Genomic data mining of the marine actinomycete *Streptomyces* sp. H-KF8 unveils insights into multi-stress related genes and metabolic pathways involved in antimicrobial synthesis

Agustina Undabarrena [1] , **Juan A Ugalde** [2, 3] , **Michael Seeger** [4] , **Beatriz Cámara** Corresp. [1]

[1] Departmento de Química y Centro de Biotecnología, Universidad Técnica Federico Santa María, Valparaiso, Chile
[2] Centro de Genética y Genómica, Facultad de Medicina Clinica Alemana, Universidad del Desarrollo, Santiago, Chile
[3] Programa de Genómica Microbiana, Facultad de Medicina Clinica Alemana, Universidad del Desarrollo, Santiago, Chile
[4] Departmento de Química & Centro de Biotecnología, Universidad Técnica Federico Santa María, Valparaiso, Chile

Corresponding Author: Beatriz Cámara
Email address: beatriz.camara@usm.cl

Streptomyces sp. H-KF8 is an actinomycete strain isolated from marine sediments of a Chilean Patagonian fjord. Morphological characterization together with the phylogenetic analysis was assessed, and complete 16S rRNA sequence showed a 99.93 % identity to S*treptomyces prasinus* NRRL B-2712^T. Antibacterial assays were performed in various culture media and revealed a carbon-source dependent activity mainly against Gram-positive bacteria. Genome mining of this antibacterial-producing bacterium unveiled the presence of 26 biosynthetic gene clusters (BGCs) for secondary metabolites, where among them, 81 % have low similarities with known BGCs. A genomic search in strain H-KF8 unveiled the presence of a wide variety of genetic determinants related to heavy metal resistance (49 genes), oxidative stress (69 genes) and antibiotic resistance (97 genes). This study revealed that the marine-derived strain H-KF8 has the capability to tolerate a diverse set of heavy metals such as copper, cobalt, mercury, chromium and nickel; as well as the highly toxic tellurite, a feature first time described for *Streptomyces*. In addition, strain H-KF8 possesses a major resistance towards oxidative stress, in comparison to the soil reference strain *Streptomyces violaceoruber* A3(2). Moreover, strain H-KF8 showed resistance to 88% of the antibiotics tested, indicating overall, a strong response to several abiotic stressors. The combination of these biological traits confirms the metabolic versatility of *Streptomyces* sp. H-KF8, a genetically well-prepared microorganism with the ability to confront the dynamics of the fiord-unique marine environment .

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Agustina Undabarrena[1], Juan Antonio Ugalde[2,3], Michael Seeger[1] & Beatriz Cámara[1]

[1] Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso, Chile
[2] Centro de Genética y Genómica, Facultad de Medicina. Clínica Alemana – Universidad del Desarrollo, Santiago, Chile
[3] Programa de Genética Microbiana, Facultad de Medicina, Clínica Alemana – Universidad del Desarrollo, Santiago, Chile.

Corresponding Author:
Beatriz Cámara[1]
Avenida España 1680, Valparaíso, 2000340, Chile
Email address: beatriz.camara@usm.cl

Abstract

Streptomyces sp. H-KF8 is an actinomycete strain isolated from marine sediments of a Chilean Patagonian fjord. Morphological characterization together with the phylogenetic analysis was assessed, and complete 16S rRNA sequence showed a 99.93 % identity to S*treptomyces prasinus* NRRL B-2712^T. Antibacterial assays were performed in various culture media and revealed a carbon-source dependent activity mainly against Gram-positive bacteria. Genome mining of this antibacterial-producing bacterium unveiled the presence of 26 biosynthetic gene clusters (BGCs) for secondary metabolites, where among them, 81 % have low similarities with known BGCs. A genomic search in strain H-KF8 unveiled the presence of a wide variety of genetic determinants related to heavy metal resistance (49 genes), oxidative stress (69 genes) and antibiotic resistance (97 genes). This study revealed that the marine-derived strain H-KF8 has the capability to tolerate a diverse set of heavy metals such as copper, cobalt, mercury, chromium and nickel; as well as the highly toxic tellurite, a feature first time described for *Streptomyces*. In addition, strain H-KF8 possesses a major resistance towards oxidative stress, in comparison to the soil reference strain *Streptomyces violaceoruber* A3(2). Moreover, strain H-KF8 showed resistance to 88% of the antibiotics tested, indicating overall, a strong response to several abiotic stressors. The combination of these biological traits confirms the metabolic versatility of *Streptomyces* sp. H-KF8, a genetically well-prepared microorganism with the ability to confront the dynamics of the fiord-unique marine environment.

Introduction

There has been a burst of genomic data in recent years due to the advances in various technologies such as next-generation sequencing. Whole genome sequencing is providing information-rich data that can hugely contribute and orientate the discovery of natural products (NPs) in microorganisms. Indeed genome mining has been positioned as a fundamental bioinformatics-approach in the NPs field (McAlpine et al., 2005; Van Lanen & Shen, 2006; Challis, 2008; Doroghazi & Metcalf, 2013; Jensen et al., 2014; Antoraz et al., 2015; Tang et al., 2015a; Katz & Baltz, 2016). NPs have clearly demonstrated to play a significant role in drug discovery, in fact 78% of antibiotics marketed during 1982 – 2002 originated from NPs (Peláez, 2006). Considering the year 2014, 25% of the approved new chemical entities were from natural or natural-derived products (Newman & Cragg, 2016). In natural environments, these metabolites also play important roles as signal molecules, facilitating intra- or inter-species interactions within microbial communities related to virulence, colonization, motility, stress response and biofilm formation (Romero et al., 2012).

Streptomyces are mycelium-forming bacteria with a complex developmental life cycle that includes sporulation and programmed cell death processes (Flärdh & Buttner, 2009; Yagüe et al., 2013). Their unsurpassed richness and diversity concerning secondary metabolism pathways has made them valuable providers for bioactive molecules, being responsible for two-thirds of all known antibiotics (Bérdy, 2012). Genome mining has become a powerful tool to unveil the biotechnological potential of *Streptomyces* species, where biosynthetic gene clusters (BGCs) can be identified (Weber et al., 2015) and even predict the chemical core structure of the molecules. Unlike other bacteria, *Streptomyces* have linear chromosomes (Chen et al., 2002) and their genome sizes are within the largest in the bacterial world (Weber et al., 2003), ranging from 6.2 Mb for *Streptomyces cattleya* NRRL 8057 (Barbe et al., 2011) to 12.7 Mb for *Streptomyces rapamycinicus* NRRL 5491 (Baranasic et al., 2013), considering complete sequenced genomes to date (Kim et al., 2015). Up to 5% of their genomes are devoted to the synthesis of secondary metabolites (Ikeda et al., 2003). The ability to produce a wide variety of bioactive molecules is based on the fact that they contain the largest numbers of BGCs such as polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), or even PKS-NRPS hybrids (Challis, 2008). The genes required for secondary metabolites biosynthesis are typically clustered together (Zazopoulos et al., 2003) and are tightly regulated both by specific regulation of each product (Bibb & Hesketh, 2009) or by pleiotropic mechanisms of regulation that can control several pathways at the same time (Martin & Liras, 2012). Due to these interesting properties, nearly 600 species and 30,000 strains of *Streptomyces* have been identified (Euzéby, 2011). To date, 653 *Streptomyces* genome assemblies are available in GenBank database (Studholme, 2016) and this number is likely to keep increasing. Although soil microorganisms from the *Streptomyces* genus have generated vast interest due to their exceptional role as antibiotic producers (Bérdy, 2012), their marine counterpart has been less explored. The marine ecosystem is highly diverse, with extreme abiotic selective pressures and immense biological diversity (Lam, 2006). In addition, many marine organisms have a sessile life style, needing chemical weapons for defense and survival (Haefner, 2003). Thus, research in NPs has been focusing on the isolation of microorganisms from corals (Hodges, Slattery & Olson, 2012; Kuang et al., 2015;

Mahmoud & Kalendar, 2016; Pham et al., 2016), sponges (Kim, Garson & Fuerst, 2005; Montalvo et al., 2005; Zhang et al., 2006; Jiang et al., 2007; Vicente et al., 2013; Sun et al., 2015), as well as marine sediments (Mincer et al., 2002; Magarvey et al., 2004; Jensen et al., 2005; León et al., 2007; Gontang, Fenical & Jensen, 2007; Duncan et al., 2014; Yuan et al., 2014). In spite of all the isolation studies associated to marine actinobacteria, relatively little is known about the molecular mechanisms behind bacterial adaptation to marine environments. It is supposed that marine actinobacteria have adapted through the development of specific biological traits (Tian et al., 2016), which has led to hypothesize that novel species from unexplored habitats may contain unique bioactive compounds (Axenov-Gribanov et al., 2016). In addition, marine habitats are under a dramatic pollution increase, where heavy metals have demonstrated to be one of the most negative causing impacts in living beings. While many metals (Fe, Zn, Mn, Co, Cu, Ni, V, Mo) are essential micronutrients for enzymes and cofactors, they still are toxic when available in high concentrations, causing adversary effects mainly by oxidative stress damage to fundamental macromolecules (Schmidt et al., 2005). In this context, marine microorganisms have developed mechanisms through molecular adaptations in order to thrive in these adverse conditions. Moreover, secondary metabolites biosynthesis are strongly influenced by the presence and concentration of certain heavy metals in *Streptomyces* genus (Locatelli, Goo & Ulanova, 2016), and also oxidative stress can regulate antibiotic production (Kim et al., 2012; Beites et al., 2014) providing evidence of a molecular crosstalk response between these stressors.

In the South Pacific region, Chile has an extensive marine coast that remains mostly unexplored. Bioprospecting of actinobacteria for the discovery of novel marine-derived NP, specifically antibiotics, has been carried out in Valparaíso Central Bay (Claverías et al., 2015) and in the remote Comau fjord in Northern Patagonia (Undabarrena et al., 2016a). Both sites proved to be a rich source for novel species of actinobacteria with antimicrobial properties. In this context, the genome of a selected marine *Streptomyces* strain was sequenced, in order to understand the genetic mechanisms underlying antibiotic biosynthesis and abiotic stress adaptation (Undabarrena et al., 2016b). In this study, we aimed to conduct a combined genomic, metabolic and physiological analysis of the marine *Streptomyces* sp. strain H-KF8 which displays an important antimicrobial activity, along with the genome mining of the BGCs encoded in its genome, and the genetic and functional response to abiotic stressors such as oxidative stress, heavy metals and antibiotics that may play an important role in the evolution of secondary metabolism genes.

