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Article

A new albomycin-producing strain of *Streptomyces globisporus* subsp. *globisporus* may provide protection for ants *Messor structor*

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Abstract: There are several well-studied examples of protective symbiosis between insect-host and symbiotic actinobacteria, producing antimicrobial metabolites to inhibit host pathogens. These mutualistic relationships are best described for some wasps and leaf-cutting ants while a huge variety of insect species still remains poorly explored. For the first time we isolated actinobacteria from harvester ant *Messor structor*, and evaluated the isolates' potential as antimicrobial producers. All isolates could be divided into two morphotypes of single and mycelial cells. We found that the highest frequency of occurrence of mycelial morphotype was observed among soldiers, and the lowest among larvae in the studied laboratory colony. The representative of this morphotype was identified as *Streptomyces globisporus* subsp. *globisporus* 4-3 by polyphasic approach. It was established using *E. coli* JW5503 pDualRep2 system, that crude broths of mycelial isolates inhibited a protein synthesis in reporter strains, but it did not disrupt the *in vitro* synthesis of proteins in cell-free extracts. Active compound was extracted, purified and identified as albomycin δ2. Pronounced ability of albomycin to inhibit the growth of entomopathogens suggests that *Streptomyces globisporus* subsp. *globisporus* may be involved in defensive symbiosis with the *Messor structor* ant against infections.

Keywords: actinobacteria; *Streptomyces globisporus* subsp. *globisporus*; albomycin; defensive symbiosis; ants; *Messor structor*

1. Introduction

The widespread use of antimicrobial compounds in medicine and agriculture has led to the emergence of multidrug-resistant pathogens, recognised now as a significant threat to human health [1,2]. Search for novel compounds possessing antimicrobial properties is still one of the ways we could overcome this global challenge, and the microorganisms are the main source in this research [3–5]. Recently the great attention was attracted by the microbes which are forming symbioses with higher organisms, in particular, plants and animals [6]. The main reason for that is the mutual evolutionary path, where microbes have proved their usefulness to the host [7,8].

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Some prokaryotes, principally the phylum of Actinomycetota [9], are involved in the formation of so-called "defensive (or protective) symbioses" [10–12] with many eukaryotic organisms. The Insecta class with the largest number of species is remarkable among them for these interactions [13,14]. Through the release of various antibiotic compounds actinobacteria protect insects, their brood and food substrate from potential pathogens and parasites [7,15–18]. Well known in this respect is the taxon of leaf-cutting ants (Atta, Acromyrmex) of the subfamily Myrmicinae. Their existence, and in particular feeding and development, depends entirely on the symbiotic actinobacteria of the genus *Pseudonocardia* [19], localized on the insect cuticle.

However, the specificity of "defensive symbioses" in other species of Formicidae remains unclear, including one of the dominant ants of the steppe zone, *Messor structor* (the steppe harvester ant). In this paper, we report on the isolation of actinobacteria from the laboratory colony of *Messor structor* ant. All of mycelial isolates demonstrated the same genotypic and phenotypic properties and were identified as S*treptomyces globisporus* subsp. *globisporus*. They produced protein synthesis inhibitor albomycin δ2, that was active against entomopathogens. The distribution of these actinobacteria among individuals from different castes suggests their essential role in maintaining the health of the ant family.

2. Materials and Methods

2.1. Ant colony rearing and microbial isolation

Prior to rearing an ant colony the special incubator was designed, which consisted of the glass tube with sterile water and poppy seeds, as the main nutrient substrate. The mated queen was maintained there during a 3 month period, until the adult ants quantity was sufficient to place the colony in a specific formicarium. The formicarium is made from the acrylic plastic with two main chambers - arena, through which the seeds are supplemented and system of chambers, where ants raise their brood. In the center of formicarium the specific watering cell is present, which maintains the humidity and water level inside the nest. Humidity is maintained between 70-90% and temperature at 24°C respectively, without pouring of the direct sunlight on the formicarium.

