The Microbe 2 (2024) 100033

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

Catechol-type siderophore producer *Streptomyces* spp. with quorum-quenching activity isolated from wild medicinal plants rhizosphere

Fatemeh Alinejad [a], Gholam Khodakaramian [a,*], Darshan Chandramowli [b], Bart Devreese [b]

[a] Department of Plant Protection, College of Agriculture, Bu-Ali Sina University, Hamadan 65174, Iran
[b] Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, Ghent B-9000, Belgium

ARTICLE INFO

Keywords:
Streptobactin
Secondary metabolites
Antibiotic production
Quorum sensing
LC-MS analyses
Biosynthetic gene clusters

ABSTRACT

Traditional medicinal plants represent a unique source for the isolation of *Streptomycetes* and antimicrobial compounds. Antimicrobial activity of *Streptomyces* isolates collected from the rhizosphere of different native medicinal plants in Iran, was investigated against Gram-positive and Gram-negative bacteria. Multi-omics analysis was performed to identify active compounds corresponding to the observed bioactivities. Since cell-to-cell communication mediated by N-acyl homoserine lactones (AHLs) is important for the virulence of Gram-negative pathogenic bacteria, quorum quenching activity of the isolates and their ability to degrade C6-HSL and C8-HSL were tested. Two highly related *Streptomyces* isolates derived from the *Helichrysum rubicundum* and *Rumex acetosa* rhizosphere, respectively, exhibited bioactivity against pathogenic indicator bacteria. Metabolite analysis of extracts obtained from liquid cultures of both isolates revealed the production of the catechol-peptide siderophores streptobactin and tribenarthin. Proteomic analysis confirmed the presence of proteins encoded by the streptobactin and lidamycin biosynthetic gene clusters. Quorum quenching activity of the strains and AHL-degrading enzyme production was confirmed using a GFP producing biosensor and LC-MS analysis. This study demonstrates catecholate siderophore production and QQ potential of two *Streptomyces* isolates from medicinal plants rhizosphere from Mishan Plain, Hamedan Province, Iran using metabolomics and proteomics analysis. Production of streptobactin by terrestrial *Streptomyces* sp. strains was confirmed for the first time.

1. Introduction

The majority of secondary metabolites with pharmacological applications are produced by members of the genus *Streptomyces*, the largest group of Actinobacteria (Barka et al., 2016). Members of this genus typically host more than 20 gene clusters involved in the biosynthesis of secondary metabolites and due to this potential, they continue to be an interesting resource for the discovery of new bioactive compounds such as antimicrobial products (De Simeis and Serra, 2021; Nett et al., 2009).

Because of morphological, physiological and metabolic diversity, *Streptomyces* bacteria are found in a wide range of environments such as in soil, seawater as well as in association with animals and plants (Wang et al., 2023; Farda et al., 2022; Goodfellow and Williams, 1983). The composition of the microbial community in the rhizosphere area is influenced by root exudates and soil geochemistry (Dennis et al., 2010). *Streptomyces* are a successful group of bacteria in colonizing this niche because of their high potential to produce bioactive compounds and of their evolution of antibiotic resistance mechanisms that provide them with a competitive advance towards other microorganisms (Schlatter et al., 2009). Indeed, there is a mutual benefit for both plant and microbe. Plants benefit mainly from the capacity of many *Streptomyces* to produce the plant hormone indole −3- acetic acid, antifungals and to induce plant defense responses i.e. induced systemic resistance (ISR) and systemic acquired resistance (SAR), whereas the bacteria may profit from plant-associated phenomena such as the presence of pollinators for their dispersal (Shepherdson et al., 2023; Tarkka et al., 2008; Viaene et al., 2016).

In this study, we describe the isolation and identification of *Streptomyces* collected from traditional medicinal plants' rhizosphere during a campaign in Hamedan Province, Iran. Traditional medicinal plants represent a rich and unique source for the isolation of *Streptomyces* and new antimicrobial compounds (Bekiesch et al., 2020; Oberhofer et al., 2019). Indeed, many *Streptomyces* are isolated from traditional plants, especially from regions with traditional medicinal practice, often in

* Corresponding author.
E-mail address: khodakaramian@basu.ac.ir (G. Khodakaramian).

https://doi.org/10.1016/j.microb.2023.100033
Received 15 July 2023; Received in revised form 7 December 2023; Accepted 29 December 2023
Available online 2 January 2024

areas with poor nutrient availability and extreme environments (Quinn et al., 2020). These bacteria were shown to produce a wide spectrum of antibiotics from different molecular origin (polyketides, peptides, aminoglycosides, siderophores …) and may be a source of novel products to contribute to human and animal health.

Therefore, metabolite extracts of the novel isolates were tested for direct antibiotic activity against both gram-negative and gram-positive model bacteria. In addition, we evaluated these isolates for their capability to interrupt the quorum sensing signaling pathway related to bacterial activities as biofilm formation, resistance and virulence. Despite being unicellular, bacteria behave collectively via quorum sensing. This cell density dependent regulatory system mediates via small signaling molecules regulating different functions such as expression of virulence factors. The signaling molecules are diverse but acyl-homoserine lactone (AHL) is the most common signal in Gram-negative bacteria (Williams, 2007; Fuqua and Greenberg, 2002). Blocking the QS system (quorum quenching (QQ)) has been considered an alternative strategy to control bacterial infections without killing them, as it puts less selective pressure on pathogens reducing the risk to develop resistance (Grandclément et al., 2015; Andersen et al., 2001). AHL-acylase and AHL-lactonase are two families of AHLs degrading enzymes that disrupt the QS system and have been reported from different Gram-positive and Gram-negative bacteria (Chen et al., 2013).

To summarize, we describe the isolation of 16 *Streptomyces* isolates that display antimicrobial activity. For two strong bioactive isolates, 16 S rRNA sequencing was performed and bioactive metabolite identification and QQ capacity of the isolates were investigated based on biological assays, metabolomic and proteomic analysis.

2. Materials and methods

2.1. Chemicals and reagents

Almost all reagents were purchased from Chem-Lab (Zedelgem, Belgium), Sigma-Aldrich (New Jersey, U.S.) and Merck (Darmstadt, Germany); unless indicated otherwise.

2.2. Soil sampling and Streptomyces isolation

Soil samples were collected from the rhizosphere of wild medicinal plants, in Mishan Plain, Hamedan Province, Iran (coordinates 34°47'54"N, 48°30'53"E) in June, 2019. Plants were selected randomly and samples were transported to the laboratory in sterile zipped bags for bacteria isolation. Soil samples were air dried at room temperature for 2 days and *Streptomyces* cultures were isolated from one gram of dried soil by serial dilution plating technique. Starch casein agar (SCA) and yeast malt agar (ISP2) were used as cultivation medium. The cultures were incubated at 28 °C for 7–14 days. Morphologically identified colonies were picked up and purified on ISP4 medium, (BD Difco) plates were incubated at 28 °C for 7 days. Spore suspension from pure cultures was prepared in 30% glycerol and stored at − 20 °C for further work (Seong et al., 2001; Shirling and Gottlieb, 1966).

2.3. Screening for antibacterial activity

2.3.1. Primary screening

Antibacterial activity of the *Streptomyces* isolates against Gram-positive (*Staphylococcus aureus* ATCC 25423, *Bacillus subtilis*) and Gram-negative (*Escherichia coli* ATCC 25923, *Pectobacterium atrosepticum*, *Agrobacterium vitis*, *Erwinia amylovora*) bacteria were tested by cross streak method on Potato dextrose agar (PDA) (Potato infusion 200 g; Dextrose 20 g; Agar 20 g in 1000 ml of dH_2O) and on Minimal Medium (MM) (L-asparagine 0.5 g; K_2HPO_4 0.5 g; $MgSO_4{\cdot}7\ H_2O$ 0.2 g; $FeSO_4.7\ H_2O$ 0.01 g; mannitol 5 g; agar 10 g in 1000 ml of dH_2O). The plates with a single streak of *Streptomyces* in the middle were incubated for 7 days at 28 °C and then inoculated with indicator bacteria by a single line at a 90∘ angle to the streak of the *Streptomyces*, the plates were again incubated for 16–24 h at 37 °C and inhibition zone was measured (Lemos et al., 1985).

2.3.2. Secondary screening (Fermentation, extraction of secondary metabolites and bioassay)

Preparation of seed culture and cultivation: one ml of spore suspension from fresh culture of the strains on ISP4 medium was inoculated into 20 ml of seed medium (0.5 g of starch, 0.5 g of glucose, 0.1 g of peptone, 0.5 g of NaCl, 0.2 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4.7\ H_2O$, 0.1 g of K_2HPO_4 and 0.2 g of $CaCO_3$ in 100 ml of distilled water) in 100 ml Erlenmeyer flasks separately and incubated at 28 °C and 150 rpm for 2 days. 5 ml of seed culture was used to inoculate 150 ml of inorganic salt medium (BPM3) (10 g of soluble starch, 2 g of $(NH_4)_2SO_4$, 1 g of K_2HPO_4, 1 g of $MgSO_4{\cdot}7\ H_2O$, 1 g of NaCl, 2 g of $CaCO_3$, 6.4 mg of $CuSO_4.5\ H_2O$, 1.1 mg of $FeSO_4.7\ H_2O$, 7.9 mg of $MnCl_2.4\ H_2O$ and 1.5 mg of $ZnSO_4.7\ H_2O$ in 1 liter of distilled water) as the main production medium in 500 ml Erlenmeyer flasks (2 flasks for each strain), the flasks was incubated at 28 °C and 150 rpm for 7 days (Kibret et al., 2018; Rajeswari et al., 2014).

Extraction and bioassays: after incubation period, mycelia and spores were removed from the fermentation broth by centrifugation at 8000 rpm for 20 min and using 0.22 μm filter. Metabolites were recovered from the broth using 150 ml mixture of chloroform and methanol (1:1, v:v) and added to the broth in 1:2 (v:v) proportion, stirred for 1 h, and centrifuged at 4500 g for 30 min. The organic phase was separated and dried in speed vac, half of the resultant dried extracts were resuspended in 100 μl of MeOH and were tested for their antimicrobial activity. Antibacterial activity of the crude extracts was tested against *B. subtilis* and *Stenotrophomonas maltophilia* using disk diffusion assay technique. The latter was selected because it is known to by highly resistant to most common antibiotics, challenging clinical management of infections of this opportunistic nosocomial pathogen (Mojica et al., 2022). 15 μl of methanolic crude extracts was applied on the 6 mm sterile filter disks, dried and placed on Nutrient agar plates (Oxoid) inoculated with 1.5 × 108 CFU/ml indicator bacteria. The plates were incubated at 37 °C and diameter of clear zone around the discs was measured after 16–24 h (Bauer et al., 1966).

2.4. Identification of the active isolates based on 16 S rRNA gene sequencing

The genomic DNA of isolates was extracted from fresh cultures grown on ISP4 medium at 28 °C for 24–48 h using alkaline lysis buffer and 16S rRNA gene fragments were amplified with the universal bacterial 16S rRNA gene primers pA and pH (Edwards et al., 1989). PCR products were run on a 1% agarose gel with Tris-borate-EDTA buffer, thereafter products were purified and sequenced with the same primers by Sanger sequencing at EUROFINS. Resulting 16S rRNA gene sequences were compared with related sequences in GenBank. Phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software version X with neighbor-joining method and 1000 bootstrap replicates (Kumar et al., 2016).