Methods and Materials

Phenotypic characterization

Underwater samples were previously collected from marine sediments from the Marine Protected Area of the Comau fjord, in the Northern Chilean Patagonia (Undabarrena et al., 2016a). Actinobacteria were isolated with several culture media and identified through 16S rRNA gene sequence (Undabarrena et al., 2016). Antimicrobial potential was screened, and a *Streptomyces* isolate was selected for complete genome sequencing (Undabarrena et al., 2016b). *Streptomyces* sp. H-KF8 (CCUG 69067) was further characterized morphologically in several media agar plates: ISP1-ISP9 (Shirling & Gottlieb, 1966), Marine Agar (MA) 2216 (Difco) and Triptic Soy Agar (TSA) (Difco N° 236950). All media, with exception of MA, were prepared with artificial sea water (ASW) (Kester et al., 1967) as the strain has a specific ASW requirement for growth (Undabarrena et al., 2016a). Plates were incubated at 30°C and visible colonies appeared after 5-7 days. Microscopic images were obtained with a Leica Zoom2000 stereoscope (Arquimed), an optical microscope L2000A (Arquimed) with 1000X magnification, and unstained low voltage electron microscopy (LVEM) for high contrast images (Delong LVEM5 microscope, Universidad Andrés Bello, Chile) after 21 days of growth in ISP3-ASW media (Vilos et al., 2013).

Phylogenetic analysis

Streptomyces sp. H-KF8 was phylogenetically analyzed based on the full sequence of the 16S rRNA gene, obtained from the genome sequence (GenBank accession number of the whole-genome shotgun project LWAB00000000). Sequence alignment and phylogenetic tree was constructed using MEGA software version 6.0 (Tamura et al., 2013) with Maximum Likelihood algorithm (Felsenstein, 1981). Bootstrap values were based on 1000 replications (Felsenstein, 1985).

Antimicrobial activity

Antimicrobial activity was evaluated previously in ISP2 and TSA-ASW agar plates (Undabarrena et al., 2016a). Here, a further evaluation of antimicrobial activity was assessed in 15 different media agar plates (ISP1-ISP9; TSA-ASW; MA; King B; Medium V (Marcone et al., 2010); LB-ASW; Actino Agar (Difco) and NaST21Cx (Magarvey et al., 2004) by cross-streak method as previously described (Claverías et al., 2015; Undabarrena et al., 2016a). Briefly, antimicrobial activity was measured against five reference bacteria: *Staphylococcus aureus* NBRC 100910^T (STAU); *Listeria monocytogenes* 07PF0776 (LIMO); *Salmonella enterica* subsp enterica LT2^T (SAEN); *Escherichia coli* FAP1 (ESCO) and *Pseudomonas aeruginosa* DSM50071^T (PSAU). Inhibition zones were ranked qualitatively as: -, no inhibition; +/-, attenuated growth of target strain; +, <50% growth inhibition of target strain; ++, 50% growth inhibition of target strain; +++, >50% growth inhibition of target strain. All experiments were performed in duplicate, using as internal control one of the reference strains. Additionally, for the double-layer method (Westerdahl et al., 1991), macrocolonies were incubated on ISP2-ASW, ISP3-ASW, TSA-ASW and MA agar plates. Macrocolonies were grown individually from five to 20 days on the same agar plate, in order to perform a time-course assay to ascertain the days of incubation where

most activity was being produced. Subsequently, 7 mL of modified-LB (7 g/L of agar instead of 15 g/L) with an aliquot of 100 µL of an overnight pre-grown STAU bacterial culture with an OD=0.3 was added above the macrocolonies of *Streptomyces* sp. H-KF8. Inhibition zones were observed after incubation of plates for 24 h at 37 °C. If inhibition zones overlapped, the experiment was repeated on separate agar plates, where only one macrocolony in the center of the plate was incubated, as shown in Figure 4.

Genome Mining and Bioinformatic analysis

Streptomyces sp. H-KF8 genome reads were *de novo* assembled using Canu (version 1.1) (Berlin et al., 2015) into 11 contigs (Undabarrena et al., 2016b). Gene calling an annotation was performed using the Prokaryotic Genome Annotation Pipeline (PGAP) at NCBI (version 3.1) (Tatusova et al., 2016). Genes were assigned to EggNOG categories (Huerta-Cepas et al., 2016) via an HMM search with HMMER3 (http://hmmer.org). Genetic determinants involved in biological traits analyzed in this report were manually established and the amino acidic signatures were validated based on domain hits through Basic Local Alignment Search Tool (BLAST) from NCBI. In addition, BGCs were identified through antibiotics & Secondary Metabolite Analysis Shell (AntiSMASH version 3.0) online platform, and compared to BGCs from literature through the Minimum information about a Biosynthetic Gene cluster (MiBiG) online database. Snapgene software (version 2.3.2) was used to visualize ORFs from each linear contig and from the BGC. Artemis software (version 16.0.0) was used to construct the graphic representation of the circular chromosome, and to assign by colors manually all the different categories of BGCs on it.

Functional response to Heavy Metals and Metalloids

For metal-resistance experiments, agar plates containing filtered salts of several metal(loid) solutions, such as copper, cobalt, zinc, cadmium, mercury, tellurite, chromate, arsenate, arsenite and nickel, were prepared. Metals were diluted to obtain the following final concentration in media plates: $CuSO_4$ (0.25 mM, 0.5 mM and 0.75 mM); $CoCl_2$ (2 mM, 4 mM and 6 mM); $ZnSO_4$ (50 mM and 100 mM); $CdCl_2$ (0.75 mM and 1.5 mM); $HgCl_2$ (20 µM, 40 µM and 60 µM); K_2TeO_3 (10 µM, 20 µM and 40 µM); K_2CrO_4 (10 µM, 17 µM and 20 µM); Na_2HAsO_4 (50 mM and 100 mM); $NaAsO_3$ (2.5 mM and 5 mM) and $NiSO_4$ (5 mM, 10 mM and 15 mM). *Streptomyces* sp. H-KF8 was evaluated after 5, 10 and 20 days of growth in TSA-ASW plates. Additionally, a special Minimal Medium (MM) used to evaluate metal resistance in *Streptomyces* spp. was prepared (Schmidt et al., 2009), modified with the addition of ASW. Experiment was performed with two biological replicates and reference values for metal concentrations were obtained from metal-tolerance literature for *Streptomyces* (Schmidt et al., 2005, 2009; Wang et al., 2006; Polti, Amoroso & Abate, 2007). Agar plates without addition of any metals were prepared as negative controls.

Functional response to Oxidative Stress

For oxidative stress experiments, tolerance to hydrogen peroxide (H_2O_2) at various concentrations (0.2 M, 0.5 M, 1 M, 2 M, and 4 M) was evaluated by directly adding 10 µL of the H_2O_2 solution to a sterile paper disk positioned on a TSA-ASW agar plate where

Streptomyces sp. H-KF8 was streaked out to grow as a thin lawn (Dela Cruz et al., 2010). The model strain *Streptomyces violaceoruber* A3(2) (DSM 40783) was used for comparison purposes. Inhibitions areas (cm^2) were observed after 5 days of growth at 30°C. Experiment was performed with three biological replicates, and standard deviation was calculated. A statistical analysis by Student's *t*-Test was carried out considering a p-value < 0.01.

Functional response Antibiotics

Susceptibility to model antibiotics of *Streptomyces* sp. H-KF8 was explored previously (Undabarrena et al., 2016a). However, in this report a further characterization was pursued. *Streptomyces* sp. H-KF8 was grown on Mueller-Hinton agar plates prepared with ASW (MH-ASW) and disks of model antibiotics were placed above. The following antibiotics were tested: Amoxicillin 25 µM, Bacitracin 0.09 IU, Novobiocin 5 µg and Erythromycin 15 µg (LabClín); Optochin (BritaniaLab); Clindamycin 2 µg, Oxacillin 1 µg, Ciprofloxacin 5 µg, Ceftriaxone 30 µg, Chloramphenicol 30 µg, Penicillin 10 UOF, Cefotaxime 30 µg, Gentamicin 10 µg and Ampicillin 10 µg (Valtek). After 5 days of incubation at 30°C, radios of the inhibition halos were measured and inhibition areas (cm^2) were calculated, and compared with critical values from Clinical and Laboratory Standards Institute (CLSI) from year 2016, to determine susceptibility or resistance against each antibiotic tested. Experiments were performed using three biological replicates, and standard deviation was calculated.

Results

Phenotypic characterization

Morphological analysis of *Streptomyces* sp. H-KF8 was carried out by strain growth in several media. Growth was observed in ISP1-ISP9 agar plates, and differences in pigmentation were noted. On ISP1, ISP2 and ISP6 media, white mycelia was observed, with appearance of grayish-spores after 14 days of growth (Fig 1A). In contrast, when *Streptomyces* sp. H-KF8 was grown on ISP3, ISP4, ISP5, ISP7 and ISP9 media, a creamy mycelia was observed, with appearance of white spores at the periphery of the colonies (Fig 1B and 1C). A different morphology was perceived when grown on MA medium. Colony size was comparatively smaller, and a dark-grey turning into black pigmentation was noticed (Fig 1D). On TSA-ASW plates, a white mycelium was observed with no change in pigmentation over time, but with presence of exudate drops in the colony surface (Fig 1E and 2A). Morphology was visualized microscopically, and typical structures of development such as hyphae and spores were observed (Fig 2B). Moreover, a complex network of intertwined hyphae and early spore chain assemblies was observed by LVEM microscopy, which is characteristic of this genus (Fig 2C).

Phylogenetic analysis

Phylogenetic relationships of *Streptomyces* sp. H-KF8 considering complete 16S rRNA gene sequence are depicted in (Fig 3). Strain H-KF8 clusters together with related microorganisms that have varied origin sources, such as from soil (highlighted in brown), plant- (green) and insect-associated (yellow). Interestingly, *Streptomyces* sp. H-KF8 is the only isolate of this clade that comes from a marine environment (blue). This suggests an evolutionary early divergence of their common ancestor. Moreover, *Streptomyces* sp. H-KF8 showed a 99.93 % of 16S rRNA gene identity with its closest type strain, *S. prasinus* NRRL B-2712^T, which was isolated from a soil sample of Mallorca, Spain, characterized to form greenish-grey spores (Ettlinger, Corbaz & Hütter, 1958). Also, it presents 99.86 % of 16S rRNA gene identity with *S. bambergiensis* NRRL B12521^T, which is known to be the producer of the antibiotic moenomycin and was isolated from soil of Bamberg, Germany (Wallhausser et al., 1965).

