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Screening of Thiopeptide-Producing Streptomycetes Isolated From the Rhizosphere Soil of *Juniperus excelsa*

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Abstract

The identification of an increasing number of drug-resistant pathogens has stimulated the development of new therapeutic agents to combat them. Microbial natural products are among the most important elements when it comes to drug discovery. Today, thiopeptide antibiotics are receiving increasing research attention due to their potent activity against Gram-positive bacteria. In this study, we demonstrated the successful use of a whole-cell microbial biosensor (*Streptomyces lividans* TK24 pMO16) for the specific detection of thiopeptide antibiotics among the native actinomycete strains isolated from the rhizosphere soil of *Juniperus excelsa* (Bieb.). Among the native strains, two strains of *Streptomyces*, namely sp. Je 1–79 and Je 1–613, were identified that were capable of producing thiopeptide antibiotics. A multilocus sequence analysis of five housekeeping genes (*gyrB*, *atpD*, *recA*, *rpoB*, and *trpB*) classified them as representatives of two different species of the genus *Streptomyces*. The thiopeptide antibiotics berninamycin A and B were identified in the extracts of the two strains by means of a dereplication analysis. The berninamycin biosynthetic gene cluster was also detected in the genome of the *Streptomyces* sp. Je 1–79 strain and showed a high level of similarity (93%) with the *ber* cluster from *S. bernensis*. Thus, the use of this whole-cell biosensor during the first stage of the screening process could serve to accelerate the specific detection of thiopeptide antibiotics.

Introduction

Today, humanity is facing the problem of the increasing spread of antibiotic resistance among microorganisms. Such antibiotic resistance could lead to the rapid spread of infectious diseases against which most currently known antibiotics are inefficient [1]. A key aspect of overcoming this antibiotic resistance crisis involves the screening of new natural compounds with antibacterial activity or new producers of previously identified natural compounds. The main candidates for this process are the actinomycetes, as they represent a good source for screening antibacterial compounds and are capable of producing various classes of antibiotics [2, 3]. Yet, the identification of the natural bioactive compounds and strains that produce actinomycetes among the other possible strains remains a very difficult task.

Numerous methods for the screening and evaluation of antimicrobial activity have been developed [4], with the use of compound-specific whole-cell biosensors being one such method. Microbial biosensors can significantly accelerate the screening process, although the most obvious benefit associated with their use is the possibility to directly detect a metabolite of interest [5]. Previously developed whole-cell biosensors have been successfully used for antibiotic screening, including a biosensor based on the LiaRS two-component system for the screening of cell wall active antibiotics [6], a biosensor based on the LnrJK two-component signaling system for the specific detection of amphotericin-like polyenes [7], and a specific biosensor for pamamycin screening [8].

The thiopeptides are a large group of highly modified, sulfur-containing peptide antibiotics [9]. Micrococcin was the first antibiotic in this group to be discovered in

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the mid-twentieth century [10]. Since then, around 100 thiopeptides have been identified [11], with their number increasing year by year [12, 13]. Most of the thiopeptides show strong activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumonia* [14]. Recent studies have also shown that some of the thiopeptides exhibit activity against multidrug-resistant clinical isolates, for example, thiostrepton against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [15] as well as micrococcin P1 against *Mycobacterium tuberculosis* [16].

A structural analysis of the thiopeptides allowed them to be classified as thiazolyl peptides [17], which consist of a macrocyclic core containing thiazoles, oxazoles, and indoles; dehydroamino acids; and a six-membered tri- or tetra-substituted nitrogen heterocycle with side chain(s) [9]. The complex structure of these antibiotics poses a serious problem in terms of their chemical synthesis, which renders the search for natural producers of thiopeptides relevant [18]. In fact, the identification of new thiopeptide producers will make it possible to obtain new compounds with improved biological or pharmacological properties. In addition, the discovery of new producers of previously identified thiopeptides also has practical implications, as they may prove to be more convenient (e.g., fast growth, a higher production, or are better suited for gene engineering manipulations) than currently utilized strains.

Our previous studies of actinomycetes isolated from the rhizosphere soil of *Juniperus excelsa* (Bieb.) from the Crimean Peninsula in Ukraine demonstrated their potential as producers of antimicrobial compounds. In particular, the producers of the previously unknown antibiotics linear polyketide juniperolide A [19] and leopolic acid A [20] were found among them, in addition to a producer of the known antibiotic lydicamycin, which showed activity against methicillin-resistant strains of *S. aureus* [21]. This confirmed the potential of actinomycete strains isolated from the rhizosphere soil of *J. excelsa* for use in the screening of producers of biologically active compounds.