2.5. Purification and identification of antibacterial compounds

The remaining half of the dried active crude extracts were dissolved in 100 μl of 5% acetonitrile in ammonium formate (10 mM) and 50 μl was fractionated to purify bioactive metabolites. Separation was carried out on a Spheri-5 RP-C18 Column (220 mm×**2**.1 mm), using an AKTA Ettan LC system equipped with a UV detector and fraction collector FRAC-950 (Cytiva). Ammonium formate (10 mM) and acetonitrile were used as mobile phase A and B, respectively. Gradient elution used 5–100% solvent B in 30 min, 10 min at 100% B, 10 min at 5% B, with 0.1 ml/min flow rate. The collected fractions were dried by vacuum centrifugation and half of each fraction was tested for their antibacterial activity using disk diffusion technique as described in the previous

section (crude extracts antimicrobial activity).

Mass spectrometric analysis of the bioactive fractions was performed using the 4800 Plus MALDI TOF/TOF Analyzer (Sciex) equipped with a Nd:YAG laser (200 Hz, 355 nm). The active fractions (a quarter of each dried active fraction dissolved in 5 µl of 0.1% TFA in 50% ACN (v/v)) and MALDI-TOF matrix (α-cyano-4-hydroxycinnamic acid (CHCA)) were mixed in a ratio 1:1(v/v). One µl of the mixture was spotted on a 384 MALDI target plate, allowed to dry at room temperature and analyzed by mass spectrometry. Reflector positive ion mode was used for mass analysis between 700–4000 Da and acquisition laser intensity was 4200 and 5500 for MS and MS/MS spectra respectively. MS/MS fragmentation was done at 1 kV.

2.6. Proteome analysis

2.6.1. Protein extraction and tryptic digestion

Pre-culture and main broth culture were prepared as described for metabolite extraction. The mycelium of the strains was collected from the three-day broth cultures by centrifugation, resuspended in 10 ml of 50 mM NH_4HCO_3 buffer in 50 ml Falcon tubes and sonicated (20 ×**3**" on and 7" off at 30%) on the ice bath, thereafter the suspension was centrifuged at 16,000 x g for 15 min at 4 °C to obtain intracellular extracts (supernatant). The protein lysate was mixed with 100% (w/v) trichloroacetic acid (TCA) solution (4:1) and incubated overnight at 4 °C. The extract was centrifuged at 16,000 x g for 30 min at 4 °C, supernatant was discarded and pellet was washed with ice cold acetone and dissolved in 50 µl of 50 mM ammonium bicarbonate containing 2 M urea. The protein concentration was assessed by Bradford analysis using the Pierce Coomassie Protein Assay Kit. Protein solutions (10 µg) were reduced with 1 M dithiothreitol (DTT) (10 min at 56 °C), alkylated with 150 mM iodoacetamide (20 min at room temperature), and digested with trypsin/LysC endoproteinase (Promega) overnight at 37 °C (1:50 w/w). Digested samples were dried and dissolved in 50 µl 20 mM ammonium formate (Saelens et al., 2022).

2.6.2. LC-MS/MS analysis of the proteome

Peptide samples of both strains were analyzed using an Eksigent Expert nanoLC 425 system, coupled online to a Triple TOF 5600 mass spectrometer (ABSciex). The peptide solutions were injected and trapped for 5 min with 8 µl/min flow rate and separated on a YMC Triart C18 column (3 µm, 0.3 ×**150** mm) using gradient elution from 3% to 40% solvent B (0.1% formic acid in ACN) for 60 min at a flow rate of 5 µl/min. The outlet of the LC column was connected to the inlet of a DuoSpray electrospray ionization source. The capillary voltage was set to 5500 and MS scans were recorded from 400 to 1200 *m/z*. The MS/MS scan range was set from 50 to 1500 *m/z*. Data were acquired in data-dependent acquisition mode. Precursors were selected for fragmentation at a threshold of 500 cps, with a charge of + 2 to + 5 and a dynamic exclusion window of 10 s

LC–MS raw data were analyzed and searched using PEAKS Xpro software (Bioinformatics Solutions Inc) against the Streptomyces proteome database downloaded from UniProt (May 16, 2022). The precursor mass and fragment ion were set at 20 ppm using monoisotopic mass and 0.2 Da respectively. Trypsin as enzyme and specific digest mode with 2 missed cleavages per peptide were selected. Methionine oxidation was allowed for the PEAKS searches. Only protein groups identified with a minimum of 2 unique peptides were accepted. For proteins detected by one unique peptide, the peptide MS/MS spectrum was manually verified by comparing the experimental spectrum with predicted fragment ions and the presence of mainly b and y type ions as expected for tryptic peptides CID spectra. The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) repository with the dataset identifier PXD042019 and PXD041994 for strains 1 M and 14 M respectively.

2.7. Screening of quorum quenching activity of the Streptomyces strain

The ability of the two strains to degrade acyl-HSLs was tested using *E. coli* JB523 as a QS-regulated GFP producer biosensor. One ml of seed culture (described above) was added to 5 ml of AB medium (Clark and Maaløe, 1967) supplemented with synthetic N-hexanoyl-L-homoserine lactone (C6-HSL) and N-octanoyl-L-homoserine lactone (C8-HSL) as a sole carbon source at a final concentration of 50 nM with pH= 7. Cultures were incubated at 28 °C and 150 rpm for 7 days (Park et al., 2005), thereafter cell free supernatant was prepared with centrifuging the cultures at 8000 g for 10 min and 0.22 µm filter then supernatants were used for fluorescence assay and LC-MS analysis.

Fluorescence based assay: 100 µl of filtrated cultures were pipetted into the wells of black 96-microwell plates in triplicate, overnight culture of E. coli JB523 (received from Prof Tom Coenye, Laboratory of Pharmaceutical Microbiology, Ghent University) in Luria broth (LB) containing 100 mg/ml tetracycline was diluted in sterile LB medium to OD600 = 0.1 and 100 µl was added to the wells, after incubation at 37 °C for 4 h with gently shaking, GFP expression was measured (excitation at 475 nm and emission at 515 nm) by a Tecan Spark multimode plate reader. AB medium and AB medium with AHLs were used as negative and positive controls respectively. Additionally, *Streptomyces* cultures in AB medium without C6-HSL and C8-HSL were used as an extra control to ensure that these signals or any similar compounds were not produced by the *Streptomyces* strains. (Medina-Martínez et al., 2007; Medina-Martínez et al., 2007). The data were analyzed with One-way Analysis of Variance (ANOVA) and Duncan's multiple range tests, using SAS 9.0. A p value of < 0.05 was required for significance.

LC-MS analysis of AHLs degradation: degradation of AHLs molecules and isolates QQ activity was investigated using LC-MS/MS. Waters NanoAcquity M-Class UPLC equipped with an IonKey source coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer was used for analysis. 5 µl of each sample were injected and trapped for 2 min with 15 µl/min flow rate on a 300 µm x 50 mm, 5 µm, 100 Å Acquity UPLC M-Class Symmetry C18 Trap Colum (Waters). IKey separation device (150 µm x 100 mm, 1.8 µm HSS T3, Waters) was used for separation using gradient elution of 3–50% solvent acetonitrile at a flow rate of 2 µl/min for 15 min

The separated samples were introduced into Waters Xevo TQ-S mass spectrometer for quantification of the analytes using positive ion electrospray and multiple reaction monitoring (MRM) mode with transitions of selected precursor ions at a set cone voltage and different collision energies. Capillary voltage, 3.6 kV; cone voltage, 35 V; source temperature, 120 °C and 0.14 ml/min flow rate of argon were set as ESI-MS/MS parameters. Data acquisition was performed by MassLynx 4.1 software and processed in Skyline version 20.2.

Fresh standard stock solutions of the C6-HSL and C8-HSL molecules were used to confirm the exact masses and retention times. Thereafter, calibration curves were generated for the molecules separately. Fresh solutions of AHLs were prepared and added into AB medium to achieve 0.002 to 0.5 µM and 0.0002 to 0.025 µM concentration for C6-HSL and C8-HSL respectively. Samples were analysed by LC-MS in MRM mode, three replicates and 5 µl injection for each sample to obtain fragments and concentration. Calibration curves were plotted using the average peak AUC of the analytes corrected for background.

3. Results

3.1. Isolation of Streptomyces and screening antimicrobial activity

In total, 16 different isolates of *Streptomyces*, based on morphology, were purified from the rhizosphere of 9 different wild medicinal plants from Mishan Plain, Hamedan Province, Iran.

In the primary screening for antimicrobial activity using cross streak method, out of 16 isolates, isolates 1 M and 14 M collected from the rhizosphere of *Helichrysum rubicundum* and *Rumex acetosa* (identified by

Dr Akbar Aliverdi, Department of Agronomy and Plant Breeding, Bu-Ali Sina University), respectively, showed strong antimicrobial activity (inhibition zone ≥ 1 cm) against both Gram-positive and Gram-negative indicator strains; *S. aureus* ATCC 25423, *B. subtilis*, *E. coli* ATCC 25923, *P. atrosepticum*, *A. vitis*, *E. amylovora* on the PDA and MM medium. These isolates were selected for further analysis.

BPM3 medium was used as liquid fermentation medium for investigation of the isolate's ability to produce any antimicrobial metabolite in the liquid medium. Crude extracts obtained from broth culture of each of selected isolates inhibited the growth of *B. subtilis* and *S. maltophilia* using disk diffusion method (Fig. 1).

3.2. Bacterial identification based on 16S rRNA gene sequencing

The two selected active isolates were confirmed as belonging to genus *Streptomyces* based on the 16 S rRNA gene sequences (~ 1500 nt) and phylogenetic analysis. The nucleotide BLAST search (Altschul et al., 1990) revealed that *Streptomyces globisporus* was the closest related species with its 16 SrRNA having 99.40% and 99.47% sequence identity to the 16S rRNA of *Streptomyces* sp. 1 M (GenBank accession no. OQ927600) and *Streptomyces* sp. 14 M (GenBank accession no. OQ927601) respectively. Alignment and comparison of 16 S rRNA nucleotide sequences of 1 M and 14 M strains using BLAST, also showed 99% sequence identity between them (data not shown). Further analysis and comparison of the two 16SrRNA sequences with those from a panel of *Streptomyces* type strains using EZBiocloud (Yoon et al., 2017) followed by phylogenetic analysis using MegaX confirmed the high relationship with *S. globisporus* and related species (Fig. 2). More detailed taxonomic analysis and genome sequences should unveil whether the two strains can be truly considered as novel species.

