterium from an extremely high-altitude cold volcano lake in Chile. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 2018, 111, 955–963, doi:10.1007/s10482-017-0994-4.
4. Vaïtilingom, M.; Attard, E.; Gaiani, N.; Sancelme, M.; Deguillaume, L.; Flossmann, A. I.; Amato, P.; Delort, A. M. Long-term features of cloud microbiology at the puy de Dôme (France). Atmos. Environ. 2012, 56, 88–100, doi:10.1016/j.atmosenv.2012.03.072.
5. Peeters, K.; Ertz, D.; Willems, A. Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. Syst. Appl. Microbiol. 2011, 34, 360–367, doi:10.1016/j.syapm.2011.02.002.
6. Lynch, R. C.; King, A. J.; Farias, M. E.; Sowell, P.; Vitry, C.; Schmidt, S. K. The potential for microbial life in the highest-elevation (>6000m.a.s.l.) mineral soils of the Atacama region. J. Geophys. Res. Biogeosciences 2012, 117, 1–10, doi:10.1029/2012JG001961.
7. Lynch, R. C.; Darcy, J. L.; Kane, N. C.; Nemergut, D. R.; Schmidt, S. K. Metagenomic evidence for metabolism of trace atmospheric gases by high-elevation desert actinobacteria. Front. Microbiol. 2014, 5, doi:10.3389/fmicb.2014.00698.
8. Rasuk, M. C.; Ferrer, G. M.; Kurth, D.; Portero, L. R.; Farías, M. E.; Albarracín, V. H. UV-resistant Actinobacteria from high-altitude andean lakes: isolation, characterization and antagonistic activities. Photochem. Photobiol. 2017, 93, 865–880, doi:10.1111/php.12759.
9. Goodfellow, M.; Fiedler, H. P. A guide to successful bioprospecting: Informed by actinobacterial systematics. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology; 2010, 98, 119–142. doi: 10.1007/s10482-010-9460-2
10. Bolger, A. M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 2014, 30, 2114–2120, doi:10.1093/bioinformatics/btu170.
11. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A. A.; Dvorkin, M.; Kulikov, A. S.; Lesin, V. M.; Nikolenko, S. I.; Pham, S.; Prjibelski, A. D.; Pyshkin, A. V.; Sirotkin, A. V.; Vyahhi, N.; Tesler, G.; Alekseyev, M. A.; Pevzner, P. A. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 2012, 19, 455–477, doi:10.1089/cmb.2012.0021.
12. Gurevich, A.; Saveliev, V.; Vyahhi, N.; Tesler, G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 2013, doi:10.1093/bioinformatics/btt086.

13. Seemann, T. Prokka: Rapid prokaryotic genome annotation. Bioinformatics 2014, 30, 2068–2069, doi:10.1093/bioinformatics/btu153.
14. Aziz, R. K.; Bartels, D.; Best, A.; DeJongh, M.; Disz, T.; Edwards, R. A.; Formsma, K.; Gerdes, S.; Glass, E. M.; Kubal, M.; Meyer, F.; Olsen, G. J.; Olson, R.; Osterman, A. L.; Overbeek, R. A.; McNeil, L. K.; Paarmann, D.; Paczian, T.; Parrello, B.; Pusch, G. D.; Reich, C.; Stevens, R.; Vassieva, O.; Vonstein, V.; Wilke, A.; Zagnitko, O. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics 2008, 9, doi:10.1186/1471-2164-9-75.
15. Overbeek, R.; Olson, R.; Pusch, G. D.; Olsen, G. J.; Davis, J. J.; Disz, T.; Edwards, R. A.; Gerdes, S.; Parrello, B.; Shukla, M.; Vonstein, V.; Wattam, A. R.; Xia, F.; Stevens, R. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014, 42: D206–D214, doi:10.1093/nar/gkt1226.
16. Weber, T.; Blin, K.; Duddela, S.; Krug, D.; Kim, H. U.; Bruccoleri, R.; Lee, S. Y.; Fischbach, M. A.; Müller, R.; Wohlleben, W.; Breitling, R.; Takano, E.; Medema, M. H. AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res. 2015, 43, W237–W243, doi:10.1093/nar/gkv437.
17. Parks, D. H.; Imelfort, M.; Skennerton, C. T.; Hugenholtz, P.; Tyson, G. W. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015, 25, 1043–1055, doi:10.1101/gr.186072.114.
18. Ankenbrand, M. J.; Keller, A. bcgTree: automatized phylogenetic tree building from bacterial core genomes. Genome 2016, doi:10.1139/gen-2015-0175.
19. Vasilyev, I.; Siniagina, M.; Malanin, S.; Boulygina, E.; Grygoryeva, T.; Yarullina, D.; Ilinskaya, O. Draft genome sequence of *Agreia bicolorata* Strain AC-1804, a producer of large amounts of carotenoid pigments, isolated from narrow reed grass infected by the phytoparasitic nematode. Genome Announc. 2015, 3: e1383-15 doi:10.1128/genomeA.01383-15.
20. Vasilenko, O. V.; Starodumova, I. P.; Dorofeeva, L. V.; Tarlachkov, S. V.; Prisyazhnaya, N. V.; Chizhov, V. N.; Subbotin, S. A.; Huntemann, M.; Clum, A.; Duffy, K.; Pillay, M.; Palaniappan, K.; Varghese, N.; Chen, I.-M. A.; Stamatis, D.; Reddy, T. B. K.; O'Malley, R.; Daum, C.; Shapiro, N.; Ivanova, N.; Kyrpides, N. C.; Woyke, T.; Whitman, W. B.; Evtushenko, L. I. Draft genome sequences of new isolates and the known species of the family *microbacteriaceae* associated with plants. Microbiol. Resour. Announc. 2018, 7, e01051-18, doi:10.1128/MRA.01051-18.
21. Eren, A. M.; Esen, Ö. C.; Quince, C.; Vineis, J. H.; Morrison, H. G.; Sogin, M. L.; Delmont, T. O. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ 2015, 3, e1319, doi:10.7717/peerj.1319.
22. Pritchard, L.; Glover, R. H.; Humphris, S.; Elphinstone, J. G.; Toth, I. K. Genomics and taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. Anal. Methods 2016, 8, 12–24. doi:10.1039/C5AY02550H
23. Buchfink, B.; Xie, C.; Huson, D. H. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 2014, 12, 59–60, doi:10.1038/nmeth.3176.

24. Magrane, M.; Consortium, U. P. UniProt Knowledgebase: A hub of integrated protein data. Database 2011, doi:10.1093/database/bar009.
25. Kelly, L. A.; Mezulis, S.; Yates, C.; Wass, M.; Sternberg, M. The Phyre2 web portal for protein modelling, prediction, and analysis. Nat. Protoc. 2015, doi:10.1038/nprot.2015-053.
26. Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. J. Mol. Graph. 1996, 14, 33–38, doi:10.1016/0263-7855(96)00018-5.
27. Tal, S.; Okon, Y. Production of the reserve material poly-ß-hydroxybutyrate and its function in Azospirillum brasilense Cd. Can. J. Microbiol. 1985, 31, 608–613, doi:10.1139/m85-115.
28. Kadouri, D.; Jurkevitch, E.; Okon, Y. Involvement of the reserve material poly-β-hydroxybutyrate in *Azospirillum brasilense* stress endurance and root colonization. Appl. Environ. Microbiol. 2003, 69, 3244–3250, doi:10.1128/AEM.69.6.3244-3250.2003.
29. Seufferheld, M. J.; Alvarez, H. M.; Farias, M. E. Role of polyphosphates in microbial adaptation to extreme environments. Appl. Environ. Microbiol. 2008, 74, 5867–5874.
30. Lanyi, J. K.; Balashov, S. P. Xanthorhodopsin: A bacteriorhodopsin-like proton pump with a carotenoid antenna. Biochim. Biophys. Acta - Bioenerg. 2008, 1777, 684–688.
31. Ernst, O. P.; Lodowski, D. T.; Elstner, M.; Hegemann, P.; Brown, L. S.; Kandori, H. Microbial and animal rhodopsins: Structures, functions, and molecular mechanisms. Chem. Rev. 2014, 114, 126–163. doi: 10.1021/cr4003769
32. Kozubek, A.; Tyman, J. H. P. Resorcinolic Lipids, the Natural Non-isoprenoid Phenolic Amphiphiles and Their Biological Activity. 1998, doi:10.1021/CR970464O.
33. Kanda, N.; Ishizaki, N.; Inoue, N.; Oshima, M.; Handa, A.; Kitahara, T. DB-2073, a new alkylresorcinol antibiotic. I. Taxonomy, isolation and characterization. J. Antibiot. (Tokyo). 1975, doi:10.7164/antibiotics.28.935.
34. Chattopadhyay, M. K. Mechanism of bacterial adaptation to low temperature. J. Biosci. 2006, 31, 157–165.
35. Young, A. J.; Lowe, G. M. Antioxidant and prooxidant properties of carotenoids. Arch. Biochem. Biophys. 2001, 385, 20–27, doi:10.1006/abbi.2000.2149.
36. Rocha, E. P. C. Codon usage bias from tRNA's point of view: Redundancy, specialization, and efficient decoding for translation optimization. Genome Res. 2004, 14, 2279–2286, doi:10.1101/gr.2896904.
37. Math, R. K.; Jin, H. M.; Kim, J. M.; Hahn, Y.; Park, W.; Madsen, E. L.; Jeon, C. O. c. PLoS One 2012, 31:157-65. doi:10.1371/journal.pone.0035784.
38. Hoang, T. T.; Sullivan, S. A.; Cusick, J. K.; Schweizer, H. P. β-Ketoacyl acyl carrier protein reductase (FabG) activity of the fatty acid biosynthetic pathway is a determining factor of 3-oxo-homoserine lactone acyl chain lengths. Microbiology 2002, 148, 3849–3856. doi:10.1099/00221287-148-12-3849

39. Methe, B. A.; Nelson, K. E.; Deming, J. W.; Momen, B.; Melamud, E.; Zhang, X.; Moult, J.; Madupu, R.; Nelson, W. C.; Dodson, R. J.; Brinkac, L. M.; Daugherty, S. C.; Durkin, A. S.; DeBoy, R. T.; Kolonay, J. F.; Sullivan, S. A.; Zhou, L.; Davidsen, T. M.; Wu, M.; Huston, A. L.; Lewis, M.; Weaver, B.; Weidman, J. F.; Khouri, H.; Utterback, T. R.; Feldblyum, T. V.; Fraser, C. M. The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc. Natl. Acad. Sci. 2005, 102, 10913–10918, doi:10.1073/pnas.0504766102.
40. Zhu, K.; Bayles, D. O.; Xiong, A.; Jayaswal, R. K.; Wilkinson, B. J. Precursor and temperature modulation of fatty acid composition and growth of *Listeria monocytogenes* cold-sensitive mutants with transposon-interrupted branched-chain α-keto acid dehydrogenase. Microbiology 2005, 151, 615–623, doi:10.1099/mic.0.27634-0.
41. Le Rudulier, D.; Strom, A. R.; Dandekar, A. M.; Smith, L. T.; Valentine, R. C. Molecular biology of osmoregulation. Science 1984, 224, 1064–1068, doi:10.1126/science.224.4653.1064.
42. Kandror, O.; DeLeon, A.; Goldberg, A. L. Trehalose synthesis is induced upon exposure of *Escherichia coli* to cold and is essential for viability at low temperatures. Proc. Natl. Acad. Sci. 2002, 99, 9727–9732, doi:10.1073/pnas.142314099.
43. Phadtare, S.; Alsina, J.; Inouye, M. Cold-shock response and cold-shock proteins. Curr. Opin. Microbiol. 1999, 2, 175–180. doi:10.1016/S1369-5274(99)80031-9
44. Chaikam, V.; Karlson, D. T. Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins. BMB Rep. 2010, 43, 1–8.
45. Schumann, W. Regulation of bacterial heat shock stimulons. Cell Stress Chaperones 2016, 21, 959–968, doi:10.1007/s12192-016-0727-z.
46. D'Amico, S.; Collins, T.; Marx, J. C.; Feller, G.; Gerday, C. Psychrophilic microorganisms: Challenges for life. EMBO Rep. 2006, 7, 385–389.
47. Leinala, E. K.; Davies, P. L.; Jia, Z. Crystal structure of β-Helical antifreeze protein points to a general ice binding model. Structure 2002, 10, 619–627, doi:10.1016/S0969-2126(02)00745-1.
48. Schumann, P.; Behrendt, U.; Ulrich, A.; Suzuki, K. I. Reclassification of *Subtercola pratensis* Behrendt et al. 2002 as *Agreia pratensis* comb. nov. Int. J. Syst. Evol. Microbiol. 2003, 53, 2041–2044, doi:10.1099/ijs.0.02664-0.
49. Kämpfer, P.; Glaeser, S. P. Prokaryotic taxonomy in the sequencing era - the polyphasic approach revisited. Environ. Microbiol. 2012 14, 291-317, doi:10.1111/j.1462-2920.2011.02615.x.

