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

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Streptomyces sp. H-KF8 is an actinobacterial strain isolated from marine sediments of a Chilean Patagonian fjord. Morphological characterization together with antibacterial activity was assessed in various culture media, revealing a carbon-source dependent activity mainly against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*). Genome mining of this antibacterial-producing bacterium revealed the presence of 26 biosynthetic gene clusters (BGCs) for secondary metabolites, where among them, 81 % have low similarities with known BGCs. In addition, a genomic search in *Streptomyces* sp. 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 *Streptomyces* sp. H-KF8 bacterium has the capability to tolerate a diverse set of heavy metals such as copper, cobalt, mercury, chromate and nickel; as well as the highly toxic tellurite, a feature first time described for *Streptomyces*. In addition, *Streptomyces* sp. H-KF8 possesses a major resistance towards oxidative stress, in comparison to the soil reference strain *Streptomyces violaceoruber* A3(2). Moreover, *Streptomyces* sp. 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.

1 **Genomic data mining of the marine actinobacteria**

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4 **antimicrobial synthesis**

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19 **Abstract**

20 *Streptomyces* sp. H-KF8 is an actinobacterial strain isolated from marine sediments of a Chilean
21 Patagonian fjord. Morphological characterization together with antibacterial activity was assessed in
22 various culture media, revealing a carbon-source dependent activity mainly against Gram-positive
23 bacteria (*S. aureus* and *L. monocytogenes*). Genome mining of this antibacterial-producing bacterium
24 revealed the presence of 26 biosynthetic gene clusters (BGCs) for secondary metabolites, where among
25 them, 65 % have low or no similarity with known BGCs. In addition, a genomic search in *Streptomyces*
26 sp. H-KF8 unveiled the presence of a wide variety of genetic determinants related to heavy metal
27 resistance (49 genes), oxidative stress (69 genes) and antibiotic resistance (97 genes). This study
28 revealed that the marine-derived *Streptomyces* sp. H-KF8 bacterium has the capability to tolerate a
29 diverse set of heavy metals such as copper, cobalt, mercury, chromate and nickel; as well as the highly
30 toxic tellurite, a feature first time described for *Streptomyces*. In addition, *Streptomyces* sp. H-KF8
31 possesses a major resistance towards oxidative stress, in comparison to the soil reference strain
32 *Streptomyces violaceoruber* A3(2). Moreover, *Streptomyces* sp. H-KF8 showed resistance to 88% of the
33 antibiotics tested, indicating overall, a strong response to several abiotic stressors. The combination of
34 these biological traits confirms the metabolic versatility of *Streptomyces* sp. H-KF8, a genetically well-
35 prepared microorganism with the ability to confront the dynamics of the fiord-unique marine
36 environment.

38 **Introduction**

39

40 There has been a burst of genomic data in recent years due to the advances in various technologies such
41 as next-generation sequencing. Whole genome sequencing is providing information-rich data that can
42 hugely contribute and orientate the discovery of natural products in microorganisms. Indeed genome
43 mining has been positioned as a fundamental bioinformatics-approach in the natural product field
44 (McAlpine et al., 2005; Van Lanen & Shen, 2006; Challis, 2008; Doroghazi & Metcalf, 2013; Jensen et al.,
45 2014; Antoraz et al, 2015; Tang et al., 2015; Katz & Baltz, 2016). Natural products have clearly
46 demonstrated to play a significant role in drug discovery, in fact 78 % of antibiotics marketed during
47 1982 – 2002 originated from natural products (Peláez, 2006). Considering the year 2014, 25 % of the
48 approved new chemical entities were from natural or natural-derived products (Newman & Cragg,
49 2016). In natural environments, these metabolites also play important roles as signal molecules,
50 facilitating intra- or inter-species interactions within microbial communities related to virulence,
51 colonization, motility, stress response and biofilm formation (Romero et al., 2012).

52

53 *Streptomyces* are mycelium-forming bacteria with a complex developmental life cycle that includes
54 sporulation and programmed cell death processes (Flärdh & Buttner, 2009; Yagüe et al., 2013). Their
55 unsurpassed richness and diversity concerning secondary metabolism pathways has made them
56 valuable providers for bioactive molecules, being responsible for two-thirds of all known antibiotics
57 (Bérdy, 2012). Genome mining has become a powerful tool to unveil the biotechnological potential of
58 *Streptomyces* species, where biosynthetic gene clusters (BGCs) can be identified (Weber et al., 2015)
59 and even predict the chemical core structure of the molecules. Unlike other bacteria, *Streptomyces* have
60 linear chromosomes (Chen et al., 2002) and their genome sizes are within the largest in the bacterial
61 world (Weber et al., 2003), ranging from 6.2 Mb for *Streptomyces cattleya* NRRL 8057 (Barbe et al.,
62 2011) to 12.7 Mb for *Streptomyces rapamycinicus* NRRL 5491 (Baranasic et al., 2013), considering
63 complete sequenced genomes to date (Kim et al., 2015). Up to 5% of their genomes are devoted to the
64 synthesis of secondary metabolites (Ikeda et al., 2003). The ability to produce a wide variety of bioactive
65 molecules is based on the fact that they contain the largest numbers of BGCs such as polyketide
66 synthases (PKS) and non-ribosomal peptide synthetases (NRPS), or even PKS-NRPS hybrids (Challis,
67 2008). The genes required for secondary metabolites biosynthesis are typically clustered together
68 (Zazopoulos et al., 2003) and are tightly regulated both by specific regulation of each product (Bibb &
69 Hesketh, 2009) or by pleiotropic mechanisms of regulation that can control several pathways at the
70 same time (Martin & Liras, 2012). Due to these interesting properties, nearly 600 species and 30,000
71 strains of *Streptomyces* have been identified (Euzéby, 2011). To date, 653 *Streptomyces* genome
72 assemblies are available in GenBank database (Studholme, 2016) and this number is likely to keep
73 increasing.

74 Although soil microorganisms from the *Streptomyces* genus have generated vast interest due to their
75 exceptional role as antibiotic producers (Bérdy, 2012), their marine counterpart has been less explored.
76 The marine ecosystem is highly diverse, with extreme abiotic selective pressures and immense biological
77 diversity (Lam, 2006). In addition, many marine organisms have a sessile life style, needing chemical
78 weapons for defense and survival (Haefner, 2003). Thus, research in natural products has been focusing
79 on the isolation of microorganisms from corals (Hodges, Slattery & Olson, 2012; Kuang et al., 2015;
80 Mahmoud & Kalendar, 2016; Pham et al., 2016), sponges (Kim, Garson & Fuerst, 2005; Montalvo et al.,
81 2005; Zhang et al., 2006; Jiang et al., 2007; Vicente et al., 2013; Sun et al., 2015), as well as marine
82 sediments (Mincer et al., 2002; Magarvey et al., 2004; Jensen et al., 2005; León et al., 2007; Gontang,
83 Fenical & Jensen, 2007; Duncan et al., 2014; Yuan et al., 2014). In spite of all the isolation studies
84 associated to marine actinobacteria, relatively little is known about the molecular mechanisms behind

85 bacterial adaptation to marine environments. It is supposed that marine actinobacteria have adapted
86 through the development of specific biological traits (Tian et al., 2016), which has led to hypothesize
87 that novel species from unexplored habitats may contain unique bioactive compounds (Axenov-
88 Gribanov et al., 2016). In addition, marine habitats are under a dramatic pollution increase, where heavy
89 metals have demonstrated to be one of the most negative causing impacts in living beings. While many
90 metals (iron, zinc, manganese, copper, cobalt, nickel, vanadium, molybdenum) are essential
91 micronutrients for enzymes and cofactors, they still are toxic when available in high concentrations,
92 causing adversary effects mainly by oxidative stress damage to fundamental macromolecules (Schmidt
93 et al., 2005). In this context, marine microorganisms have developed mechanisms through molecular
94 adaptations in order to thrive in these adverse conditions. Moreover, secondary metabolites
95 biosynthesis are strongly influenced by the presence and concentration of certain heavy metals in
96 *Streptomyces* genus (Locatelli, Goo & Ulanova, 2016), and also oxidative stress can regulate antibiotic
97 production (Kim et al., 2012; Beites et al., 2014) providing evidence of a molecular crosstalk response
98 between these stressors.
99
100 In the South Pacific region, Chile has an extensive marine coast that remains mostly unexplored.
101 Bioprospecting of actinobacteria for the discovery of novel marine-derived natural products, specifically
102 antibiotics, has been carried out in Valparaíso Central Bay (Claverías et al., 2015) and in the Comau fjord
103 in Northern Patagonia (Undabarrena et al., 2016a). Both sites proved to be a rich source for novel
104 species of actinobacteria with antimicrobial properties. In this context, the genome of a selected
105 antimicrobial-producer marine *Streptomyces* strain from Comau fjord was sequenced (Undabarrena et
106 al.,2016b). In this study, we aimed to conduct a combined genomic, metabolic and physiological analysis
107 of the marine *Streptomyces* sp. H-KF8 bacterium, through the further exploration of its antimicrobial
108 activity and the genome mining of the BGCs encoded in its genome. In addition, the genetic and
109 functional response to abiotic stressors such as oxidative stress, heavy metals and antibiotics, which
110 may play an important role in the evolution of secondary metabolism genes, was evaluated in
111 *Streptomyces* sp. H-KF.
112

Methods and Materials

Bacterium selection

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). Fjords are especially attractive due to its unique biogeographic characteristics, being a deep narrow inlet with significantly eroded bottom and communication with open sea (Bredholdt et al., 2007). Comau fjord is one of the deepest; it has a high precipitation rate crucial for fresh water input; where water surface temperatures ranges between 5 and >20 °C, sustaining a thermohaline circulation (Bustamante, 2009; Sobarzo, 2009). As microorganisms of these ecosystems may display remarkable genetic features of tolerance to the dynamics of these abiotic stressors, marine actinobacteria were isolated with several culture media and identified through 16S rRNA gene sequence (Undabarrena et al., 2016a). Antimicrobial potential was screened using two strategies, including assessing the antimicrobial activity of crude extracts derived from liquid cultures (Undabarrena et al., 2016a). *Streptomyces* sp. H-KF8 was selected due to its antimicrobial activities against *S. aureus, L. monocytogenes* and *E. coli* for whole genome sequencing, representing the first genome of Chilean marine actinobacteria (Undabarrena et al., 2016b).