In the present study, we demonstrated the successful use of a microbial biosensor for the specific detection of thiopeptide antibiotics among the native actinomycete strains isolated from the rhizosphere soil of *J. excelsa.* As a consequence, we identified new berninamycin producers using both metabolite profiling and genome mining. The data obtained in this study extend the list of existing biosensor systems that can be successfully used for the targeted screening of biologically active compounds in various natural niches. Moreover, the data demonstrate that the utilized biosensor can be used for the identification of thiopeptide antibiotics.

Materials and Methods

Strains and Growth Conditions

The 372 actinomycete-like strains used in this study were isolated from the rhizosphere soil of *J. excelsa* collected from Kischka Mountain on the Crimean Peninsula in Ukraine [Global Positioning System (GPS) coordinates: N 44°24′02.07" E 33°59′32.96"] [22]. The strains were deposited in the Microbial Culture Collection of Antibiotic Producers (MCCAP) of Ivan Franko National University of Lviv.

Oatmeal medium (OM) (20 g/L oat flour and 20 g/L agar; pH 7.2) was used for the cultivation of the actinomycete strains. Liquid tryptic soy broth (TSB) (Sigma-Aldrich, St. Louis, MO, USA) was used for the isolation of the total DNA. Bennett's agar (10 g/L glucose, 1 g/L soy peptone, 1 g/L yeast extract, and 2 g/L tryptone; pH 7.3) was used for thiopeptides screening. In addition, SG medium (20.0 g/L glucose, 10 g/L soy peptone, and 2 g/L $CaCO_3$; pH 7.2) was used to produce the secondary metabolites.

Screening of the Thiopeptides

The screening assay was performed against the strain-biosensor *S. lividans* TK24 pMO16, which contains the integrative plasmid pMO16, in which the expression of the neomycin-resistance (*neo*) gene is under the transcription control of the thiostrepton-inducible promoter (*tipA*) [23]. The strain-biosensor was grown on OM medium for seven days. The spores of the strain-biosensor were washed from the surface of the OM agar plates with a saline solution (0.9% sodium chloride) and then collected. Next, 100 µL of a tenfold dilution of the spore suspension were plated on Bennett's agar plates supplemented with 50 µg/mL of kanamycin. After that, agar plugs of the studied strains of actinomycetes were cut from lawns grown on OM medium for seven days and stacked onto the plates of freshly plated strain-biosensor. The plates were incubated for five to seven days at 28 °C. The occurrence of a growth halo of the strain-biosensor around the agar plugs indicated the production of thiopeptides. *S. sioyaensis* NRRL-B5408 (a siomycin producer) and *S. coelicolor* M145 (does not produce thiopeptide antibiotics) agar plugs were used as the positive and negative controls, respectively. All the experiments were performed in triplicate.

Metabolite Extraction and Analysis

The production of secondary metabolites by the Je 1–79 and Je 1–613 strains was studied using liquid SG medium.

The pre-cultures of these strains were inoculated in 10 mL TSB medium and then cultured for two days at 28 °C and 180 rpm. Next, 1 mL of pre-culture was inoculated into 50 mL of SG medium and cultured for six days at 28 °C and 180 rpm. The fermented broths were separated by means of centrifugation (8000 rpm for 5 min). An equal volume of ethyl acetate was used for the metabolite extraction from the liquid phase. An acetone:methanol (1:1) mixture (15 mL) for extraction from biomass was also used. The received extracts were mixed and evaporated using an IKA RV-8 rotary evaporator (IKA, Königswinter, Germany) at 40 °C and dissolved in 1 mL of methanol. The obtained metabolic extracts were also tested for their ability to induce the growth of the strain-biosensor by means of the disk diffusion method. For this purpose, 25 µl of the extracts were applied to paper disks (diameter = 6 mm) and dried. The ability of the metabolic extracts to induce the growth of the biosensor strain was then determined as described above.

To identify the compounds, the extracts were separated using a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) and a 10-sm ACQUITY UPLC R BEH C18 column (1.7 µm) (Waters, Milford, MA, USA). The mobile phase was determined to be composed of two solvents, namely acetonitrile solution contains 0.1% (v/v) formic acid and water solution contains 0.1% (v/v) formic acid. The solvent concentrations varied in a linear gradient from 5% to 95% for 18 min at a flow rate of 0.6 mL/min. The mass detection was performed in the positive mode with a detection range of 150–2000 m/z. The HPLC system was coupled with either an amaZon speed mass spectrometer or a maXis high-resolution LC-QTOF system (Bruker, USA) to allow for the mass spectrometry analysis of the extracts. Bruker Compass Data Analysis version 4.2 software (Bruker, Billerica, MA, USA) was used to conduct the data analysis. The compound screening was performed using the DNP (Dictionary of Natural Products) database version 10.0 [24] on the basis of the following parameters: accurate molecular mass, ultraviolet (UV) spectra, fragmentation analysis, and biological source [25]. The compounds were considered to be similar when the difference in the accurate mass was less than 5 ppm and the UV spectra were identical.