Chapter VI: Genomic potential of natural product biosynthesis by seven Actinobacteria isolated from the Atacama Desert

Alvaro S. Villalobos[a], Jutta Wiese[a], Sven Künzel[b], Johannes F. Imhoff [a],*

[a] Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

[b] Max Planck Institut für Evolutionsbiologie, Plön

* Corresponding author at

Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Tel.: 0049-431-600 4450

Fax: 0049-431-600 4482

Data in preparation for publication

Abstract

Actinobacteria strains obtained from the Atacama Desert show taxonomic novelty and a high potential in the production of antibiotics. We evaluated the genomic potential for natural products production of seven actinobacteria, *Streptomyces* sp. AD26, *Streptomyces* sp. Huas28, *Kocuria* sp. Huas7, *Nocardiopsis* sp. Huas15, *Kribbella* sp AD5, *Superstesspora tarapancensis* Llam7^{T} and *Subtercola vilae* DB165^{T}. antiSMASH 3.0 predicted that strains affiliating to *Streptomyces* and *Superstesspora* had the higher abundance and diversity of natural product biosynthetic gene clusters among the isolates. Moreover, the strains *Kribbella* sp AD5, *Superstesspora tarapacensis* Llam7^{T}, and *Streptomyces* sp. AD26 and Huas28 contain natural product biosynthetic gene clusters with no synteny homology with the database, highlighting their potential in the production of novel compounds.

Keyword: Natural product biosynthetic gene clusters; PKS; NRPS; Actinobacteria; Genomics

Introduction

The phylum Actinobacteria is one of the largest taxonomic units among the major lineages within the Bacteria domain (Ludwig et al. 2012). Actinobacteria are Gram-positive filamentous bacteria with a high guanine-plus-cytosine (G+C) content in their genomes. Actinobacteria are of great importance in the field of biotechnology as producers of a vast variety of bioactive secondary metabolites with extensive industrial, medical, and agricultural applications. Within members of the class Actinobacteria, the genus *Streptomyces* represents the richest source of natural products. The first antibiotics discovered in Actinobacteria were actinomycin from a culture of *Streptomyces antibioticus* in 1940 (Waksman and Woodruff 1940), streptothricin from *Streptomyces lavendulae* in 1942 (Wakman and Woodruff 1942), and streptomycin from *Streptomyces griseus* in 1944 (Schatz and Waksman 1944). Actinobacteria strains in general are the source of approx. 45% of all microbial bioactive secondary metabolites. Specifically, strains affiliated with the *Streptomyces* genus have been the major source of clinical antibiotics and are responsible for more than 80% of all antibiotics of actinobacterial origin (Bérdy 2005).

Most of the classes of clinical antibiotics were discovered and produced by Actinobacteria, such as aminoglycosides (Busscher et al. 2005), angucyclines (Kharel et al. 2012), ansamycins (Kang et al. 2012), anthracyclines (Minotti 2004), β-lactams (Liras 1999), clavulanic acid (Jensen and

Paradkar 1999), chloramphenicol (Vining and Stuttard 1995), glutarimides (K'ominek 1975), glycopeptides (Van Bambeke 2006), lipopeptides (Baltz 2010), lantibiotics (Willey and van der Donk 2007), macrolides (Gaynor and Mankin 2003), oxazolidinones (Mulinos 1955), streptogramins (Johnston et al. 2002), and tetracyclines (Okami and Hotta 1988).

However, the discovery of novel compounds has been limited due the culture conditions employed in the screening of strains. From all *Streptomyces* isolated randomly from soil, approx. 1% are able to produce streptomycin and 0.1% actinomycin, while erythromycin and vancomycin are produced in around one out of 10^7 and daptomycin in one out of around 10^9 soil isolates, and (Baltz 2007). Despite this limitation in the screening process, the natural product production capacity of an individual actinobacterial strain varies enormously. In some cases, actinobacterial strains are able to produce a single compound, while others produce a range of different metabolites and even compound classes. Even though, a huge number of compounds have been discovered from Actinobacteria, it is predicted that only around the 10% of the natural products that can be synthesised by these bacterial class have been discovered (Watve et al. 2001).

Recently, due the application of genomic sequencing technologies and new tools for the annotation of natural product biosynthetic gene clusters (Weber et al. 2015)the interest of Actinobacteria as a source of natural products has bloomed once again. Whole genomes of representative strains of Actinobacteria such as *Rhodococcus* sp. RHA1 (McLeod et al. 2006), *Saccharopolyspora erythraea* NRRL 23338 (Oliynyk et al. 2007), *Salinispora tropica* CNB-440 (Udwary et al. 2007), and *Streptomyces coelicolor* A(3)2 (Bentley et al. 2002) showed that each genome contained around 20 or more natural product biosynthetic gene clusters for the production of known or predicted secondary metabolites. In the specific case of *Streptomyces coelicolor* A(3)2, *which* is a model system for studying antibiotic production and known for the production of actinorhodin (Rudd and Hopwood 1979), undecylprodigiosin (Feitelson et al. 1985), a calcium-dependent antibiotic (Hopwood and Wright 1983), and methylenomycin (Rudd and Hopwood 1979). Nevertheless, the genome sequence of *S. coelicolor* A(3)2 showed that the potential as a producer of natural products was underestimated, revealing over 20 biosynthetic gene clusters for natural products (Challis and Hopwood 2003), including the genes involved in the production of a cryptic polyketide (Pawlik et al. 2007).

To date, the majority of the 20263 genome sequences of Actinobacteria comprise the orders *Corynebacteriales* (14450), *Micrococcales* (1690), *Streptomycetales* (1392), *Propionibacteriales*

(528), *Pseudonocardiales* (260) and *Micromonosporales* (248) (https://www.patricbrc.org/), showing that the capacity to harbour a large number of biosynthetic clusters is a regular property of most strains affiliated with the Actinobacteria class (Doroghazi and Metcalf 2013).

During the last three years, we isolated several Actinobacteria affiliated with different genera with high potential in the production of novel natural products, putative novel species, and remarkable characteristics to survive to extreme environments. We selected two strains isolated from rhizosphere samples: the strain AD5 isolated from *Cristaria integerrima* represent a putative novel species of *Kribbella*, genus from which only 3 natural products compounds have been described, and genome analyses of representatives of this genus showed more than 10 natural product biosynthetic gene clusters (Pukall et al. 2010); we also selected *Streptomyces* sp. AD26 isolated from *Cumulupuntia boliviana* which showed promising antibiotic activity against *Bacillus subtilis* and *Stapylococcus lentus.* From the hypersaline lake Salar de Huasco, we selected three strains. The strain Huas7 affiliated with *Kocuria* genus has the capacity to utilise complex carbon sources such as cellulose, chitin, starch, and it has the potential to produce different nanoparticles with unknown composition; the strain Huas15 represent a putative novel species of *Nocardiopsis*, showed antibiotic activity against *Bacillus subtilis*, *Staphylococcus lentus* and *Pseudomonas fluorescens*, it can grow in 150 g/L of NaCl and 250 g/L of Tropic marine salt, genome analyses of *Nocardiopsis* strains showed a high number of natural product biosynthetic gene clusters (Sun et al. 2010; Horn et al. 2015); the strain Huas28 is a putative novel species of Streptomyces genus, showed antibiotic activity against *Bacillus subtilis*, and it can grow till 100 g/L of NaCl and 200 g/L of Tropic marine salt, salt tolerance that is not shared among all *Streptomyces* representatives. We also analysed the genomic potential in the production of natural products of the two strains described as novel species (Chapter III and IV). The strain *Superstesspora tarapacensis* is a novel genus obtained from Salar de Llamará, it showed antibiotic activity against *Pseudomonas fluorescens* and *Escherischia coli*, it belongs to *Micromonosporaceae* family which contain several genera well known in the production of natural products, such as *Micromonospora*, *Salinispora*, *Verrucosispora*, and *Actinoplanes*; the strain *Subtercola vilae* obtained from Llullaillaco Volcano Lake genome was analysed (Chapter V) showing the presence of natural product biosynthetic gene clusters.

Materials and methods

The strains *Kocuria* sp. Huas7, Nocardiopsis sp. Huas15, *Streptomyces* sp. Huas28, and *Superstesspora tarapacensis* Llam7^{T} were isolated according to Villalobos et al. 2018 (see Chapter II). *Streptomyces* sp. AD26 and *Kribbella* sp. AD5 were obtained using the methodology presented in Chapter I and *Subtercola vilae* DB165^{T} was isolated according to Villalobos et al. 2018 (see Chapter IV).

DNA was extracted using DNeasy®Blood&Tissue Kit (Qiagen). The quality and quantity of the extracted DNA was evaluated by 0.8 % (w/v) agarose gel electrophoresis. The genomic DNA library was generated using Nextera XT (Illumina Inc.) according to the manufacturer's instructions. After fragmentation, size-selection was performed using NucleoMag NGS Clean-up and Size Select (Macherey-Nagel) to obtain a library with median insert-size around 400 bp. After PCR enrichment, the library was validated with a high-sensitivity DNA chip and Bioanalyzer 2100 (both Agilent Technologies, Inc.) and additionally quantified using the Qubit dsDNA HS assay (Life Technologies). Four sequencing runs were performed on a NextSeq device using v2 2 × 250 bp chemistry.

The raw paired-end sequences data were subjected to the Trimmomatic software for adapter and quality trimming (mean Phred quality score ≥ 30) (Bolger et al. 2014), filtering of sequences containing ambiguous bases and a minimum length of 200 bp. The filtered data were assembled with SPAdes assembler using enabled precorrection and k-mer sizes ranging from 15 to 127 (step size of 10) (Bankevich et al. 2012). The assemblies obtained were analysed using QUAST (Gurevich et al. 2013), whereas 127-kmers showed the bests quality.

Open reading frames were identified using Prodigal in Prokka and barrnap for rRNA genes (Seemann 2014). In addition, gene prediction and functional annotation was performed with the Integrated Microbial-Genomes Expert Review and the Rapid Annotation using Subsystem Technology webserver (Aziz et al. 2008; Overbeek et al. 2014). The genome completeness was analysed with CheckM (Parks et al. 2015). Natural product biosynthetic gene clusters (BGC) were identified by antiSMASH 3.0 (Weber et al. 2015) using ClusterFinder algorithm for BGC border prediction, minimum cluster size in CDS of 5, minimum number of biosynthesis-related PFAM domain of 5, and a minimum ClusterFinder probability of 60%.

Phylogenetic relationship of the seven strains was prepared using the complete 16S rRNA gene sequences derived from the respective genomes. Similarities between the strains and related type strains were obtained from EzTaxon e-server (Kim et al. 2012). The closest relatives of the strains and *Rubrobacter aplysinae* DSM 27440^T as outgroup were aligned using SINA aligner (v1.2.11) (Pruesse et al. 2012). Phylogenetic trees were constructed using neighbor-joining (Saitou and Nei 1987) and maximum-likelihood methods (Felsenstein 1981) applying bootstrap method with 1000 repeats with MEGA software version 6.0 (Tamura et al. 2013). NJplot was used to draw the phylogenetic trees expressed in the Newick phylogenetic tree format (Perrière and Gouy 1996).

For scanning electron microscopy all the strains were grown for 21 days at 26 °C. The strains *Kocuria* sp. Huas7, Nocardiopsis sp. Huas15, *Streptomyces* sp. Huas28, and *Superstesspora tarapacensis* Llam7^T were cultivated in SYP agar plates (Villalobos et al. 2018; Chapter II), while *Streptomyces* sp. AD26 and *Kribbella* sp. AD5 were grown for in SGG agar plates (Chapter I). Three colonies were cut from the agar plate, dehydrated by an ascending ethanol series (50 %, 70 %, 90 % and three times 100 % for 10 min each) (Boyde and Wood 1969), critical-point dried with carbon dioxide, and sputter-coated with Au/Pb. Finally, the samples were examined with scanning electron microscope.