Phenotypic characterization

Streptomyces sp. H-KF8 was 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., 2016). Plates were incubated at 30 °C and visible colonies appeared after 5-7 days. Microscopic images were obtained with a Leica Zoom2000 stereoscope (Arquimed), Gram-staining was performed with an optical microscope L2000A (Arquimed) with 1000X magnification, and unstained low voltage electron microscopy (LVEM) was used for high contrast images (Delong LVEM5 microscope, Universidad Andrés Bello, Chile) after 21 days of *Streptomyces* sp. H-KF8 growth in ISP3-ASW media (Vilos et al., 2013).

Antimicrobial activity

Antimicrobial activity was evaluated previously in ISP2 and TSA-ASW agar plates, and activity was corroborated by liquid culture derived crude extracts (Undabarrena et al., 2016a). In this study, a further evaluation of antimicrobial activity was assessed in 15 different media agar plates, to explore the relation between *Streptomyces* sp. H.KF8 morphology and antimicrobial activity. Various media were employed: ISP1-ISP9; MA; King B; Medium V (Marcone et al., 2010); LB-ASW; Actino Agar (Difco) and NaST21Cx (Magarvey et al., 2004), using cross-streak method as previously described (Haber and Ilan, 2014). The assay was slightly modified for marine actinobacteria by our group (Claverías et al., 2015; Undabarrena et al., 2016a). Antimicrobial activity was measured against five reference bacteria: *Staphylococcus aureus* NBRC 100910[T]; *Listeria monocytogenes* 07PF0776; *Salmonella enterica* subsp enterica LT2[T]; *Escherichia coli* FAP1 and *Pseudomonas aeruginosa* DSM50071[T]. Briefly, inhibition zones were seen as part of the bacterial line where the reference bacteria did not grow, and ranked qualitatively as: -, no inhibition; +/-, attenuated growth of target bacterium; +, <50% growth inhibition of target bacterium (1-5 mm of the line); ++, 50% growth inhibition of target bacterium (6-10 mm of the line); +++, >50% growth inhibition of target bacterium (≥11 mm of the line). All experiments were performed in duplicate, using as internal control one of the reference bacteria.

159 Additionally, the double-layer method (Westerdahl et al., 1991) was employed, in order to perform a
160 time-course assay to ascertain the days of incubation where most activity was being produced.
161 *Streptomyces* sp. H-KF8 macrocolonies were incubated on ISP2-ASW, ISP3-ASW, TSA-ASW and MA agar
162 plates. Macrocolonies were grown individually from five to 20 days on the same agar plate, and
163 subsequently, 7 mL of modified-LB (7 g/L of agar instead of 15 g/L) with an aliquot of 100 µL of an
164 overnight pre-grown *S. aureus* bacterial culture with an OD=0.3 was added above the macrocolonies of
165 *Streptomyces* sp. H-KF8. Inhibition zones were observed after incubation of plates for 24 h at 37 °C. If
166 inhibition zones overlapped, the experiment was repeated on separate agar plates, where only one
167 macrocolony in the center of the plate was incubated, as shown in Figure 2.
168
169

170 ***Genome Mining and Bioinformatic analysis***

171

172 *Streptomyces* sp. H-KF8 whole genome sequencing was performed by Illumina and PacBio (Undabarrena
173 et al., 2016b). Genome reads were *de novo* assembled using Canu (version 1.1) (Berlin et al., 2015) into
174 11 contigs, representing one linear chromosome of 7,684,888 bp genome. Full genome sequencing
175 details can be found elsewhere (Undabarrena et al., 2016b). Gene calling an annotation was performed
176 using the Prokaryotic Genome Annotation Pipeline (PGAP) at NCBI (version 3.1) (Tatusova et al., 2016).
177 Genes were assigned to EggNOG categories (Huerta-Cepas et al., 2016) via an HMM search with
178 HMMER3 (http://hmmer.org). Genetic determinants involved in biological traits analyzed in this report
179 were manually established and the amino acidic signatures were validated based on domain hits
180 through Basic Local Alignment Search Tool (BLAST) from NCBI. Also, BGCs were identified through
181 AntiSMASH (version 3.0) online platform. Snapgene software (version 2.3.2) was used to visualize ORFs
182 related to functional biological traits from each linear contig. Artemis software (version 16.0.0) was used
183 to construct the graphic representation of the circular chromosome, and to assign by colors manually all
184 the different categories of BGCs on it.

185

186 ***Functional response to Heavy Metal(loid)s***

187

188 For metal-resistance experiments, agar plates containing filtered salts of several metal(loid) solutions
189 were prepared. Metals were diluted to obtain the following final concentration in media plates: $CuSO_4$
190 (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); $CdCl_2$
191 (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
192 (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
193 mM, 10 mM and 15 mM). *Streptomyces* sp. H-KF8 was evaluated after 5, 10 and 20 days of growth in
194 TSA-ASW plates. Additionally, a special Minimal Medium (MM) used to evaluate metal resistance in
195 *Streptomyces* spp. was prepared (Schmidt et al., 2009), modified with the addition of ASW. Experiment
196 was performed with two biological replicates. Reference values for metal concentrations were decided
197 based on metal-tolerance *Streptomyces* obtained from literature (Schmidt et al., 2005, 2009; Wang et
198 al., 2006; Polti, Amoroso & Abate, 2007). Agar plates without addition of any metals were prepared as
199 negative controls.

200

201 ***Functional response to Oxidative Stress***

202

203 For oxidative stress experiments, tolerance to hydrogen peroxide (H_2O_2) at various concentrations (0.2
204 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
205 paper disk positioned on a TSA-ASW agar plate where *Streptomyces* sp. H-KF8 was streaked out to grow
206 as a thin lawn (Dela Cruz et al., 2010). The model strain *Streptomyces violaceoruber* A3(2) (DSM 40783)

207 was used to test the tolerance response. Inhibitions areas (cm^2) were observed after 5 days of growth at
208 30 °C. Experiment was performed with three biological replicates, and standard deviation was
209 calculated. A statistical analysis by Student's *t*-Test was carried out considering a p-value <0.01.
210
211 ***Functional response Antibiotics***
212
213 Susceptibility to model antibiotics of *Streptomyces* sp. H-KF8 was explored previously (Undabarrena et
214 al., 2016a). However, in this report a further characterization was pursued. *Streptomyces* sp. H-KF8 was
215 grown on Mueller-Hinton agar plates prepared with ASW (MH-ASW) and commercial standard disks of
216 model antibiotics were placed above. The following antibiotics were tested: Amoxicillin 25 µM,
217 Bacitracin 0.09 IU, Novobiocin 5 µg and Erythromycin 15 µg (LabClín); Optochin 5 µg (BritaniaLab);
218 Clindamycin 2 µg, Oxacillin 1 µg, Ciprofloxacin 5 µg, Ceftriaxone 30 µg, Chloramphenicol 30 µg, Penicillin
219 10 UOF, Cefotaxime 30 µg, Gentamicin 10 µg and Ampicillin 10 µg (Valtek). After 5 days of incubation at
220 30 °C, radios of the inhibition halos were measured, and inhibition areas (cm^2) were calculated. Data
221 was compared with standarized cut off values from Clinical and Laboratory Standards Institute (CLSI)
222 from year 2016, to determine susceptibility or resistance against each antibiotic tested. Experiments
223 were performed using three biological replicates, and standard deviation was calculated for each
224 antibiotic.

225   **Results**

226

227   ***Phenotypic characterization***

228   Morphological analysis of *Streptomyces* sp. H-KF8 was carried out by strain growth in several media,
229   containing different carbon sources (Fig 1; inset colony morphology). Growth of *Streptomyces* sp. H-KF8
230   was observed in the standard ISP1-ISP9 agar plates, although differences in growth rates and
231   pigmentation were noticed (Fig 1A-1F). On ISP1 (yeast extract, pancreatic digest of casein), ISP2 (yeast
232   extract, malt extract, dextrose) and ISP6 (peptone, yeast extract and iron) media, white mycelia was
233   observed, with appearance of grayish-spores after 14 days of growth. In contrast, when *Streptomyces*
234   sp. H-KF8 was grown on ISP3 (outmeal), ISP4 (soluble starch and inorganic salts), ISP5 (glycerol and
235   asparagine) and ISP9 (glucose) media, creamy mycelia was observed, with appearance of white spores at
236   the periphery of the colonies. In contrast, poor growth was observed in ISP7 (tyrosine) medium. A
237   different morphology was perceived when *Streptomyces* sp. H-KF8 was grown on MA medium (Fig 1G).
238   Colony size was comparatively smaller (5.06 ± 1.1 mm in ISP2 vs 3.12 ± 0.78 mm in MA; *p*<0.01), and a
239   dark-grey turning into black pigmentation was noticed within 5 days of growth. On TSA-ASW plates, a
240   white mycelium was observed with no change in pigmentation over time, but with presence of exudate
241   drops in the colony surface (Fig 1H). Additionally, morphology was visualized microscopically, and typical
242   *Streptomyces* structures of development such as hyphae and spores were observed (Fig 2). Exudate
243   drops were appreciated in ISP2 medium during late growth phase (Fig 2A), spores were identified with
244   optical microscopy (Fig 2B) and hyphae with Gram staining (Fig 2B). Moreover, the complex network of
245   intertwined hyphae and early spore chain assemblies was observed by LVEM microscopy, which is a
246   distinctive feature of *Streptomyces* genus (Fig 2D).