PCR Amplification and Sequencing of the 16S rRNA and Housekeeping Genes

For the total DNA isolation, the strains were cultivated in TSB medium for three days at a temperature of 28 ºC and a shaking rate of 180 rpm. The total DNA was isolated by means of the salting out procedure, as described in [26]. The amplification of the 16S rRNA gene was conducted using the following primers: 8F (5′-AGAGTT TGATYMTGGCTCAG-3′) and 1510R (5′-TACGGYTAC CTTGTTACGACTT-3′). The polymerase chain reaction (PCR) was performed in a total volume of 50 µl, which contained 2.0 µl of genomic DNA (~ 50 ng), 0.5 µl of each primer (100 pmol), 1.0 µl of deoxynucleotide triphosphates (10.0 mM each), 5 µl of 10 × PCR buffer, 0.5 µl of DNA polymerase (1 U/µl), 2.5 µl of dimethyl sulfoxide, and 38.0 µl of MilliQ grade water. The PCR parameters involved initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing of the primers at 53 °C for 30 s, and extension at 72 °C for 90 s. A final extension was conducted at 72 °C for 10 min. The PCR amplification and sequencing of five housekeeping genes, namely *gyrB*, *atpD*, *recA*, *rpoB*, and *trpB*, were performed using the primers and amplification conditions described in [27]. The received PCR products were visualized in 1% agarose gel and then purified using a QIAquick Gel Extraction Kit (Qiagen, Venlo, Netherlands). Next, they were sequenced with forward and reverse primers by GATC Biotech (European Genome and Diagnostics Centre, Constance, Germany). The sequences of the 16S rRNA genes of the Je 1–79 and Je 1–613 isolates were deposited in GenBank (accession numbers OL636373 and OL636374, respectively). The GenBank accession numbers of the housekeeping genes sequences (*gyrB*, *atpD*, *recA*, *rpoB*, and *trpB*) of the Je 1–79 and Je 1–613 isolates are listed in Table S1.

Phylogenetic and Multilocus Sequence Analyses

The phylogenetic analysis of the 16S rRNA gene sequences of the strains was performed using RDP Release 11 [28]. The most closely related *Streptomyces* species to the 16S rRNA and other housekeeping genes were identified based on BLAST search data in the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and then obtained from GenBank. The phylogenetic trees were constructed using the neighbor joining (NJ) algorithm [29] in MEGA X [30]. The evolutionary distances were computed using the Kimura two-parameter method [31], while the robustness of the tree topology was evaluated by means of the bootstrap test (1000 replicates) [32].

The multilocus sequence analysis (MLSA), which included the five housekeeping gene sequences, was performed as previously described [27]. The resulting sequences were adjusted as follows: *atpD*—496 bp, *gyrB*—441 bp, *recA*—504 bp, *rpoB*—540 bp, and *trpB*—567 bp. The artificial sequence of the five housekeeping genes was created in the following order: atpD-gyrB-recA-rpoB-trpB, thereby forming a final size of 2548 bp for both strains. The concatenated sequences were then aligned using MUSCLE [33].

Genome Sequencing and Bioinformatics Analysis of *Streptomyces* sp. Je 1–79

For the isolation of the total DNA, the *Streptomyces* sp. Je 1–79 strain was grown in TSB medium for four days at a temperature of 28 ºC and a shaking rate of 180 rpm. The salting out procedure was used to obtain the total DNA [26]. The RNA-free genomic DNA of the Je 1–79 strain was sequenced using Illumina for the short-read sequencing and PacBio Sequel II/Ile, Nanopore for the long-read sequencing on the Novogene platform (Cambridge, UK). The Illumina sequencing data were de novo assembled using Newbler v3.0 (454 Life Sciences, Branford, CT) with the default settings. The Burrows-Wheeler Aligner tool was used for mapping the reads [34]. The gene prediction and genome annotation were performed using Prodigal [35] and the NCBI Prokaryotic Genome Annotation Pipeline. The secondary metabolite gene clusters were analyzed using the antiSMASH genome mining tool [36]. The analysis of the genetic data was performed using Geneious 11.0.3 [37].

The genome sequences of the *Streptomyces* sp. Je 1–79 strain has been deposited at DDBJ/ENA/GenBank under the accession number JAMGSL000000000.

Results

Screening of Thiopeptides Using a Whole-Cell Biosensor

A total of 372 actinomycete-like strains isolated from the rhizosphere soil of *J. excelsa* were tested with regard to the production of thiopeptide antibiotics. Among the tested strains of actinomycetes, we identified two strains (i.e., Je 1–79 and Je 1–613) that induced the growth of the strain-biosensor around the agar plugs on the plates with 50 µg/mL of kanamycin. The crude extracts of the secondary metabolites of these strains obtained following cultivation in SG medium also induced the growth of the strain-biosensor (Fig. 1).