3.3. Purification and identification of bioactive compound

Both bioactive extracts were fractionated by RP HPLC on a C18 column in gradient mode to separate and partially purify compounds

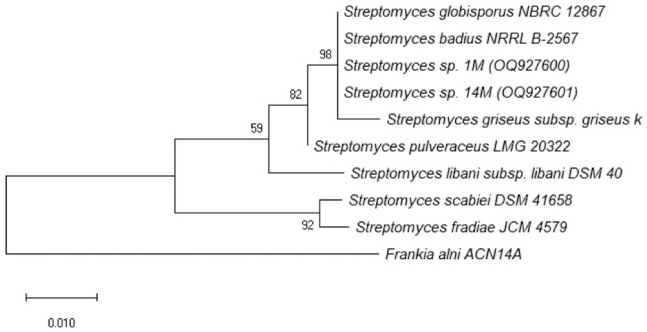

Fig. 2. Phylogenetic tree based on comparison of 16 S rRNA gene sequences of novel isolates 1 M and 14 M with a selection of *Streptomyces* type strains using Maximum-Likelihood Tree construction in MEGA X. Bootstrap values was calculated from 1000 replicates and the tree was rooted with *Frankia alni* ACN14a (Edwards et al., 1989).

responsible for the antibacterial activity against indicator bacteria. In total, 21 and 16 fractions were collected from the 1 M and 14 M crude extracts, respectively, and based on bioassay testing, *Streptomyces* sp. 1 M fraction 7 (collected at 24.9–25.9 min) and *Streptomyces* sp. 14 M fraction 5 (collected at 26.9–28.9 min) were identified as most active fractions (Fig. 3). These fractions prevented the growth of *B. subtilis* and *S. maltophilia* on the plates and inhibition zones (9 mm ≤ inhibition zone ≤ 12 mm) were observed around the paper disks (Fig. 4).

The metabolite identification was carried out using MALDI-TOF mass spectrometry analysis. *Streptomyces* sp. 14 M fraction 5 in positive ion mode displays a set of peaks with *m/z* values of 1180.70, 1198.46 and 1242.62 ($[M + H]^+$) as main peaks (Fig. 5). MS/MS spectrum of each precursor ion was analysed in detail and the combination of precursor/fragment masses was compared with public data available from the library of Streptomyces compounds (Streptome DB), GNPS spectrum library and information available in the literature. Based on this information it was found that these compounds may correspond to

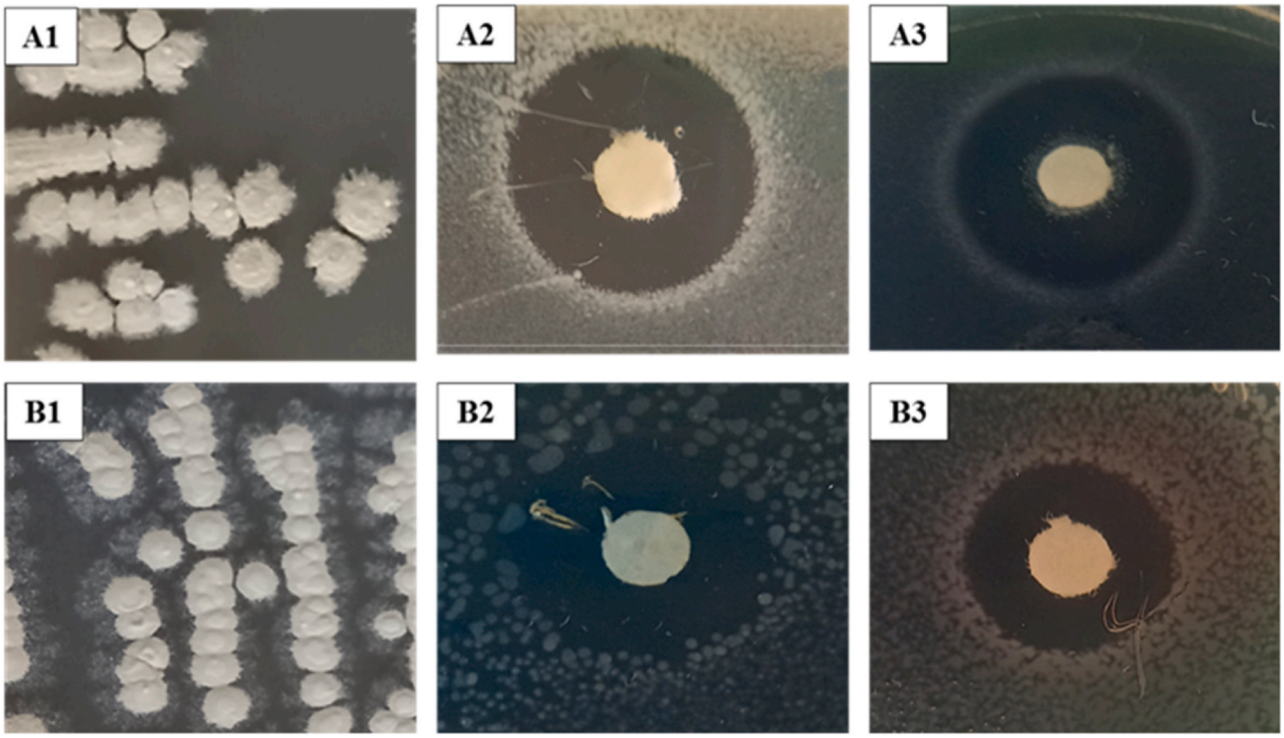

Fig. 1. *Streptomyces* sp. 1 M morphology on ISP4 medium (A1) and its crude extract antibacterial activity against *Bacillus subtilis* (A2) and *Stenotrophomonas maltophilia* (A3); *Streptomyces* sp. 14 M morphology on ISP4 medium (B1) and its crude extract antibacterial activity against *B. subtilis* (B2) and *S. maltophilia* (B3).

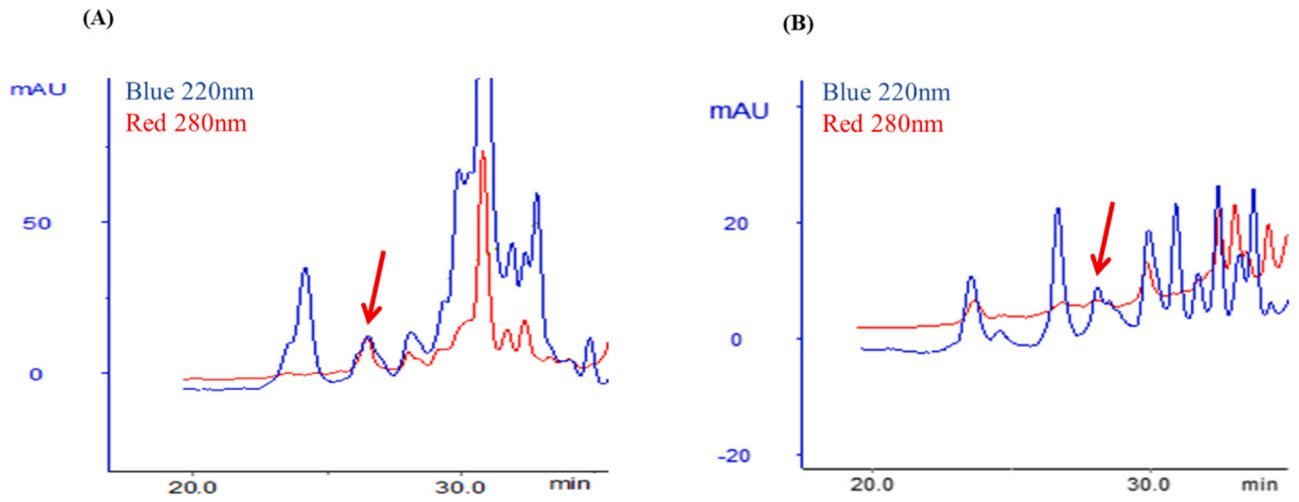

Fig. 3. Detail of HPLC chromatogram of crude methanol/chloroform extracts of *Streptomyces* sp. 1 M (A) and *Streptomyces* sp. 14 M (B) and their active peaks/fractions corresponding to the antimicrobial activity.

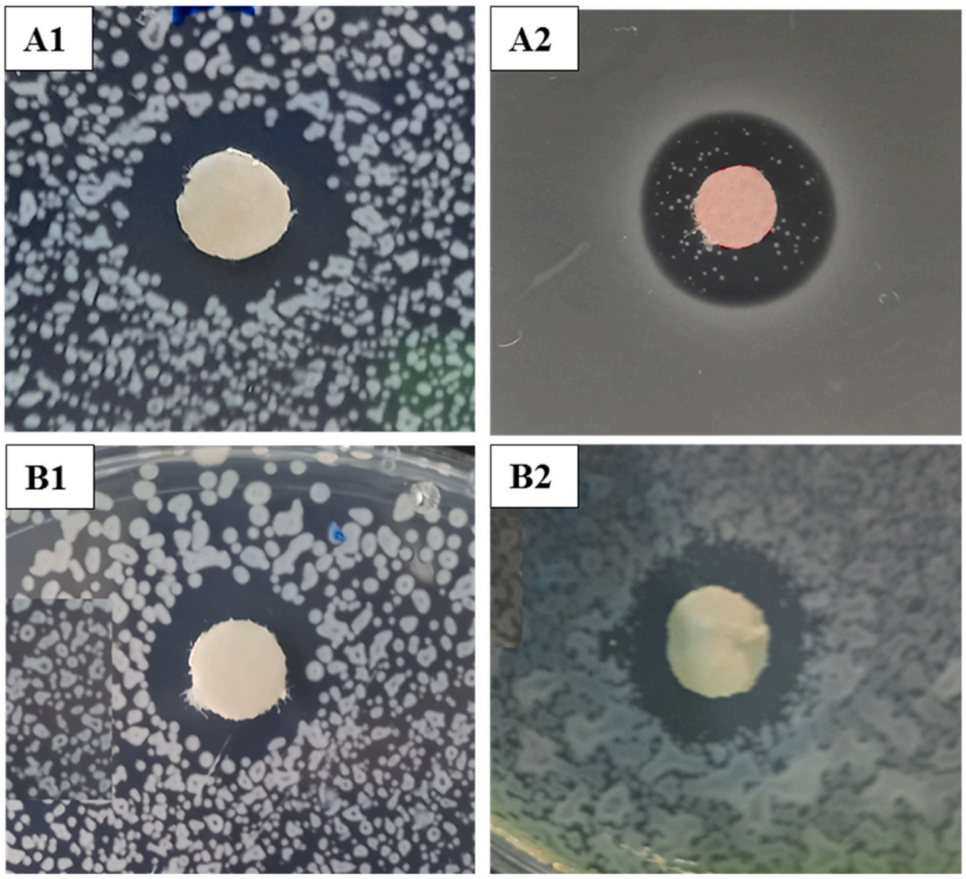

Fig. 4. Antimicrobial activity of the *Streptomyces* sp. 1 M fraction 7 against *Bacillus subtilis* (A1), *Stenotrophomonas maltophilia* (A2); and *Streptomyces* sp. 14 M fraction 5 against *B. subtilis* (B1), *S. maltophilia* (B2).

streptobactin, tribenarthin and an unknown compound probably related to the two other compounds in view of the highly comparable mass shifts in the MS/MS spectrum (Matsuo et al., 2011).

Identification of the compound with *m/z* 1180.70 as streptobactin (calculated monoisotopic mass MH^+: 1180.49) is based on the MALDI TOF/TOF MS/MS spectrum (Fig. 6). The main fragment ion displays at *m/z* 787.38 and is attributed to the loss of a unit of -Thr-Arg- 2,3-dihydroxybenzoyl (DHBA) with formula $C_{17}H_{23}N_5O_6$. This fragment appears at *m/z* 394.22 [M + H]+ . The presence of *m/z* fragments 1044.55 and 888.43 is due to the loss of -DHBA and -Arg-DHBA respectively while the appearance of *m/z* fragment 495.26 is the result of separating of Arg-DHBA from *m/z* 787.3833 part. As such, the similarity of the fragmentation pattern of the precursor ion 1180.70 with that of streptobactin is confident (Matsuo et al., 2011).