247   ***Antimicrobial activity***

248   Antimicrobial activity of *Streptomyces* sp. H-KF8 was further characterized using agar media with
249   different carbon sources (Table 1). In general, antimicrobial activity was more evident against Gram-
250   positive reference bacteria (*S. aureus* and *L. monocytogenes*), although inhibition against *E. coli* was also
251   observed in most media, which is consistent with results obtained from *Streptomyces* sp. H-KF8 crude
252   extracts (Undabarrena et al., 2016a). *P. aeruginosa* was the reference bacterium less inhibited. Among
253   the 15 different media used, inhibition of at least one reference bacterium was noted in 87% of the
254   media. Best media for antimicrobial activity were ISP1, ISP2, ISP6, and V media, where inhibition of four
255   of the five reference bacteria was observed. Notably, in ISP2 medium a unique attenuation of *P.*
256   *aeruginosa* growth was observed. Alternatively, a time-course assay using the double-layer method was
257   performed to visualize the starting day of the antimicrobial activity, in four media that presented
258   inhibition. Even though at day 5 a relatively scarce colony growth of *Streptomyces* sp. H-KF8 was
259   observed in ISP2 medium, at day 6 it was possible to visualize a modest inhibition against *S. aureus* (Fig
260   3A). Yet, inhibition zone increased as incubation time for *Streptomyces* sp. H-KF8 extended, as shown in
261   Fig 3B, showing a maximum halo size at day 15 (Fig 3C), suggesting a tight relation between growth and
262   antimicrobial activity which is also correlated to the carbon source of the media tested.

263   ***Bioinformatic analysis and Genome mining for BGCs***

264   Whole genome sequencing and genome features were previously described (Undabarrena et al.,2016b).
265   Briefly, *Streptomyces* sp. H-KF8 genome was assembled into 11 contigs, with a total genome length of
266   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
267   tRNAs and 6 16S rRNAs. Genes with coding sequences were grouped into COGs categories, although 808
268   genes remain ungrouped. Description and gene percentage of each category is depicted in Table 2. For
269   *Streptomyces* sp. H-KF8, the most abundant categories were transcription (522 genes), carbohydrate
270   transport and metabolism (362 genes), and amino acid transport and metabolism (362 genes). The
271   *Streptomyces* sp. H-KF8 categorized genes were compared to the model *Streptomyces violaceoruber*
272   A3(2) isolated from soil (Bentley et al., 2002) and the marine *Streptomyces* sp. TP-A0598 (Komaki et al.,
273   2015), in order to observe if these features could be considered as source-derived traits (Fig 4). As there
274   are scarce reports available on marine *Streptomyces* genomes that include COGs detailed annotation,
275   *Streptomyces* sp. TP-A0598 is one of the few that have these characteristics, and therefore selected for
276   comparison. While all three strains showed the same tendency in the categories previously named in
277   terms of abundancy, differences were observed in terms of percentage in transcription and
278   carbohydrate metabolism categories, where *S. violaceoruber* A3(2) strain was slightly higher. On the
279   other hand, both marine strains (*Streptomyces* sp. H-KF8 and *Streptomyces* sp. TP-A0598) showed higher
280   number of genes related to categories of post-translational modification, protein turnover and
281   chaperone functions, as well as in secondary metabolism and translation categories.

282   Secondary metabolism category comprises 2.3 % of the *Streptomyces* sp. H-KF8 genome, being slightly
283   higher when compared to both strains, the soil-derived *S. violaceoruber* A3(2), and the marine-derived
284   *Streptomyces sp.* TP-A0598, accounting for 1.9 % and 2.0 % of their genomes, respectively. A
285   bioinformatics analysis was performed using the antiSMASH tool to detect biosynthetic gene clusters
286   (BGCs) present in *Streptomyces* sp. H-KF8 that may explain the antimicrobial activity observed, and a
287   total of 26 BGCs were detected (Undabarrena et al., 2016b). In this report, we show that the spatial
288   distribution of the 26 BGCs are evenly allocated throughout the contigs of *Streptomyces* sp. H-KF8
289   genome (Fig 5), which were grouped into 11 different types (NRPS, PKS, hybrids, terpenes, RiPP, ectoine,
290   melanine, siderophores, lantipeptides and butyrolatones). Furthermore, a comparison of the BGCs
291   present in *Streptomyces* sp. H-KF8 with other known BGCs deposited into the MIBiG database, was
292   performed (Table 3). In this line, *Streptomyces* sp. H-KF8 bears two NRPSs BGCs with very low similarity
293   to BGCs involved in the synthesis of the lipoglycopeptide antibiotic mannopeptimycin, produced by *S.*
294   *hygroscopicus* (Magarvey et al., 2006); and the streptolydigin antibiotic, which interferes with the RNA
295   elongation by inhibition of the bacterial RNA polymerase (Olano et al., 2009), with 7% and 13% of gene
296   similarity, respectively (Table 3). The two PKSs predicted in *Streptomyces* sp. H-KF8 genome corresponds
297   to the type II spore pigment BGC showing 83 % of gene similarity, and also another BGC where only 6 %
298   of gene similarity to the antibacterial kirromycin BGC from *S. collinus* Tü 365 was found (Weber et al.,
299   2008) (Table 3). A total of eight hybrid clusters, where four of them are PKS-NRPS hybrids were also
300   predicted, which presented low gene similarities with other known BGCs, except for one NRPS-PKS type
301   I cluster (Table 3). In addition, other BGCs found in *Streptomyces* sp. KF8 included five terpenes BGCs,
302   two lantipeptides and two ribosomally synthesized and post-translationally modified peptides (RiPPs)

303   such as the lassopeptide and bacteriocin BGCs. In general, only six BGCs from *Streptomyces* sp. H-KF8
304   genome displayed 100 % gene similarity to their most related known cluster. Examples of these consists
305   on the BGC for the previously mentioned antibiotics moenomycin (Ostash, Saghatelian & Walker, 2007)
306   and albaflavenone (Zhao et al., 2008) (Table 3). Additionally, BGCs for the aromatic carotene
307   isorenieratene, involved in anoxigenic photosynthesis in *S. griseus* (Krügel et al., 1999), the conserved
308   osmolite ectoine, that may provide protection from osmotic stress (Prabhu et al., 2004; Graf et al., 2008)
309   and the melanin pigment clusters (Guo et al., 2014; Sivaperumal, Kamala & Rajaram, 2015) were
310   observed with 100 % similarity. Most of the BGCs (65 %) presented low similarity to BGCs of known
311   compounds, evidencing the potential of *Streptomyces* sp. H-KF8 strain to produce novel bioactive
312   molecules.

313   Due to the dynamics of environmental parameters from the unique isolation site of *Streptomyces* sp. H-
314   KF8, genome mining of pathways involved in response to abiotic stressors such as heavy metals,
315   oxidative stress and antibiotics were also analyzed in this study, in order to unveil genetic determinants
316   that may explain tolerance to these stressful environmental conditions.

317   ***Functional response to Heavy Metals and Metalloids***

318   Genetic determinants involved in heavy metal-resistance in *Streptomyces* sp. H-KF8 were analyzed by
319   genome mining, and at least 49 predicted genes may be playing a role in such tolerance (Fig 6A).
320   Amongst these, the most abundant genes were related to tellurite, followed by arsenate, copper and
321   mercury, and, to a lesser extent, chromate, nickel and cobalt tolerance (Fig 6A). Tellurite resistance
322   genetic determinants involved seven *terD* genes, four *terB* genes, two *yceC* genes, one *terC* gene and
323   one *tehB* gene that encodes a tellurite methyltransferase*.* In addition, 11 genetic determinants for
324   arsenic tolerance were found, involving three *arsC* genes encoding arsenate reductases, two genes *arsA*
325   encoding arsenical pump-driving ATPases, five genes *arsR* encoding arsenical transcriptional regulators,
326   and the arsenical resistance protein encoding gene *acr3*. Genetic determinants encoding for copper
327   resistance genes, included *copA* and *mco* genes encoding multicopper oxidases, *copD* encoding a copper
328   resistance protein, two genes *ycnJ* encoding for copper transport proteins, and two genes for the
329   copper-sensing transcriptional regulator, *csoR*. Mercury resistance genes consisted in the mercury
330   reductase encoding gene *merA,* and the mercury transcriptional regulator *merR*. In addition, the *czcD*
331   and *rcnA* genes coding for efflux pumps for cadmium, zinc, cobalt and nickel, respectively, together with
332   the *chrR* gene encoding a chromate reductase, and general heavy metal tolerance such as the *hmt1*
333   gene and seven genes encoding for *merR*-family transcriptional regulators, were also found. Considering
334   all the genetic determinants listed above, we attempted to determine if *Streptomyces* sp. H-KF8 was
335   able to grow on various metal-containing media. *Streptomyces* sp. H-KF8 was able to tolerate copper-,
336   cobalt-, mercury-, tellurite-, chromate- and nickel-containing media, as shown in Fig 6B for the
337   maximum concentrations tested. Despite the arsenic tolerance-related genes present in *Streptomyces*
338   sp. H-KF8 genome, comprising 27 % of the total number of metal-related genes, no evident growth of
339   *Streptomyces* sp. H-KF8 was perceived in this metalloid-containing medium, even in the two different
340   toxic forms of arsenic tested: arsenate and arsenite. Also, no growth was observed in media containing
341   cadmium or zinc.