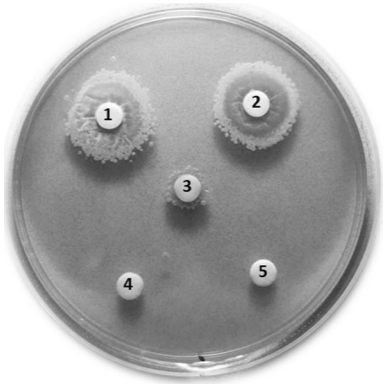


Fig. 1 The growth of the strain-biosensor *S. lividans* TK24 pMO16 (on Bennett's agar supplemented with 50 µg/mL kanamycin) around paper disks with the following crude extracts: (1) *Streptomyces* sp. Je 1–79, (2) *Streptomyces* sp. Je 1–613, (3) positive control (*S. sioyaensis* NRRL-B5408), (4) negative control 1 (*S. coelicolor* M145), and (5) negative control 2 (SG medium)

Identification and Dereplication of the Thiopeptides

The secondary metabolites of the Je 1–79 and Je 1–613 strains were analyzed to identify any compounds capable of activating resistance to kanamycin within the strain-biosensor. SG medium was used to obtain the extracts of the secondary metabolites of these strains. The high-performance liquid chromatography mass spectrometry (HPLC–MS) analysis of the extracts revealed the presence of a peak at a retention time (RT) of 10.1 min and mass m/z 1146.3484 $[M+H]^+$ in both strains (Fig. 2A, B). It should be noted that while this peak was identical in the two strains, the production level in the Je 1–79 strain was higher than that in the Je 1–613 strain. In addition, this peak exhibited similar UV absorption signals at λ_{max} 230 nm, 258 nm, and 320 nm (Fig. 2C). The dereplication of the monoisotopic masses 1145.3411 in the DNP database yielded a result and was identified as thiopeptide antibiotic berninamycin A. The berninamycin A derivative, namely berninamycin B [RT of 11.2 min and mass m/z 1130.3534 $(M+H)^+$], was also found on the both chromatograms (Fig. 2). However, the production level of berninamycin B was lower than that of berninamycin A.

A fragmentation analysis was performed to confirm the determination of the berninamycin A. For this purpose, a tandem mass spectrometry (MS^2) analysis was performed to determine the elementary composition of key fragments (Fig. 3). The fragmentation of the linear peptide side chain of the berninamycin A occurred via the cleavage of the NH–CO bond between the dehydroalanines, which formed a fragment ion peak at m/z 1067.41 $[M+H]^+$. The formation of a fragment ion peak at m/z 1007.46 $[M+H]^+$ via the cleavage of the $CH_2=C$–NH bond between the Dha II and the Pyr was also observed. The cleavage of the peptide ring system by means of the fragmentation of the CO–NH peptide bonds led to the following fragment identification: fragment of two amino acids OxaB-DhaIV [m/z 912.28 $(M+H)^+$], three DhaIV-L-Hyval-OxaA [m/z 812.29 $(M+H)^+$], four DhaIV-L-Hyval-OxaA-DhaIII [m/z 743.32 $(M+H)^+$], and five DhaIV-L-Hyval-OxaA-DhaIII-OxaB [m/z 579.27 $(M+H)^+$]. In addition, fragments formed following the loss of –OH [m/z 1129.43 $(M+H)^+$] were also observed.