Antimicrobial activity

Antimicrobial activity of *Streptomyces* sp. H-KF8 was further characterized using agar media with different carbon sources (Table 1). In general, antimicrobial activity was more evident against Gram-positive reference bacteria (STAU and LIMO), although inhibition against ESCO was also observed in most media. PSAU was the reference bacterium less inhibited. Among the 15 different media used, inhibition of at least one reference bacteria was noted in 87% of the media. Best media for antimicrobial activity were ISP1, ISP2, ISP6, and V media, where inhibition of four of the five reference bacteria was observed. Notably, in ISP2 medium a unique attenuation of PSAU growth was observed. Alternatively, a time-course assay using the double-layer method was performed to visualize the starting day of the antimicrobial activity, in the four of the media with best results previously tested. Even though at day 5 a relatively scarce colony growth of strain

H-KF8 was observed in ISP2 medium, at day 6 it was possible to visualize a modest inhibition against STAU (Fig 4A). Yet, inhibition zone increased as incubation time for strain H-KF8 extended, as shown in Fig 4B, showing a maximum halo size at day 15 (Fig 4C).

Bioinformatic analysis and Genome mining for BGCs

Whole genome sequencing and genome features were previously described (Undabarrena et al., 2016b). Briefly, *Streptomyces* sp. H-KF8 genome was assembled into 11 contigs, with a total genome length of 7,684,888 bp, and a G+C content of 72.1 %. A total of 6,574 genes are represented among 6,486 CDS, 67 tRNAs and 6 16S rRNAs. Genes with coding sequences were grouped into COGs categories, and 808 genes were not grouped in COGs. Description and gene percentage of each category is depicted in Table 2. For *Streptomyces* sp. H-KF8, the most abundant categories were transcription (522 genes), carbohydrate transport and metabolism (362 genes), and amino acid transport and metabolism (362 genes). The *Streptomyces* sp. H-KF8 categorized genes were compared to the model *Streptomyces violaceoruber* A3(2) isolated from soil (Bentley et al., 2002) and the marine *Streptomyces sp.* TP-A0598 (Komaki et al., 2015), in order to obtain insights of marine adaptation (Fig 5). While all three strains showed the same tendency in the categories previously named in terms of abundancy, differences were observed in terms of percentage in transcription and carbohydrate metabolism categories, where *S. violaceoruber* A3(2) strain was slightly higher. On the other hand, both marine strains showed higher number of genes for post-translational modification, protein turnover and chaperone function category, as well as in secondary metabolism and translation categories.
Secondary metabolism category comprises 2.3 % of the *Streptomyces* sp. H-KF8 genome, being slightly higher than when compared to both the *S. violaceoruber* A3(2), and *Streptomyces sp.* TP-A0598 strains, with 1.9 % and 2.0 % respectively. In this line, a bioinformatic analysis was performed using the antiSMASH tool to detect biosynthetic gene clusters (BGCs) that may explain the antimicrobial activity observed in *Streptomyces* sp. H-KF8, and a total of 26 BGCs were detected. In this report, we show that the spatial distribution of the BGCs are evenly allocated throughout the contigs of *Streptomyces* sp. H-KF8 genome (Fig 6). Furthermore, a comparison of these BGCs to already known BGCs through MiBiG database was performed. In this line, *Streptomyces* sp. H-KF8 bears two NRPSs BGCs with very low similarity to BGCs involved in the synthesis of the lipoglycopeptide antibiotic mannopeptimycin produced by *S. hygroscopicus* (Magarvey et al., 2006) and the streptolydigin antibiotic which interferes with the RNA elongation by inhibition of the bacterial RNA polymerase (Olano et al., 2009), with 7% and 13% of gene similarity, respectively (Table 3). The two PKSs predicted in *Streptomyces* sp. H-KF8 genome corresponds to the type II spore pigment BGC and also another BGC where only 6 % of gene similarity to the antibacterial kirromycin BGC from *S. collinus* Tü 365 was found (Weber et al., 2008) (Table 3). A total of eight hybrid clusters, where four of them are PKS-NRPS hybrids were also predicted (Table 3). In addition, other BGCs included five terpenes, two lantipeptides and two ribosomally synthesized and post-translationally modified peptides (RiPPs) such as the lassopeptide and bacteriocin BGCs. In general, only five BGCs from *Streptomyces* sp. H-KF8 genome displayed 100 % gene similarity to their most related known cluster. Examples of these consists on the BGC for the previously mentioned antibiotics moenomycin (Ostash, Saghatelian & Walker, 2007) and

albaflavenone (Zhao et al., 2008) (Table 3). Additionally, BGCs for the aromatic carotene isorenieratene, involved in anoxigenic photosynthesis in *S. griseus* (Krügel et al., 1999), the conserved osmolite ectoine, that may provide protection from osmotic stress (Prabhu et al., 2004; Graf et al., 2008) and the melanin pigment clusters (Guo et al., 2014; Sivaperumal, Kamala & Rajaram, 2015) were observed with 100 % similarity. The remaining of the BGCs presented low similarity to BGCs of known compounds, evidencing the potential of *Streptomyces* sp. H-KF8 strain to produce novel bioactive molecules.
Moreover, genome mining of pathways involved in response to abiotic stressors such as heavy metals, oxidative stress and antibiotics were analyzed, in order to unveil genetic determinants that may explain tolerance to stressful environmental conditions.

Functional response to Heavy Metals and Metalloids

Genetic determinants involved in heavy metal-resistance in *Streptomyces* sp. H-KF8 were analyzed by genome mining, and at least 49 genes may be playing a role in such tolerance (Fig 7A). Amongst these, the most abundant genes were related to tellurite, followed by arsenic, copper and mercury, and, to a lesser extent, chromate, nickel and cobalt tolerance. Tellurite resistance genes involved seven *terD* genes, four *terB* genes, two *yceC* genes, one *terC* gene and one *tehB* gene encoding a tellurite methyltransferase. In addition, 11 genetic determinants for arsenic tolerance were found, involving three *arsC* genes encoding arsenate reductases, two genes *arsA* encoding arsenical pump-driving ATPases, five genes *arsR* encoding arsenical transcriptional regulators, and the arsenical resistance protein encoding gene *acr3*. Genetic determinants encoding for copper resistance genes, included *copA* and *mco* genes encoding multicopper oxidases, *copD* encoding a copper resistance protein, two genes *ycnJ* encoding for copper transport proteins, and two genes for the copper-sensing transcriptional regulator, *csoR*. Mercury resistance genes consisted in the mercury reductase encoding gene *merA*, and the mercury transcriptional regulator *merR*. In addition, the *czcD* and *rcnA* genes coding for efflux pumps for cadmium, zinc, cobalt and nickel, respectively, together with the *chrR* gene encoding a chromate reductase, and general heavy metal tolerance such as the *hmt1* gene, were also found. Considering all the genetic determinants listed above, we attempted to determine if *Streptomyces* sp. H-KF8 was able to grow on various metal-containing media. *Streptomyces* sp. H-KF8 was able to tolerate copper, cobalt, mercury, tellurite, chromium and nickel, as shown in Fig 7B. Despite the arsenic tolerance-related genes present in strain H-KF8 genome, comprising 27 % of the total number of metal-related genes, no evident growth of *Streptomyces* sp. H-KF8 was perceived, even in the two different toxic forms of arsenic: arsenate and arsenite. Also, no growth was observed in media containing cadmium or zinc (Fig 7B).

Functional response to Oxidative Stress

A significant amount of genes (69 genes) that may participate in the detoxification of reactive oxygen species (ROS) were found within the *Streptomyces* sp. H-KF8 genome. Genes for mycothiol biosynthesis (20 genes), thioredoxin and thioredoxin reductases system (11 genes), alkyl hydroperoxide reductases (nine genes), glutaredoxin and glutathione peroxidase system (four genes), catalases (three genes), and superoxide dismutases (three genes), among others, were identified (Fig 8A). Interestingly, genes involved in osmotic stress detoxification of chlorinated and brominated compounds such as

three *bpo* genes encoding for bromoperoxidases, one *cpo* gene encoding for a chloroperoxidase and one gene encoding for a chlorite dismutase were also present in *Streptomyces* sp. H-KF8 genome. Concerning transcriptional regulators controlling the redox balance, transcriptional factors from *perR*, *rex*, *lysR* and *soxR* families, were also present. Response of *Streptomyces* sp. H-KF8 to H_2O_2 was compared to *S. violaceoruber* A3(2). At various H_2O_2 concentrations, *Streptomyces* sp. H-KF8 displayed smaller inhibition areas than *S. violaceoruber* A3(2) (Fig 8B and 8C, respectively). A significant difference at 1, 2 and 4 M concentrations of H_2O_2 indicates a major resistance response of *Streptomyces* sp. H-KF8 towards H_2O_2 toxicity (Fig 8D).

Functional response to Antibiotics

Antibiotic-producing *Streptomyces* strains usually code resistant genes to protect themselves against the noxious action of the synthetized compound. Resistance of *Streptomyces* sp. H-KF8 to commercial antibiotics with different biological targets was explored. Genome mining revealed more than 90 genes that could be involved in antibiotic resistance. The most abundant genes encode for bleomycin resistance proteins (24 genes). Specific resistance genes related to modification and inactivation of antibiotics such as aminoglycoside phosphotransferases (eight genes), β-lactamases (three genes), metallo-β-lactamases (three genes), and one gene for erythromycin esterase and penicillin amidase, respectively, were identified. In addition, genes for efflux of toxic compounds including multidrug resistance proteins (20 genes), daunorubicin/doxorubicin ABC transporter permeases (15 genes), multidrug ABC transporters (seven genes) and one gene encoding for a multidrug MFS transporter, were detected. Among the transcriptional regulators, the TetR-family transcriptional regulators were the most abundant, with 10 genes. Also, the MarR-family transcriptional regulator and three *marR* genes encoding for multiple antibiotic resistance proteins were identified (Fig 9A). In the functional assay against 16 different antibiotics tested, *Streptomyces* sp. H-KF8 exhibited an 88 % of resistance-response, being susceptible only to the antibiotics: novobiocin, which targets the DNA gyrase, and gentamicin, which inhibits protein synthesis by irreversibly binding to the 30S subunit of the bacterial ribosome (Fig 9B).