342   ***Functional response to Oxidative Stress***

343   A significant amount of genes (69 genes) that may participate in the detoxification of reactive oxygen
344   species (ROS) were found within the *Streptomyces* sp. H-KF8 genome (Fig 7A). Genes for mycothiol
345   biosynthesis (20 genes), thioredoxin and thioredoxin reductases system (11 genes), alkyl hydroperoxide
346   reductases (nine genes), glutaredoxin and glutathione peroxidase system (four genes), catalases (three
347   genes), and superoxide dismutases (three genes), among others, were identified (Fig 7A). Interestingly,
348   genes involved in osmotic stress detoxification of chlorinated and brominated compounds such as three
349   *bpo* genes encoding for bromoperoxidases, one *cpo* gene encoding for a chloroperoxidase and one gene
350   encoding for a chlorite dismutase were also present in *Streptomyces* sp. H-KF8 genome (Fig 7A).
351   Concerning transcriptional regulators controlling the redox balance, transcriptional factors from *perR*,
352   *rex*, *lysR* and *soxR* families, were also present. Due to an important genetic content of oxidative stress
353   related genes, response of *Streptomyces* sp. H-KF8 to the toxic H_2O_2 was tested, and compared to the
354   model streptomycete *S. violaceoruber* A3(2). At various H_2O_2 concentrations, *Streptomyces* sp. H-KF8
355   displayed smaller susceptibility areas against the toxic, in comparison with *S. violaceoruber* A3(2) (Fig 7B
356   and 7C, respectively). A significant difference of the suceptibility areas among the two strains was
357   observed at concentrations of 1 M, 2 M and 4 M of H_2O_2, indicating a major resistance response of
358   *Streptomyces* sp. H-KF8 towards H_2O_2 toxicity (Fig 7D).

359   ***Functional response to Antibiotics***

360   Antibiotic-producing *Streptomyces* strains usually encode resistance genes within their BGCs to protect
361   themselves against the noxious action of the synthetized compound (Zotchev, 2014). In this line,
362   resistance of *Streptomyces* sp. H-KF8 to commercial antibiotics with different biological targets was
363   explored. Genome mining revealed more than 90 genes that could be involved in antibiotic resistance.
364   The most abundant genes encode for bleomycin resistance proteins (24 genes). Specific resistance genes
365   related to modification and inactivation of antibiotics such as aminoglycoside phosphotransferases
366   (eight genes), β-lactamases (three genes), metallo-β-lactamases (three genes), and one gene for
367   erythromycin esterase and penicillin amidase, respectively, were identified (Fig 8A). In addition, genes
368   for efflux of toxic compounds including multidrug resistance proteins (20 genes),
369   daunorubicin/doxorubicin ABC transporter permeases (15 genes), multidrug ABC transporters (seven
370   genes) and one gene encoding for a multidrug MFS transporter, were detected (Fig 8A). Among the
371   transcriptional regulators, the TetR-family transcriptional regulators were the most abundant, with 10
372   genes. Also, the marR-family transcriptional regulator and three *marR* genes encoding for multiple
373   antibiotic resistance proteins were identified (Fig 8A). In the functional assay against 16 different
374   antibiotics tested, *Streptomyces* sp. H-KF8 exhibited an 88 % of resistance-response, being susceptible to
375   only two antibiotics: novobiocin, which targets the DNA gyrase, and gentamicin, which inhibits protein
376   synthesis by irreversibly binding to the 30S subunit of the bacterial ribosome (Fig 8B).

377   **Discussion**

378   In this report, phenotypic analysis of *Streptomyces* sp. H-KF8 in several agar media was assessed,
379   revealing in general one week of incubation time to obtain colonies and two weeks for sporulation;
380   although growth rates, sporulation rates and pigmentation differs throughout the different media used.
381   Antimicrobial production in *Streptomyces* sp. H-KF8 was enhanced in late growth phase (> 10 days) and
382   favoured in media where sporulation was observed. *Streptomyces* genus is characterized for slow
383   growth and a complex developmental life cycle (Flärdh & Buttner, 2009). Physiological differentiation is
384   tightly linked to secondary metabolism and hence, sporulation capacities of *Streptomyces* might
385   enhance the discovery of new compounds (Chater, 2013; Kalan et al., 2013; Zhu et al., 2015). In
386   addition, antibiotics synthesis is regulated by environmental nutrients, such as carbon sources. Media
387   carbon source has an important effect on antibiotic production, being demonstrated that when bacteria
388   are grown with a preferred carbon source, secondary metabolism seems repressed (Sánchez et al.,
389   2010). This may explain the differences in inhibition patterns observed for the *Streptomyces* sp. H-KF8
390   antagonistic assays displayed in the various media tested, showing a maximum inhibition halo against *S.
391   aureus* after 15 days of incubation. Due to the interesting antibacterial activity of *Streptomyces* sp. H-
392   KF8, its whole-genome was sequenced and previously reported (Undabarrena et al., 2016b). Thus, in this
393   study an extended genome analysis for *Streptomyces* sp. H-KF8 was performed, in order to gain insights
394   into the mechanisms by which it displays antibiotic biosynthesis and resistance to multiple stressors.

395   Genome mining has been used in various fields to describe the exploitation of genomic information for
396   the discovery of new processes, targets and products (Challis, 2008). Through genome sequencing and
397   bioinformatic analysis using antiSMASH platform (Medema et al., 2011; Blin et al., 2013; Weber et al.,
398   2015), it is possible to address the secondary metabolic potential of a strain by identification of its
399   biosynthesis gene clusters (BGCs) (Iftime et al., 2016). A total of 26 BGCs were previously detected in
400   *Streptomyces* sp. H-KF8 genome (Undabarrena et al., 2016b). In this report, an extended genetic analysis
401   including the distribution of these BGCs along *Streptomyces* sp. H-KF8 genome was determined and
402   comparison with known BGCs from the Minimum Information about a Biosynthetic Gene cluster (MIBiG)
403   database, which compiles a total of 1,170 experimentally characterized known gene clusters (Medema
404   et al., 2015) was aimed. *Streptomyces* sp. H-KF8 BGCs include two PKSs, two NRPSs and four hybrid PKS-
405   NRPS, four other hybrids, five terpenes, two lantipeptides, one bacteriocin, lassopeptide, siderophore,
406   butyrolactone, ectoine, melanin, and one with unknown classification. Notably, *Streptomyces* sp. H-KF8
407   presented only six BGCs with 100% similarity to a known cluster; suggesting that most secondary
408   metabolites produced by *Streptomyces* sp. H-KF8 are yet to be elucidated, and can contribute to the
409   discovery of novel natural products. In this context, genome mining has proven to be a fundamental tool
410   for genome-based natural product discovery (Jensen et al., 2014), and has guided the discovery of novel
411   natural products from several marine actinobacteria (Gulder & Moore, 2010; Tang et al., 2015b). Among
412   these are the aromatic polyketide angucyclinone antibiotic (Zhang et al., 2012) and polyene macrolides
413   with antifungal activity (Tang et al., 2015a). Moreover, marine *Streptomyces* metabolites are produced
414   by different metabolic pathways in comparison to their terrestrial counterparts (Li et al., 2011; Lee et al.,
415   2014; Barakat & Beltagy, 2015). These metabolites emerge as a result of the unique and dynamic
416   conditions of the ocean, such as high hydrostatic pressure, low temperature, variation in salinity, and

417   depletion of micronutrients proper of the marine environment (Das, Lyla & Khan, 2006; Lam, 2006; de
418   Carvalho & Fernandes, 2010). Despite that marine adaptations are scarcely studied, recent comparative
419   genomics of marine-derived *Streptomyces* unveiled an enrichment in TrK and BCCT transporters, along
420   with the observation that their genomes are generally smaller in size and have a slightly higher GC
421   content in comparison to *Streptomyces* from other environmental sources (Tian et al., 2016).
422   *Streptomyces* sp. H-KF8 genome is consistent with these findings, holding distinctive biological and
423   genomic signatures acknowledged for marine *Streptomyces* strains. Therefore, its metabolite
424   biosynthesis may be under marine abiotic selective pressures, hence modulating secondary metabolism
425   production.

426   Comparative genomics encompassing completely sequenced *Streptomyces* obtained from several
427   isolation sources revealed that the most abundant COG categories were transcription (K), followed by
428   carbohydrate metabolism (G) and amino acid metabolism (E) (Kim et al., 2015). This is in agreement with
429   the most abundant categories found in the *Streptomyces* sp. H-KF8 genome, which also could explain
430   the versatility of *Streptomyces* sp. H-KF8 to grow in several media with different carbon sources.
431   Furthermore, in marine-derived *Streptomyces,* a higher proportion of genes belonging to the COG
432   categories of translation (J) and post-translational modification, protein turnover and chaperones (O)
433   was observed (Tian et al., 2016). Accordingly, the (J) and (O) COGs categories were also overrepresented
434   in both marine strains analyzed, *Streptomyces* sp. H-KF8 and *Streptomyces* sp. TP-A0598 (Komaki et al.,
435   2015), in comparison to the terrestrial *Streptomyces violaceoruber* A3(2) (Bentley et al., 2002). This may
436   indicate an important role of protein metabolism in marine environments, probably due to the active
437   responses against abiotic stressors and the dynamics that microorganisms have to overcome to survive
438   in these extreme ecosystems. In addition, our analysis showed an increase in the categories of cell cycle
439   control, cell division, chromosome portioning (D), secondary metabolism (Q) and defense mechanisms
440   (V), for both marine strains in comparison to *Streptomyces violaceoruber* A3(2). Percentage of the COG
441   category for defense mechanisms (V) in *Streptomyces* sp. H-KF8 was interestingly higher (2,81 %) than in
442   *Streptomyces* sp. TP-A0598 (1,8 %), and comparatively similar with what was observed for deep-sea
443   bacteria (3,0 %) (Qin et al., 2011). As the defense mechanism category includes genes for resistance to
444   heavy metals, osmotic and oxidative stress as well as antibiotics, the functionality of these biological
445   traits was evaluated for *Streptomyces* sp. H-KF8, and notably, an important resistance to these multiple
446   stressors was evidenced.