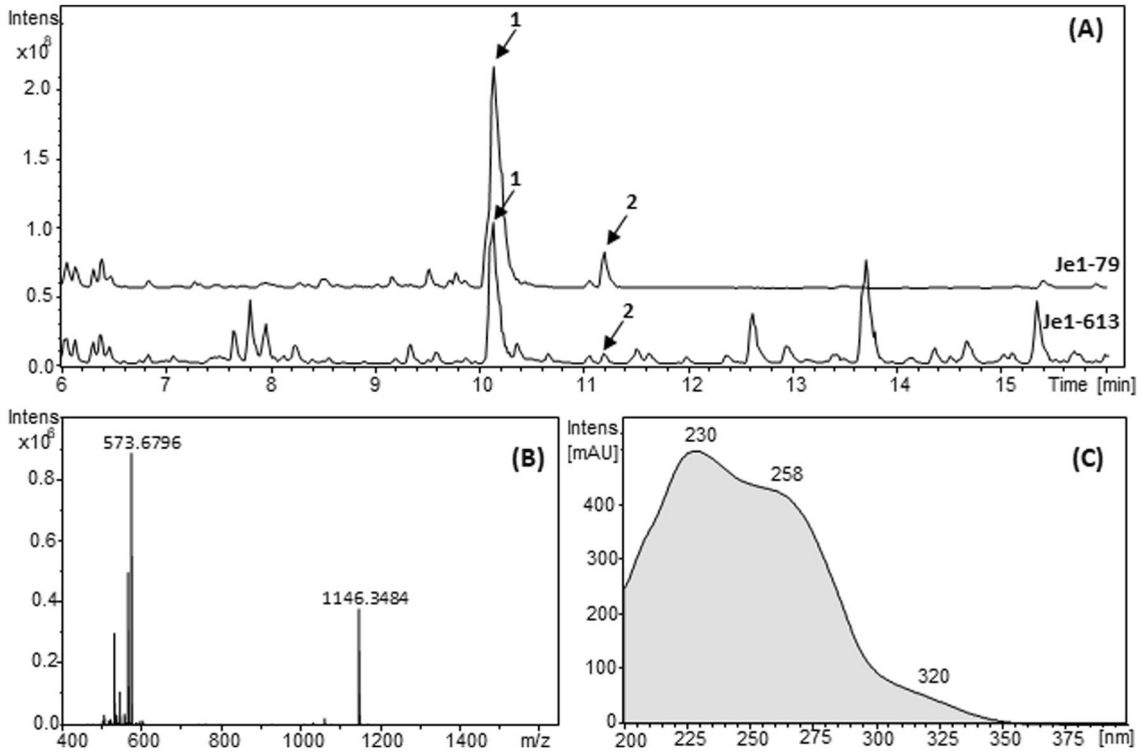


Fig. 2 HPLC–MS analysis of the berninamycin production. **A** Base peak chromatograms of the crude extracts from the *Streptomyces* sp. Je 1–79 and Je 1–613 strains. The peaks corresponding to berninamycin A and B are marked by 1 and 2, respectively. **B** The mass spectrum of berninamycin A [m/z 1146.3484 $(M+H)^+$], with the m/z 573.6796 corresponding to the $[M+2H]^+$ ion. **C** The UV spectrum of berninamycin A

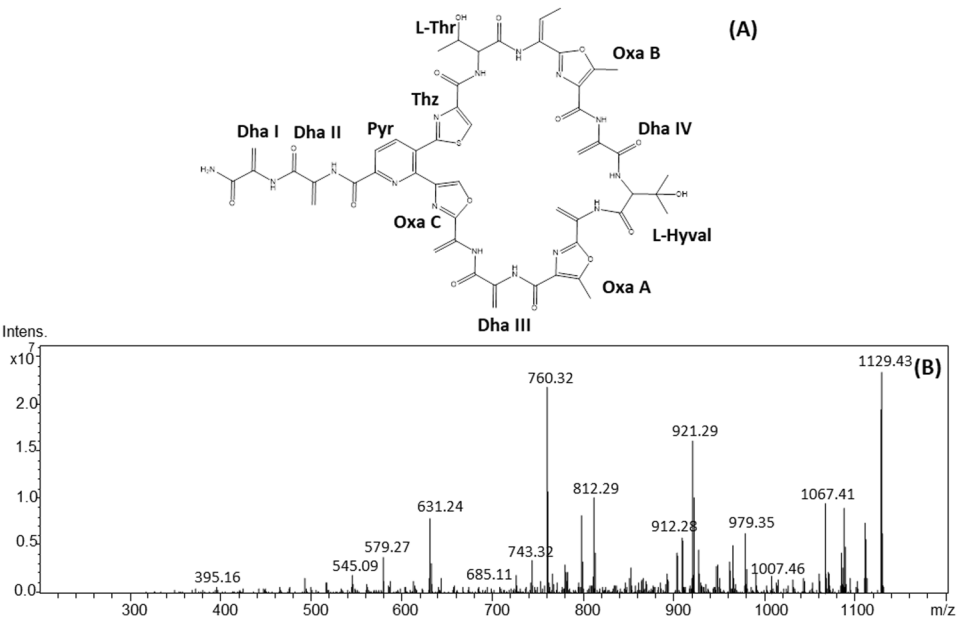


Fig. 3 Structure of berninamycin A (**A**) and $MS.^2$ spectrum of berninamycin A (**B**)

Phylogenetic and MLSA Analyses of the Berninamycin Producers

The phylogenetic analysis using the RDB Classifier program based on the 16S rRNA gene sequences of the Je 1–79 and Je 1–613 strains revealed these strains to belong to the genus *Streptomyces*. The Je 1–79 and Je 1–613 strains showed the highest sequence similarity to the *S. lateritius* FCS09 strain (similarity of 99.93%) and *S. candidus* strain PM425C strain (similarity of 99.93%), respectively. The NJ phylogenetic tree based on the 16S rRNA gene sequences revealed that the Je 1–79 and Je 1–613 strains formed tight clades with their closest neighbors. These strains are located in different clades, and no strain was also grouped with the tested producers of thiopeptide antibiotics (Fig. 4).