This is supported by the fact that, as described in (Matsuo et al., 2011), the compound with *m/z* 1180.70 is accompanied with a

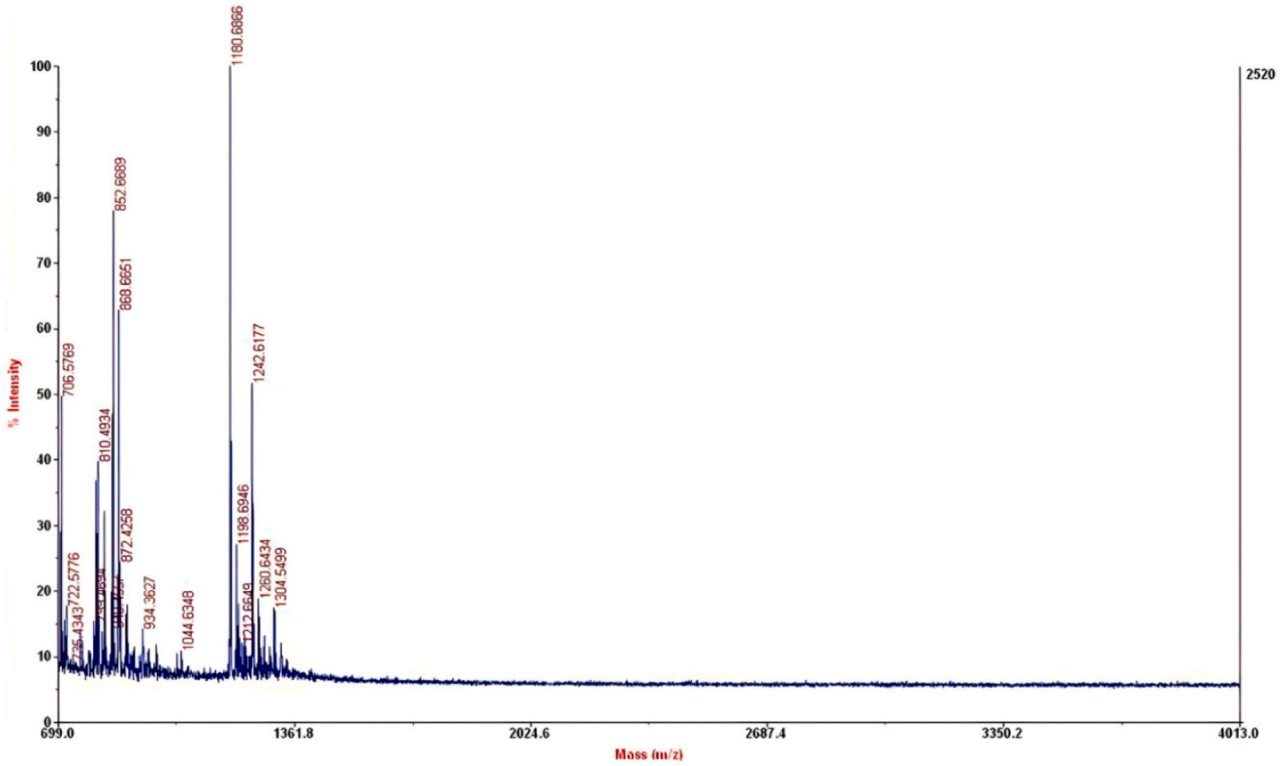

Fig. 5. MALDI TOF/TOF–MS spectrum of *Streptomyces* sp. 14 M fraction 5 containing antimicrobial compound(s) $[M + H]^+$.

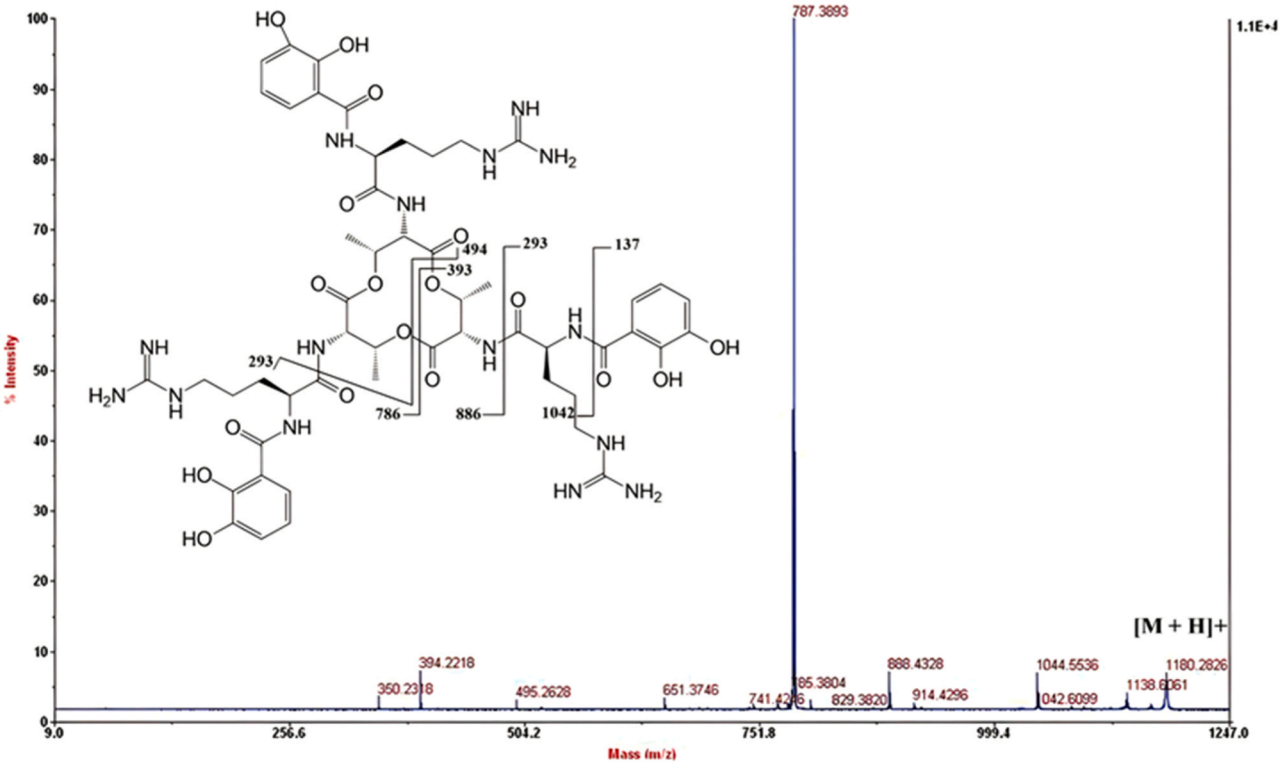

Fig. 6. MALDI TOF/TOF mass spectrum of streptobactin (m/z 1180.46 [M + H]+) with molecular structure and mass fragment analysis diagram.

component with m/z 1198.46 $[M + H]^+$ that was identified as tribenarthin (calculated monoisotopic mass, $[M+H]^+$: 1198.51). MS/MS revealed an ion with m/z 805.40 as the main fragment. This is explained as a result of the cleavage of one of the ester bonds resulting in the loss of a unit of benarthin from the linear trimeric tribenarthin structure. Also other observed fragments indicate that this precursor and its MS/MS pattern are highly similar as reported MS and MS/MS data reported for tribenarthin (Fig. 7) (Matsuo et al., 2011).

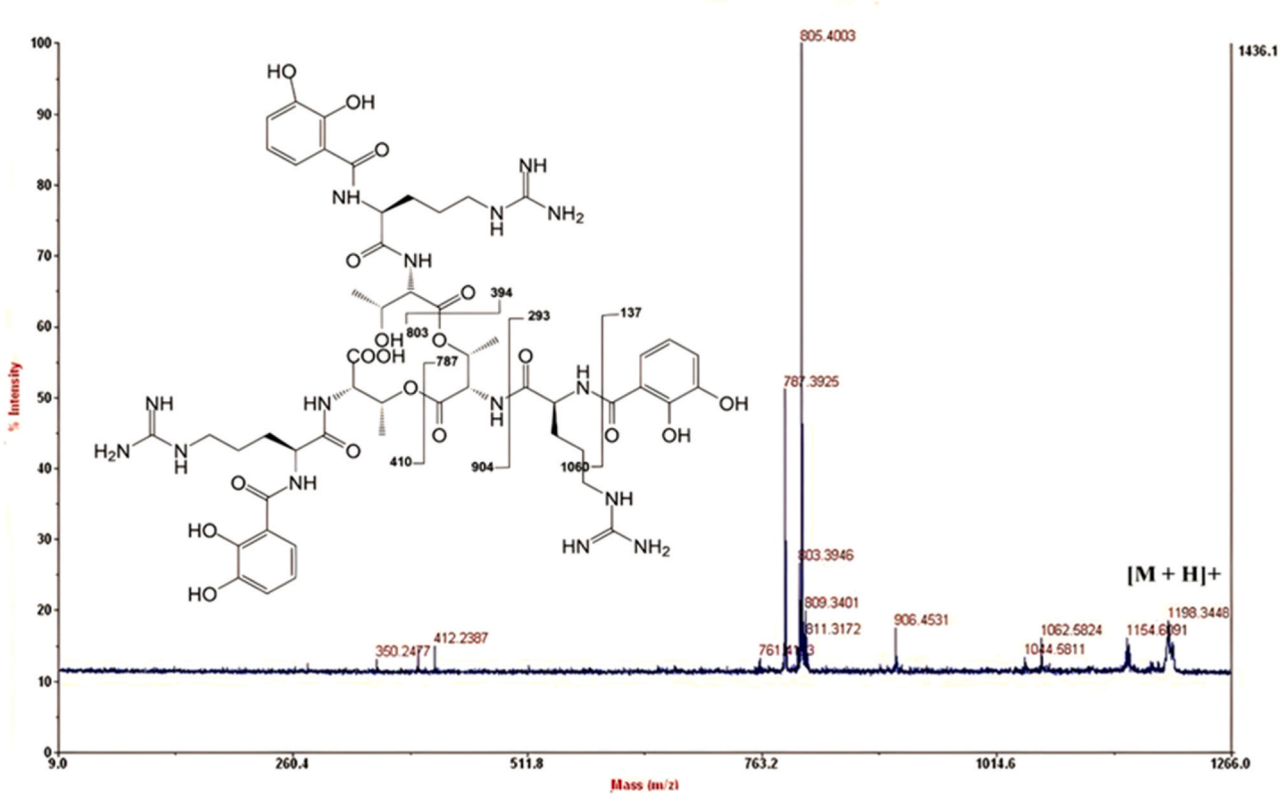

Fig. 7. MALDI TOF/TOF MS/MS spectrum of tribenarthin (precursor *m/z* 1198.46 $[M + H]^+$) with molecular structure and mass fragment analysis diagram.

The third component with *m/z* 1242. 62 is probably related to streptobactin as well as its main mass shift after MS/MS refers again to the loss of -Thr-Arg- 2,3-dihydroxybenzoyl (DHBA) (Fig. 8). The mass difference of 62 Da with streptobactin could not be explained so far.