447   Environmental pollution by heavy metals can arise due to anthropogenic and/or geogenic sources.
448   Although metal-resistant strains isolated from contaminated areas have been described (Amoroso et al.,
449   2001; Schmidt et al., 2005, 2009; Polti, Amoroso & Abate, 2007; Albarracin et al., 2008; Haferburg et al.,
450   2008; Siñeriz, Kothe & Abate, 2009; Lin et al., 2011; El Baz et al., 2015), there is limited information
451   about the physiology of *Streptomyces* in presence of environmental metal pollutants. Due to the
452   naturally high concentrations of certain heavy metals in Chilean northern Patagonia (Guevara et al.,
453   2004; Revenga et al., 2012; Hermanns & Biester, 2013) product of the highly active seismic and volcanic
454   activity (Pantoja, Luis Iriarte & Daneri, 2011), the ability of *Streptomyces* sp. H-KF8 to grow in several
455   metal(loid)s supplemented media was evaluated. Surprisingly, resistance to copper, cobalt, mercury,
456   tellurite, chromate and nickel was revealed.

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

470   Additionally, resistance to mercury at a concentration of 60 μM was observed for *Streptomyces* sp. H-
471   KF8. In general, bacteria capable of resisting mercury above 20 μM, should possess specific
472   detoxification systems, as mercury is one of the most toxic elements on earth and produces several
473   health concerns for macroorganisms (Das, Dash & Chakraborty, 2016). In bacteria, two different
474   resistance operons are known, the basic narrow-spectrum *mer* operon *merRTPA* for inorganic mercury,
475   and the broad-spectrum operon that additionally contains *merB*, which provides protection against
476   organo-mercurial compounds (Barkay, Miller & Summers, 2003). In addition, it was recently
477   demonstrated that mercury resistance mechanisms could also be involved in tellurite cross-resistance
478   (Rodriguez-Rojas et al., 2015). Studies in *Streptomyces* includes *S. lividans* 132, that carries two
479   divergently transcribed operons named *merAB* and *merRTP* in the chromosome (Sedlmeier &
480   Altenbuchner, 1992; Brünker et al., 1996; Rother, Mattes & Altenbuchner, 1999), and two *Streptomyces*
481   spp. strains isolated from estuarine sediments where these genes were also observed in giant linear
482   plasmids (Ravel, Schrempf & Hill, 1998; Ravel et al., 2000). Interestingly, the genetic operons mentioned
483   above were not detected in *Streptomyces* sp. H-KF8, despite the fact that a mercury-resistance
484   phenotype was evidenced. Instead, the presence of two mercury-related genes, the transcriptional
485   regulator *merR* and the mercuric reductase *merA*, may be playing a role in such resistance. MerA is a
486   flavoprotein NADPH-dependent enzyme responsible for the reduction of mercury(II) to the elemental
487   and less toxic volatile mercury(0) (Barkay, Miller & Summers, 2003). Similarly, evidence of functional
488   operons conformed either by *merA* or *merRA* have been previously reported in archaea (Boyd & Barkay,
489   2012).

490   However, no evident growth was observed in the presence of arsenate or arsenite, although
491   *Streptomyces* sp. H-KF8 bears at least 11 genetic determinants that could involved in its detoxification.
492   In general, the arsenic resistance operon consists of *arsRABCD* genes, where *arsC* encodes for an
493   arsenate reductase that converts arsenate to arsenite, which is then exported through the ArsAB
494   ATPase-efflux pump. In *Streptomyces* sp. H-KF8, *arsA*, *arsC* and *arsR* genes are present, but lack the *arsB*
495   gene, which encodes an arsenite antiporter, crucial for anchoring ArsA to the inner membrane with
496   concomitant detoxification of arsenite. Absence of the *arsB* gene may explain the sensitivity of

497   *Streptomyces* sp. H-KF8 towards these toxics. Arsenic resistance genes are generally widespread
498   amongst both Gram-positive and Gram-negative bacteria, reflecting its broad distribution in the
499   environment (Silver & Phung, 2005). In fact, these genes were also conserved in several marine
500   streptomycetes from the South China Sea (Tian et al., 2016).

501   *Streptomyces* sp. H-KF8 displayed a notorious copper-resistant phenotype, concordant with the
502   detection of three *copA* genes encoding for multicopper oxidases that may be responsible for the
503   oxidation of Cu(I) to its less toxic form Cu(II) (Hobman & Crossman, 2014). Copper is an essential metal
504   for living beings, but is extremely toxic at higher concentrations (Gaetke & Chow, 2003). Moreover, Chile
505   is the major copper-producing country in the world, due its geological nature (Wacaster, 2015). Hence,
506   the widespread of copper resistant genetic determinants that has been demonstrated in Chilean marine
507   sediments (Besaury et al., 2013) is expected.

508   Resistance to nickel and cobalt in *Streptomyces* sp. H-KF8 might be given by the *rcnA* gene that
509   participates in the efflux system of these metals. Highly nickel- and cobalt-resistant *Streptomyces* were
510   found in an acid mine drainage, where growth in media containing up to 10 mM nickel(II) or 3 mM
511   cobalt(II) was observed (Schmidt et al., 2005). In this report, *Streptomyces* sp. H-KF8 was able to grow
512   even at higher concentrations: 15 mM nickel(II) and 6 mM cobalt(II), respectively. Furthermore,
513   chromate toxicity (20 mM) might be overcome in *Streptomyces* sp. H-KF8 due to the presence of the
514   *chrR* gene encoding a chromate reductase involved in the enzymatic reduction of chromate to the less
515   harmful chromite cation (Das, Dash & Chakraborty, 2016). Previously reported *Streptomyces* chromate-
516   resistant strains isolated from sugar cane plant were able to grow in 17 mM, where also chromate-
517   removing activity was demonstrated (Polti, Amoroso & Abate, 2007).

518   Metal exposure and adverse abiotic environmental factors produces a general condition of oxidative
519   stress in microorganisms. As oxidative stress is hazardous for fundamental macromolecules, bacteria
520   have evolved several mechanisms to protect themselves from these environmental stresses. In
521   *Streptomyces* sp. H-KF8, an exceptional response to several concentrations of H_2O_2 was observed,
522   compared to the model *Streptomyces violaceoruber* A3(2) which was more susceptible towards the
523   toxic. Consequently, a wide number of genetic determinants related to ROS response were present in
524   the *Streptomyces* sp. H-KF8 genome. Remarkably, a high number of thioredoxins (*trx*) and alkyl
525   hydroperoxide reductases (*ahp*) genes (nine of each) were found in *Streptomyces* sp. H-KF8, in
526   comparison with *Streptomyces violaceoruber* A3(2) where five and one genes were described,
527   respectively. The *ahp* and *trx* are fundamental H_2O_2-inducible genes that encodes for enzymes known to
528   participate in the bacterial response to oxidative stress, which are regulated by *oxyR* in *E. coli* (Storz &
529   Imlay, 1999; Seaver & Imlay, 2001; Chiang & Schellhorn, 2012). The *oxyR* regulon is not present in
530   *Streptomyces* sp. H-KF8, but instead two copies of the *perR* regulator fulfill its role in Gram-positive
531   bacteria (Ricci et al., 2002; Dubbs & Mongkolsuk, 2012). Also, the *ohrR* transcriptional regulator that
532   senses organic peroxide (ROOH) and sodium hypochlorite (NaOCl) (Dubbs & Mongkolsuk, 2012) was
533   found in *Streptomyces* sp. H-KF8. In addition, several genes regulated by the *soxR* transcriptional
534   regulatory system such as glutaredoxin and glutathione peroxidase, superoxide dismutases (*sod),*
535   catalases (*kat*) and thioredoxin reductases were recognized in *Streptomyces* sp. H-KF8 genome, which
536   overall may be accounting for it resistance through H_2O_2 exposure. Even more, the chromate reductase

537 (*chrR*) previously mentioned, could also provide additional protection against H_2O_2 (Das, Dash &
538 Chakraborty, 2016). Interestingly, unusual genes encoding for bromoperoxidases, chloroperoxidases and
539 chlorite dismutases, involved in osmotic stress detoxification of brominated and chlorinated toxic
540 compounds which are abundant in the marine environments (Sander et al., 2003; Bouwman et al.,
541 2012), were also present in *Streptomyces* sp. H-KF8 genome. On the other hand, *Streptomyces*
542 *violaceoruber* A3(2) possess only one chloroperoxidase, suggesting that this might represent another
543 marine adaptation trait for *Streptomyces* sp. H-KF8. Osmotic and oxidative stress response seems to be
544 regulated via a network of sigma factors in *Streptomyces violaceoruber* A3(2), that controls the
545 activation of several oxidative defense proteins, chaperones and systems that provide osmolytes and
546 mycothiol (Lee et al., 2005). Consistently, a high amount of genes for mycothiol biosynthesis was
547 identified in *Streptomyces* sp. H-KF8. Mycothiol is the major low-molecular-weight thiol present in
548 actinobacteria, and serves as a buffer to advert disulfide stress, in complement of the enzymatic system
549 presented above (Buchmeier & Fahey, 2006; den Hengst & Buttner, 2008).