411 **Discussion**
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413 In this report, phenotypic analysis of the fljord-derived marine *Streptomyces* sp. H-KF8 in
414 several agar media was assessed, revealing in general one week of incubation time to obtain
415 colonies and two weeks for sporulation; although growth rates, sporulation rates and
416 pigmentation differs throughout the different media used. *Streptomyces* genus is
417 characterized for slow growth and a complex developmental life cycle (Flärdh & Buttner,
418 2009). Physiological differentiation is tightly linked to secondary metabolism and hence,
419 sporulation capacities of *Streptomyces* might enhance the discovery of new compounds
420 (Chater, 2013; Kalan et al., 2013; Zhu et al., 2015). Therefore, in this report an extended
421 evaluation of morphological features along with antimicrobial activities in 15 different
422 media was pursued, showing a maximum inhibition halo against STAU when *Streptomyces*
423 sp. H-KF8 was growth for 15 days, in agreement with sporulation rates. As *Streptomyces*
424 are ubiquitous in different natural environments, they must compete with other fast-
425 growing microorganisms for nutrients in order to survive. To confront this, *Streptomyces*
426 are armed with a wide range of secondary metabolites, which may act as antibacterial
427 and/or antifungal substances that help them to thrive adverse conditions (Ruiz et al., 2010).
428 In this context, marine actinobacteria have demonstrated to provide a huge resource for
429 structurally diverse NP with biotechnological activities (Subramani & Aalbersberg, 2012;
430 Valliappan, Sun & Li, 2014). Antibiotics synthesis is regulated by environmental nutrients
431 such as carbon sources. Consequently, media carbon source has an important effect on
432 antibiotic production, being demonstrated that when bacteria are grown with a preferred
433 carbon source, secondary metabolism seems repressed (Sánchez et al., 2010). This may
434 explain the differences in inhibition patterns observed for *Streptomyces* sp. H-KF8 in the 15
435 different media tested. Due to the interesting antibacterial activity displayed, its whole-
436 genome was sequenced and primary features were previously reported (Undabarrena et al.,
437 2016b). Thus, in this report a further analysis of genome mining for *Streptomyces* sp. H-
438 KF8 was performed, in order to gain insights into the mechanisms by which it displays
439 antibiotic biosynthesis and resistance to multiple stressors.
440 The term ‘genome mining’ has been used in various fields to describe the exploitation of
441 genomic information for the discovery of new processes, targets and products (Challis,
442 2008). Through genome sequencing and bioinformatic analysis using antiSMASH platform
443 (Medema et al., 2011; Blin et al., 2013; Weber et al., 2015), it is possible to address the
444 secondary metabolic potential of a strain by identification of its biosynthesis gene clusters
445 (BGCs) (Iftime et al., 2016). Previously, 26 BGCs were detected in *Streptomyces* sp. H-
446 KF8 genome (Undabarrena et al., 2016b). These include two NRPSs, two PKSs and four
447 hybrid PKS-NRPS, among others. In this report, the distribution of these BGCs along
448 *Streptomyces* sp. H-KF8 genome was determined, in addition with a comparison with
449 known BGCs through the MiBiG database was aimed, which compiles a total of 1,170
450 experimentally characterized known gene clusters (Medema et al., 2015). Notably,
451 *Streptomyces* sp. H-KF8 presented only five BGCs with full similarity to their most similar
452 known cluster; suggesting that most secondary metabolites produced by *Streptomyces* sp.
453 H-KF8 are yet to be elucidated, and can contribute to the discovery of novel NP. In this
454 context, genome mining has proven to be a fundamental tool for genome-based NP
455 discovery (Jensen et al., 2014), and has guided the discovery of novel NP from several
456 marine actinobacteria (Gulder & Moore, 2010; Tang et al., 2015b). Among these are the
457 aromatic polyketide angucyclinone antibiotic (Zhang et al., 2012) and polyene macrolides

with antifungal activity (Tang et al., 2015a). Moreover, metabolites are produced by different metabolic pathways in comparison to their terrestrial counterparts (Li et al., 2011; Lee et al., 2014; Barakat & Beltagy, 2015). These metabolites emerge as a result of the unique and dynamic conditions of the ocean, such as high hydrostatic pressure, low temperature, variation in salinity, and depletion of micronutrients proper of the marine environment (Das, Lyla & Khan, 2006; Lam, 2006; de Carvalho & Fernandes, 2010). Despite that marine environmental adaptations are scarcely studied, recent comparative genomics of marine-derived *Streptomyces* unveiled an enrichment in TrK and BCCT transporters, along with the observation that these genomes are generally smaller in size and have a slightly higher GC content in comparison to *Streptomyces* from other environmental sources (Tian et al., 2016). *Streptomyces* strain H-KF8 genome is consistent with these findings, holding distinctive biological and genomic signatures acknowledged for marine *Streptomyces* strains. Therefore, it is suggested that its metabolite biosynthesis may be under marine abiotic selective pressures, which may be modulating secondary metabolism production.

Another study of comparative genomics of completely sequenced *Streptomyces* obtained from several isolation sources revealed that the most abundant COG categories were transcription (K), followed by carbohydrate metabolism (G) and amino acid metabolism (E) (Kim et al., 2015). This is in agreement with the most abundant categories found in the *Streptomyces* strain H-KF8 genome. Furthermore, in marine-derived *Streptomyces* a higher proportion of genes belonging to COG categories of translation (J), and post-translational modification, protein turnover and chaperones (O) was observed (Tian et al., 2016). Accordingly, in this report the (J) and (O) COGs categories were also overrepresented in both marine strains analyzed, *Streptomyces* strain H-KF8 and *Streptomyces* sp. TP-A0598 (Komaki et al., 2015), in comparison to the terrestrial *Streptomyces violaceoruber* A3(2) (Bentley et al., 2002). This may indicate an important role of protein metabolism in marine environments, probably due to the active responses against abiotic stressors and the dynamics that microorganisms have to overcome to survive in these extreme ecosystems. In addition, our analysis showed an increase in the categories of cell cycle control, cell division, chromosome portioning (D), secondary metabolism (Q) and defense mechanisms (V), for both marine strains in comparison to *Streptomyces violaceoruber* A3(2). Percentage of the COG category for defense mechanisms (V) in *Streptomyces* strain H-KF8 was interestingly higher (2,81 %) than in *Streptomyces* sp. TP-A0598 (1,8 %), and comparatively similar with what was observed for deep-sea bacteria (3,0 %) (Qin et al., 2011). As the defense mechanism category includes genes for resistance to heavy metals, osmotic and oxidative stress as well as antibiotics, the functionality of these biological traits was evaluated for *Streptomyces* strain H-KF8, and notably, an important resistance to these multiple stressors was evidenced.

Environmental pollution by heavy metals can arise due to anthropogenic and/or geogenic sources. Although some metal-resistant strains isolated from contaminated areas have been described (Amoroso et al., 2001; Schmidt et al., 2005, 2009; Polti, Amoroso & Abate, 2007; Albarracin et al., 2008; Haferburg et al., 2008; Siñeriz, Kothe & Abate, 2009; Lin et al., 2011; El Baz et al., 2015), there is limited information about the physiology of *Streptomyces* in presence of environmental metal pollutants. Due to the naturally high concentrations of certain heavy metals in Chilean northern patagonia (Guevara et al., 2004; Revenga et al., 2012; Hermanns & Biester, 2013) product of the highly active seismic and volcanic activity (Pantoja, Luis Iriarte & Daneri, 2011), the ability of *Streptomyces* sp. H-

KF8 to grow in several metal(loid)s supplemented media was evaluated. Surprisingly, resistance to copper, cobalt, mercury, tellurite, chromate and nickel was revealed. Interestingly, the most abundant genes in *Streptomyces* sp. H-KF8 were related to tellurite resistance, involving the tellurite methyltransferase (encoded by *tehB*) and several tellurite resistance genes (*terB, terC, terD, yceC*). Although the *ter* operon has been described previously (Taylor, 1999), specification of its mechanism of action remains obscure (Chasteen et al., 2009). Mainly, it has been shown that tellurite detoxification is via enzymatic reduction by several flavoprotein-mediated non-specific metabolic enzymes (Arenas-Salinas et al., 2016), or by non-enzymatic mechanisms mediated by intracellular thiols like glutathione (Turner et al., 2001). Either way, tellurite reduction generates oxygen reactive species (ROS), especially superoxide anion (O_2^-), which is deleterious to fundamental cell macromolecules producing protein oxidation, lipid peroxidation and DNA damage (Pérez et al., 2007; Tremaroli, Fedi & Zannoni, 2007). Surprisingly, *Streptomyces* sp. H-KF8 did not show black pigmentation after tellurite exposure, which is a distinctive phenotype that indicates tellurite reduction to elemental tellurium (Taylor, 1999), suggesting that other mechanisms of resistance could be involved in *Streptomyces* sp. H-KF8. To our knowledge, this is the first tellurite-resistant *Streptomyces* strain described so far.

Additionally, resistance to mercury at a concentration of 60 µM was observed for *Streptomyces* sp. H-KF8. In general, bacteria capable of resisting mercury above 20 µM, should possess specific detoxification systems, as mercury is one of the most toxic elements on earth and produces several health concerns for macroorganisms as well as microorganisms (Das, Dash & Chakraborty, 2016). In bacteria, two different resistance operons are known, the basic narrow-spectrum *mer* operon *merRTPA* for inorganic mercury, and the broad-spectrum operon that additionally contains *merB*, which provides protection against organo-mercurial compounds (Barkay, Miller & Summers, 2003). In addition, it was recently demonstrated that mercury resistance mechanisms could also be involved in tellurite cross-resistance (Rodriguez-Rojas et al., 2015). Studies in *Streptomyces* includes *S. lividans* 132, that carries two divergently transcribed operons named *merAB* and *merRTP* in the chromosome (Sedlmeier & Altenbuchner, 1992; Brünker et al., 1996; Rother, Mattes & Altenbuchner, 1999), and two *Streptomyces* spp. strains isolated from estuarine sediments where these genes were also observed in giant linear plasmids (Ravel, Schrempf & Hill, 1998; Ravel et al., 2000). Interestingly, the genetic operons mentioned above were not detected in *Streptomyces* sp. H-KF8, despite the fact that a mercury-resistance phenotype was evidenced. Instead, the presence of two mercury-related genes, the transcriptional regulator *merR* and the mercuric reductase *merA*, may be playing a role in such resistance. MerA is a flavoprotein NADPH-dependent enzyme responsible for the reduction of Hg^{2+} to the elemental and less toxic volatile Hg^0 (Barkay, Miller & Summers, 2003). Similarly, evidence of functional operons conformed either by solely *merA* or *merRA* have been previously reported in archaea (Boyd & Barkay, 2012). However, no evident growth was observed in the presence of arsenate or arsenite, although *Streptomyces* sp. H-KF8 bears at least 11 genetic determinants that could be involved in its detoxification. In general, the arsenic resistance operon consists of *arsRABCD* genes, where *arsC* encodes for an arsenate reductase that converts As^{5+} to As^{3+}, which is then exported through the ArsAB ATPase-efflux pump. The two other genes play a minor role by acting as a *trans*-responsive transcriptional repressor (*arsR*), and as a metallochaperone for the arsenite efflux system (*arsD*) (Hobman & Crossman, 2014). In *Streptomyces* sp. H-KF8,