To clarify the phylogenetic relationships between the Je 1–79 and Je1-613 strains, partial sequences of five housekeeping genes of these strains, namely *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB*, were obtained. The BLAST analysis of the housekeeping genes showed that the closest relatives of the Je 1–79 strain were the *S. exfoliatus* A1013Y strain (98.89% and 98.55% *rpoB* and *gyrB* gene sequence similarity, respectively), the *S. exfoliatus* AS 4.1407 strain (98.64% *atpD* gene sequence similarity), the *S. omiyaensis* NRRL B-1587 strain (96.63% *recA* gene sequence similarity), and the *S. cacaoi* subsp. *asoensis* NRRL B-16592 strain (97.2% *trpB* gene sequences similarity). In terms of Je 1–613 strain, the closest relatives were the *S. spiroverticillatus* NBRC 12,821 strain (89.71% *gyrB* gene sequence similarity), the *S. candidus* NRRL ISP-5151 strain (99.32% and 98.41% *atpD* and *recA* gene sequence similarity, respectively), the *S. griseoluteus* NRRL B-1315 strain (94.02% *trpB* gene sequences similarity), and *Streptomyces* sp. Doro_SunPrairie_70 (97.96% *rpoB* gene sequences similarity).

The atpD-gyrB-recA-rpoB-trpB concatenated sequence's similarity with the Je 1–79 and 1–613 strains was determined to be 89.3%. Moreover, the pairwise distance calculated between these concatenated sequences was 0.108, which was clearly above 0.007, the threshold for species determination.

Identification and Analysis of the Berninamycin Gene Cluster From the Je 1–79 Strain

The *Streptomyces* sp. Je 1–79 genome was sequenced and assembled into 30 contigs. AntiSMASH software was used to identify potential clusters of secondary metabolite biosynthesis genes within the Je 1–79 genome. According to the analysis, the Je 1–79 genome contains 28 clusters of secondary metabolite (Table S2). Among the identified clusters, one gene cluster was annotated as a thiopeptide-like gene cluster. The BLAST analysis of this cluster among the known clusters revealed 93% similarity with the berninamycin A (*ber*) biosynthetic gene cluster obtained from *S. bernensis* (GenBank: KC894738). All the genes of the identified *ber*-like cluster from the Je 1–79 genome showed a high level of similarity with the corresponding genes of the *ber* cluster, while the gene cluster organization of these clusters was found to be identical. For example, the similarity between the structural *berA* and Orf 10 genes indicated 100% sequence homology, while the similarity between the *berB* and Orf 11 genes, *berC* and Orf 12 genes, and *berD* and Orf 9 genes in both clusters from Je 1–79 and *S. bernensis* indicated 83%, 86%, and 75% sequence homology,

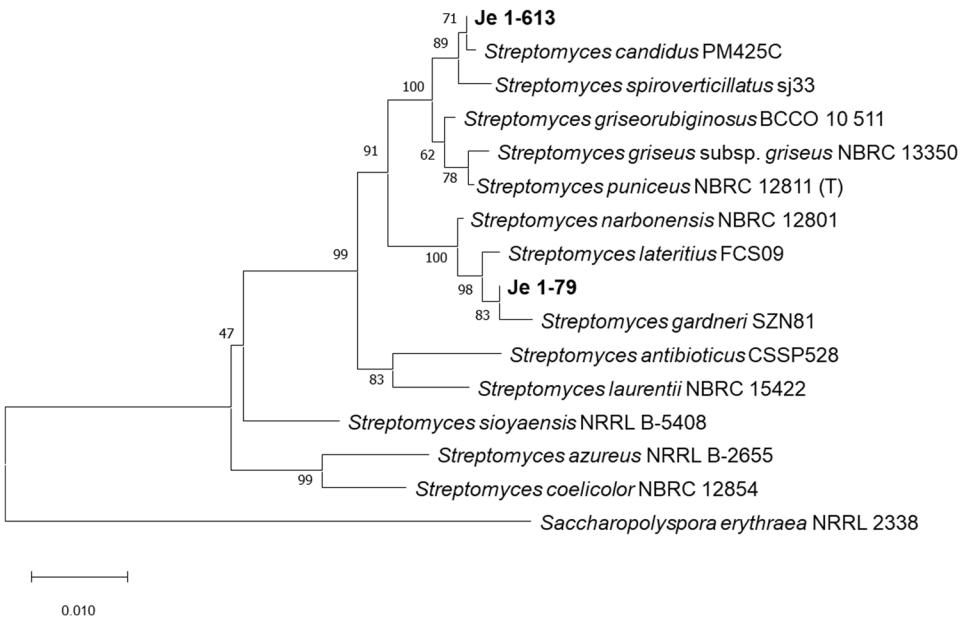
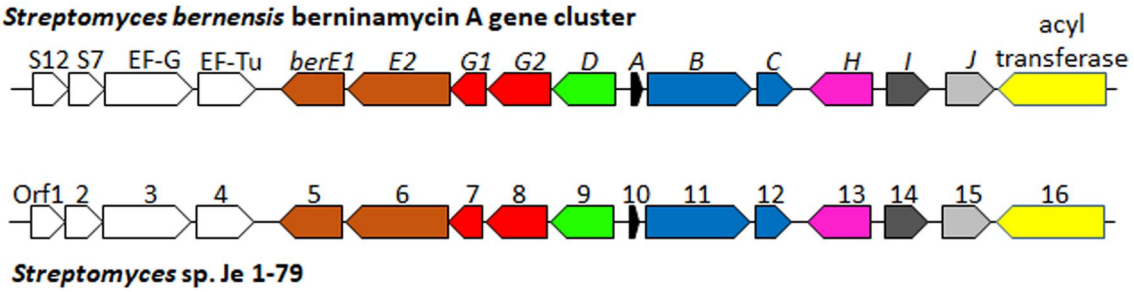