Results

Among the genomes of all strains, the isolates *Streptomyces* sp. AD26, *Kribbella* sp. AD5, and *Superstesspora tarapacensis* Llam7^T have the largest genome, respectively (Table 1). Interestingly, the genome size in *Streptomyces* strains sequenced in this work (AD26 and Huas28) varies, showing a difference of about 2.7 Mb. In terms of gene number, *Kribbella* sp. AD5 contains 7980 genes from which 7878 are coding sequences, while *Streptomyces* AD26 has the highest number of tRNAs (86).

The strains affiliated with six actinobacterial families, *Micrococcaceae* (Huas7), *Microbactericeae* (DB165^T), *Micromonosporaceae* (Llam7^T), *Nocardiopsaceae* (Huas15), *Nocardioidaceae* (AD5), and *Streptomycetaceae* (AD26 and Huas28). According to preliminary phylogenetic data based in the 16S rRNA gene sequence, *Kribbella* sp. AD5, *Nocardiopsis* sp. Huas15, and *Streptomyces* sp. Huas28 clustered separately from their next related type strains

(Figure 1). *Subtercola vilae* DB165^{T} represents a new species and *Superstesspora tarapacensis* Llam7^{T} a new species and genus (this thesis Chapter III and IV).

While the cell shape of *Streptomyces* sp. strains AD26 and Huas28, *Superstesspora tarapacensis* Llam7^{T}, *Kribbella* sp. AD5, and *Nocardiopsis* sp. Huas15 consisted in long filaments (Figure 2), cells of *Kocuria* sp. Huas7 were short cocci that do not form cell aggregations and of *Subtercola vilae* DB165^{T} short rods (Figure 2).

Table 1. Genome statistics of sequenced strains.

Attribute	*Kocuria* sp. Huas7	*Nocardiopsis* sp. Huas15	*Streptomyces* sp. Huas28	*Kribbella* sp. AD5	*Streptomyces* sp. AD26	*Superstesspora tarapacensis* Llam7^{T}	*Subtercola vilae* DB165^{T}
Genome size (bp)	5043546	5211693	5721425	8399753	8488708	6694035	4043135
Contigs	1176	1318	659	268	668	1013	103
G+C content (%)	70.37	69.25	68.66	68.01	68.46	69.51	65.1
Genes	4873	4885	5234	7980	7142	6507	3879
CDS	4750	4790	5104	7878	6965	6366	3797
rRNA	6	3	7	3	8	3	5
tRNA	78	62	70	65	86	74	59
Misc RNA	38	29	52	33	82	63	17
tmRNA	1	1	1	1	1	1	1
Completeness (%)	96.55	93.51	94.87	100	99.21	97.98	99.5

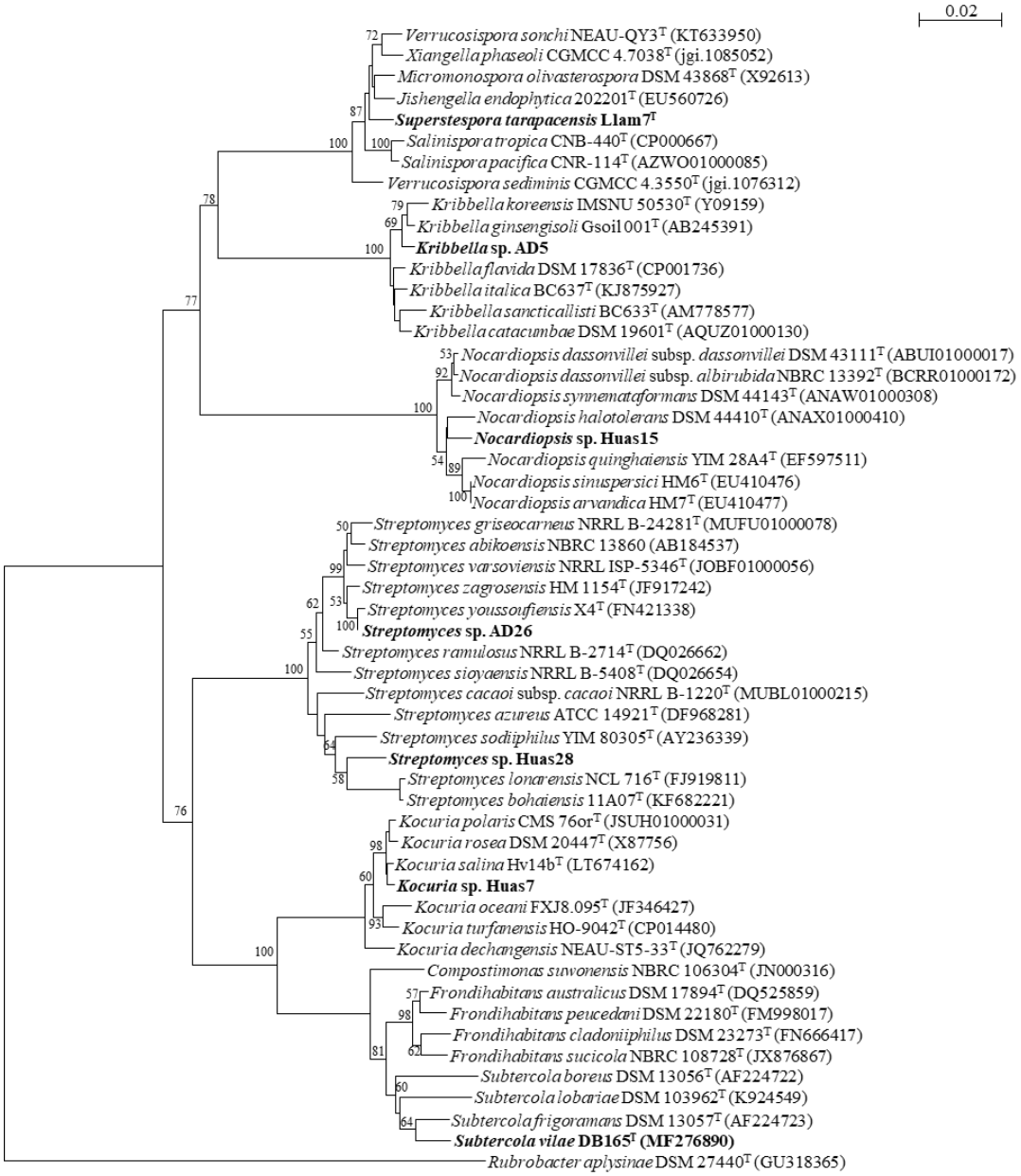


Figure 1. Neighbour-joining phylogenetic tree based on the complete 16S rRNA gene of the selected strains, their next related type strains, and *Rubrobacter aplysinae* DSM 2744^T as outgroup. Numbers in the nodes represent bootstrap support (%) based in the analysis of 1000 bootstrap replications. Only bootstrap values ≥ 50% are indicated. Genebank accession numbers of the sequences are given in parentheses. Bar indicates 0.02 substitutions per site.

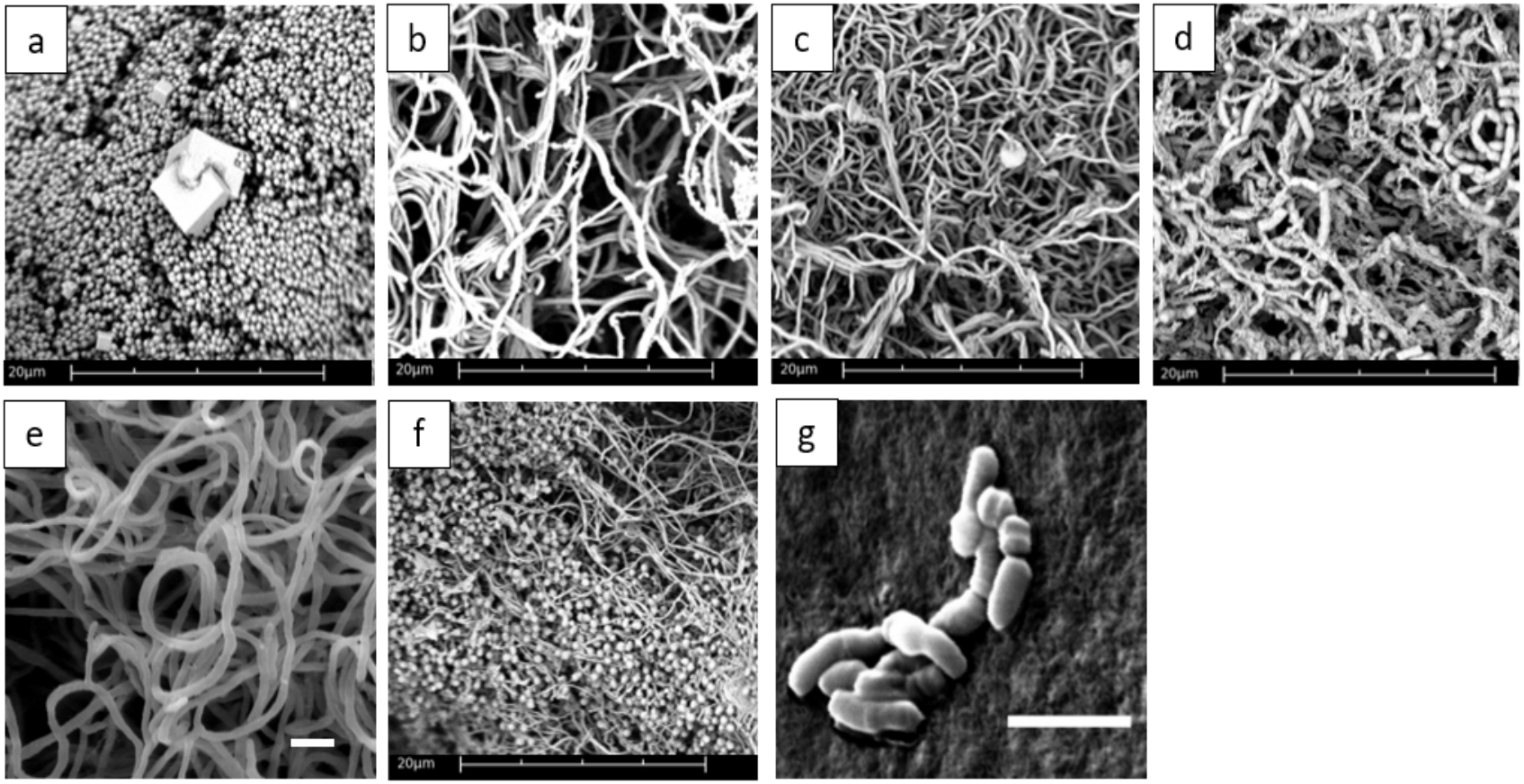


Figure 2. Scanning electron micrograph of the actinobacterial strains from this study. a) *Kocuria* sp. Huas7. b) *Nocardiopsis* sp. Huas15. c) *Streptomyces* sp. Huas28. d) *Kribbella* sp. AD5. e) *Streptomyces* sp. AD26. f) *Superstesspora tarapacensis* Llam7^{T}. g) *Subtercola vilae* DB165^{T}. Scale bar in e and g indicate 2 µm.

The natural product biosynthetic gene clusters (BGCs) analysis of the sequenced draft genomes revealed that the strains affiliating to the genus *Streptomyces* displayed the most abundant and diverse repertoire (Table 2). Specifically, *Streptomyces* sp. AD26 contained 46 natural product biosynthetic gene clusters sorted in 16 BGCs types, from which 11 represent type 1 polyketide synthases, followed by 7 non-ribosomal peptide synthetases and 7 terpene biosynthetic clusters. *Superstesspora tarapacensis* Llam7^{T} follows as third with abundant BGCs types and ranks as second (together with *Streptomyces* sp. Huas28) in diversity with 12 different BGCs types.