Similar spectra corresponding to streptobactin and tribenarthin were observed in the case of fraction 7 of Streptomyces sp. 1 M, but with different intensities. The difference in the retention time and the shape of the chromatogram peaks in the two strains seems to be related to the

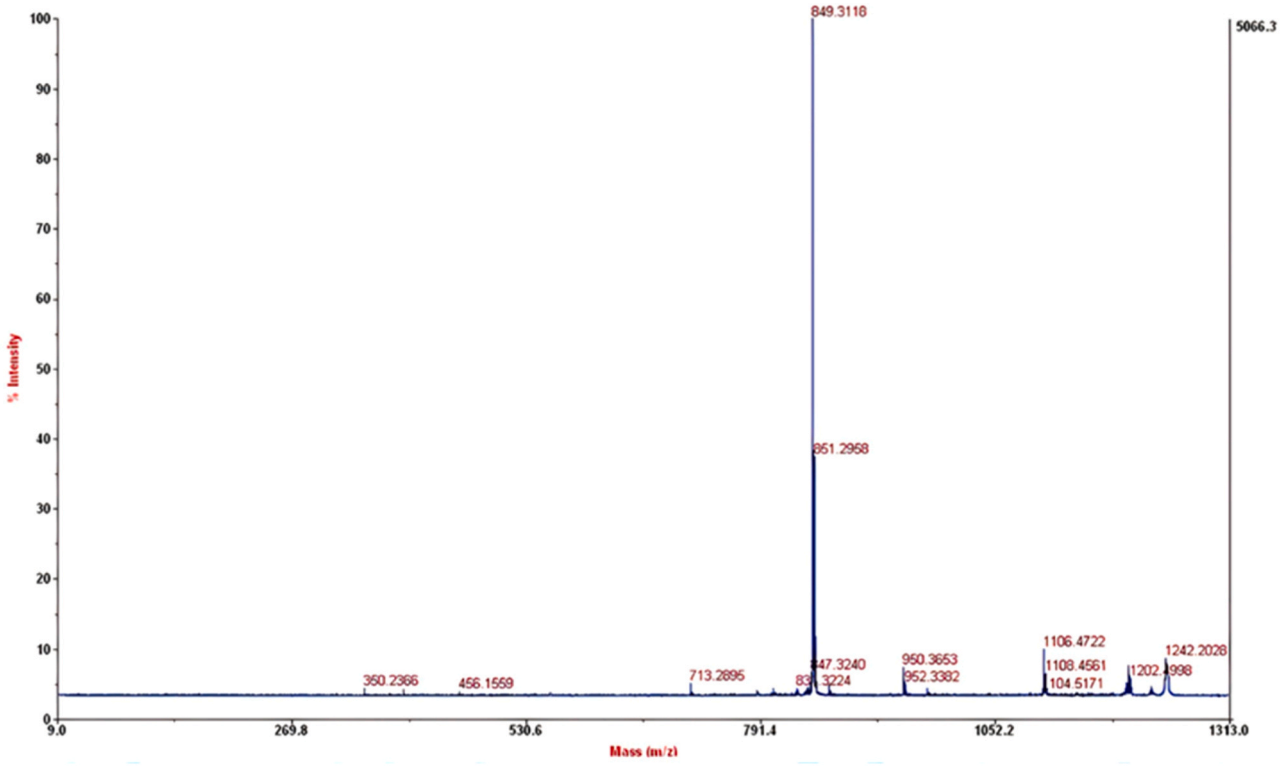

Fig. 8. MALDI TOF/TOF mass spectrum of *m/z* 1242 $[M+H]^+$.

absence of the unknown compound with *m/z* 1242.62 $[M + H]^+$ in fraction 7 of *Streptomyces* sp. 1 M (Fig. 9). Additional offline ESI-MS analysis of all active fractions in the mass range, 100–2000 *m/z* was performed and no other signals than those attributed to the siderophores were detected (data not shown).

Streptobactin and its related compounds benarthin, dibenarthin and tribenarthin were first isolated from the fermentation broth of the marine derived *Streptomyces* sp. YM5–799 that are produced under iron-limited conditions (Matsuo et al., 2011). Streptobactin ($C_{17}H_{23}N_5O_6$) is a cyclic trimeric structure of benarthin ((2,3-dihydroxybenzoyl) Arg-Thr)) unities with three ester bonds while tribenarthin consists of three units of benarthin in a linear structure (Matsuo et al., 2011). Streptobactin, like paenibactin, bacillibactin and enterobactin, is a catecholate type siderophore that is synthesised by a nonribosomal peptide synthetase (NRPS) that uses oxygen atoms as iron-binding ligands to chelate Fe^{3+} in iron-limited conditions (Wen et al., 2011; Wilson et al., 2006; O'Brien and Gibson, 1970).

The streptobactin biosynthesis gene cluster (BGC) was first identified in *Streptomyces* sp. ATCC 700974 by Patzer and Braun (Patzer and Braun, 2010). This gene cluster has 13 genes with different functions including DHBA biosynthesis genes (dhbABC), NRPS/NRPS related genes (dhbEG, griED), siderophore export gene (griC) and siderophore uptake/utilization genes (griABFGH). The streptobactin gene cluster was also reported from lichen isolate *Streptomyces* sp. YIM 130001(Schneider et al., 2018), endophytic *Streptomyces* sp. CBMAI 2042 (Paulo et al., 2019) and the marine sponge derived *Streptomyces* sp. ADI98–10 (Guerrero-Garzón et al., 2020). Recently benarthin and dibenarthin and its BGC were also identified in *Planobispora rosea* based on the metabolomics and transcriptomics data respectively (Del Carratore et al., 2021).

To validate that this organism is indeed capable of producing streptobactin and related compounds, we performed a proteomic analysis to search for proteins that are encoded by the streptobactin BGC. Investigation of the proteomic data of the strains confirmed the presence of griG (griseobactin-binding lipoprotein) (Table 1). This protein was only detected via a single unique peptide (GGPLAADVVLADYVK) but the MS/MS spectrum was manually verified (data not shown). The peptide is confirmed for this iron-siderophore transporter substrate-binding protein as proteotypic using the Unipept server (Mesuere et al., 2016). Note that no genome sequence for each of the strains is available, but data were searched against a *Streptomyces* protein database extracted from Uniprot in May 2022.

Table 1
Identified protein encoded by streptobactin biosynthetic gene clusters via *Streptomyces* sp. 1 M and *Streptomyces* sp. 14 M strains proteomic analysis. Peaks score from 14 M strain data.

Accession	Gene	Description	Avg. Mass	Peaks Score
D2TVG2_9ACTN	griG	Griseobactin-binding lipoprotein	35276	52.11

Since both strains are highly related to *S. globisporus* based on 16 S rRNA, and proteomics data showed a large number of hits with strain C-1027, known as an antitumor antibiotic lidamycin producer, we checked for the presence of proteins from the lidamycin gene cluster in the proteomics data (Li et al., 2016). Indeed, 8 proteins encoded in the lidamycin biosynthetic gene cluster were found in the proteomic data of both strains suggesting that they are capable of producing this compound, or a related compound, as well (Table 2).

Also other proteins belonging to biosynthetic gene clusters for secondary metabolites were found in the proteomic data including a lantipeptide synthetase protein, nosiheptide resistance regulatory protein, bleomycin resistance protein and erythromycin biosynthesis sensory transduction protein indicating the large potential of these strains to produce various secondary metabolites (supplementary table 1).

3.4. Quorum quenching activity

Quorum quenching (QQ) activity of the strains was first investigated using a microtiter assay with *E. coli* JB523 as a biosensor that produces GFP in response to exogenous C6-HSL and C8-HSL (Andersen et al.,

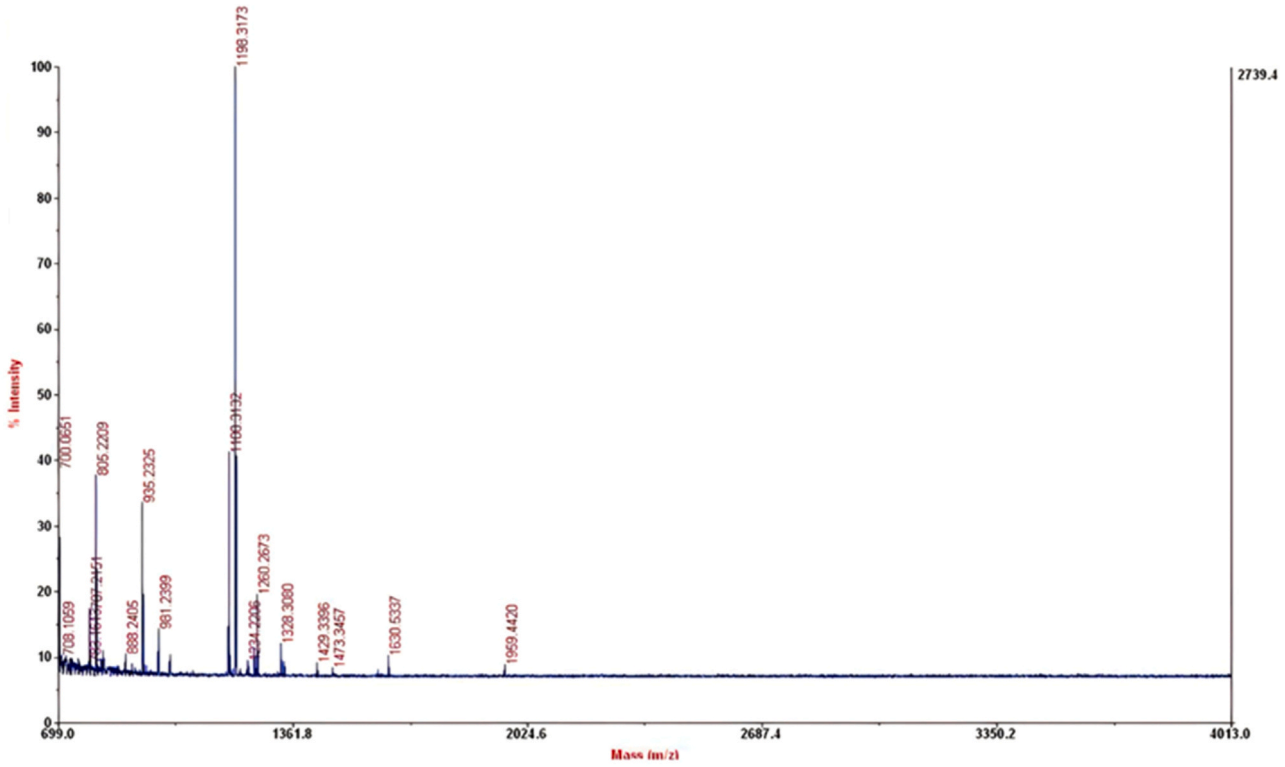

Fig. 9. MALDI TOF/TOF–MS spectrum of *Streptomyces* sp. 1 M fraction 7 containing antimicrobial compound $[M + H]^+$.

Table 2
Identified proteins encoded by lidamycin biosynthetic gene clusters via Streptomyces sp. 1 M and Streptomyces sp. 14 M strains proteomic analysis. Peaks score refer to 14 M dataset, except for epoxide hydrolase that was only found in 1 M dataset.