Fig. 4 NJ tree based on the 16S rRNA gene sequences of the Je 1–79 and Je 1–613 strains (in bold), their closest neighbors, a few known thiopeptide producers, and selected *Streptomyces*-type strains. *Saccharopolyspora erythraea* NRRL 2338 was used as an outgroup. Bar indicates 0.01 substitutions per nucleotide position



Gene in Je 1-79	Homologue in the berninamycin A gene cluster	Percentage coverage	Percentage identity
Orf 1	S12 ribosomal protein	100	100
Orf 2	S7 ribosomal protein	100	97
Orf 3	Elongation factor G	100	92
Orf 4	Elongation factor Tu	100	96
Orf 5	*berE1*	99	81
Orf 6	*berE2*	100	81
Orf 7	*berG1*	100	78
Orf 8	*berG2*	100	86
Orf 9	*berD*	100	75
Orf 10	*berA*	100	100
Orf 11	*berB*	100	83
Orf 12	*berC*	79	86
Orf 13	*berH*	82	74
Orf 14	*berI*	98	86
Orf 15	*berJ*	98	79
Orf 16	Acyl transferase	83	58

Fig. 5 Comparison of the biosynthetic gene clusters encoding berninamycin A from *S. bernensis* (top) and *Streptomyces* sp. Je 1–79 (bottom)

respectively. Moreover, as in the *ber* cluster, the *ber*-like cluster from the Je 1–79 strain contains ribosomal and translationally associated proteins and an acyltransferase at the edges (Fig. 5).

Discussion

Whole-cell biosensors play a significant role in the discovery of antimicrobial compounds, which is particularly relevant given the rate of development of antibiotic resistance among pathogenic microorganisms. Thiopeptide antibiotics have attracted the attention of researchers due to showing high levels of activity against various antibiotic-resistant microorganisms, suggesting that they could be considered candidates for their control [38, 39]. The microbial whole-cell biosensor *S. lividans* TK24 pMO16 used in this study is based on the *tipA* promoter fused to the gene *neo*. This *tipA* promoter controls the expression of the resistance gene *neo* and induces kanamycin-resistant growth in the presence of thiopeptide antibiotics [23]. Thus, the induction of strain-biosensor growth around the agar plugs with the test strains indicates the production of thiopeptide antibiotics. In addition, this biosensor has previously been successfully used to screen producers associated with an increased level of siomycin production in *S. sioyaensis* [40]. However, this

biosensor has not yet been used to screen thiopeptide producers among natural actinomycetes strains. These natural strains of actinomycetes are usually a good reservoir of biologically active compounds, including antibiotics [41]. Therefore, in the present study, we demonstrated for the first time the use of the microbial biosensor *S. lividans* TK24 pMO16 for the screening of thiopeptide antibiotics among natural actinomycete strains. To achieve this, previously isolated actinomycetes strains from the rhizosphere soil of *J. excelsa* were used [22]. Among the tested actinomycete strains, we identified two strains (i.e., Je 1–79 and Je 1–613) that induced the growth of the strain-biosensor. The dereplication analysis of the crude extract of these strains resulted in the annotation of the thiopeptide antibiotics berninamycin A and B. The berninamycins are macrocyclic pyridine-containing thiopeptide antibiotics, which were first isolated from *S. bernensis* [42].