The queen was collected during the mating season in July 2017 in the Astrakhan region, Russia (46°51'13.5"N 47°59'06.2"E). Isolation of actinobacteria strains was performed only after the stabilisation of the population number of at least 50 specimens.

Actinobacterial strains were isolated from bodies of larvae, pupae and imago workers and soldiers castes of *Messor structor*. A total of 14 individuals from each group were examined. Every specimen was washed three times in sterile distilled water and then crushed by tissue microhomogenizer with sterile saline solution. Aliquots of this mixtures and their 10-fold dilutions were spread over mineral agar 1 [20] and Organic agar 79 [21] supplemented nystatin and nalidixic acid at final concentrations 250 μg/mL and 10 μg/mL, accordingly, and incubated for 14 days at 28°C [22]. Actinobacteria isolates were purified and maintained on ISP 3 slants [23], and preserved as suspension of mycelial fragments and spores in 20% glycerol at -20°C.

2.2. 16S rRNA phylogeny of isolated strains

The extraction of genomic DNA of isolates, PCR amplification and were achieved using procedures described elsewhere [24]. Both the pair of universal primers F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′-TACGGYTACCTTGTTACGACTT-3′), and actinobacterial primers 243F (5′-GGATGAGCCCGCGGCCTA-3′) and A3R (5′-CCAGCCCCACCTTCGAC-3′) were used. The amplicons were purified and sequenced using a commercial service (EvroGen). All sequences were identified by searching close

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relatives with BLAST service (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and submitted in GenBank with the assignment of access numbers.

2.3. Genome Features and Phylogenomic analysis

Genome of strain 4-3 was sequenced de novo by Skoltech Genomics Core Facility, using the Illumina HiSeq 4000 platform. The quality control and adapter trimming was done by the bbDuk tool from the bbMap suite v38.42 (https://sourceforge.net/projects/bbmap/). Genome assembly was performed by SPAdes v3.13.0 [25]. Genome was annotated using RASTtk pipeline implemented on the PATRIC web service [26]. Assembly is available in the European Nucleotide Archive with project accession PRJEB51905.

Values of average nucleotide identity (ANI), genome completeness and quality were evaluated using a web service MiGA (http://microbial-genomes.org/), and *in silico* digital DNA:DNA hybridization (DDH) values were calculated by using GGDC method, with the recommended formula 2, available at the TYGS web service (https://tygs.dsmz.de/) [27].

Phylogenomic analysis was performed using Type (Strain) Genome Server (https://tygs.dsmz.de/). The phylogenomic tree inferred with FastME 2.1.6.1 [28] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5.

The full-length 16S rRNA gene sequences of strain 4-3 was extracted from the whole genome sequence (PRJEB51905) and was compared to sequences of related *Streptomyces globisporus* subsp. *globisporus* and some actinobacteria species, firstly isolated from insects. Evolutionary trees based on 16S rRNA gene sequences were inferred with the Neighbour-Joining [29], Maximum-Parsimony [30] and Maximum-Likelihood [31] treemaking algorithms after CLUSTAL W alignment by using MEGA software version X [32].

2.4. Analysis of bioactive compound biosynthetic gene clusters

Secondary metabolite biosynthetic gene clusters in complete genome strain 4-3 and its neighbors were identified with the bacterial version of antiSMASH 6.1.0 (https://antismash.secondarymetabolites.org/). Homologous regions on each genome were identified using NCBI Blastn (https://blast.ncbi.nlm.nih. gov/).

2.5. Phenotypic characterization

Cultural characteristics of strain 4-3 were observed on the range of ISP 2-ISP 7 media [23] after cultivation up to 14 days at 28°C. The RAL Classic Standard was used to determine the designations of colony colors. The shape of spore chains and spore surface of strain 4-3 on ISP 3 after cultivation at 28°C for 14 days were studied using light microscopy (Fisherbrand AX-502, Thermo Fisher Scientific) and scanning electron microscopy (JSM-6380LA, JEOL).