Accession	Description	Avg. Mass	Peaks Score	U Peptides
A0A0U3ME95_STRGL	Epoxide hydrolase	42751	61.76	3
A0A0U3M6Q4_STRGL	MFS transporter	52953	50.79	1
A0A0U3M9S7_STRGL	Phenylacetate–CoA ligase	49000	124.34	7
A0A0U3CA27_STRGL	Hydrolase	28611	149.92	6
A0A0U3MEB4_STRGL	Tyrosine 2 3-aminomutase	57955	146.6	6
A0A0U3KHX7_STRGL	Methyltransferase	35228	80.06	2
A0A0U3KRH8_STRGL	Acyl-CoA dehydrogenase	42277	55.29	1
A0A0U3CA48_STRGL	Wyosine base formation domain-containing protein	28706	39.25	1

2001; Jafra and Van Der Wolf, 2004). Sterile supernatant of the *Streptomyces* strains culture in AB medium was inoculated with the reporter strain and induction of GFP expression was measured using a microtitre plate fluorescence reader.

One-way ANOVA indicated significant differences between the samples and control groups (P-value < 0.05 and P-value was 0.0001). Since fluorescence intensity is an indicator to confirm the presence of AHL signals, the lower fluorescence intensity in the supernatant samples compared to the positive control indicates a lower concentration in the medium. Based on the statistical results, both strains showed a significant reduction in amount of AHLs signals and GFP expression compared to the positive controls, strain 14 M showed higher degradation capacity for C6-AHL and C8-AHL than strain 1 M (Fig. 10).

Since the biosensor strain showed good growth in the presence of culture supernatants of the *Streptomyces* strains, it seems that growth inhibition compounds were not produced or less produced in the AB medium used for the growth of the biosensor, whereas Bpm3 medium was used for the antibiotic assay. Alternatively, the biosensor is less sensitive to the secreted bactericidal compounds produced by the two strains.

3.5. Detection and quantification of AHLs molecules by LC-MS

In addition to the biosensor assay, LC-MS analysis was used to assess the strain's QQ capacity by measuring reduction of AHLs molecules added to AB culture medium as sole carbon source. Therefore, a targeted LC-MS involving MRM analysis was set up to monitor the presence of C6-HSL and C8-HSL in the filtered culture supernatants. We used two fragment ions related to cleavage of the lactone ring from the acyl side chain (*m/z* fragments 101 and 98 of C6-HSL and 102 and 127 of C8-HSL) as representative transitions in the MRM experiment.

First, a calibration curve was derived based on a dilution series of C6-HSL and C8-HSL molecules, respectively and different slopes were observed for low and high concentration ranges of two molecules. This was used to determine the retention time and dose response by determining the peak areas. Calibration curves were plotted and used for quantification (Fig. 11).

The C6-HSL retention time was determined as 8.3 min for calibration standards, positive control and *Streptomyces* spp. 1 M and 14 M culture supernatants. In the case of C8-HSL, it was 10.6 min

By comparing their peak height with positive control samples, the reduction and degradation of both C6-HSL and C8-HSL molecules by strains were confirmed as lower concentrations of autoinducer molecules were observed in the culture supernatants of strains (Fig. 12). These results are in agreement with the biosensor assay results, therefore, *Streptomyces* sp. 1 M and *Streptomyces* sp. 14 M are confirmed as QQ isolates and it seems that they secrete QQ enzymes.

We chased for the presence of acyl-homoserine lactonases or acylases, the best described QQ enzymes, but they could not be found in the proteome data (Fetzner, 2015). However, several beta-lactamase-like enzymes were identified. β-lactam acylases, like AHL acylase (groups A and B), belong to the Ntn hydrolase family proteins. They are known as bifunctional enzymes that cleave amide bonds in β-lactam antibiotics such as penicillin G and AHL molecules and cause resistance to β-lactams and quenching of the QS system (Wang et al., 2019; Yasutake et al., 2017).

4. Discussion

Streptomyces bacteria are known for their exceptional ability to produce various secondary metabolites that are used as anti-microbial and anti-cancer compounds and due to their high potential represent

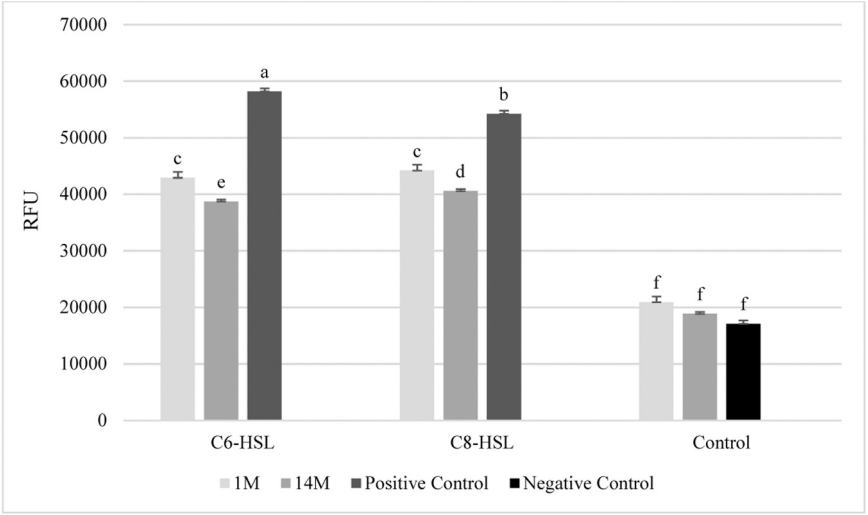

Fig. 10. Expression of quorum sensing-regulated green fluorescent protein (GFP) in *Escherichia coli* JB523 induced by C6-HSL and C8-HSL molecules in the cell free supernatant of the *Streptomyces* sp. 1 M and *Streptomyces* sp .14 M cultures in AB medium enriched by AHL molecules; negative control (AB medium); positive controls (AB medium enriched by AHL molecules (50 nM)). The results (one-way ANOVA, Duncan's test, $P < 0.05$) showed a decrease in the expression of GFP in the samples compared to the controls. Results are the mean of three replicates; error bars represent standard deviation.

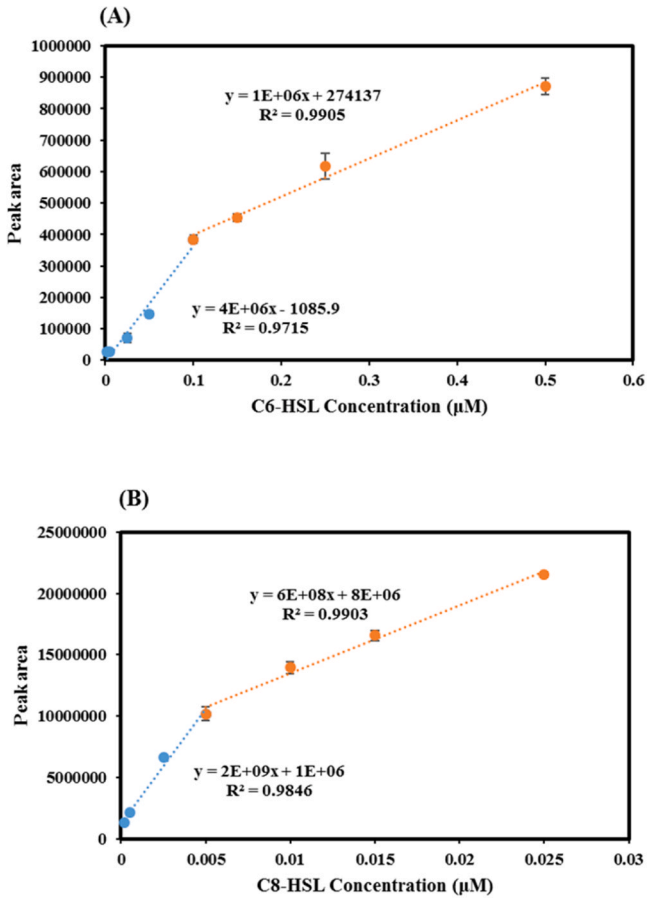

Fig. 11. LC-MS analysis of C6-HSL and C8-HSL degradation; AHLs molecules calibration curves were obtained by using standard solutions with 0.002 to 0.5 µM and 0.0002 to 0.025 µM concentration of C6-HSL and C8-HSL respectively, error bars represent standard deviation. (A and B).

an interesting source for discovering new drugs and metabolites to overcome increasing antibiotic resistance (de Lima Procópio et al., 2012). The results of this study show that the isolated *Streptomyces* sp. 1 M and 14 M strains from the rhizosphere soil of medicinal plants exhibit potent growth inhibitory activity against Gram-positive and Gram-negative pathogenic bacteria.

Based on bioassays, metabolomics and proteomics analysis using mass spectrometry streptobactin and tribenarthin siderophores were responsible for strain's bacterial growth inhibitory activity, and their QQ ability. Additionally, we have strong evidence for the production of a lidamycin-like compound. To date, marine-derived *Streptomyces* sp. YM5–799 isolated from an algae sample in Japan has been reported to produce streptobactin (Matsuo et al., 2011). To our best knowledge, this is the first report of streptobactin production by a terrestrial *Streptomyces* that confirmed via metabolomics and proteomics analysis although the gene cluster synthesizing this catechol-peptide siderophore has been identified in several strains (Schneider et al., 2018; Paulo et al., 2019; Guerrero-Garzón et al., 2020; Del Carratore et al., 2021). Iron is an essential element for bacterial life processes, and although it is one of the most abundant metals on the earth, it is usually found in insoluble iron (III) oxide forms in the environment that bacteria cannot use. To overcome this restriction, most bacterial genera have developed different strategies such as the secretion of siderophores to chelate and absorb iron from the environment (Boukhalfa and Crumbliss, 2002). Siderophores are structurally diverse and classified into three groups, i.e. catechols, hydroxamates, and carboxylates based on the metal chelating motifs (Zajdowicz et al., 2012). Streptobactin (griseobactin) is a catechol-type siderophore and its related compounds, dibenarthin and tribenarthin were originally isolated from the marine-derived *Streptomyces* sp. YM5–799. Streptobactin is a cyclic trilactone siderophore with 2, 3-dihydroxybenzoyl (DHB) functional group that conjugates arginine and threonine amino acids (Matsuo et al., 2011; Patzer and Braun, 2010).

Streptobactin like other triscatechol siderophores i.e. enterobactin (O'Brien and Gibson, 1970), bacillibactin (Wilson et al., 2006), salmochelin (Bister et al., 2004), cyclic trichrysobactin (Sandy and Butler, 2011; Persmark et al., 1989) and paenibactin (Wen et al., 2011) has a cyclic structure with a different peptide-domain whiles trivanchrobactin (Sandy et al., 2010), turnerbactin (Han et al., 2013) and di/tribenarthin (Matsuo et al., 2011) have a linear structure. Since the related compounds to streptobactin, benarthin and dibenarthin, have been reported from *P. rosea* and many BGCs can produce different types of metabolite, it is difficult to conclude that the production of streptobactin is limited to the genus *Streptomyces* or other members of Actinomycetes capable produce this iron-chelating siderophore (Fischbach and Clardy, 2007). On the other hand, the same bioactive profile of the 1 M and 14 M strains as well as the production of similar siderophores by *P. rosea* can confirm the horizontal gene transfer between bacteria (Hopwood, 2006).

The QQ ability of both *Streptomyces* strains has also been investigated and confirmed. The exact identity of the responsible enzyme could not be revealed from the proteomics data yet, but we found several beta-lactam hydrolases that may be candidates. AhlM AHL-acylase with cleaving penicillin G activity was the first reported in Gram-positive bacteria and *Streptomyces* (Park et al., 2005) and recently SlPA, penicillin acylase has been derived from *S. lavendulae* (Velasco-Bucheli et al., 2020).

Totally, the results of this study can be a motivation for isolating these bacteria from new environments or finding ways to stimulate the expression of silent genes synthesis of such active compounds.