Natural product biosynthetic gene clusters exhibiting 100% of homology in its synteny with the database were uncommon in the analysed draft genomes. From a total of 162 BGCs identified in all the strains, only 6 gene clusters have 100% of synteny homology, from which 2 of them are ectoine biosynthetic gene clusters. In *Kribbella* sp. AD5 we detected the biosynthetic gene cluster to produce geosmin (Supplementary Table 4), while in *Superstesspora tarapacensis* Llam7^{T} the gene cluster to produce sioxanthin was detected (Supplementary Table 6). The gene cluster of the type 3 polyketide alkylresorcinol was found in *Kribbella* sp. AD5 and *Subtercola vilae* DB165^{T} (Supplementary Table 4 and 7), however in both strains the gene clusters contain several additional biosynthetic genes suggesting that the alkylresorcinol core structure might suffer further modifications.

Table 2. Number of predicted natural product gene clusters sorted in different classes in the draft genome of the sequenced strains.

Gene cluster class	*Kocuria* sp. Huas7	*Nocardiopsis* sp. Huas15	*Streptomyces* sp. Huas28	*Kribbella* sp. AD5	*Streptomyces* sp. AD26	*Superstespora tarapacensis* Llam7^{T}	*Subtercola vilae* DB165^{T}
Amglyccycl	-	-	1	-	-	-	-
Amglyccycl-Nrps	-	-	2	-	-	-	-
Arylpolyene	-	-	-	1	1	-	-
Bacteriocin	-	-	1	-	2	2	-
Butyrolactone	-	-	-	1	1	-	-
Ectoine	1	2	1	-	1	-	-
Indole-Nrps	-	-	1	-	-	-	-
Ladderane	-	-	-	-	1	1	-
Lantipeptide	1	1	4	4	-	-	-
Lantipeptide-Nrps	-	-	-	-	-	1	-
Nrps	1	1	13	-	7	14	-
Other	3	1	2	1	4	1	1
Otherks	1	1	-	-	1	-	-
Siderophore	1	1	3	2	3	2	-
T1pks	1	2	7	-	11	4	-
T1pks-Nrps	-	-	-	1	1	1	-
T2pks	-	-	1	1	-	1	-
T3pks	1	-	2	1	2	1	1
T3pks-T1pks	-	-	-	-	-	1	-
Terpene	1	1	5	1	7	5	1
Terpene-T1pks	-	-	-	-	1	-	-
Thiopeptide	-	-	-	1	-	1	-
Transatpks	-	-	-	-	2	-	-
Transatpks-T1pks	-	-	-	-	1	-	-
Total	11	10	43	14	46	35	3

A total of 11 complete BGCs showed no synteny homology with any biosynthetic gene cluster in the antiSMASH database, suggesting the production of natural products with novel structure. Among these BGCs, *Streptomyces* sp. Huas28 encodes a cryptic lantipeptide cluster of 30.7 kb (Cluster 2), a bacteriocin that comprise 11.4 kb (Cluster 11), and a terpene cluster of 10.9 kb (Cluster 15) (Supplementary Table 3). On the other hand, *Kribbella* sp. AD5 codifies a thiopeptide gene cluster of 22.2 kb (Cluster 3), a lantipeptide gene cluster of 22.7 kb (Cluster 5), and a siderophore gene cluster of 15.2 kb (Cluster 10) (Supplementary Table 4). The *Streptomyces* sp. AD26 encodes a NRPS gene cluster of 58.7 kb (Cluster 9), a terpene biosynthetic cluster of 21.1 kb (Cluster 15), a siderophore gene cluster that comprises 7.6 kb (Cluster 16), and a butyrolactone gene cluster 8.2 kb (Cluster 23) (Supplementary Table 5). Two gene clusters encoded by *Superstesspora tarapacensis* Llam7^{T} have no homology with clusters of the database, the first one is a NRPS gene cluster with a size of 30.6 kb (Cluster 4) and the second is a siderophore (Cluster 22) (Supplementary Table 6).

According to the high level of synteny homology of the clusters with the database of antiSMASH (≥55%), *Streptomyces* sp. Huas28 might be able to produce compounds with scaffolds similar to 7-prenylisatin, jinggangmycin, and vicenistatin (Supplementary Table 3); while *Streptomyces* sp. AD26 could produce isorenieratene, piericidin A1, hopene, paenibactin, and flaviolin like compounds (Supplementary Table 5). *Superstesspora tarapacensis* Llam7^{T} has the potential to produce compounds with similar structure as kendomycin, sioxanthin, and desferrioxamine B (Supplementary Table 6).

Conclusions

The potential to produce a huge variety of natural products of 7 Actinobacteria isolated from extreme environments of the Atacama Desert was presented. *Streptomyces* spp. AD26 and Huas28 as well as *Superstesspora tarapacensis* Llam7^{T} showed a promising potential due to the high diversity and abundance of different BGCs types. In addition, both *Streptomyces* strains (AD26 and Huas28), *Kribbella* sp. AD5, and *Superstesspora tarapacensis* Llam7^{T} encode biosynthetic gene clusters that showed no synteny homology with natural product biosynthetic gene clusters of the database, suggesting that these strains might be able to produce compounds with a novel structure. The phylogenetic affiliation of the strains is an important for the selection of strains in genome mining of natural products gene clusters. Strains belonging to the genus *Streptomyces* and strains affiliating to *Micromonosporaceae* family are the actinobacteria with more compounds described till the date, which can be attributed to their large number of natural product biosynthetic gene clusters, and their cosmopolitan distribution. Furthermore, the genome sequences of the seven isolates provide a large pool of diverse and in part novel biosynthetic gene clusters of natural products and give new insights into the genomics of Actinobacteria.

References

Aziz RK, Bartels D, Best A, et al (2008) The RAST Server: Rapid annotations using subsystems technology. BMC Genomics 9:75. doi: 10.1186/1471-2164-9-75

Baltz RH (2010) Genomics and the ancient origins of the daptomycin biosynthetic gene cluster. J Antibiot (Tokyo) 63:506–511. doi: 10.1038/ja.2010.82

Banerjee SK, Shanker R (2002) Comments on the pyridine catalysed reaction of alcohols with lead tetraacetate in benzene containing 1% acetic acid (v/v). Asian J Chem 14:512–514. doi: 10.2174/1389450023347678

Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell Sequencing. J Comput Biol 19:455–477. doi: 10.1089/cmb.2012.0021

Bérdy J (2005) Bioactive microbial metabolites: A personal view. J. Antibiot. (Tokyo). 58:1–26. doi:10.1038/ja.2005.1

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. doi: 10.1093/bioinformatics/btu170

Boyde A, Wood C (1969) Preparation of animal tissues for surface-scanning electron microscopy. J Microsc 90:221–249. doi: 10.1111/j.1365-2818.1969.tb00709.x

Busscher GF, Rutjes FPJT, van Delft FL (2005) 2-Deoxystreptamine: Central scaffold of aminoglycoside antibiotics. Chem. Rev. 105:775–791.

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc Natl Acad Sci 100:14555–14561. doi: 10.1073/pnas.1934677100

Doroghazi JR, Metcalf WW (2013) Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. BMC Genomics 14:611. doi: 10.1186/1471-2164-14-611

Feitelson JS, Malpartida F, Hopwood DA (1985) Genetic and Biochemical Characterization of the red Gene Cluster of *Streptomyces coelicolor* A3(2). Microbiology 131:2431–2441. doi: 10.1099/00221287-131-9-2431

Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. J Mol Evol 17:368–376. doi: 10.1007/BF01734359

Gaynor M, Mankin A (2003) Macrolide Antibiotics: binding site, mechanism of action, resistance. Curr Top Med Chem 3:949–960. doi: 10.2174/1568026033452159

Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. doi: 10.1093/bioinformatics/btt086

Hopwood DA, Wright HM (1983) CDA is a new chromosomally-determined antibiotic from *Streptomyces coelicolor* A3(2). Microbiology 129:3575–3579. doi: 10.1099/00221287-129-12-3575

Horn H, Cheng C, Edrada-Ebel R, et al (2015) Draft genome sequences of three chemically rich actinomycetes isolated from Mediterranean sponges. Mar Genomics 24:285–287. doi: 10.1016/J.MARGEN.2015.10.003

K'ominek LA (1975) Cycloheximide production by *Streptomyces griseus*: control mechanisms of cycloheximide biosynthesis. Antimicrob Agents Chemother 7:856–6. doi: 10.1128/AAC.7.6.856

Kang Q, Shen Y, Bai L (2012) Biosynthesis of 3,5-AHBA-derived natural products. Nat. Prod. Rep. 29:243–263. doi:10.1039/c2np00019a

Kharel MK, Pahari P, Shepherd MD, et al (2012) Angucyclines: Biosynthesis, mode-of-action, new natural products, and synthesis. Nat. Prod. Rep. 29:264–325. doi:10.1039/c1np00068c

Kim OS, Cho YJ, Lee K, et al (2012) Introducing EzTaxon-e: A prokaryotic 16s rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. doi: 10.1099/ijs.0.038075-0

Lin Y, Song M, Stone CA, Shaw SJ (2013) A comprehensive study on the curing kinetics and network formation of cyanate ester resin/clay nanocomposites. Thermochim Acta 552:77–86. doi: 10.1016/j.tca.2012.11.009

Liras P (1999) Biosynthesis and molecular genetics of cephamycins. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 75:109–124. doi: 10.1023/A:1001804925843

Ludwig W, Euzéby J, Schumann P, et al (2012) Road map of the phylum Actinobacteria. In: Bergey's Manual of Systematic Bacteriology. pp 1–28 Eds: Goodfellow M. et al.

McLeod MP, Warren RL, Hsiao WWL, et al (2006) The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci 103:15582–15587. doi: 10.1073/pnas.0607048103

Minotti G (2004) Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. Pharmacol Rev 56:185–229. doi: 10.1124/pr.56.2.6

Mulinos MG (1955) Cycloserine: an antibiotic paradox. Antibiot Annu 3:131–5.

Okami Y, Hotta K (1988) Search and Discovery of New Antibiotics. In: Actinobacteria in Biotechnology. Elsevier, pp 33–67

Oliynyk M, Samborskyy M, Lester JB, et al (2007) Complete genome sequence of the erythromycin-producing bacterium *Saccharopolyspora erythraea* NRRL23338. Nat Biotechnol 25:447–453. doi: 10.1038/nbt1297

Overbeek R, Olson R, Pusch GD, et al (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42: D206–D214 doi: 10.1093/nar/gkt1226

Parks DH, Imelfort M, Skennerton CT, et al (2015) CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. doi: 10.1101/gr.186072.114

Pawlik K, Kotowska M, Chater KF, et al (2007) A cryptic type I polyketide synthase (cpk) gene cluster in *Streptomyces coelicolor* A3(2). Arch Microbiol 187:87–99. doi: 10.1007/s00203-006-0176-7

Perrière G, Gouy M (1996) WWW-Query: An on-line retrieval system for biological sequence banks. Biochimie 78:364–369. doi: 10.1016/0300-9084(96)84768-7

Prates RO (2013) Relato de Experiência de Ensino de IHC. CEUR Workshop Proc 1042:37–40. doi: 10.1038/417141a

Pruesse E, Peplies J, Glöckner FO (2012) SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. doi: 10.1093/bioinformatics/bts252

Pukall R, Lapidus A, Glavina Del Rio T, et al (2010) Complete genome sequence of *Kribbella flavida* type strain (IFO 14399). Stand Genomic Sci 2:186–193. doi: 10.4056/sigs.731321

Rudd BA, Hopwood DA (1979) Genetics of actinorhodin biosynthesis by *Streptomyces coelicolor* A3(2). J Gen Microbiol 114:35–43. doi: 10.1099/00221287-114-1-35

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. doi: 10.1093/oxfordjournals.molbev.a040454

Schatz A, Waksman SA (1944) Effect of Streptomycin and other antibiotic substances upon *Mycobacterium tuberculosis* and related organisms. Exp Biol Med 57:244–248. doi: 10.3181/00379727-57-14769

Schulz PC, Morini MA (1997) Styrene solubilization in micelles of dodecyltrimethylammonium hydroxide. Colloid Polym Sci 275:604–607. doi: 10.1007/s003960050125

Seemann T (2014) Prokka: Rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. doi: 10.1093/bioinformatics/btu153

Sun H, Lapidus A, Nolan M, et al (2010) Complete genome sequence of *Nocardiopsis dassonvillei* type strain (IMRU 509). Stand Genomic Sci 3:325–336. doi: 10.4056/sigs.1363462

Tamura K, Stecher G, Peterson D, et al (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi: 10.1093/molbev/mst197

Udwary DW, Zeigler L, Asolkar RN, et al (2007) Genome sequencing reveals complex secondary metabolome in the marine actinomycete *Salinispora tropica*. Proc Natl Acad Sci 104:10376–10381. doi: 10.1073/pnas.0700962104

Vining LC, Stuttard C (1995) Chloramphenicol. Genet Biochem Antibiot Prod 505–530. doi: 10.1016/B978-0-7506-9095-9.50028-9

Wakman SA, Woodruff HB (1942) Selective antibiotic action of various substances of microbial origin. J Bacteriol. 44:373–384.