Carbon source utilization was assessed on basal medium ISP 9 [23] with addition of 0.04% solution of bromocresol purple at 28 °C for 14 days. Enzyme activities were estimated using a paper indicator system (NPO Microgen, Russia) according to the manufacturer's recommendations at 28 °C for 7 days. The degradation of casein, starch and cellulose was estimated on clearing of the insoluble compounds around areas of growth [33].

2.6. Screening of the antimicrobial potential

The ability of actinobacteria isolates to inhibit bacterial growth was assessed by the agar diffusion method. The isolates were challenged against different clinically significant microorganisms: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* CBS 8836 and *Aspergillus niger* INA 00760. Anti-entomopathogenic activity was also investigated against: *Bacillus thuringiensis* VKM B-6650, *Paenibacillus alvei* VKM B-502, *Beauveria bassiana* VKM F-1357, *Entomophthora coronata* VKM F-1359.

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Test bacteria, yeast and fungi strains were individually inoculated in Luria–Bertani agar, dextrose–peptone-yeast agar and potato dextrose agar, accordingly. Agar plugs of each actinobacterial isolate (2-week-old cultures in ISP6) placed on the surface of the inoculated media. The plates were incubated at 37°C and after 24-48 h the inhibition zones were checked.

The two *E. coli* reporter strains: BW25113 wild-type pDualRep2 and JW5503 ΔtolC pDualRep2 [34] were used in this work as previously described [35]. Briefly, 100 µl of cultural broth were placed into wells in agar that had the lawn of a reporter strain. Two control antibiotics, erythromycin (Ery, 2 µg) and levofloxacin (Lev, 0.05 µg), were additionally applied to an agar plate. Plates were incubated at 37°C overnight and then scanned by ChemiDoc (Bio-Rad) in the modes 'Cy3-blot' for RFP and 'Cy5-blot' for Katushka2S. Expression of the rfp gene occurred in the case of activation of the SOS-response system of the cell, and katushka2S, in the case of a violation of translation, when the ribosome was stalled on the mRNA template. When scanning, the signal from two black and white images was superimposed on each other with the assignment of green for the signal from the RFP protein and red for Katushka2S.

2.7. In vitro translation

In vitro translation reactions were performed in the presence of HPLC 4-3 fraction (1/10 of final volume) in 5 µl using the PURExpress (NEB) system supplemented with 100 ng Fluc mRNA and 0.05 mM d-luciferin. Chemiluminescence was recorded with a VictorX5 multi-reader.

2.8. Purification and identification of Albomycin

To obtain sufficient amount of active compound for detailed bioactivity studies, strain 4-3 was cultured in four 750-mL Erlenmeyer flasks with 250 mL of liquid ISP 6 at 28°C for 14 days under static conditions. 1 liter of culture liquid 4-3 was used for gravity-force reverse-phase chromatography on the sorbent LPS500H (polyvinylbenzene, pore size 50-1000 Å) eluting with 10, 20, 30, 40, 50, 75 and 100% acetonitrile solutions. The pDualrep2 double reporter system was used to analyze the activity of the lps fractions. The most active fractions were 10-20% acetonitrile, which induced the expression of reporter protein Katushka2S. The 10% acetonitrile fraction was further purified by high performance liquid chromatography (HPLC) (Agilent 1260, isocratic elution 4% of MeCN, 10 mM AcONH4, 1 ml/min, 25C) using a Phenomenex HPLC column (Luna 5 µm C18 (2) 100 Å, 4.6*250 mm), the collected fractions were analyzed using the reporter pDualrep2.