5. Conclusion

This study demonstrates the catecholate siderophores production and QQ potential of the two *Streptomyces* strains isolated from medicinal plants rhizosphere from Mishan Plain, Hamedan Province, Iran using metabolomics and proteomics analysis. The production of streptobactin by terrestrial *Streptomyces* sp. strains was confirmed for the first time.

Funding

This work was supported by funding from Ghent University Bijzonder Onderzoeksfonds for mass spectrometric equipment and operational costs of the Proteomics Expertise Center (grants 01XP1517, 01B08915).

CRediT authorship contribution statement

Devreese Bart: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Chandramowli Darshan:** Formal analysis, Investigation, Methodology, Validation. **Khodakaramian Gholam:** Conceptualization, Resources, Supervision, Writing – review & editing. **Alinejad Fatemeh:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Investigation, Validation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

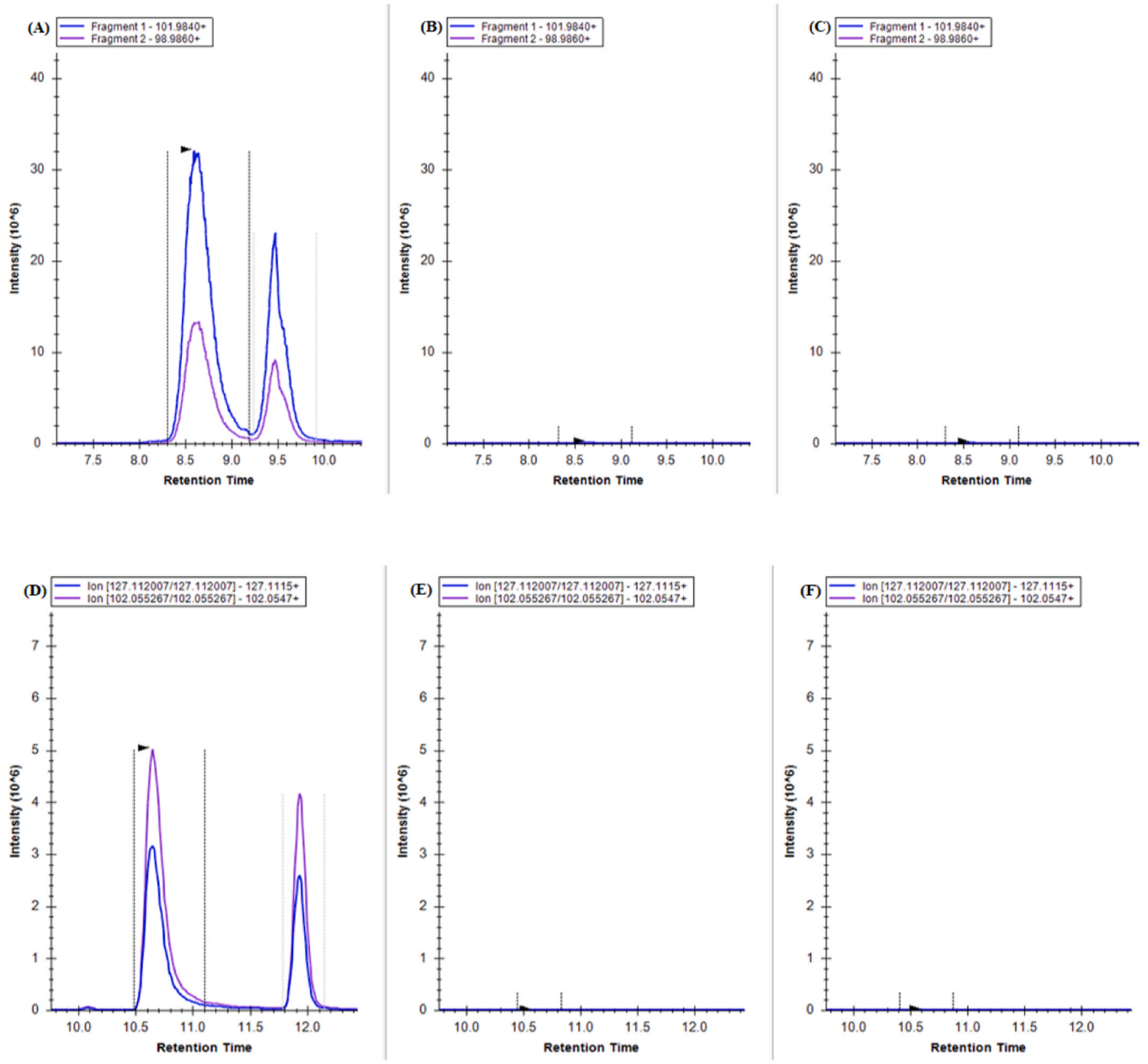

Fig. 12. LC-MS analysis of C6-HSL and C8-HSL degradation. C6-HSL (M+H *m/z* 200 with the *m/z* 101 and 98 product ions) mass spectrometry retention time and concentration in: Positive control (A) *Streptomyces* sp. 1 M (B) *Streptomyces* sp. 14 M (C) cultures supernatants samples. C8-HSL (M+H *m/z* 228 with the *m/z* 127 and 102 product ions) mass spectrometry retention time and concentration in; Positive control (D) *Streptomyces* sp. 1 M (E) *Streptomyces* sp. 14 M (F) cultures supernatants samples.

Data availability

The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE [30] partner repository with the dataset identifier PXD042019 (project accession), 10.6019/PXD042019 (project DOI) and PXD041994 (project accession), 10.6019/PXD041994 (project DOI) for strains 1 M and 14 M respectively. 16 S rRNA data are submitted to Genbank: 16 S rRNA of *Streptomyces* sp. 1 M (GenBank accession no. OQ927600) and *Streptomyces* sp. 14 M (GenBank accession no. OQ927601).

Acknowledgments

The authors thank Prof. Dieter Deforce for access to the LC-MS facility and Dr Simon Daled for performing the proteomics LC-MS analysis. Liesbeth Lebbe and Prof. Anne Willems are acknowledged for support and advice in the 16S rRNA and phylogenetic analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2023.100033.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Andersen, J.B., Heydorn, A., Hentzer, M., Eberl, L., Geisenberger, O., Christensen, B.B., Molin, S., Givskov, M., 2001. GFP-based N-acyl homoserine-lactone sensor systems