Waksman SA, Woodruff HB (1940) Bacteriostatic and Bactericidal Substances Produced by a Soil Actinomyces. Exp Biol Med 45:609–614. doi: 10.3181/00379727-45-11768

Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus *Streptomyces*? Arch. Microbiol. 176:386–390.

Weber T, Blin K, Duddela S, et al (2015) AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. doi: 10.1093/nar/gkv437

Willey JM, van der Donk WA (2007) Lantibiotics: peptides of diverse structure and function. Annu Rev Microbiol 61:477–501. doi: 10.1146/annurev.micro.61.080706.093501

General discussion and conclusions

The aim of the present study was the characterisation of Actinobacteria from four different environments in the Atacama Desert (Salar de Llamará, Salar de Huasco, rhizosphere from Socaire, and Llullaillaco Volcano Lake) regarding their taxonomic novelty, antibacterial activity, genomic adaptations for surviving in extreme environments, and the synthesis of natural products. The Actinobacteria strains were obtained using different culture media, in the attempt to mimic the physicochemical conditions of each environment. The selection of strictly Actinobacteria isolates resulted in more than the half of the isolates showing antibacterial activity (against Gram-positive and Gram-negative bacteria). The taxonomic novelty of the strains is initially suggested by the low similarity of the 16S rRNA gene as compared with the next related type strains (Chapters I and II), and for two strains (isolated from Salar de Llamará and Llullaillaco Volcano Lake) this novelty was validated through the polyphasic characterisation ending up with a novel genus *Superstesspora tarapacensis* and the novel species *Subtercola vilae* (Villalobos et al. 2018) (Chapters III and IV). Moreover, the adaptations of *Subtercola vilae* involved in its survival in cold environments were identified by combining different approaches of functional annotation, protein modelling, and pangenomics, leading to the identification of different proteins involved in the degradation of different reactive oxygen species (ROS), two putative novel ice-binding proteins, and to the characterisation of genomic differences and similarities of *S. vilae* and next related strains. On the other hand, the genomic characterisation of seven Actinobacteria isolates allows to identify 162 biosynthetic gene clusters involved in the production of natural products, including known compounds such as geosmin, ectoine, and clusters whose gene synteny show no homology with the databases.

Diversity of Actinobacteria isolates from Socaire, Salar de Llamará and Salar de Huasco

After only a few studies on Actinobacteria in the Atacama Desert (Okoro et al. 2009; Carro et al. 2018) this is the first comprehensive and in-depth study on Actinobacteria diversity.

In this thesis, Actinobacteria diversity was studied in three different locations of the Atacama Desert: i) the rhizosphere of different plants closed to Socaire, ii) two hypersaline lakes of Salar de Llamará, which is in the central depression of the Atacama Desert, and iii) Salar de Huasco, located in the Altiplano.

A much higher diversity than in previous studies was found with seventy-nine isolates affiliating to sixteen genera (Okoro et al. 2009; Carro et al. 2018). Strains were identified as members of the genera *Nocardia, Kribbella, Pseudonocardia, Arthrobacter Pseudarthrobacter, Rhodococcus, Kocuria, Nocardiopsis, Plantactinospora, Actinoplanes, Nonomuraea, Blastococcus, Microbacterium* and *Superstesspora*.

Of all the isolates obtained in this study, only one (Soc15) showed similarity to an isolate previously obtained from Atacama Desert (Goodfellow et al. 2017) (Chapter I). Isolates from the different locations in this study revealed no identity between the sites. Essentially, this demonstrates the high diversity of Actinobacteria in the Atacama Desert and the specific compositions of its different environments.

The culture medium composition plays an important role in the selective isolation of specific taxa such as Actinobacteria. Highly diverse Actinobacteria (different genera and species) were isolated by the utilisation of a variety of culture media with different carbon and nitrogen sources. In particular, the medium containing trehalose, proline and histidine yielded a high degree of genus diversity and taxonomic novelty. Traditional culture media used for the isolation of Actinobacteria include high concentration of complex carbon sources such as starch or chitin, resulting in the high diversity of known species of Actinobacteria (Vickers et al. 1984). For example, media recommended for the isolation of *Streptomyces*, such as starch-casein agar (SCA), contains 20 g of starch, which is far from mimicking the oligotrophic conditions of Atacama Desert. However, the use of this and similar media has led to the stunning diversity of known *Streptomyces* species, which exceeds 846 type strains (www.bacterio.net). In this study, the use of SCA medium yielded a lower number of isolates in comparison to the trehalose-proline-histidine medium, which contained only a quarter of the nutrient concentrations (Chapter I). Using this latter medium, we also obtained a wider array of different genera (Chapters I and II).

The physicochemical characteristics of the environment play an important role in the microbial community composition (Fierer et al. 2012). Hence, contrasting characteristics of the environments studied thus far in the Atacama Desert might be reflected in actinobacterial diversity. Actinobacteria are well known for having a cosmopolitan distribution (Ludwig et al. 2012); however, this wide distribution can only be applied to lower taxonomic levels (e.g. genus) (DeLong et al. 2001). In this respect, the 16S rRNA gene sequences can help indicate the degree

of similarity of the new isolates with those obtained earlier from similar environments. The majority of samples used previously for the isolation of Actinobacteria from the Atacama Desert were described as hyper-arid soils (Okoro et al. 2009; Santhanam et al. 2012; Idris et al. 2017; Goodfellow et al. 2017; Carro et al. 2018), while in the present study Actinobacteria from different sources were studied. Salar de Llamará strains were obtained only from microbial mats samples, which are characterised by their high amount of nutrients (carbon, nitrogen, phosphate, etc.), contrasting with previous studies that focused only on hyper-arid soils. These isolates showed high similarity with Actinobacteria obtained from environments with similar physicochemical characteristics, such as mangroves (Thawai et al. 2007, 2008; Xie et al. 2011) and marine sediments (Maldonado et al. 2005). Regarding rhizosphere samples from Socaire, due to nutrients provided by the plants, the Actinobacteria diversity showed identity with plant-associated Actinobacteria characterised in direct and indirect growth promotion (Verma et al. 2015; Singh et al. 2016; Yadav et al. 2018). Salar de Huasco is described as a cold environment in which the water and sediments contain high concentrations of salt; isolates obtained from these samples showed high similarity with strains obtained from cold deserts (Mayilraj 2006), saline in-land sediments (Yang et al. 2008; Gao et al. 2016), marine sediments (Bredholdt et al. 2007; Engelhardt et al. 2010), and alkaline soils (Schippers et al. 2002; Ueda et al. 2008), among others. Even though their 16S rRNA gene showed similarity with Actinobacteria strains obtained from these environments, most of the strains obtained in our study show a 16S rRNA gene similarity level below the threshold of putative novel species (Yarza et al. 2014).

Characterisation of novel of isolates

In accordance with 16S rRNA gene sequence similarities of >98.7%, thirty-nine (49.3%) of the strains obtained in this thesis represent novel species. The percentage of novel species obtained from the hypersaline lakes was higher (15; 47%) compared to the rhizosphere samples (13; 28%) (Chapters I and II). Under-explored environments such as the Salares in Atacama Desert represent a good source of novel species, which is demonstrated by the results from Laguna de Chaxa of Salar de Atacama (Santhanam et al. 2012, 2013; Busarakam et al. 2014). Furthermore, we identified one of the strains (Llam7) obtained from Salar de Llamará as a new taxon, confirming that Salares are environments that contain unique biodiversity. Using BLAST analysis of the 16S rRNA gene sequence of this strain, it could not be identified as belonging to one of the known genera of the *Micromonosporaceae* family (96 – 98%). Its placement on the

phylogenetic trees demonstrated that the strain represented a novel genus (Chapter II). Chemotaxonomic evidence of different diagnostic characteristics indicated differences with all members of the *Micromonosporaceae* family. Clearly, characteristics such as diamino acids in its peptidoglycan, hydrogenated menaquinone length, whole-sugar content, polar lipids, and fatty acid composition, as well as its physiological characteristics, differentiates Llam7^T from *Salinispora*, *Jishengella*, *Micromonospora* and *Verrucosispora* genera. *Superstesspora tarapacensis* was proposed as the name for this bacterium (Chapter III).

Another isolate obtained during this study (strain DB165^T), representing a novel species of the genus *Subtercola*, was characterised. It was isolated from Llullaillaco Volcano Lake (6710 meters above sea level), located in the Andes Mountains at the border of the Atacama Desert. Llullaillaco Volcano Lake has been characterised as a cold oligotrophic environment with high incidence of UV radiation; analyses of microbial communities in Llullaillaco soil have shown that more than the 90% of the population is comprised of *Pseudonocardia* bacteria (Actinobacteria) (Lynch et al. 2012, 2014). Llullaillaco Volcano Lake has not been studied, making the characterisation of the new Actinobacterium interesting as a model microorganism. The new isolate showed high similarity not only to *Subtercola* species, but also to type strains of *Agreia* and *Frondihabitans* (96 – 97%). Therefore, the identification only by 16S rRNA gene sequence analysis is problematic. Chemotaxonomic analyses reveal differences in the composition of dimethyl acetals and diamino acids present in the peptidoglycan (Schumann et al. 2003), differentiating strain DB165^T from *Agreia* and *Frondihabitans*. In addition, characteristics such as fatty acid profile, polar lipids, and enzymatic activity showed clear differences between the strain DB165^T and the type strains of *Subtercola* genus. Hence strain DB165^T was described as a new species named *Subtercola vilae* (Villalobos et al. 2018)(Chapter IV).

The metabolic properties and mechanisms involved in the survival of *Subtercola vilae* were studied by analysing its genome sequence (Chapter V). The mechanisms that *S. vilae* might use to cope with the cold environment of Llullaillaco Volcano Lake were described. The genes involved in low temperature adaptation are similar to other cold-adapted microorganisms, and apparently are well conserved in closely related strains (Methe et al. 2005; Math et al. 2012). Most significantly, we identified two proteins that contain a functional ice-binding motif (Leinala et al. 2002). This suggests that *S. vilae* produces proteins to interact with the ice. Despite the presence

of the ice-binding motif, half of the protein showed no similarity with the database. Thus, we propose that *S. vilae* contains novel ice-binding proteins which function in cold adaptation.

Antibiotic activity of isolates and secondary metabolites biosynthetic gene clusters

As Actinobacteria are well known to produce a large variety of antibiotics, and the need for new antibiotics is well documented, one of the goals of this thesis was the study of antibiotic production in the new isolates obtained. This was considered a promising approach, because under-explored environments and novel species are expected to represent valuable sources for new antibiotic compounds.

In this study, more than the half (46 of 79) of the strains showed antibiotic activity against Gram-positive and Gram-negative bacteria (Chapters I and II). The isolation of strains is an essential step in analysing their metabolic capacity to synthesise natural products. Metabolic characteristic can be explored using different approaches; for large culture collections, the selection of culture media proven to yield a high amount and chemical diversity of compounds can be used (e.g: oatmeal and starch glucose glycine medium for *Streptomyces*) (Goodfellow and Fiedler 2010), while for small culture collections a more intensive program such as OSMAC (one strain many compounds) can be used (Bode et al. 2002). This consists utilising several culture media for each strain to improve the production of secondary metabolites. Nowadays, due to the accessibility of genome sequence technologies, the mining of natural product biosynthetic gene clusters from the strain can reveal its genomic potential through the production of secondary metabolites (Doroghazi and Metcalf 2013). Compared with metagenomic approaches, the availability of an actinobacterial culture ensures the production of sufficient genomic DNA for cloning and expression of selected gene clusters.

Nevertheless, the research involved in the screening and purification of potentially novel compounds is an extensive and expensive process. For that reason, the selection of few candidates for an in-depth analysis is crucial. Different databases provide valuable information needed to select good candidates for genome sequencing. Dictionary of Natural Products, for instance, is one of the most extensive databases that describes natural products produced by microorganisms; the List of Prokaryotic Names with Standing Nomenclature (LPNS; www.Bacterio.net), meanwhile, is a curated database of bacterial type strains. Using this information, a rational selection of strains can be made.