The growth of the strain-biosensor around the discs with crude extracts of the identified strains was markedly better than that of the control strain (Fig. 1). This observation may indicate a difference in thiopeptide production or thiopeptide-dependent biosensor sensitivity because the control strain produces siomycin while the Je 1–79 and Je 1–613 strains produce berninamycin. We also observed a difference in berninamycin production between the Je 1–79 and Je 1–613 strains on HPLC chromatograms (Fig. 2), but there was no obvious difference in their biosensor sensitivity. On this basis, we suggest that berninamycin induces the *tipA* promoter better than siomycin. Chiu et al. [43] demonstrated that berninamycin A is capable of inducing the transcriptional activator TipA in *Streptomyces* (at a level of 1.2 ng/ml) at a rather low concentration compared to other thiopeptides. The authors also showed that the minimal induction concentrations of thiopeptides depend on the number of dehydroalanine residues in the tail. Thus, we assume that the sensitivity of the biosensor used in this work depends not only on the concentration of thiopeptides in the medium but also on their structure. Therefore, thiopeptides with the presence of dehydroalanine residues will better induce the biosensor under study. However, this finding does not diminish the potential use of this biosensor for screening thiopeptide antibiotic producers.

As the Je 1–79 and Je 1–613 strains produced the same thiopeptide antibiotic, although morphologically different (Fig. S1), we decided to phylogenetically identify these strains. Based on the 16S rRNA gene sequence analysis, these strains belong to the genus *Streptomyces*. However, an analysis of the 16S rRNA gene sequence is insufficient to separate closely related species, especially within the *Streptomyces* genus. Thus, an MLSA analysis of five housekeeping genes was performed to more accurately establish the taxonomic relationship between these strains [27]. The affiliation of the Je 1–79 and Je 1–613 strains with different *Streptomyces* species according to different housekeeping genes suggested the two strains to be phylogenetically distinct from each other. Moreover, the pairwise distance calculated between the atpD-gyrB-recA-rpoB-trpB concatenated sequences of these strains was higher than 0.007, which conforms to 70% DNA–DNA homology and passes the threshold for species determination [44]. Therefore, we suggest that the Je 1–79 and Je 1–613 strains should be considered representatives of two different species of the genus *Streptomyces*.

The ability to produce the same antibiotic by different strains may suggest a relatively distant common ancestor and the vertical transmission of the *ber* cluster or the same region of existence and the horizontal transmission of the gene cluster [45]. Given the phylogenetic interactions between the studied strains and their relative distance in housekeeping genes, this indicates a possible horizontal transmission of the *ber* cluster. It is also possible that the two strains have a relatively distant common ancestor, and the *ber* clusters may have co-evolved together with the housekeeping genes as they evolved independently. A detailed analysis of the evolutionary patterns between *ber* clusters and phylogenetic marker genes in the identified strains will provide a better understanding of the evolution and distribution of the *ber* cluster in the study environment and will be the focus of future investigations.

Additional convincing evidence for the berninamycin production was the identification of the biosynthesis gene cluster. Due to both strains producing the same thiopeptide antibiotic, we decided to sequence the genome of only one of them. The thiopeptide-like biosynthetic gene cluster was identified in the genome of the Je 1–79 strain, and it showed similarity with the *ber* cluster from *S. bernensis*. The structural gene *berA*, which encodes the precursor peptide BerA, as well as the *berB*, *berC*, and *berD* genes, which are probably involved in the formation of the macrocycle [46], indicated particularly high similarity between the clusters of the two strains. The presence of a biosynthetic gene cluster with a high level of similarity with the *ber* cluster in the Je 1–79 genome confirms the effectiveness of the strain-biosensor *S. lividans* TK24 pMO16 in terms of the screening of thiopeptide antibiotics. Thus, we believe that the strain-biosensor S. lividans TK24 pMO16 is an effective tool for screening thiopeptide producers in natural biotopes.

Conclusion

In this study, we successfully used a microbial biosensor for the screening of thiostrepton-like thiopeptide antibiotics among the actinomycete strains isolated from the rhizosphere soil of *J. excelsa*, which led to the identification of two producers of thiostrepton-like thiopeptide antibiotics.

Moreover, our dereplication analysis of the crude extracts of these strains led to the identification of the thiopeptide antibiotics berninamycin A and B. The thiopeptide-like gene cluster was identified in the genome of the Je 1–79 strain and showed a high level of similarity with the gene cluster of berninamycin A from *S. bernensis*. The two strains were deposited in the MCCAP of Ivan Franko National University of Lviv (collection numbers Lv 224 for *Streptomyces* sp. Je 1–79 and Lv 521 for *Streptomyces* sp. Je 1–613). Therefore, this study found that the use of the strain-biosensor *S. lividans* TK24 pMO16 during the first stage of the screening process can serve to accelerate the specific detection of thiopeptide antibiotics.

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Authors' Contributions OG conceived and planned the experiments. ST and OG performed the screening of the thiopeptides. MM performed the metabolite extraction and identified the berninamycins. ST performed the phylogenetic and multilocus sequence analyses, the genome sequencing, and the identification of the berninamycin gene cluster. ST and OG both processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. VF and AL aided in interpreting the results and also worked on the manuscript. All the authors read and approved the manuscript.

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Declarations

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Ethics Approval Not applicable.

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