for detection of bacterial communication. Appl. Environ. Microbiol. 67, 575–585. https://doi.org/10.1128/AEM.67.2.575-585.2001.
Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y., Wezeld, G.P.V., 2016. Taxonomy, physiology, and natural products of actinobacteria. Am. Soc. Microbiol. 80, 1–43. https://doi.org/10.1128/MMBR.00019-15.
Bauer, A., Kirby, W.M., Sherris, J., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493.
Bekiesch, P., Zehl, M., Domingo-Contreras, E., Martín, J., Pérez-Victoria, I., Reyes, F., Kaplan, A., Rückert, C., Busche, T., Kalinowski, J., Zotchev, B.Z., 2020. Viennamycins: lipopeptides produced by a *Streptomyces* sp. J. Nat. Prod. 83, 2381–2389. https://doi.org/10.1021/acs.jnatprod.0c00152.
Bister, B., Bischoff, D., Nicholson, G.J., Valdebenito, M., Schneider, K., Winkelmann, G., Hantke, K., Süssmuth, R.D., 2004. The structure of salmochelins: C-glucosylated enterobactins of *Salmonella enterica*. BioMetals 17, 471–481. https://doi.org/10.1023/B:BIOM.0000029432.69418.6a.
Boukhalfa, H., Crumbliss, A.L., 2002. Chemical aspects of siderophore mediated iron transport. BioMetals 15, 325–339. https://doi.org/10.1023/A:1020218608266.
Chen, F., Gao, Y., Chen, X., Yu, Z., Li, X., 2013. Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. Int. J. Mol. Sci. 14, 17477–17500. https://doi.org/10.3390/ijms140917477.
Clark, D.J., Maaløe, O., 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. 23, 99–112 doi: org/10.1016/S0022-2836(67)80070-6.
de Lima Procópio, R.E., da Silva, I.R., Martins, M.K., de Azevedo, J.L., de Araújo, J.M., 2012. Antibiotics produced by *Streptomyces*. Braz. J. Infect. Dis. 16, 466–471. https://doi.org/10.1016/j.bjid.2012.08.014.
De Simeis, D., Serra, S., 2021. Actinomycetes: a never-ending source of bioactive compounds—an overview on antibiotics production. Antibiotics 10, 483. https://doi.org/10.3390/antibiotics10050483.
Del Carratore, F., Iorio, M., Pérez-Bonilla, M., Schmidt, K., Pérez-Redondo, R., Sosio, M., Macdonald, S.J., Gyulev, I.S., Tsigkinopoulou, A., Thomas, G.H., Genilloud, O., Rodríguez-García, A., Donadio, S., Breitling, R., Takano, E., 2021. Multi-omics Study of *Planobispora rosea*, Producer of the Thiopeptide Antibiotic GE2270A. e0034121 MSystems 6. https://doi.org/10.1128/msystems.00341-21.
Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? Fems. Microbiol. Ecol. 72, 313–327. https://doi.org/10.1111/j.1574-6941.2010.00860.x.
Edwards, U., Rogall, T., Blocker, H., Emde, M., Bottger, E.C., 1989. Isolation and direct complete nucleotide determination of entire genes. characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17, 7843–7853. https://doi.org/10.1093/nar/17.19.7843.
Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., Pellegrini, M., 2022. Actinomycetes from caves: an overview of their diversity, biotechnological properties, and insights for their use in soil environments. Microorganisms 10, 453. https://doi.org/10.3390/microorganisms10020453.
Fetzner, S., 2015. Quorum quenching enzymes. J. Biotechnol. 201, 2–14. https://doi.org/10.1016/j.jbiotec.2014.09.001.
Fischbach, M.A., Clardy, J., 2007. One pathway, many products. Nat. Chem. Biol. 3, 353–355. https://doi.org/10.1038/nchembio0707-353.
Fuqua, C., Greenberg, E.P., 2002. Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell. Biol. 3, 685–695. https://doi.org/10.1038/nrm907.
Goodfellow, M., Williams, S.T., 1983. Ecology of actinomycetes. Annu. Rev. Microbiol. 37, 189–216. https://doi.org/10.1146/ANNUREV.MI.37.100183.001201.
Grandclément, C., Tannières, M., Moréra, S., Dessaux, Y., Faure, D., 2015. Quorum quenching: role in nature and applied developments. Fems. Microbiol. Rev. 40, 86–116. https://doi.org/10.1093/femsre/fuv038.
Guerrero-Garzón, J.F., Zehl, M., Schneider, O., Rückert, C., Busche, T., Kalinowski, J., Bredholt, H., Zotchev, S.B., 2020. *Streptomyces* spp. from the marine sponge antho dichotoma: analyses of secondary metabolite biosynthesis gene clusters and some of their products. Front. Microbiol. 11, 1–15. https://doi.org/10.3389/fmicb.2020.00437.
Han, A.W., Sandy, M., Fishman, B., Trindade-silva, A.E., Soares, C.A.G., Distel, D.L., Butler, A., Haygood, M.G., 2013. Turnerbactin, a novel triscatecholate siderophore from the shipworm endosymbiont *Teredinibacter turnerae* T7901. e76151 PLoS. One 8. https://doi.org/10.1371/journal.pone.0076151.
Hopwood, D.A., 2006. Soil to genomics: the *Streptomyces* chromosome. Annu. Rev. Genet. 40, 1–23. https://doi.org/10.1146/annurev.genet.40.110405.090639.
Jafra, S., Van Der Wolf, J.M., 2004. Fast screening method for detection of acyl-HSL-degrading soil isolates. J. Microbiol. Methods 57, 415–420. https://doi.org/10.1016/j.mimet.2004.01.015.
Kibret, M., Guerrero-Garzón, J.F., Urban, E., Zehl, M., Wronski, V.K., Rückert, C., 2018. *Streptomyces* spp. From Ethiopia producing antimicrobial compounds: characterization via bioassays, genome analyses, and mass spectrometry. Front. Microbiol. 9, 1270. https://doi.org/10.3389/fmicb.2018.01270.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054. Epub 2016 Mar 22.
Lemos, M.L., Toranzo, A.E., Barja, J.L., 1985. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microb. Ecol. 11, 149–163. https://doi.org/10.1007/BF02010487.
Li, X., Lei, X., Zhang, C., Jian, Z., Shi, Y., Wang, S., Wang, L., Hong, B., 2016. Complete genome sequence of *Streptomyces globisporus* C-1027, the producer of an enediyne antibiotic lidamycin. J. Biotechnol. 222, 9–10. https://doi.org/10.1016/j.jbiotec.2016.02.004.
Matsuo, Y., Kanoh, K., Jang, J.K., Adachi, K., Matsuda, S., Miki, O., Kato, T., Shizuri, Y., 2011. Streptobactin, a tricatechol-type siderophore from marine-derived *Streptomyces* sp. YM5-799. J. Nat. Prod. 74, 2371–2376. https://doi.org/10.1021/np200290j.
Medina-Martínez, M.S., Uyttendaele, M., Meireman, S., Debevere, J., 2007. Relevance of N-acyl-L-homoserine lactone production by *Yersinia enterocolitica* fresh foods. J. Appl. Microbiol. 102, 1150–1158. https://doi.org/10.1111/j.1365-2672.2006.03143.x.
Medina-Martínez, M.S., Uyttendaele, M., Rajkovic, A., Nadal, P., Debevere, J., 2007. Degradation of N-acyl-L-homoserine lactones by *Bacillus cereus* in culture media and pork extract. Appl. Environ. Microbiol. 73, 2329–2332. https://doi.org/10.1128/AEM.01993-06.
Mesuere, B., Van der Jeugt, F., Devreese, B., Vandamme, P., Dawyndt, P., 2016. The unique peptidome: taxon-specific tryptic peptides as biomarkers for targeted metaproteomics. Proteomics 16, 2313–2318. https://doi.org/10.1002/pmic.201600023.
Mojica, M.F., Humphries, R., Lipuma, J.J., Mathers, A.J., Rao, G.G., Shelburne, S.A., Fouts, D.E., Duin, D.V., Bonomo, R.A., 2022. Clinical challenges treating *Stenotrophomonas maltophilia* infections: an update. Jac. Antimicrob. Resist. 4, 1–19. https://doi.org/10.1093/jacamr/dlac040.
Nett, M., Ikeda, H., Moore, B.S., 2009. Genomic basis for natural product biosynthetic diversity in the actinomycetes. Nat. Prod. Rep. 26, 1362–1384. https://doi.org/10.1039/b817069j.
O'Brien, I.G., Gibson, F., 1970. The structure of enterochelin and related 2,3-dihydroxy-N-benzoyne conjugates from *Escherichia coli*. Biochim. Biophys. Acta 215, 393–402. https://doi.org/10.1016/0304-4165(70)90038-3.
Oberhofer, M., Hess, J., Leutgeb, M., Gössnitzer, F., Rattei, T., Wawrosch, C., Zotchev, B. Z., 2019. Exploring actinobacteria associated with rhizosphere and endosphere of the native alpine medicinal plant *Leontopodium nivale* subspecies alpinum. Front. Microbiol. 10, 1–13. https://doi.org/10.3389/fmicb.2019.02531.
Park, S.Y., Kang, H.O., Jang, H.S., Lee, J.K., Koo, B.T., Yum, D.Y., 2005. Identification of extracellular N-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. Appl. Environ. Microbiol. 71, 2632–2641 doi: org/10.1128/AEM.71.5.2632-2641.2005.
Patzer, S.I., Braun, V., 2010. Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of *Streptomyces* sp. ATCC 700974. J. Bacteriol. 192, 426–435. https://doi.org/10.1128/JB.01250-09.
Paulo, B.S., Sigrist, R., Angolini, C.F.F., Eberlin, M.N., De Oliveira, L.G., 2019. Gene deletion leads to improved valinomycin production by *Streptomyces* sp. CBMAI 2042. J. Braz. Chem. Soc. 30, 673–679. https://doi.org/10.21577/0103-5053.20180207.
Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D.J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., Vizcaíno, J.A., 2022. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. D543-D552 Nucleic Acids Res. 7. https://doi.org/10.1093/nar/gkab1038.
Persmark, M., Expert, D., Neilands, J.B., 1989. Isolation, characterization, and synthesis of chrysobactin, a compound with siderophore activity from *Erwinia chrysanthemi*. J. Biol. Chem. 264, 3187–3193. https://doi.org/10.1016/s0021-9258(18)94049-4.
Quinn, G.A., Banat, A.M., Abdelhameed, A.M., Banat, I.M., 2020. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. J. Med. Microbiol. 69, 1040–1048. https://doi.org/10.1099/jmm.0.001232.
Rajeswari, P., Jose, P.A., Amiya, R., Jebakumar, S.R.D., 2014. Characterization of saltern based *Streptomyces* sp. and statistical media optimization for its improved antibacterial activity. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00753.
Saelens, G., Planckaert, S., Martínez-Sernández, V., Ubeira, F.M., Devreese, B., Gabriël, S., 2022. Targeted proteomics and specific immunoassays reveal the presence of shared allergens between the zoonotic nematodes *Anisakis simplex* and *Pseudoterranova decipiens*. Sci. Rep. 12, 1–12. https://doi.org/10.1038/s41598-022-08113-3.
Sandy, M., Butler, A., 2011. Chrysobactin siderophores produced by *Dickeya chrysanthemi* ec16. J. Nat. Prod. 74, 1207–1212. https://doi.org/10.1021/np200126z.
Sandy, M., Han, A., Blunt, J., Munro, M., Haygood, M., Butler, A., 2010. Vanchrobactin and anguibactin siderophores produced by *Vibrio* sp. DS40M4. J. Nat. Prod. 73, 1038-43. doi: 10.1021/np900750g.
Schlatter, D., Fubuh, A., Xiao, K., Hernandez, D., Hobbie, S., Kinkel, L., 2009. Resource amendments influence density and competitive phenotypes of *Streptomyces* in soil. Microb. Ecol. 57, 413–420. https://doi.org/10.1007/s00248-008-9433-4.
Schneider, O., Simic, N., Aachmann, F.L., Rückert, C., Kristiansen, K.A., Kalinowski, J., Jiang, Y., Wang, L., Jiang, C.L., Lale, R., Zotchev, S.B., 2018. Genome mining of *Streptomyces* sp. YIM 130001 isolated from lichen affords new thiopeptide antibiotic. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.03139.
Seong, C.N., Choi, J.H., Baik, K.S., 2001. An improved selective isolation of rare actinomycetes from forest soil. J. Microbiol. 39, 17–23.
Shepherdson, E.M., Baglio, C.R., Elliot, M.A., 2023. *Streptomyces* behavior and competition in the natural environment, 102257 Curr. Opin. Microbiol. 71. https://doi.org/10.1016/j.mib.2022.102257.
Shirling, E.B., Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16, 313–340. https://doi.org/10.1099/00207713-16-3-313.
Tarkka, M.T., Lehr, N.A., Hampp, R., Schrey, S.D., 2008. Plant behavior upon contact with Streptomycetes. Plant. Signal. Behav. 3, 917–919. https://doi.org/10.4161/psb.5996.
Velasco-Bucheli, R., Hormigo, D., Fernández-Lucas, J., Torres-Ayuso, P., Alfaro-Ureña, Y., Saborido, A.I., Serrano-Aguirre, L., García, J.L., Ramón, F., Acebal, C.,

Santos, A., Arroyo, M., Mata, I.D.L., 2020. Penicillin acylase from *Streptomyces lavendulae* and aculeacin a acylase from *Actinoplanes utahensis*: two versatile enzymes as useful tools for quorum quenching processes. Catalysts 10, 730. https://doi.org/10.3390/catal10070730.

Viaene, T., Langendries, S., Beirinckx, S., Maes, M., Goormachtig, S., 2016. *Streptomyces* as a plant's best friend? FEMS Microbiol. Ecol. 92, 1–10. https://doi.org/10.1093/femsec/fiw119.

Wang, Y., Yang, D., Yu, Z., 2023. New lactones produced by *Streptomyces* sp. SN5431 and their antifungal activity against *Bipolaris maydis*. Microorganisms 11, 616. https://doi.org/10.3390/microorganisms11030616.

Wang, Y.L., Liu, S., Yu, Z.J., Lei, Y., Huang, M.Y., Yan, Y.H., Ma, Q., Zheng, Y., Deng, H., Sun, Y., Wu, C., Yu, Y., Chen, Q., Wang, Z., Wu, Y., Li, G.B., 2019. Structure-based development of (1-(3′-Mercaptopropanamido)methyl)boronic acid derived broad-spectrum, dual-action inhibitors of metallo- and Serine-β-lactamases. J. Med. Chem. 62, 7160–7184. https://doi.org/10.1021/acs.jmedchem.9b00735.

Wen, Y., Wu, X., Teng, Y., Qian, C., Zhan, Z., Zhao, Y., Li, O., 2011. Identification and analysis of the gene cluster involved in biosynthesis of paenibactin, a catecholate siderophore produced by *Paenibacillus elgii* B69. Environ. Microbiol. 13, 2726–2737. https://doi.org/10.1111/j.1462-2920.2011.02542.x.

Williams, P., 2007. Quorum sensing, communication and cross-kingdom signalling in the bacterial world. Microbiology 153, 3923–3938. https://doi.org/10.1099/mic.0.2007/012856-0.

Wilson, M.K., Abergel, R.J., Raymond, K.N., Arceneaux, J.E.L., Byers, B.R., 2006. Siderophores of *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. Biochem. Biophys. Res. Commun. 348, 320–325. https://doi.org/10.1016/j.bbrc.2006.07.055.

Yasutake, Y., Kusada, H., Ebuchi, T., Hanada, S., Kamagata, Y., Tamura, T., Kimura, N., 2017. Bifunctional quorum-quenching and antibiotic-acylase MacQ forms a 170-kDa capsule-shaped molecule containing spacer polypeptides. Sci. Rep. 7 (1), 11. https://doi.org/10.1038/s41598-017-09399-4.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617. https://doi.org/10.1099/ijsem.0.001755.

Zajdowicz, S., Haller, J.C., Krafft, A.E., Hunsucker, S.W., Mant, C.T., Duncan, M.W., Hodg-es, R.S., Jones, D.N.M., Holmes, R.K., 2012. Purification and structural characterization of siderophore (corynebactin) from *Corynebacterium diphtheriae*. PLoS. One 7, e34591. https://doi.org/10.1371/journal.pone.0034591.