In this thesis, a total of sixteen actinobacteria genera were isolated. According to the Dictionary of Natural Products (CRC press, 2015) the genera *Streptomyces*, *Micromonospora*, and *Nocardia* stand out as proven producers with a high number of natural products described (Table 1). However, this might be attributed to their cosmopolitan distribution and the extensive knowledge regarding their selective isolation, as is indicated by the high number of type strains described for each of these genera. Interestingly, the genus *Arthrobacter* produced a lower number of compounds in comparison to *Micromonospora*. This can be attributed to their genomic potential for production of natural products, reflected in the number of biosynthetic gene clusters present in each genus. In *Micromonospora* the number of these clusters is evidently higher than *Arthrobacter*. A clear example of this potential is the genus *Salinispora*, which only contains three type species, but during intensive studies of their representatives, fifty-six natural product compounds have been described. Accordingly, strains affiliating to genera such *Nonomuraea*, *Nocardiopsis*, *Kribbella*, among others with a similar number of biosynthetic gene clusters such as *Streptomyces*, *Nocardia*, and *Micromonospora* represent an under-explored source of novel natural products.

Table 1. Potential of natural products discovered in Actinobacteria.

Genus	Strains isolated in this study	Type strains described for each genus[a]	Compounds described according to DNP[b]	Number of NP gene clusters [c]
Streptomyces	26	846	7389	25
Micromonospora	7	72	422	17
Salinispora	5*	3	56	16
Plantactinospora	3	6	0	15
Actinoplanes	1	48	133	18
Nocardia	11	110	316	44
Nocardiopsis	9	50	88	13
Rhodococcus	3	65	28	25
Nonomuraea	1	42	32	33
Kribbella	1	27	3	12
Pseudonocardia	1	59	30	12
Arthrobacter	1	95	61	8
Pseudarthrobacter	3	10	0	5
Blastococcus	2	4	0	5
Kocuria	2	23	2	5
Microbacterium	1	97	5	3

*Closest next related strain to *Superstesspora tarapacensis* strain

[a] Type validated strains according to the List of prokaryotic names and standing nomenclature (http://www.bacterio.net/)

[b] Compounds associated to each genus in the Dictionary of Natural Products 2015

[c] Natural products clusters detected in complete genomes ([d]) using AntiSMASH 3.0 (Weber et al. 2015)

In our effort to explore the genomic potential in the production of natural products of strains isolated during this study, the genomes of 7 strains were sequenced (Chapter VI). The selection of the strains followed the criteria mentioned previously. According to their proven potential in the production of natural products, we selected two *Streptomyces* strains and *Superstesspora tarapacensis* strains (strain described as a novel genus in Chapter III), which are closely related to *Salinispora* and *Micromonospora*. Strains affiliated with *Nocardiopsis*, *Kribbella*, and *Kocuria* genera represent a good target for natural product discovery due to the high number of biosynthetic gene clusters encoded in their genomes, low number of species described, and few compounds described. In addition, the genome of *S. vilae* was also analysed (Chapter V).

We annotated a total of 162 natural product biosynthetic gene clusters from all the isolates. *Streptomyces* strains AD26 and Huas28 showed the highest number of clusters (46, 43 respectively) among all strains, approximately twice the number of clusters codified in

Streptomyces coelicolor and *Streptomyces ambofaciens* genomes (Bentley et al. 2002; Thibessard et al. 2015). From all the clusters annotated in the seven actinobacterial genomes, only six showed 100% of synteny homology with known compounds. Some of the natural product biosynthetic gene clusters show a lower percentage of synteny homology (>50%), which indicates that the metabolites synthesised by these clusters might present a similar chemical scaffold with their similar hits from the database. We obtained novel natural product biosynthetic gene clusters that show a new arrangement of their gene synteny, showing low synteny homology with known natural product biosynthetic gene clusters. These clusters were found in two under-explored genera, *Kribbella* sp. AD5 and *Superstesspora tarapacensis* Llam7, and in the two sequenced *Streptomyces* (AD26 and Huas28), suggesting that even when a high number of natural products have been discovered from this genus, the potential for the production of novel natural products is present in isolates from Atacama Desert environments. These biosynthetic gene clusters encode for lantipeptide, bacteriocin, and terpene compounds in *Streptomyces* sp. Huas28; thiopeptide and siderophore in *Kribbella* sp. AD5; non-ribosomal peptide, terpene, siderophore, and butyrolactone in *Streptomyces* sp. AD26; and non-ribosomal peptide and siderophore in *Superstesspora tarapacensis* Llam7. The results obtained from the genome analyses suggest a high degree of novelty in the biosynthetic gene clusters of the sequenced strains, though it is important to consider that only a small fraction of known compounds have their biosynthetic gene pathways resolved.

Conclusions

Atacama Desert environments exhibit a wide diversity of Actinobacteria, and are apparently unique for each biome studied so far. Actinobacteria isolates from the rhizosphere of plants are affiliated with groups commonly found to promote plant growth, while the ones found in the Salares de Llamará and Huasco showed high 16S rRNA gene similarity with Actinobacteria found in biomes with similar physicochemical conditions. More than half of the isolates are putative novel species, from which we described the novel genus and species *Superstesspora tarapacensis* from microbial mats of the hypersaline Salar de Llamará and the new species *Subtercola vilae* from Llullaillaco Volcano Lake. The majority of the Actinobacteria isolated from Atacama Desert were active in the production of antibiotics. Those studied by genome analysis showed a rich diversity of natural product biosynthetic gene clusters, most of which had

low similarity with clusters available in the databases. The mechanisms of *Subtercola vilae* to survive the cold conditions at Llullaillaco Volcano were deduced from the genome, and revealed a high number of genes involved in the degradation of reactive oxygen species, in membrane modulation, and in gene regulation at lower temperatures, as well as the presence of two novel ice-binding proteins.

Future studies shall focus on the structure elucidation of compounds produced by the isolated strains and heterologous expression of biosynthetic gene clusters which are necessary to transform the potential of the Actinobacteria into biotechnological products.

References

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: Possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627. doi: 10.1002/1439-7633(20020703)3:7

Bredholdt H, Galatenko OA, Engelhardt K, et al (2007) Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: Isolation, diversity and biological activity. Environ Microbiol 9:2756–2764. doi: 10.1111/j.1462-2920.2007.01387.x

Busarakam K, Bull AT, Girard G, et al (2014) *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 105:849–861. doi: 10.1007/s10482-014-0139-y

Carro L, Razmilic V, Nouioui I, et al (2018) Hunting for cultivable *Micromonospora* strains in soils of the Atacama Desert. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:1375–1387. doi: 10.1007/s10482-018-1049-1

DeLong EF, Pace NR, Kane M (2001) Environmental diversity of bacteria and archaea. Syst Biol 50:470–478. doi: 10.1080/10635150118513

Doroghazi JR, Metcalf WW (2013) Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. BMC Genomics 14:611. doi: 10.1186/1471-2164-14-611

Engelhardt K, Degnes KF, Kemmler M, et al (2010) Production of a new thiopeptide antibiotic, TP-1161, by a marine *Nocardiopsis* species. Appl Environ Microbiol 76:4969–4976. doi: 10.1128/AEM.00741-10

Fierer N, Leff JW, Adams BJ, et al (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci 109:21390–21395. doi: 10.1073/pnas.1215210110

Gao MY, Qi H, Li JS, et al (2017) A new polysubstituted cyclopentene derivative from *Streptomyces* sp. HS-NF-1046. J Antibiot (Tokyo) 70:216–218. doi: 10.1038/ja.2016.111

Goodfellow M, Busarakam K, Idris H, et al (2017) *Streptomyces asenjonii* sp. nov., isolated from hyper-arid Atacama Desert soils and emended description of *Streptomyces* viridosporus Pridham et al. 1958. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 110:1133–1148. doi: 10.1007/s10482-017-0886-7

Goodfellow M, Fiedler H-PP (2010) A guide to successful bioprospecting: Informed by actinobacterial systematics. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 98:119–142

Idris H, Nouioui I, Asenjo JA, et al (2017) *Lentzea chajnantorensis* sp. nov., an actinobacterium from a very high altitude Cerro Chajnantor gravel soil in northern Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 110:795–802. doi: 10.1007/s10482-017-0851-5

Leinala EK, Davies PL, Jia Z (2002) Crystal structure of β-Helical antifreeze protein points to a general ice binding model. Structure 10:619–627. doi: 10.1016/S0969-2126(02)00745-1

Ludwig W, Euzéby J, Schumann P, et al (2012) Road map of the phylum Actinobacteria. In: Bergey's Manual® of Systematic Bacteriology. pp 1–28

Lynch RC, Darcy JL, Kane NC, et al (2014) Metagenomic evidence for metabolism of trace atmospheric gases by high-elevation desert actinobacteria. Front Microbiol. 5:698 doi: 10.3389/fmicb.2014.00698

Lynch RC, King AJ, Farías ME, et al (2012) The potential for microbial life in the highest-elevation (>6000m.a.s.l.) mineral soils of the Atacama region. J Geophys Res Biogeosciences 117:1–10. doi: 10.1029/2012JG001961

Maldonado LA, Fenical W, Jensen PR, et al (2005) *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. Int J Syst Evol Microbiol 55:1759–1766. doi: 10.1099/ijs.0.63625-0

Math RK, Jin HM, Kim JM, et al (2012) Comparative genomics reveals adaptation by *Alteromonas* sp. SN2 to marine tidal-flat conditions: Cold tolerance and aromatic hydrocarbon metabolism. PLoS One. doi: 10.1371/journal.pone.0035784

Mayilraj S, Kroppenstedt RM, Suresh K, Saini HS (2006) *Kocuria himachalensis* sp. nov., an actinobacterium isolated from the Indian Himalayas. Int J Syst Evol Microbiol 56:1971–1975. doi: 10.1099/ijs.0.63915-0

Methe BA, Nelson KE, Deming JW, et al (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci 102:10913–10918. doi: 10.1073/pnas.0504766102

Okoro CK, Brown R, Jones AL, et al (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 95:121–133. doi: 10.1007/s10482-008-9295-2

Prates RO (2013) Relato de Experiência de Ensino de IHC. CEUR Workshop Proc 1042:37–40. doi: 10.1038/417141a

Santhanam R, Okoro CK, Rong X, et al (2012) *Streptomyces atacamensis* sp. nov., isolated from an extreme hyper-arid soil of the Atacama Desert, Chile. Int J Syst Evol Microbiol 62:2680–2684. doi: 10.1099/ijs.0.038463-0

Santhanam R, Rong X, Huang Y, et al (2013) *Streptomyces bullii* sp. nov., isolated from a hyper-arid Atacama Desert soil. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 103:367–373. doi: 10.1007/s10482-012-9816-x

Schippers A, Bosecker K, Willscher S, et al (2002) *Nocardiopsis metallicus* sp. nov., a metalleaching actinomycete isolated from an alkaline slag dump. Int J Syst Evol Microbiol 52:2291–2295. doi: 10.1099/ijs.0.02279-0

Schumann P, Behrendt U, Ulrich A, Suzuki KI (2003) Reclassification of *Subtercola pratensis* Behrendt et al. 2002 as *Agreia pratensis* comb. nov. Int J Syst Evol Microbiol 53:2041–2044. doi: 10.1099/ijs.0.02664-0

Singh RN, Gaba S, Yadav AN, et al (2016) First high quality draft genome sequence of a plant growth promoting and cold active enzyme producing psychrotrophic *Arthrobacter agilis* strain L77. Stand Genomic Sci. doi: 10.1186/s40793-016-0176-4

Thawai C, Tanasupawat S, Kudo T (2008) *Micromonospora pattaloongensis* sp. nov., isolated from a Thai mangrove forest. Int J Syst Evol Microbiol 58:1516–1521. doi: 10.1099/ijs.0.65410-0

Thawai C, Tanasupawat S, Suwanborirux K, et al (2007) *Micromonospora narathiwatensis* sp . nov ., from Thai peat swamp forest soils. J Gen Appl Microbiol 293:287–293. doi: 10.2323/jgam.53.287