arsA, *arsC* and *arsR* genes are present, but lack the *arsB* gene, which encodes an arsenite antiporter, crucial for anchoring ArsA to the inner membrane with concomitant detoxification of arsenite. Absence of the *arsB* gene may explain the sensitivity of *Streptomyces* sp. H-KF8 towards these toxics. Arsenic resistance genes are generally widespread amongst both Gram-positive and Gram-negative bacteria, reflecting its broad distribution in the environment (Silver & Phung, 2005). In fact, these genes were also conserved in several marine streptomycetes from the South China Sea (Tian et al., 2016).
Streptomyces sp. H-KF8 displayed a notorious copper-resistant phenotype, concordant with the detection of three *copA* genes encoding for multicopper oxidases that may be responsible for the oxidation of Cu^{+} to its less toxic form Cu^{2+} (Hobman & Crossman, 2014). Copper is an essential metal for living beings, but at higher concentrations it is extremely toxic (Gaetke & Chow, 2003). For this reason, it has been used as antimicrobial, algicidal, pesticidal and antifungal in agriculture (Russell, 2005) thus becoming an important environmental pollutant. Moreover, Chile is the major copper-producing country in the world, due its geological nature (Wacaster, 2015). Hence, the widespread of copper resistant genetic determinants that has been demonstrated in Chilean marine sediments (Besaury et al., 2013) is expected.
Resistance to nickel and cobalt in *Streptomyces* sp. H-KF8 might be given by the *rcnA* gene that participates in the efflux system of these metals. Highly nickel- and cobalt-resistant *Streptomyces* were found in an acid mine drainage, where growth in media containing up to 10 mM Ni or 3 mM Co was observed (Schmidt et al., 2005). In this report, *Streptomyces* sp. H-KF8 was able to grow even at higher concentrations: 15 mM Ni and 6 mM Co, respectively. Furthermore, chromate toxicity (20 mM) might be overcome in *Streptomyces* sp. H-KF8 due to the presence of the *chrR* gene encoding a chromate reductase involved in the enzymatic reduction of Cr^{6+} to the less harmful Cr^{3+} cation (Das, Dash & Chakraborty, 2016). Previously reported *Streptomyces* chromate-resistant strains isolated from sugar cane plant were able to grow in 17 mM, where also chromate-removing activity was demonstrated (Polti, Amoroso & Abate, 2007).
On the other hand, *Streptomyces* sp. H-KF8 does not grow when exposed to zinc or cadmium, although it bears the *czcD* gene; probably due to the absence of the rest of the genes of the *czcCBAD* operon. This operon involves a transporter (CzcA), a membrane fusion protein (CzcB), the integral outer membrane protein (CzcC) and the transcriptional regulator (CzcD). This operon is known to play a role in the detoxification of cobalt, zinc and cadmium by transporting them across the cytoplasmic membrane, periplasm and outer membrane (Rensing, Pribyl & Nies, 1997).
Metal exposure and adverse abiotic environmental factors produces a general condition of oxidative stress in microorganisms. As oxidative stress is hazardous for fundamental macromolecules, bacteria have evolved several mechanisms to protect themselves from these environmental stresses. In *Streptomyces* sp. H-KF8, an exceptional response to several concentrations of H_2O_2 was observed, compared to the model *Streptomyces violaceoruber* A3(2) which was more susceptible towards the toxic. Consequently, a wide number of genetic determinants related to ROS response were present in the *Streptomyces* sp. H-KF8 genome. Remarkably, a high number of thioredoxins (*trx*) and alkyl hydroperoxide reductases (*ahp*) genes (nine of each) were found in *Streptomyces* sp. H-KF8, in comparison with *Streptomyces violaceoruber* A3(2) where five and one genes were described, respectively. The *ahp* and *trx* are fundamental H_2O_2-inducible genes that encodes for enzymes known to participate in the bacterial response to oxidative stress,

599 which are regulated by *oxyR* in *E. coli* (Storz & Imlay, 1999; Seaver & Imlay, 2001;
600 Chiang & Schellhorn, 2012). The *oxyR* regulon is not present in *Streptomyces* sp. H-KF8,
601 but instead two copies of the *perR* regulator fulfill its role in Gram-positive bacteria (Ricci
602 et al., 2002; Dubbs & Mongkolsuk, 2012). Also, the *ohrR* transcriptional regulator that
603 senses organic peroxide (ROOH) and sodium hypochlorite (NaOCl) (Dubbs &
604 Mongkolsuk, 2012) was found in *Streptomyces* sp. H-KF8. In addition, several genes
605 regulated by the *soxR* transcriptional regulatory system such as glutaredoxin and
606 glutathione peroxidase, superoxide dismutases (*sod),* catalases (*kat*) and thioredoxin
607 reductases were recognized in *Streptomyces* sp. H-KF8 genome, which overall may be
608 accounting for it resistance through H_2O_2 exposure. Even more, the chromate reductase
609 (*chrR*) previously mentioned, could also provide additional protection against H_2O_2 (Das,
610 Dash & Chakraborty, 2016). Interestingly, unusual genes encoding for bromoperoxidases,
611 chloroperoxidases and chlorite dismutases, involved in osmotic stress detoxification of
612 brominated and chlorinated toxic compounds which are abundant in the marine
613 environments (Sander et al., 2003; Bouwman et al., 2012), were also present in
614 *Streptomyces* sp. H-KF8 genome. On the other hand, *Streptomyces violaceoruber* A3(2)
615 possess only one chloroperoxidase, suggesting that this might represent another marine
616 adaptation trait for *Streptomyces* sp. H-KF8. Osmotic and oxidative stress response seems
617 to be regulated via a network of sigma factors in *Streptomyces violaceoruber* A3(2), that
618 controls the activation of several oxidative defense proteins, chaperones and systems that
619 provide osmolytes and mycothiol (Lee et al., 2005). Consistently, a high amount of genes
620 for mycothiol biosynthesis was identified in *Streptomyces* sp. H-KF8. Mycothiol is the
621 major low-molecular-weight (LMW) thiol present in actinobacteria, and serves as a buffer
622 to advert disulfide stress, in complement of the enzymatic system presented above
623 (Buchmeier & Fahey, 2006; den Hengst & Buttner, 2008).
624 Recently, evidence of heavy metal driving co-selection of antibiotic resistance in both
625 natural environments (Seiler & Berendonk, 2012) and contaminated ones (Li, Li & Zhang,
626 2015; Henriques et al., 2016) have been reported. In this line, isolation of *Streptomyces*
627 with both metal and antibiotic co-resistances have been described (Van Nostrand et al.,
628 2007). Also, co-evolution of resistance within closely related antibiotic-producing bacteria
629 has been demonstrated for *Streptomyces* (Laskaris et al., 2010). Hence, the antibiotic
630 response against pharmaceutical compounds was investigated in *Streptomyces* sp. H-KF8,
631 and resistance was observed to all antibiotics tested, with exception of gentamicin and
632 novobiocin. The phenomena of widespread distribution antibiotic resistance genes in
633 natural environments is consequence of improper use of antibiotics in medical treatment, as
634 well as by an indiscriminate use in agriculture, livestock and aquaculture (Brown et al.,
635 2006). Phenomena such as the grasshopper effect may also contribute to the rapid transport
636 of toxics around the globe through atmospheric and oceanic currents (Sadler & Connell,
637 2012).
638 Overall, our study shows the response of a marine *Streptomyces* strain H-KF8 against
639 several abiotic stressors such as heavy metals, oxidative stress and antibiotics, along with
640 the genome mining of the biosynthetic gene clusters that could be involved in the
641 antimicrobial activity observed. Altogether, these biological features may enable
642 *Streptomyces* strain H-KF8 to thrive in the complex marine environment.
643

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1083 Table 1. Antibacterial activity of *Streptomyces* sp. H-KF8 in several culture media.

Medium	Inhibition of bacterial strains*				
	STAU	LIMO	PSAU	SAEN	ESCO
ISP1	+++	+++	-	+++	+++
ISP2	+++	+/-	+/-	-	+
ISP3	+	+	-	-	+
ISP4	+	-	-	-	-
ISP5	+	-	-	-	-
ISP6	++	+++	-	+	+
ISP7	+++	-	-	+/-	++
ISP9	+++	+++	-	-	-
TSA-ASW	+++	+/-	-	-	+
MA	+++	+++	-	-	++
King B	-	+/-	-	-	-
Medium V	++	++	-	+/-	+++
LB-ASW	+++	++	-	+/-	-
Actino Agar	-	-	-	-	-
NaST21Cx	-	-	-	-	-

1084 *-, no inhibition; +/-, attenuated growth; +, <50% growth inhibition; ++, 50% growth
1085 inhibition; +++, >50% growth inhibition
1086

1087 Table 2. COGs distribution of genes with coding sequences in *Streptomyces* sp. H-KF8.

COG functional categories	Abbreviation	N° of genes	Percentage (%)
Energy production and conversion	C	275	4.18
Cell division and chromosome partitioning	D	41	0.62
Amino acid transport and metabolism	E	322	4.90
Nucleotide transport and metabolism	F	89	1.35
Carbohydrate transport and metabolism	G	362	5.51
Coenzyme transport and metabolism	H	136	2.07
Lipid metabolism	I	142	2.16
Translation	J	168	2.56
Transcription	K	522	7.94
DNA replication and repair	L	217	3.30
Cell envelope biogenesis, outer membrane	M	169	2.57
Cell motility	N	0	0.00
Post-translational modification, protein turnover, chaperones	O	135	2.05
Inorganic ion transport and metabolism	P	223	3.39
Secondary metabolism	Q	148	2.25
General function prediction only	R	238	3.62
Function unknown	S	2111	32.11
Signal transduction	T	283	4.30
Defense mechanisms	V	185	2.81
Not in COGs	-	808	12.29