Fractions with antibacterial activity corresponding to an individual peak on chromatograms were collected and the active compound was identified using ultra-high performance liquid chromatography - electrospray ionization - high resolution mass spectrometry (UPLC-ESI-HRMS). Analysis was carried out on an Ultimate 3000 RSLCnano HPLC system connected to an Orbitrap Fusion Lumos mass spectrometer (ThermoFisher Scientific). Sample of the active compound was separated on Luna Omega C18 100 x 2.1 mm 1.6 um columns at 0.2 ml/min flow rate and at RT. Separation was done by a gradient elution in two components mixture from initial 5% to 20 % of component B for 10 min. Component A was 0.1% formic acid plus 10mM formate ammonium in water and component B was 0.1% formic acid in 100% MeCN and 10% 10 mM formate ammonium in water. UV data were registered at 290 nm. MS1 and MS2 spectra were collected in positive ion mode and recorded at 30K and 15K resolution respectively with HCD fragmentation.

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3. Results

3.1. Isolation of actinobacteria strains, associated with Messor structor ants

All bacterial strains isolated from *Messor structor* individuals were divided into two morphotypes: one of which formed branching mycelium (labeled as 4-3), and the other gram-positive cocci (L1). Results demonstrated, 4-3 had more association with the caste of soldiers making 89% frequency of occurrence among this group. While workers' caste, pupae and larvae showed less specific results - 50%, 21% and 7% respectively. On the contrary, L1 bacteria were found in the vast majority of individuals from these groups besides soldiers (Table S1).

The analysis of 16S rRNA sequences and comparison them with GenBank database demonstrated that 4-3 strain belong to *Streptomyces* genus. The closest strains were *Streptomyces globisporus* subsp. *globisporus* DSM 40136 (formerly a type strain of *Streptomyces albovinaceus*), *Streptomyces globisporus* subsp. *globisporus* DSM 40199^T, *Streptomyces rubiginosohelvolus* DSM 40176^T and *Streptomyces pluricolorescens* DSM 40019^T. The BLAST analysis of L1 sequence revealed 100% similarity with *Staphylococcus gallinarum* DSM 20610^T.

3.2. Genome features and phylogenomic analysis of Streptomyces sp. strain 4-3

Phylogenomic analysis based on whole-genome sequences showed that strain 4-3 formed a well supported monophyletic clade with *S. globisporus* subsp. *globisporus* DSM 40199^T and *S. globisporus* subsp. *globisporus* DSM 40136 with 95% bootstrap value (Fig. 1).