Thibessard A, Haas D, Gerbaud C, et al (2015) Complete genome sequence of *Streptomyces ambofaciens* ATCC 23877, the spiramycin producer. J Biotechnol 214:117–118. doi: 10.1016/j.jbiotec.2015.09.020

Ueda K, Tagami Y, Kamihara Y, et al (2008) Isolation of bacteria whose growth is dependent on high levels of CO_2 and implications of their potential diversity. Appl Environ Microbiol 74:4535–4538. doi: 10.1128/AEM.00491-08

Verma P, Yadav AN, Khannam KS, et al (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann Microbiol 65:1885–1899. doi: 10.1007/s13213-014-1027-4

Vickers JC, Williams ST, Ross GW (1984) a Taxonomic Approach to selective isolation of *Streptomycetes* from soil. Biol Biochem Biomed Asp Actinomycetes 553–561. doi: 10.1016/B978-0-12-528620-6.50045-8

Villalobos AS, Wiese J, Aguilar P, et al (2018) *Subtercola vilae* sp. nov., a novel actinobacterium from an extremely high-altitude cold volcano lake in Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:955–963. doi: 10.1007/s10482-017-0994-4

Weber T, Blin K, Duddela S, et al (2015) AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. doi: 10.1093/nar/gkv437

Xie QY, Wang C, Wang R, et al (2011) *Jishengella endophytica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. Int J Syst Evol Microbiol 61:1153–1159. doi: 10.1099/ijs.0.025288-0

Yadav AN, Verma P, Kumar S, et al (2018) Actinobacteria from rhizosphere: Molecular Diversity, distributions, and potential biotechnological applications. in: new and future developments in microbial biotechnology and bioengineering: Actinobacteria: Diversity and Biotechnological Applications. pp 13–41

Yang R, Zhang L-P, Guo L-G, et al (2008) *Nocardiopsis valliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. Int J Syst Evol Microbiol 58:1542–1546. doi: 10.1099/ijs.0.65090-0

Yarza P, Yilmaz P, Pruesse E, et al (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635–645. doi: 10.1038/nrmicro3330

Individual scientific contributions to multiple-author publications

Results of this thesis were prepared or submitted for publication:

Villalobos AS, Wiese J, Aguilar P, Dorador C, Imhoff JF. *Subtercola vilae* sp. nov., a novel actinobacterium from an extremely high-altitude cold volcano lake in Chile. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2018;111(6):955–63. Published, **Chapter IV**.

Villalobos AS, Wiese J, Imhoff JF, Dorador C, Keller A, Hentschel U. Cold-adaptation of *Subtercola vilae* DB165^{T} an isolate from a high-altitude cold volcano lake as revealed by its genome analysis. Submitted to Microorganisms (in revision), **Chapter V**.

Villalobos AS, Wiese J, Imhoff JF, Rahn T, Dorador C. *Superstesspora tarapacensis* gen. nov., sp. nov., a new member of the *Micromonosporaceae* family from the hypersaline Salar de Llamará, Chile. To be submitted to International Journal of Systematic and Evolutionary Microbiology, **Chapter III.**

Contribution of the author to the different chapters of this thesis:

Chapter I: Rhizosphere samples from Socaire were taken by Prof. Johannes F. Imhoff during December 2008. Cultivation of Actinobacteria, purification, identification, phylogenetic analyses and antibiotic tests were performed by Alvaro S. Villalobos. Preparation of the manuscript was done by Alvaro S. Villalobos under the supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Chapter II: Samples of the Salares were taken in collaboration with Dr. Cristina Dorador Lab (2015/2016); specifically, Salar de Huasco samples were taken by Eduardo Acosta and Pablo Aguilar, and Salar de Llamará samples were taken by Alvaro S. Villalobos and Pablo Aran. Cultivation of Actinobacteria, purification, identification, phylogenetic analyses and antibiotic tests were performed by Alvaro S. Villalobos. The manuscript was written by Alvaro S. Villalobos in supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Chapter III: The strain DB165^{T} was isolated by Pablo Aguilar under the supervision of Dr. Cristina Dorador in 2012. Alvaro S. Villalobos performed all physiological test assays, the identification by 16S rRNA and its phylogenetic tree, and the preparation of the biomass used for the chemotaxonomic analyses under supervision of Dr. Jutta Wiese. Chemotaxonomic analyses

were performed in DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen. The genome was sequenced in Universität Würzburg Biozentrum by PD. Dr. Alexander Keller, while the assembly and G+C content calculation was done by Alvaro S. Villalobos. The manuscript was written by Alvaro S. Villalobos in supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Chapter IV: The strain Llam7^{T} was isolated by Alvaro S. Villalobos. Alvaro S. Villalobos, Tanja Rahn and Dr. Jutta Wiese performed the physiological test assays. Alvaro S. Villalobos prepared the biomass used for the chemotaxonomic analyses. Chemotaxonomic analyses were performed in DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen. The genome was sequenced by Dr. Sven Künzel in Max Planck Institut für Evolutionsbiologie, Plön. The assembly and G+C content calculation, genome annotation, and phylogenetic analyses were done by Alvaro S. Villalobos. The manuscript was written by Alvaro S. Villalobos in supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Chapter V: The genome annotation, protein modelling of the ice-binding proteins and the pangenome analysis, was done by Alvaro S. Villalobos. Phylogenetic analysis was performed by PD. Dr. Alexander Keller. The manuscript was written by Alvaro S. Villalobos in supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Chapter VI: Genomic DNA was extraction was performed by Tanja Rahn. The genomes were sequenced by Dr. Sven Künzel in Max Planck Institut für Evolutionsbiologie, Plön. Genome assembly, annotation, and natural product biosynthetic gene clusters analyses were performed by Alvaro S. Villalobos. The manuscript was written by Alvaro S. Villalobos in supervision of Dr. Jutta Wiese and Prof. Dr. Johannes F. Imhoff.

Acknowledgements

A mi familia por su apoyo incondicional y permanente.

Especially, I would like to thank Prof. Dr. Johannes F. Imhoff for giving me this wonderful opportunity to work in his research group, for his academic support during the past years. I am deeply thankful to have had Dr. Jutta Wiese as supervisor, who introduced and guided me to the beautiful research field of microbial taxonomy and for her competent support which made it possible to organise myself and my research better and finish my PhD thesis. I am grateful to all the members and former members of Imhoff's group who kindly helped me methodologically and with all the small talks, in particular to Tanja Rahn for all the technical support and friendship since the first day I arrived at GEOMAR, to Dr. Rolf Schmaljohann and Ignacio Sottorf for their help with SEM and HPLC respectively.

Furthermore, I wish to thank Prof. Dr. Ute Hentschel for the support and the opportunity to work in the Marine Mikrobiologie group after Prof. Dr. Johannes F. Imhoff retirement. I want to thank the entire Sponges group, in special to Hannes Horn, Martin Jahn, Beate Slaby, Kristina Bayer, Lucia Pita, Kathrin Busch, Lara Schmittman, and Erick Borchert for always having the time to discuss science and for those small talks at the lab hallway.

Special thanks to my former professor and friend Dr. Cristina Dorador, who gave me the opportunity to learn microbiology and encouraged me to follow the scientific career. I would like to thank Cristina Doradors group for their direct and indirect help brought during my field trip to Salar de Llamará and Salar de Huasco, in special to Pablo Aran, Pablo Aguilar, Eduardo Acosta, Alfonso Araya and Sergio Mujica.

I express my gratitude to all the colleagues who contributed to my research with their assistance, to Dr. Hannes Horn who introduced me to the bioinformatic pipeline behind genome assembly, to Dr. Alexander Keller and Dr. Sven Künzel for the genome sequencing of my strains, to Martin Jahn and Beate Slaby for always being awailable when I needed help with "Jazz", to Prof. Dr. Frank Sönnichsen for his help in the mass and NMR analyses of several purified compounds, that will make possible to resolve their chemical structure.

All my work presented in this thesis would not be possible without all my friends that helped me improve my English. In specific to Holly McKelvey who took the time to proofread of my thesis and Lara for help with the Zusammenfassung.

At last, I would like to thank the DAAD (Deutscher Akademischer Austauschdienst) and CONICYT (Comité Nacional de Ciencia y Tecnología, Chile) for providing me with the fellowship to live and do my research in Germany.

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt habe und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde weder im Ganzen noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt und entstand unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft.

Kiel, den 03.12.2018 _____

Alvaro S. Villalobos

Supplementary material for chapter VI

Supplementary Tables 1-7. Predicted biosynthetic gene clusters of all sequenced strains.

Supplementary Table 1. Predicted biosynthetic gene clusters of *Kocuria* sp. Huas7.

Cluster	Gene cluster type	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1*	Siderophore	12.0	Desferrioxamine B biosynthetic gene cluster (60% of genes show similarity)	BGC0000941 c1
Cluster 2	Terpene	15.6	Carotenoid_biosynthetic_gene_cluster (50% of genes show similarity)	BGC0000644_c1
Cluster 3	Other	12.2	Neocarzilin_biosynthetic_gene_cluster (14% of genes show similarity)	BGC0000111_c1
Cluster 4	Other	11.6	-	-
Cluster 5	Other	8.2	-	-
Cluster 6	T3pks	4.7	-	-
Cluster 7	Nrps	4.5	-	-
Cluster 8	T1pks	4.2	Chlorothricin_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000036_c1
Cluster 9	Otherks	2.0	-	-
Cluster 10	Lantipeptide	1.2	-	-
Cluster 11	Ectoine	1.0	-	-

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 2. Predicted biosynthetic gene clusters of *Nocardiopsis* sp. Huas15.

Cluster	Gene cluster type	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1	ectoine	5.4	-	-
Cluster 2	t1pks	5.7	ECO-02301_biosynthetic_gene_cluster (32% of genes show similarity)	BGC0000052_c1
Cluster 3	lantipeptide	4.3	-	-
Cluster 4	t1pks	4.1	-	-
Cluster 5	nrps	3.2	-	-
Cluster 6	Other ks	2.7	-	-
Cluster 7	ectoine	2.1	-	-
Cluster 8	nrps	2.0	-	-
Cluster 9	other	1.5	-	-
Cluster 10	siderophore	1.1	-	-
Cluster 11	terpene	1.0	-	-

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 3. Predicted biosynthetic gene clusters of *Streptomyces* sp. Huas28.

Cluster	Gene cluster type	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1	amglyccycl-nrps	45.6	Incednine biosynthetic gene cluster (8% of genes show similarity)	BGC0000078 c1
Cluster 2*	lantipeptide	30.7	-	-
Cluster 3*	terpene	17.6	Xiamycin_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000665_c1
Cluster 4	t1pks	26.8	SWA-2138_biosynthetic_gene_cluster (28% of genes show similarity)	BGC0000597_c1
Cluster 5	nrps	24.4	Telomycin_biosynthetic_gene_cluster (5% of genes show similarity)	BGC0001406_c1
Cluster 6	indole-nrps	21.3	7-prenylisatin_biosynthetic_gene_cluster (40% of genes show similarity)	BGC0001294_c1
Cluster 7	nrps	20.4	Kirromycin_biosynthetic_gene_cluster (1% of genes show similarity)	BGC0001070_c1
Cluster 8	amglyccycl	13.5	Jinggangmycin_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000701_c1
Cluster 9	nrps	18.1	-	-
Cluster 10	t1pks	16.9	Vicenistatin_biosynthetic_gene_cluster (60% of genes show similarity)	BGC0000167_c1
Cluster 11*	bacteriocin	11.4	-	-
Cluster 12*	ectoine	10.2	Ectoine_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000853_c1
Cluster 13	nrps	14.2	-	-
Cluster 14	lantipeptide	13.5	-	-
Cluster 15*	terpene	10.9	-	-
Cluster 16	lantipeptide	11.9	-	-
Cluster 17	other	11.2	-	-
Cluster 18	t1pks	10.8	-	-
Cluster 19	t3pks	10.8	Herboxidiene_biosynthetic_gene_cluster (2% of genes show similarity)	BGC0001065_c1
Cluster 20	nrps	9.5	-	-
Cluster 21	t1pks	9.3	Elaiophylin_biosynthetic_gene_cluster (20% of genes show similarity)	BGC0000053_c1
Cluster 22	lantipeptide	9.0	SapB_biosynthetic_gene_cluster (75% of genes show similarity)	BGC0000551_c1
Cluster 23	nrps	9.0	Kedarcidin_biosynthetic_gene_cluster (2% of genes show similarity)	BGC0000081_c1
Cluster 24	t2pks	8.9	Rabelomycin_biosynthetic_gene_cluster (16% of genes show similarity)	BGC0000262_c1
Cluster 25	siderophore	8.7	Desferrioxamine_B_biosynthetic_gene_cluster (50% of genes show similarity)	BGC0000940_c1