550 Recently, evidence of heavy metal driving co-selection of antibiotic resistance in both natural
551 environments (Seiler & Berendonk, 2012) and contaminated ones (Li, Li & Zhang, 2015; Henriques et al.,
552 2016) have been reported. In this line, isolation of *Streptomyces* with both metal and antibiotic co-
553 resistances have been described (Van Nostrand et al., 2007). In addition, co-evolution of resistance
554 within closely related antibiotic-producing bacteria has been demonstrated for *Streptomyces* (Laskaris et
555 al., 2010). Hence, the antibiotic response against pharmaceutical compounds was investigated in
556 *Streptomyces* sp. H-KF8, and resistance was observed to all antibiotics tested, with exception of
557 gentamicin and novobiocin. Resistance to almost all antibiotics tested, could be due to the presence of
558 multiple BGCs with different mode of action. A typical BGC cluster that produces a bioactive compound
559 is generally coupled to its corresponding resistance gene (Zotchev, 2014). The phenomena of
560 widespread distribution antibiotic resistance genes in natural environments is consequence of improper
561 use of antibiotics in medical treatment, as well as by an indiscriminate use in agriculture, livestock and
562 aquaculture (Brown et al., 2006). Phenomena such as the grasshopper effect may also contribute to the
563 rapid transport of toxics around the globe through atmospheric and oceanic currents (Sadler & Connell,
564 2012).

565 Overall, our study shows the response of a marine *Streptomyces* sp. H-KF8 against several abiotic
566 stressors such as heavy metals, oxidative stress and antibiotics, along with the genome mining of the
567 biosynthetic gene clusters that could be involved in the antimicrobial activity observed. Altogether,
568 these biological features may enable *Streptomyces* sp. H-KF8 to thrive in the complex fjord marine
569 environment.

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968   **Tables**

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

Medium	Bacterial strains[a]				
	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	-	-	-	-	-

970   *-, no inhibition; +/-, attenuated growth; +, <50% growth inhibition (1-5 mm); ++, 50% growth inhibition
971   (6-10 mm); +++, >50% growth inhibition (≥11 mm)
972   [a]STAU, *S. aureus*; LIMO*, L. monocytogenes*; PSAU, *P. aeruginosa*; SAEN, *S. enterica*; ESCO, *E. coli*.

973 **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

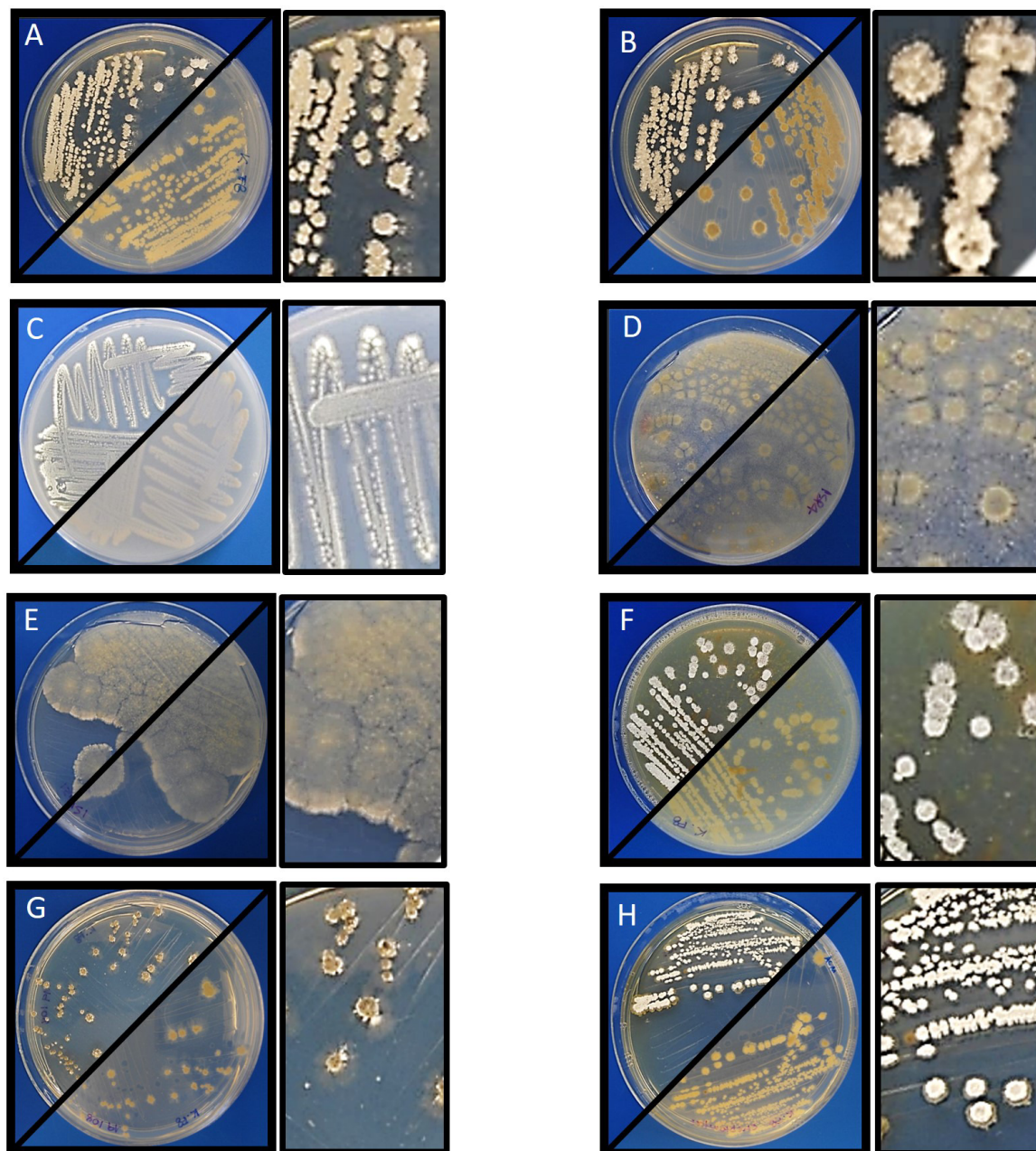
974

975

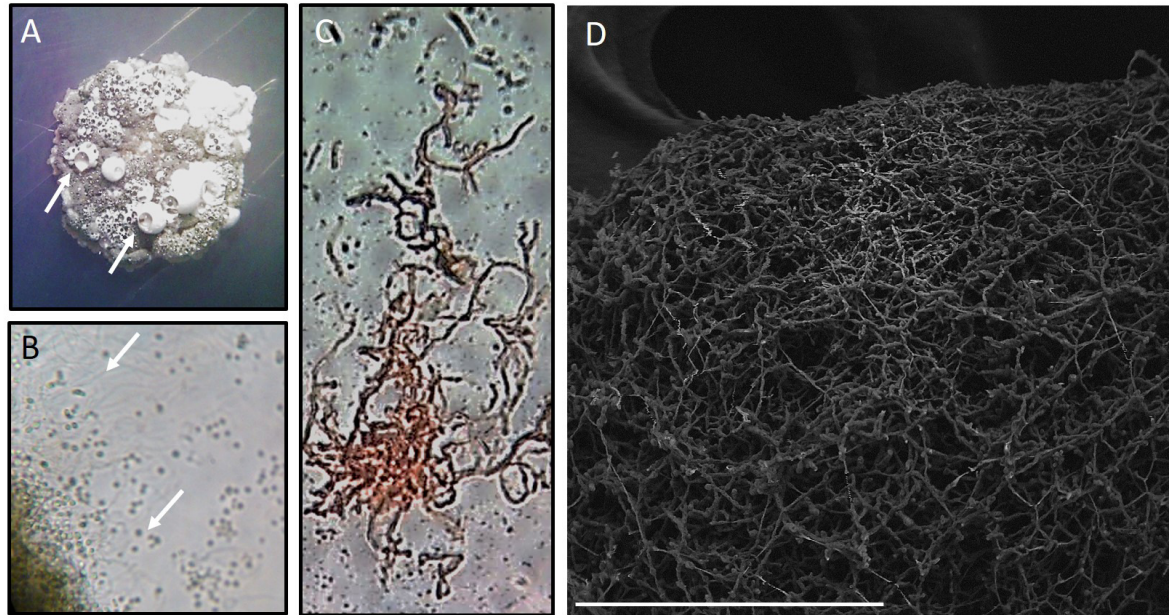
976   **Table 3.** Biosynthetic gene clusters (BGCs) for secondary metabolites in *Streptomyces* sp. H-KF8.