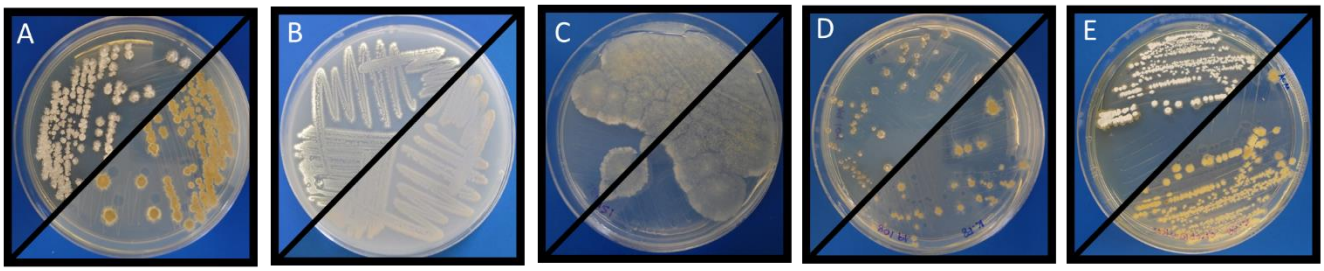
1088
1089

1090 Table 3. Biosynthetic gene clusters for secondary metabolites in *Streptomyces* sp. H-KF8.

antiSMASH type descriptor	Scaffold	Length (bp)	Predicted product (%[a])	MIBiG-ID
NRPS	tig_02	81285	Streptolydigin (13%)	BGC0001046
NRPS	tig_02	79174	Mannopeptimycin (7%)	BGC0000388
PKS T1	tig_138	33925	Kirromycin (6%)	BGC0001070
PKS T2	tig_139	42512	Spore pigment (83%)	BGC0000271
NRPS-PKS T1	tig_138	50808	SGR PTMS (100%)	BGC0001043
NRPS-PKS T1	tig_139	52764	Neomycin (5%)	BGC0000710
NRPS-PKS T1	tig_02	56103	Himastatin (12%)	BGC0001117
NRPS-PKS T3	tig_02	54318	Furaquinocin A (21%)	BGC0001078
Terpene-Siderophore	tig_02	50603	Isorenieratene (100%)	BGC0000664
Nucleoside-Phosphoglycolipid	tig_00	35469	Moenomycin (100%)	BGC0000805
Oligosaccharide-PKS T1	tig_16	42574	Stambomycin (52%)	BGC0000151
Lantipeptide-PKS T1	tig_138	61004	unknown	-
Terpene	tig_02	26858	Hopene (76%)	BGC0000663
Terpene	tig_00	20992	unknown	-
Terpene	tig_02	21253	unknown	-
Terpene	tig_02	22162	unknown	-
Terpene	tig_138	21220	Albaflavenone (100%)	BGC0000660
Lantipeptide	tig_02	21819	unknown	-
Lantipeptide	tig_139	24585	unknown	-
Bacteriocin	tig_02	11412	unknown	-
Lassopeptide	tig_10	22692	unknown	-
Siderophore	tig_139	11808	Desferrioxiamine B (83%)	BGC0000940
Butyrolactone	tig_14	11073	Griseoviridin/Viridogrisein (11%)	BGC0000459
Ectoine	tig_139	10398	Ectoine (100%)	BGC0000853
Melanin	tig_139	10509	Melanin (100%)	BGC0000910
Other	tig_00	43290	Stenothricin (13%)	BGC0000431

1091 [a] Percentage of genes from known BGCs that show similarity to genes predicted for BGCs
1092 from strain H-KF8.
1093

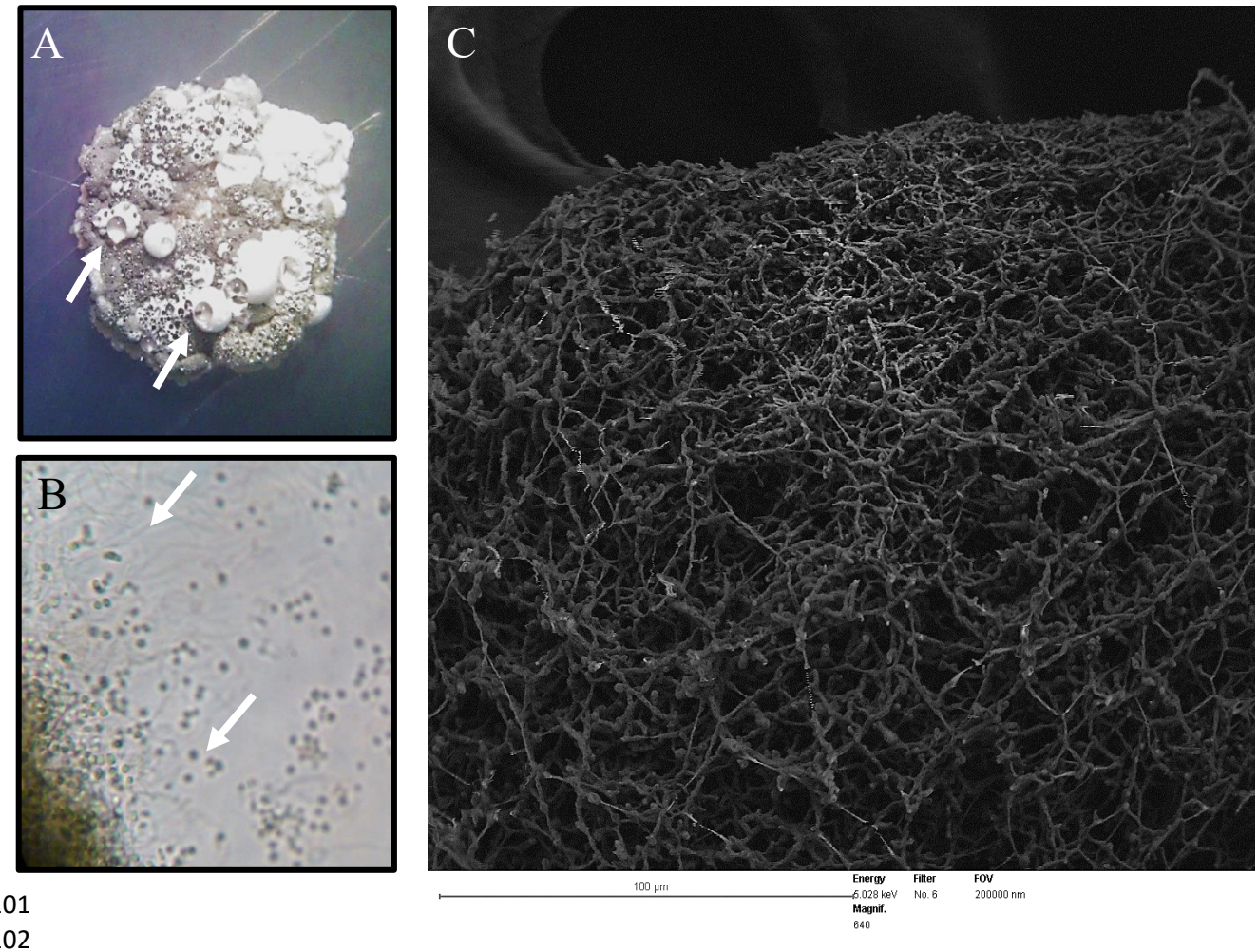
1094 Fig. 1



1095

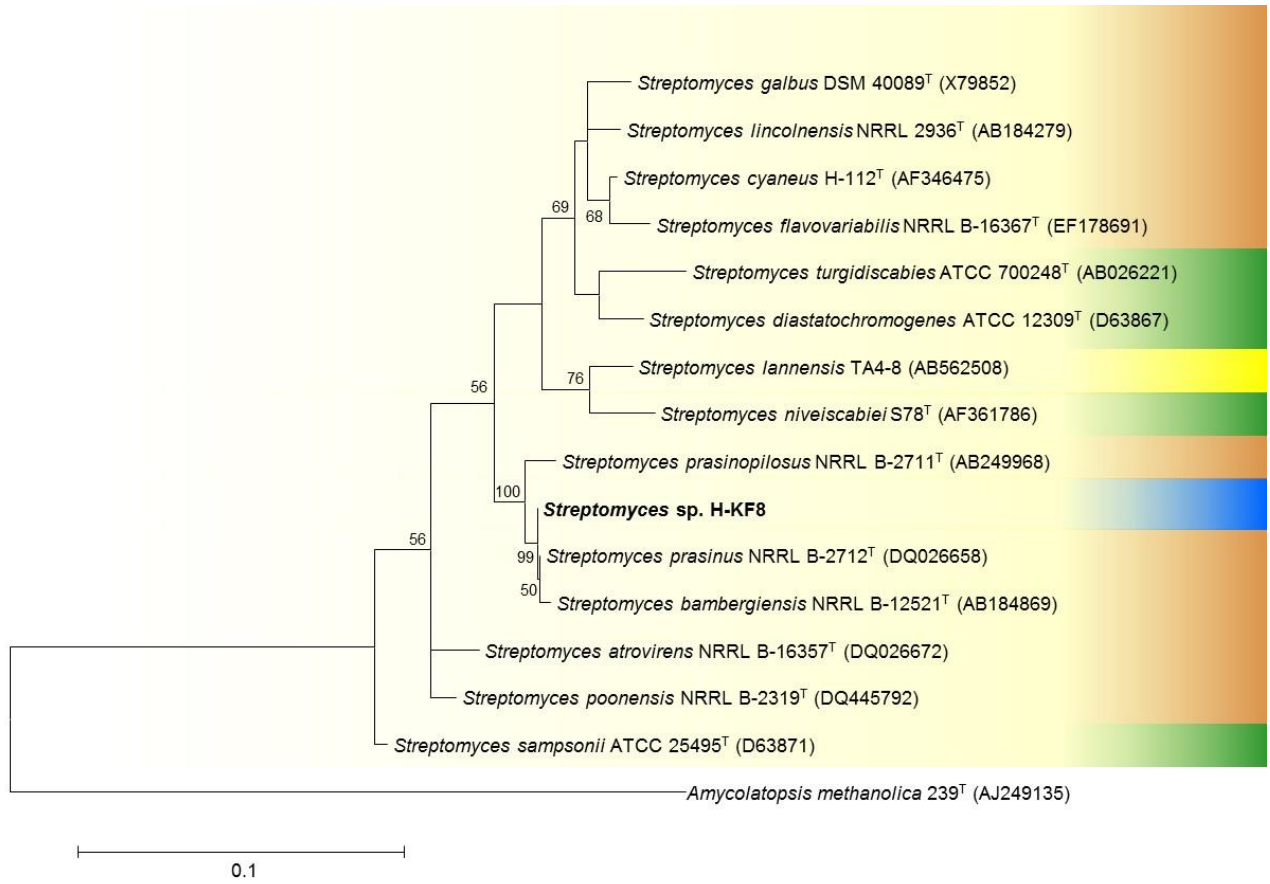
1096 Figure 1. **Morphology of *Streptomyces* sp. strain H-KF8.** Macrocolony showing anverse
1097 and reverse growth in **A)** ISP2-ASW; **B)** Marine Agar (MA); **C)** TSA-ASW; **D)** ISP5-ASW
1098 and **E)** ISP3-ASW.