Cluster 26	t1pks	6.8	-	-
Cluster 27	nrps	6.5	-	-
Cluster 28	other	6.3	Diazepinomicin_biosynthetic_gene_cluster (12% of genes show similarity)	BGC0000679_c1
Cluster 29	terpene	5.7	Hopene_biosynthetic_gene_cluster (23% of genes show similarity)	BGC0000663_c1
Cluster 30	nrps	5.6	-	-
Cluster 31	siderophore	5.5	-	-
Cluster 32	nrps	5.4	-	-
Cluster 33	nrps	4.8	Skyllamycin_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0000429_c1
Cluster 34	nrps	3.9	-	-
Cluster 35	t3pks	3.8	Alkyl-O-Dihydrogeranyl-Methoxyhydroquinones_biosynthetic_gene (57% of genes	BGC0001077_c1
Cluster 36	t1pks	3.5	Halstoctacosanolide_biosynthetic_gene_cluster (77% of genes show similarity)	BGC0000073_c1
Cluster 37	t1pks	3.5	Apoptolidin_biosynthetic_gene_cluster (17% of genes show similarity)	BGC0000021_c1
Cluster 38	siderophore	3.1	-	-
Cluster 39	terpene	2.0	-	-
Cluster 40	terpene	1.9	Carotenoid_biosynthetic_gene_cluster (18% of genes show similarity)	BGC0000633_c1
Cluster 41	nrps	1.1	-	-
Cluster 42	nrps	1.0	-	-

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 4. Predicted biosynthetic gene clusters of *Kribbella* sp. AD5.

Cluster	Gene cluster	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1	lantipeptide	49.4	Catenulipeptin biosynthetic gene cluster (60% of genes show similarity)	BGC0000501 c1
Cluster 2*	terpene	22.2	Geosmin_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000661_c1
Cluster 3*	thiopeptide	22.2	-	-
Cluster 4*	butyrolactone	10.9	Glycopeptidolipid_biosynthetic_gene_cluster (7% of genes show similarity)	BGC0000769_c1
Cluster 5*	lantipeptide	22.7	-	-
Cluster 6*	lantipeptide	22.5	Catenulipeptin_biosynthetic_gene_cluster (60% of genes show similarity)	BGC0000501_c1
Cluster 7*	siderophore	11.9	Desferrioxamine_B_biosynthetic_gene_cluster (40% of genes show similarity)	BGC0000941_c1
Cluster 8	other	28.6	-	-
Cluster 9	t3pks	30.1	Alkylresorcinol_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000282_c1
Cluster 10*	siderophore	15.2	-	-
Cluster 11	t1pks-nrps	40.8	Actagardine_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0000495_c1
Cluster 12*	lantipeptide	17.1	Catenulipeptin_biosynthetic_gene_cluster (60% of genes show similarity)	BGC0000501_c1
Cluster 13*	t2pks	35.4	Dactylocycline_biosynthetic_gene_cluster (18% of genes show similarity)	BGC0000216_c1
Cluster 14*	arylpolyene	22.1	Avilamycin_A_biosynthetic_gene_cluster (5% of genes show similarity)	BGC0000026_c1

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 5. Predicted biosynthetic gene clusters of *Streptomyces* sp. AD26.

Cluster	Gene cluster	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1*	t1pks	46.3	Tetronasin biosynthetic gene cluster (9% of genes show similarity)	BGC0000163 c1
Cluster 2	terpene-t1pks	55.4	Isorenieratene_biosynthetic_gene_cluster (85% of genes show similarity)	BGC0000664_c1
Cluster 3	t1pks-nrps	92.5	ECO-02301_biosynthetic_gene_cluster (42% of genes show similarity)	BGC0000052_c1
Cluster 4	transatpks-t1pks	45.1	Difficidin_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000176_c1
Cluster 5*	t1pks	34.2	Piericidin_A1_biosynthetic_gene_cluster (91% of genes show similarity)	BGC0000124_c1
Cluster 6*	terpene	26.7	Hopene_biosynthetic_gene_cluster (61% of genes show similarity)	BGC0000663_c1
Cluster 7	nrps	40.3	Stenothricin_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000431_c1
Cluster 8	t1pks	60.4	Fostriecin_biosynthetic_gene_cluster (28% of genes show similarity)	BGC0000060_c1
Cluster 9*	nrps	58.7	-	-
Cluster 10*	terpene	18.8	Merochlorin_biosynthetic_gene_cluster (7% of genes show similarity)	BGC0001083_c1
Cluster 11	t1pks	37.2	Candicidin_biosynthetic_gene_cluster (23% of genes show similarity)	BGC0000034_c1
Cluster 12*	otherks	24.1	-	-
Cluster 13*	other	30.1	Echosides_biosynthetic_gene_cluster (76% of genes show similarity)	BGC0000340_c1
Cluster 14	nrps	40.4	GE81112_biosynthetic_gene_cluster (14% of genes show similarity)	BGC0000360_c1
Cluster 15*	terpene	21.1	-	-
Cluster 16*	siderophore	7.6	-	-
Cluster 17	arylpolyene	35.7	Kedarcidin_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0000081_c1
Cluster 18*	t3pks	28.5	Azinomycin_B_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0000960_c1
Cluster 19	nrps	28.3	Paenibactin_biosynthetic_gene_cluster (66% of genes show similarity)	BGC0000401_c1
Cluster 20	terpene	21.8	-	-
Cluster 21*	t3pks	26.7	Flaviolin_biosynthetic_gene_cluster (75% of genes show similarity)	BGC0000902_c1
Cluster 22	t1pks	23.3	Aculeximycin_biosynthetic_gene_cluster (26% of genes show similarity)	BGC0000002_c1
Cluster 23*	butyrolactone	8.2	-	-
Cluster 24	other	21.8	A-503083_biosynthetic_gene_cluster (3% of genes show similarity)	BGC0000288_c1
Cluster 25	nrps	21.5	Arylomycin_biosynthetic_gene_cluster (33% of genes show similarity)	BGC0000306_c1

Cluster 26	t1pks	21.1	Nigericin_biosynthetic_gene_cluster (77% of genes show similarity)	BGC0000114_c1
Cluster 27	nrps	18.5	Myxochelin_biosynthetic_gene_cluster (16% of genes show similarity)	BGC0001345_c1
Cluster 28*	terpene	14.2	-	-
Cluster 29	other	17.5	-	-
Cluster 30	terpene	12.8	Xiamycin_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000666_c1
Cluster 31	t1pks	14.5	Pimaricin_biosynthetic_gene_cluster (29% of genes show similarity)	BGC0000125_c1
Cluster 32*	ectoine	10.4	Ectoine_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000853_c1
Cluster 33	t1pks	11.8	Nigericin_biosynthetic_gene_cluster (61% of genes show similarity)	BGC0000114_c1
Cluster 34	other	11.5	-	-
Cluster 35	terpene	10.1	Isorenieratene_biosynthetic_gene_cluster (18% of genes show similarity)	BGC0001227_c1
Cluster 36	nrps	9.5	-	-
Cluster 37	transatpks	8.9	-	-
Cluster 38	t1pks	8.7	Lasalocid_biosynthetic_gene_cluster (13% of genes show similarity)	BGC0000087_c1
Cluster 39	ladderane	8.6	-	-
Cluster 40	transatpks	8.2	Bryostatin_biosynthetic_gene_cluster (60% of genes show similarity)	BGC0000174_c1
Cluster 41*	siderophore	7.4	-	-
Cluster 42	t1pks	6.6	-	-
Cluster 43	siderophore	5.6	-	-
Cluster 44	t1pks	4.5	-	-
Cluster 45	bacteriocin	2.5	-	-
Cluster 46	bacteriocin	1.6	-	-

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 6. Predicted biosynthetic gene clusters of *Superstesspora tarapacensis* Llam7^{T}.

Cluster	Gene cluster	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1*	t3pks-t1pks	58.2	Kendomycin biosynthetic gene cluster (55% of genes show similarity)	BGC0001066 c1
Cluster 2*	bacteriocin	10.8	Collismycin_A_biosynthetic_gene_cluster (7% of genes show similarity)	BGC0000973_c1
Cluster 3*	thiopeptide	34.5	Yatakemycin_biosynthetic_gene_cluster (9% of genes show similarity)	BGC0000466_c1
Cluster 4*	nrps	30.6	-	-
Cluster 5*	nrps	31.3	Fluorometabolite_biosynthetic_gene_cluster (33% of genes show similarity)	BGC0000903_c1
Cluster 6	nrps	46.0	Pacidamycin_biosynthetic_gene_cluster (22% of genes show similarity)	BGC0000951_c1
Cluster 7	t1pks-nrps	31.8	Lasalocid_biosynthetic_gene_cluster (18% of genes show similarity)	BGC0000087_c1
Cluster 8	lantipeptide-nrps	22.7	-	-
Cluster 9	nrps	21.4	Polyoxypeptin_biosynthetic_gene_cluster (27% of genes show similarity)	BGC0001036_c1
Cluster 10	nrps	17.7	-	-
Cluster 11	t1pks	17.3	Salinomycin_biosynthetic_gene_cluster (12% of genes show similarity)	BGC0000144_c1
Cluster 12	nrps	14.7	Polyoxypeptin_biosynthetic_gene_cluster (24% of genes show similarity)	BGC0001036_c1
Cluster 13	nrps	13.8	-	-
Cluster 14	terpene	13.0	Phosphonoglycans_biosynthetic_gene_cluster (3% of genes show similarity)	BGC0000806_c1
Cluster 15	terpene	12.3	-	-
Cluster 16	siderophore	11.6	Desferrioxamine_B_biosynthetic_gene_cluster (83% of genes show similarity)	BGC0000940_c1
Cluster 17	terpene	11.5	-	-
Cluster 18	t1pks	10.3	Naphthomycin_biosynthetic_gene_cluster (15% of genes show similarity)	BGC0000106_c1
Cluster 19	t2pks	10.0	Spore_pigment_biosynthetic_gene_cluster (33% of genes show similarity)	BGC0000271_c1
Cluster 20	other	9.5	-	-
Cluster 21	nrps	8.5	-	-
Cluster 22*	siderophore	8.0	-	-
Cluster 23	t1pks	7.2	Heronamide_biosynthetic_gene_cluster (33% of genes show similarity)	BGC0001349_c1
Cluster 24	nrps	6.2	-	-
Cluster 25	t3pks	5.7	Alkyl-O-Dihydrogeranyl-Methoxyhydroquinones_biosynthetic_gene (28% of	BGC0001077_c1

Cluster 26	nrps	5.4	-	-
Cluster 27	nrps	5.2	Thiocoraline_biosynthetic_gene_cluster (7% of genes show similarity)	BGC0000445_c1
Cluster 28	terpene	4.9	Sioxanthin_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0001087_c4
Cluster 29	bacteriocin	4.8	Lymphostin_biosynthetic_gene_cluster (30% of genes show similarity)	BGC0001007_c1
Cluster 30	nrps	4.6	Coelibactin_biosynthetic_gene_cluster (18% of genes show similarity)	BGC0000324_c1
Cluster 31	terpene	3.2	-	-
Cluster 32	t1pks	2.8	-	-
Cluster 33	nrps	2.7	-	-
Cluster 34	nrps	2.6	-	-
Cluster 35	ladderane	1.5	Skyllamycin_biosynthetic_gene_cluster (4% of genes show similarity)	BGC0000429_c1

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.

Supplementary Table 7. Predicted biosynthetic gene clusters of *Subtercola vilae* DB165^{T}.

Cluster	Gene cluster type	Size (kb)	Compound with gene cluster of highest homology	MIBiG BGC-ID
Cluster 1	terpene	20.8	Carotenoid biosynthetic gene cluster (37% of genes show similarity)	BGC0000637 c1
Cluster 2	t3pks	40.9	Alkylresorcinol_biosynthetic_gene_cluster (100% of genes show similarity)	BGC0000282_c1
Cluster 3	other	17.1	-	-

*Indicates that the biosynthetic gene cluster is complete, therefore it is not split into different contigs.