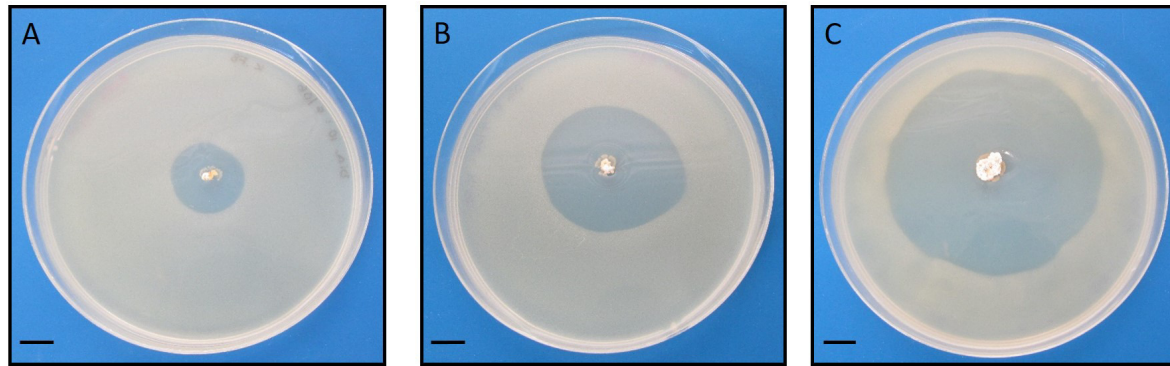
antiSMASH type descriptor	Scaffold	Length (bp)	Predicted product (%[a])	MIBiG-ID
NRPS	tig_02	81285	Streptolydigin (13%)	BGC0001046
NRPS	tig_02	79174	Mannopeptimycin (7%)	BGC0000388
PKS T1	tig_138	33925	Kirromycin (6%)	BGC0001070
PKS T2	tig_139	42512	Spore Pigment (83%)	BGC0000271
NRPS-PKS T1	tig_138	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

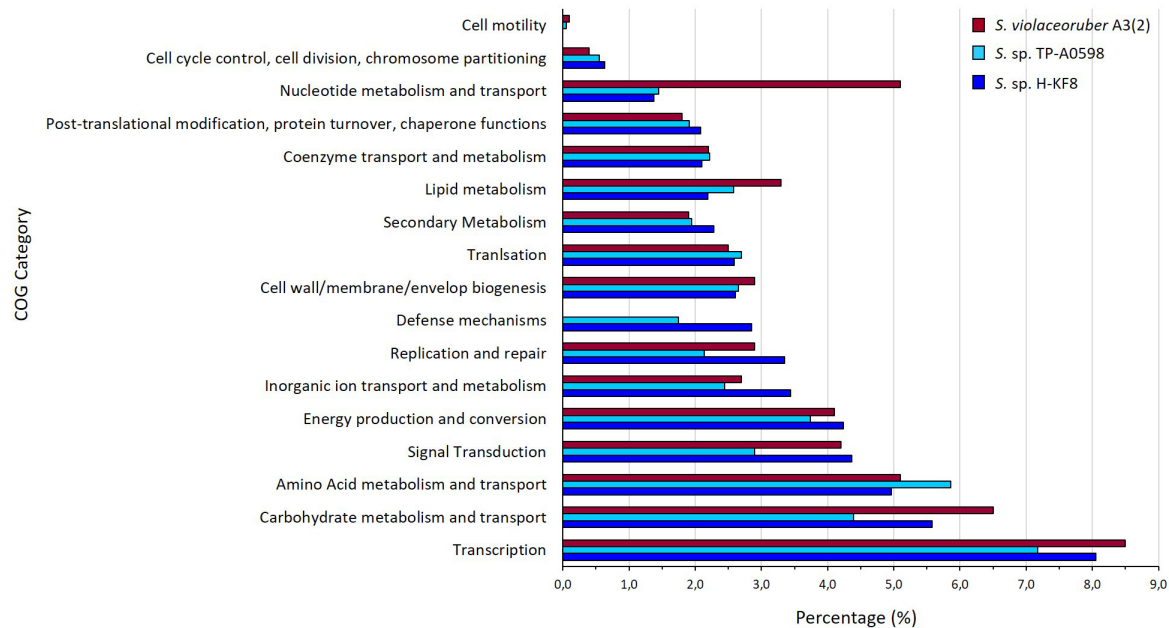
977[a] Percentage of genes from known BGCs that show similarity to genes predicted for BGCs from *Streptomyces* sp.
978H-KF8.

979   **Figures**

980

981   Figure 1. **Morphology of *Streptomyces* sp. H-KF8.** Macrocolony showing anverse and reverse growth in
982   several media. Inset shows a zoom of colony morphology. **A)** ISP1-ASW; **B)** ISP2-ASW); **C)** ISP3-ASW; **D)**
983   ISP4-ASW; **E)** ISP5-ASW; **F)** ISP6-ASW; **G)** Marine Agar (MA); **H)** TSA-ASW.

984

985 Figure 2. **Microscopy of *Streptomyces* sp. H-KF8. A)** Stereoscope zoom of a macrocolony grown in ISP2-
986 ASW agar plate. Arrows shows exudates. **B)** Optic Microscopy image at 1000X. Arrows indicate hyphae
987 and spores, respectively. **C)** *Streptomyces* sp. H-KF8 gram staining, showing hyphae. **D)** Scanning
988 Electron Microscopy (LVEM) image of *Streptomyces* sp. H-KF8 grown on ISP3-ASW agar plates for 21
989 days. Bar represent 100 µm.

990

991 Figure 3. **Antibacterial activity of *Streptomyces* sp. H-KF8**. Photographs depict inhibition zone against
992 *Staphylococcus aureus*. Bar represents 1 mm. Time course was performed using the double-layer
993 method, at various incubation days: **A)** 6 days; **B)** 9 days; **C)** 15 days.

994

995
996 Figure 4. **Comparative genomics of COGs categories.** Percentage of each COG category is shown for the
997 different *Streptomyces* species, where blue is *Streptomyces* sp. H-KF8; light blue is the marine-derived
998 *Streptomyces* sp. TP-A0598; and red is the soil-derived *Streptomyces violaceoruber* A3(2).

Legend:
- NRPS
- Terpenes
- Hybrids
- RiPP
- Other
- PKS
- Ectoine
- Melanine
- Siderophores
- Lantipeptide
- Butyrolactone

999

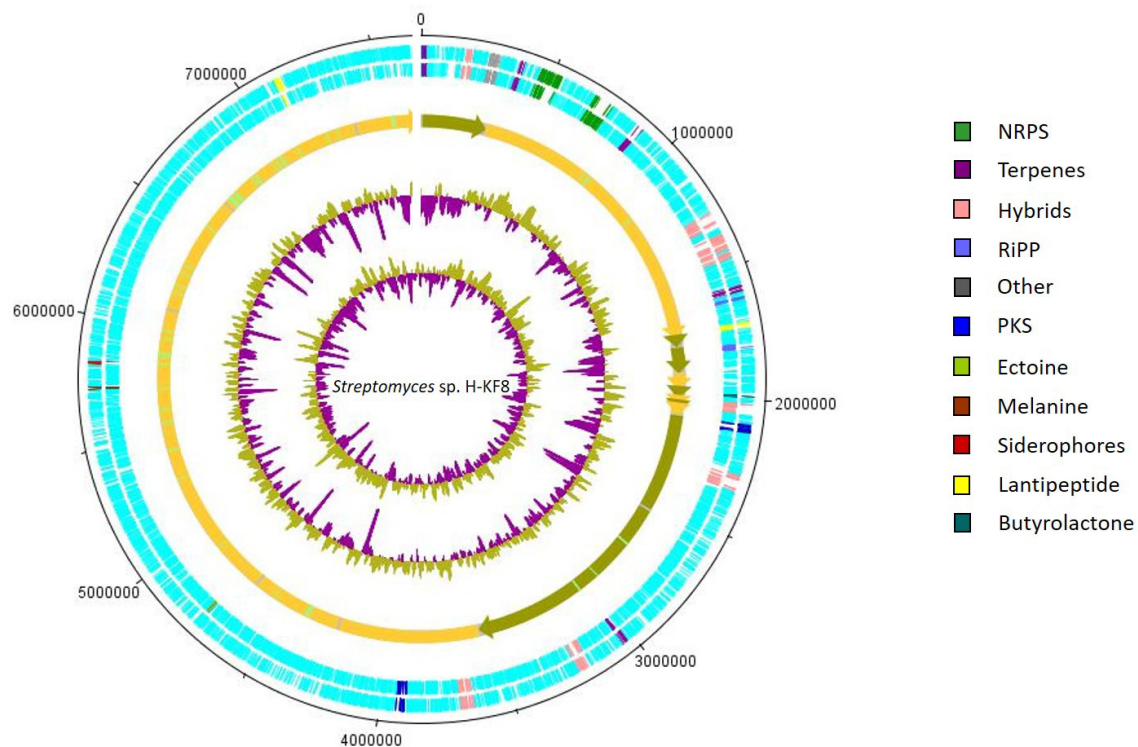



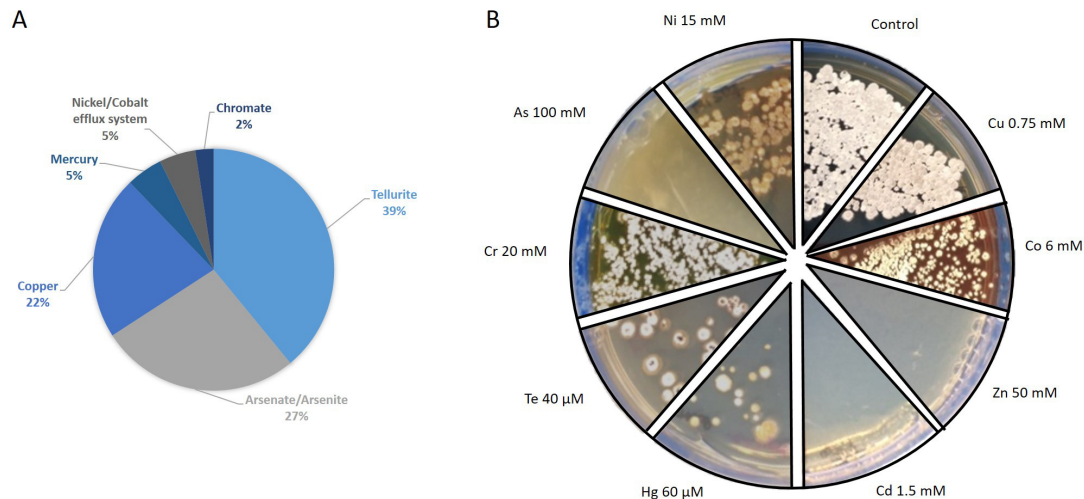
Figure 5. **Representation of chromosome features and BGCs of *Streptomyces* sp. H-KF8**. Colors depict the different classification types of secondary metabolism gene clusters along the sequenced genome. NRPS, Non-ribosomal peptide synthetase; PKS, polyketide synthase; RiPP, ribosomally synthesized and post-translationally modified peptides. From outside inward: DNA strands reverse and forward; contigs; GC content; GC skew.