1099 Fig. 2
1100



1101
1102
1103 Figure 2. **Microscopy of *Streptomyces* sp. strain H-KF8. A)** Stereoscope zoom of a
1104 macrocolony grown in ISP2-ASW agar plate. Arrows shows exudates. **B)** Optic
1105 Microscopy (OM) image at 1000X. Arrows indicate hyphae and spores, respectively. **C)**
1106 Low Voltage Electron Microscopy (LVEM) image of strain H-KF8 grown on ISP3-ASW
1107 agar plates for 21 days.

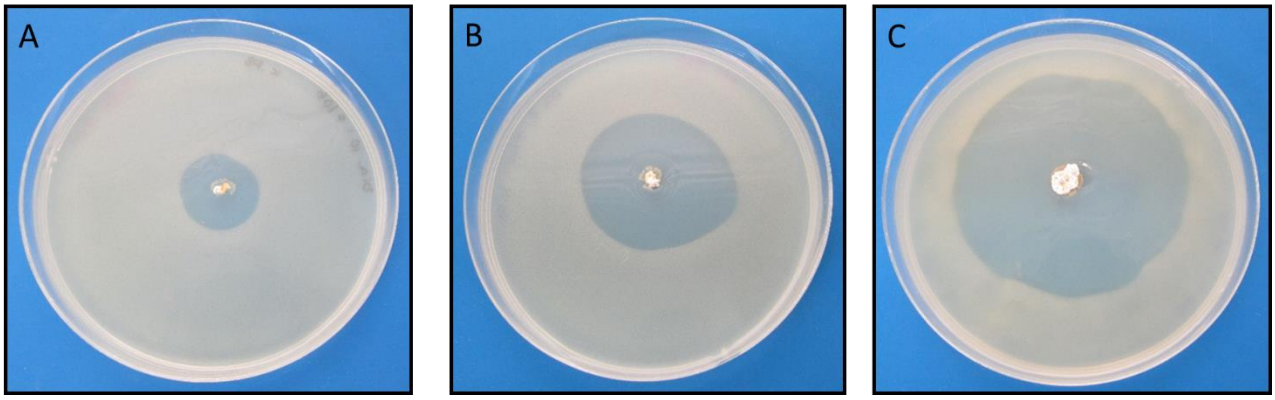
1108 Fig. 3



1109

1110 Figure 3. **Phylogenetic tree of *Streptomyces* sp. H-KF8.** Maximum Likelihood tree of
1111 complete 16S rRNA gene showing close representatives of the *Streptomyces* genus. Node
1112 numbers represent the percentage of bootstrap replicates (1000 resampling). Values below
1113 50% are not shown. Sequence positions 14-1544 were considered, according to the
1114 *Escherichia coli* K12 (AP012306) 16S rRNA gene sequence numbering. Arrow points to
1115 the outgroup *E. coli* K12. GenBank accession numbers of 16S rRNA sequences are given in
1116 parentheses. Scale bar corresponds to 0.1 substitutions per nucleotide positions. Colours
1117 depict the isolation source of each strain, where brown is soil, green is plant-associated,
1118 yellow is insect-associated and blue is marine environment.
1119

1120 Fig. 4



1121

1122 Figure 4. **Antibacterial activity of isolate *Streptomyces* sp. H-KF8.** Photographs depict
1123 inhibition zone against *Staphylococcus aureus*. Time course was performed using the
1124 double-layer method, at various incubation days: **A)** 6; **B)** 9 and **C)** 15 days.
1125

Fig. 5

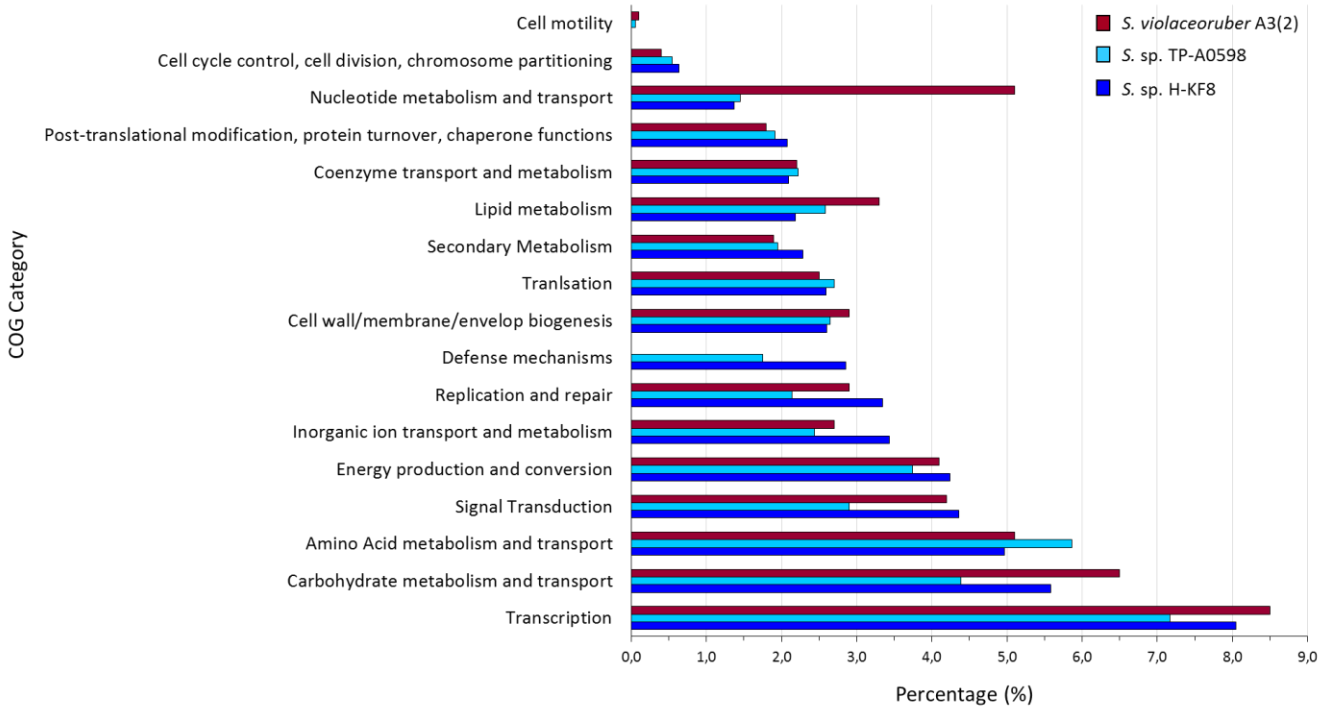


Figure 5. **Comparative genomics of COGs categories.** Percentage of each COG category is shown for the different *Streptomyces* species, where blue is *Streptomyces* sp. H-KF8; light blue is *Streptomyces* sp. TP-A0598; and red is *Streptomyces violaceoruber* A3(2).

Fig. 6

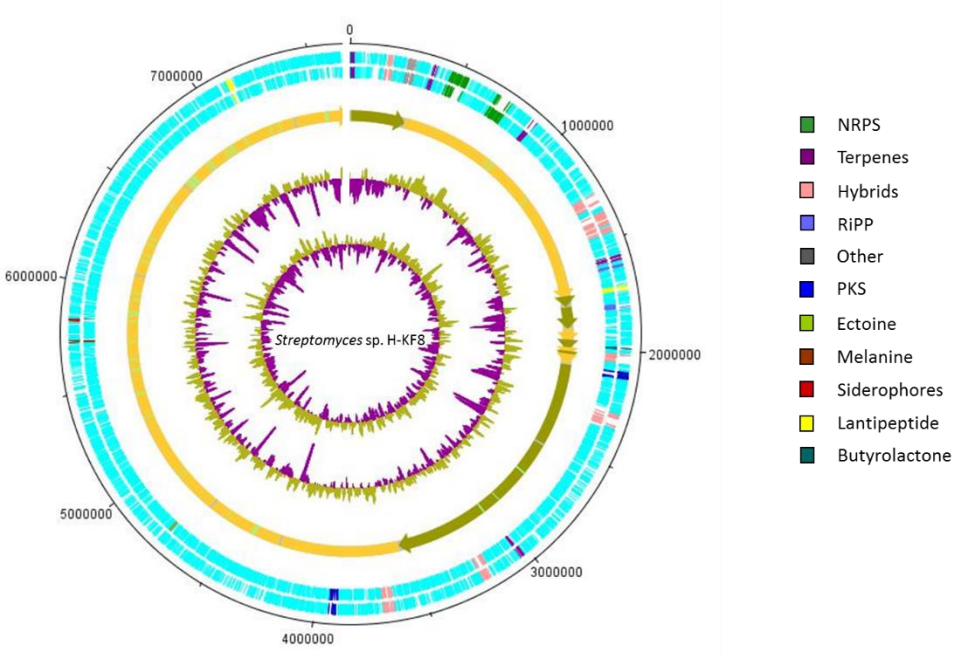


Figure 6. **Representation of chromosome features and BGC of *Streptomyces* sp H-KF8.** Colors depict the different classification types of secondary metabolism gene clusters along the sequenced genome. NRPS, Non-ribosomal peptide synthetase; PKS, polyketide synthase; RiPP, ribosomally synthesized and post-translationally modified peptides. From outside inward: DNA strands reverse and forward; contigs; GC content; GC skew.