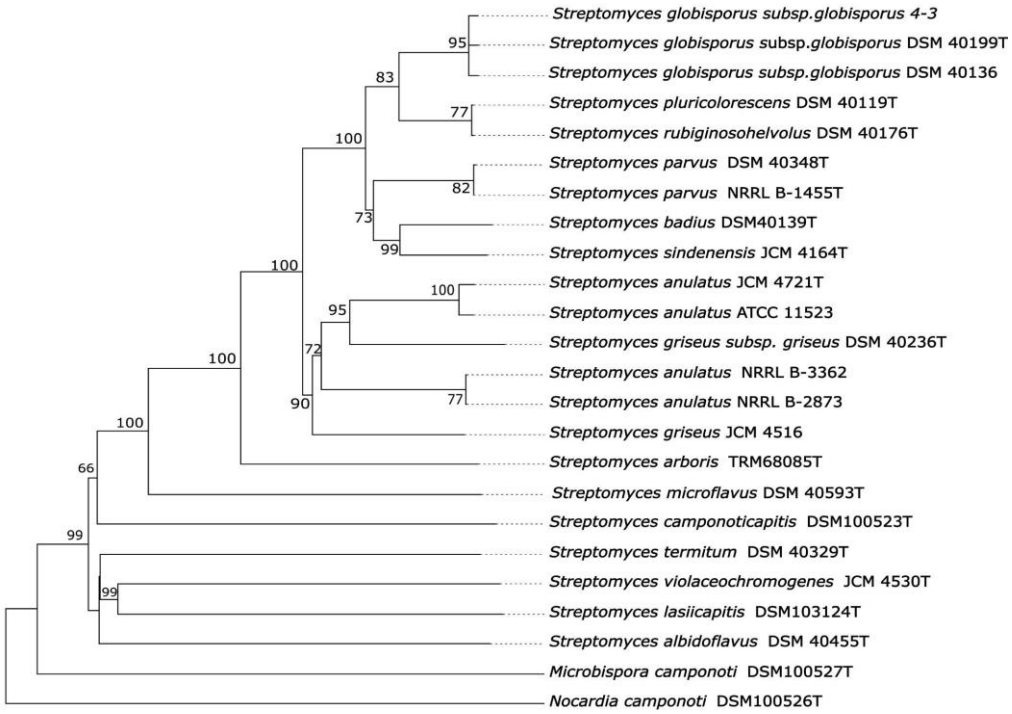

Figure 1. Phylogenetic tree based on whole-genome sequences from 4-3, related type strains and actinobacteria isolated from ants. The branch lengths are scaled in terms of GBDP distance formula d5. Numbers above branches are GBDP pseudo-bootstrap support values> 60% from 100 replications, with an average branch support of 81.4%. *Nocardia camponoti* DSM 100526^T as outgroup [27].

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The complete genome size of strain 4-3 was 7,941,828 bp with DNA G + C content of 71.6%, which was consistent with the G + C content of the genus *Streptomyces* [36]. The closest neighbors *S. globisporus* subp. *globisporus* DSM 40199^T and *S. globisporus* subp. *globisporus* DSM 40136 are characterized by similar genome size and G+C content (Table S2).

The ANI and *in silico* dDDH values between strains 4-3 and *S. globisporus* subp. *globisporus* DSM 40136 and *S. globisporus* subp. *globisporus* DSM 40199^T were above the recommended threshold of 96% and 70% (Table 2) needed for species separation [27,37]. Based on this, the strain 4-3 most likely belongs to the species *Streptomyces globisporus* subp. *globisporus*.

Table 2. Genome relatedness of 4-3 and *Streptomyces* type-strains

Subject strain	ANI,%	dDDH (in %)	G+C content difference (in %)
Streptomyces globisporus subsp. globisporus DSM 40136	99.3	96.3	0.14
Streptomyces globisporus subsp. globisporus DSM 40199^T	99.4	95.9	0.12
Streptomyces rubiginosohelvolus DSM 40176^T	96.3	66.2	0.18
Streptomyces pluricolorescens DSM 40019^T	96.2	66.1	0.14
Streptomyces parvus NRRL B-1455^T	94.8	56.2	0.01
Streptomyces parvus JCM 4069^T	94.7	55.7	0.05
Streptomyces sindenensis JCM 4164^T	94.2	52.9	0.25
Streptomyces badius JCM 4350^T	94.2	52.5	0.11
Streptomyces anulatus JCM 4721^T	92.1	41.8	0.15

Neighbor-Joining phylogenetic analysis demonstrated that 4-3 was most closely related to type and not type strains of *Streptomyces globisporus* subsp. *globisporus*: DSM 40199^T, DSM 40139, C-1027, TFH56 as well as to *S. rubiginosohelvolus* DSM 40176^T, *S. pluricolorescens* DSM 40019^T, *S. sindenensis* DSM 40255^T, *S. anulatus* DSM 40361^T, *S. griseus subsp. griseus* ATCC 13273 and formed a share clade with 100% bootstrap value (Fig. S1). However, type strains of actinobacterial species, first isolated from ants and other insects, did not form well supported clades with 4-3. This relationship was also supported in the phylogenetic trees generated with maximum-parsimony and maximum-likelihood methods (Figs. S2 and S3, available in Supplementary Material).