1005

Figure 6. **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 show maximum concentration of metal(loids) where growth of *Streptomyces* sp. H-KF8 was observed. Concentrations below these values also presented growth. Control, agar plate without any metal.

1011

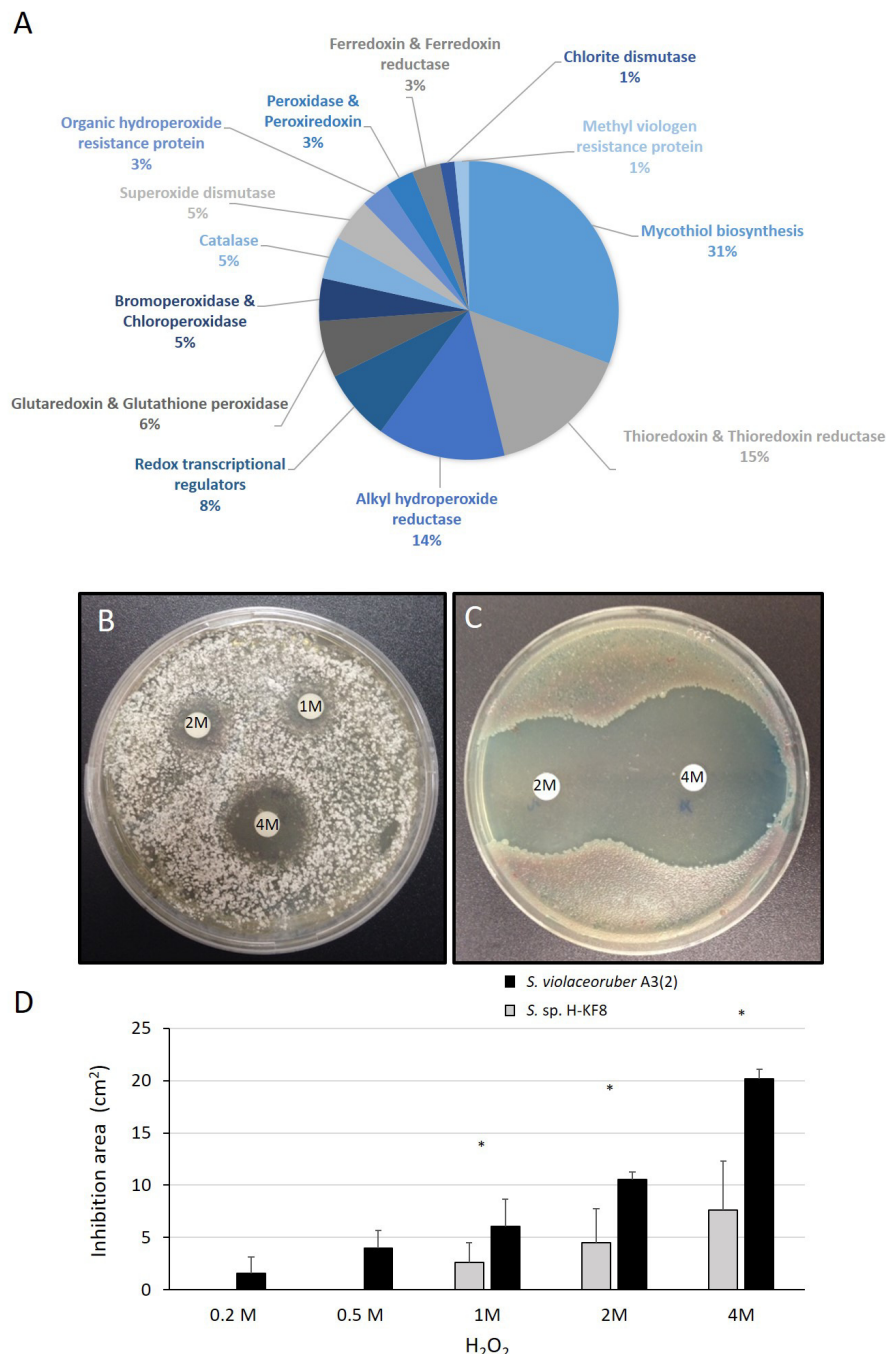



Figure 7. **Oxidative stress response of *Streptomyces* sp. H-KF8**. **A)** Genetic determinants involved in oxidative stress-resistance observed by genome mining. Functional response of **B)** *Streptomyces* sp. H-KF8 and **C)** *Streptomyces violaceoruber* A3(2) respectively, showing comparative inhibition zones with hydrogen peroxide where the concentration of hydrogen peroxide used in each disk is shown. **D)** Quantitative assay of inhibition area of both *Streptomyces* strains facing several concentrations of hydrogen peroxide. Asterisks indicate significant differences between strains (*t*-Test considering a p-value <0.01).

A

B

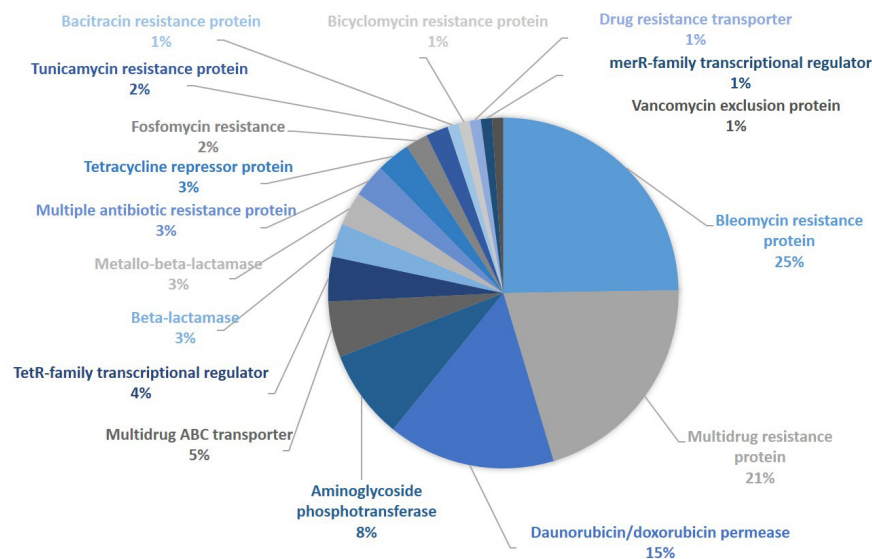
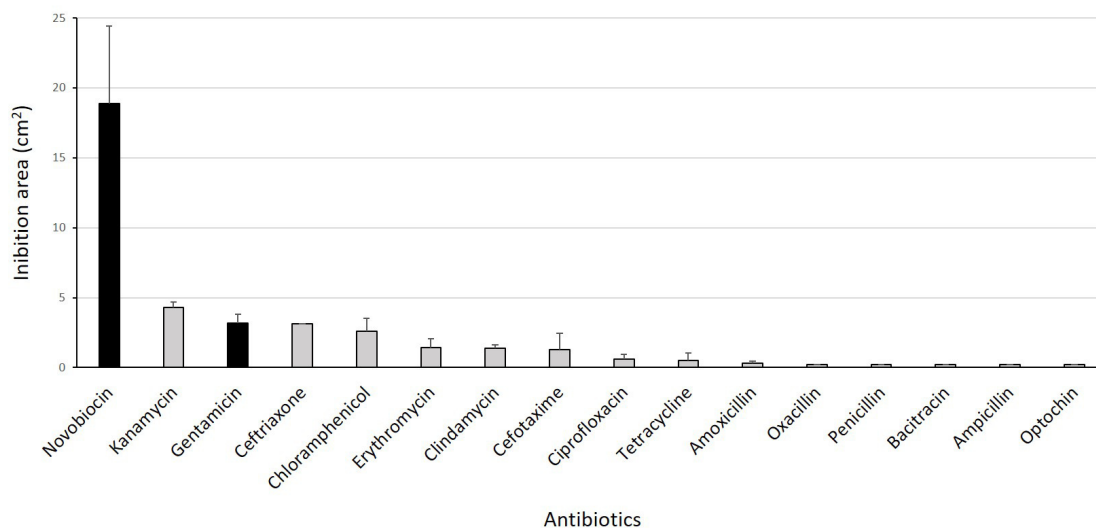



Fig. 8. **Antibiotic-resistance response in *Streptomyces* sp. H-KF8. A)** Genetic determinants involved in antibiotic-resistance observed by genome mining. **B)** Functional response of antibiotic-resistance in MH-ASW agar plates. Black columns indicate susceptibility to the antibiotic tested and grey columns indicate resistance to the antibiotic tested.