Fig.7

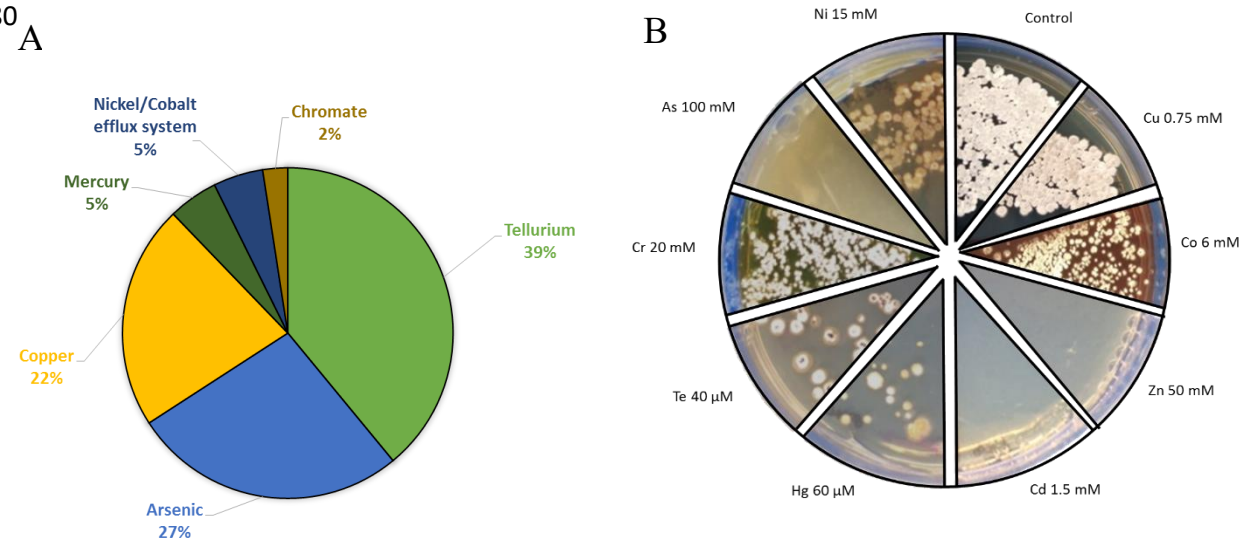


Figure 7. **Metal-tolerance response in *Streptomyces* sp H-KF8. A)** Genetic determinants involved in metal-resistance observed by genome mining. **B)** Functional response of metal-resistance in TSA-ASW agar plates. Images shows maximum concentration where growth is observed. Control, agar plate without any metal.

Fig. 8

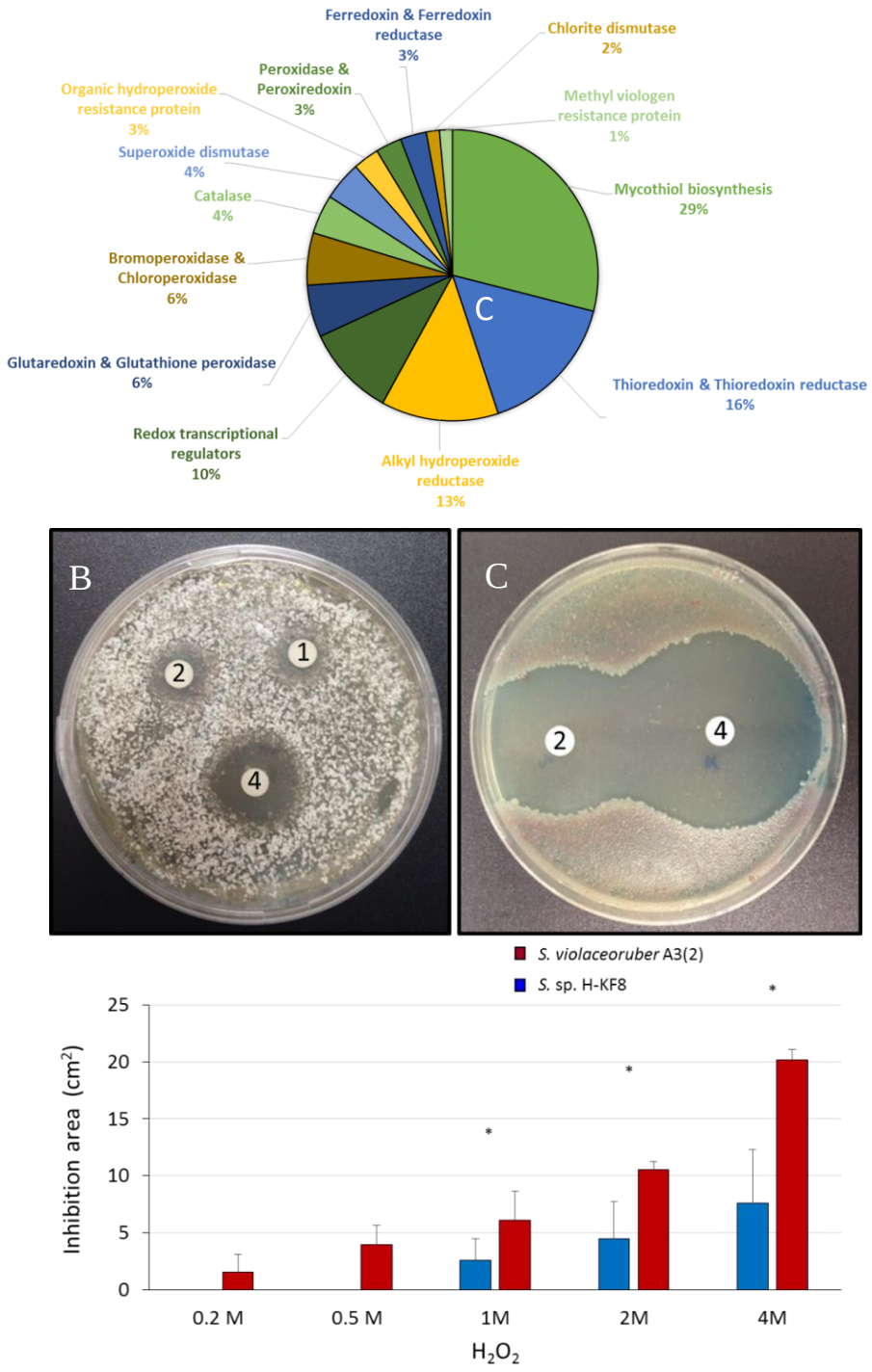


Figure 8. **Oxidative stress response of isolate *Streptomyces* sp. H-KF8. A)** Genetic determinants involved in oxidative stress-resistance observed by genome mining. Functional response of **B)** *Streptomyces* sp. H-KF8 and **C)** *Streptomyces violaceoruber* A3(2) respectively, showing comparative inhibition zones with hydrogen peroxide. **D)** Quantitative assay of inhibition area of both *Streptomyces* strains facing several concentrations of hydrogen peroxide. Asterisks indicate significative differences between strains (P value < 0.1).

Fig. 9

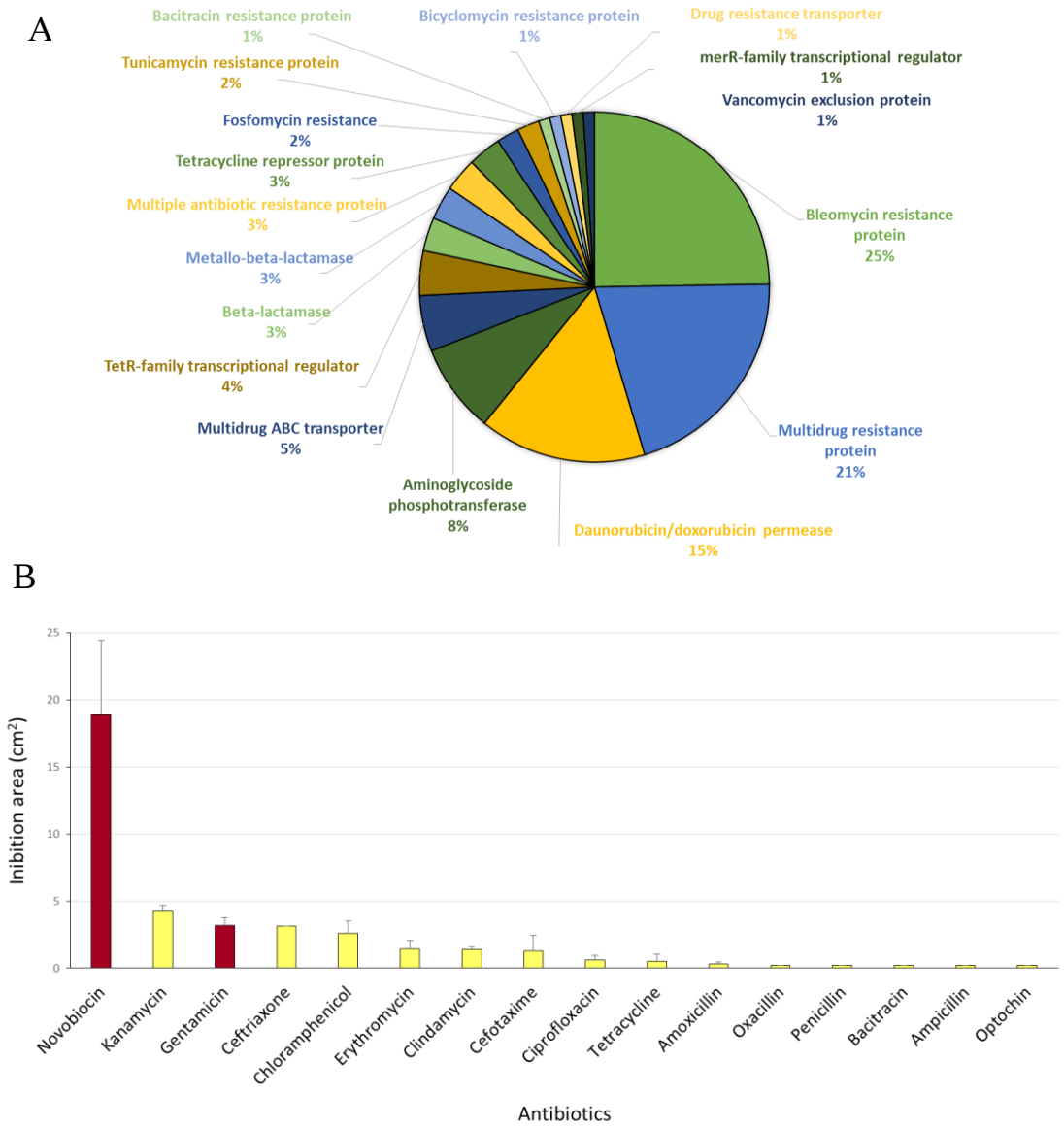


Fig. 9. **Antibiotic-resistance response in *Streptomyces* sp H-KF8. A)** Genetic determinants involved in antibiotic-resistance observed by genome mining. **B)** Functional response of antibiotic-resistance in MH-ASW agar plates. Red columns indicate susceptibility to the antibiotic tested and yellow columns indicate resistance to the antibiotic tested.