3.3. Phenotypic characterization of Streptomyces sp. strain 4-3

To further evaluate the features of the 4-3 strain using polyphasic taxonomy approach, the cultural, morphological and physiological properties of 4-3 were compared with ones of the type strains of *Streptomyces globisporus* subsp. *globisporus*. Results demonstrated the identity of these organisms in morphology -- the shape of sporophores and spore surface (Table S3, Fig. 2), and high similarity of their cultural characteristics on the series of ISP media (Table 3, Fig. S4).

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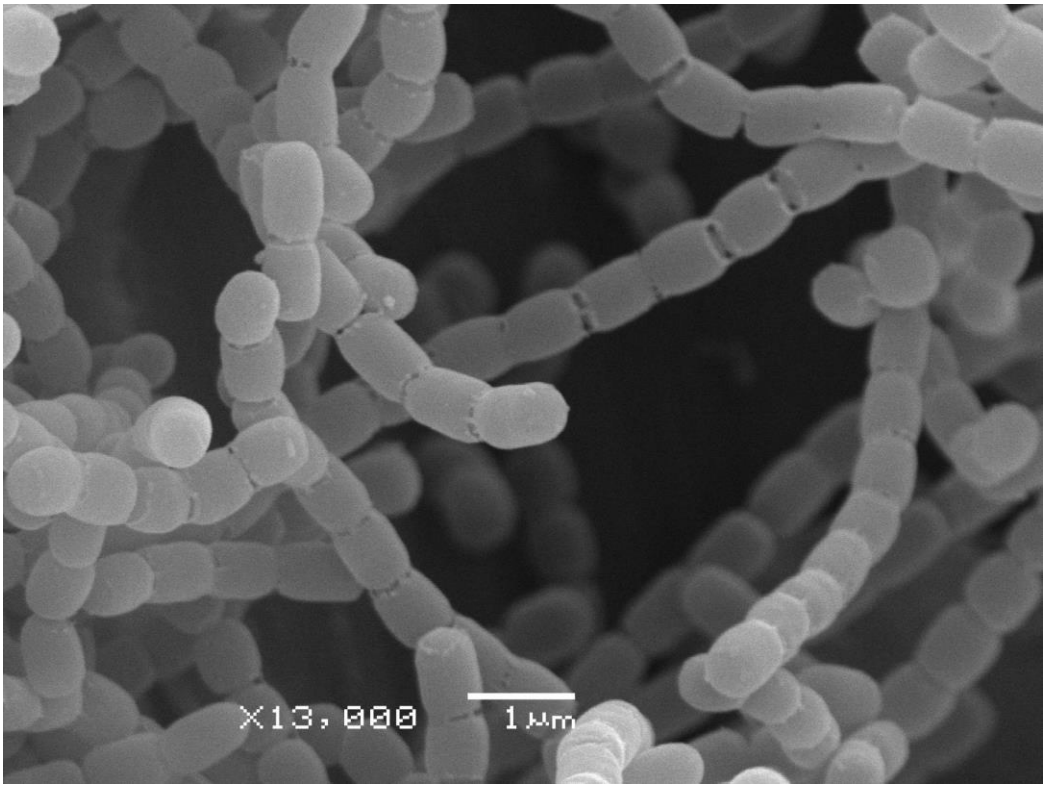

Figure 2. Scanning electron micrograph of strain *Streptomyces globisporus* subsp. *globisporus* 4-3, showing spore surface after incubation on ISP 3 medium at 28°C for 14 days.

However, it should be noted some differences in biochemical and physiological properties of strain 4-3 and closest type strains of *S. globisporus* subsp. *globisporus* (Table S3). For example, the production acid from glucose and xylose was positive in strain 4-3 (Fig. S5), whereas the other type strains showed negative results. In the decomposition of polymers, strain 4-3 was unable to use cellulose as a sole carbon source, whereas the other type strains utilized it. Also, enzyme assay of 4-3 were negative for L-ornithine decarboxylase and L-arginine decarboxylase, in contrast, DSM 40199^T and DSM 40136 demonstrated positive responses (Table S3). However, most of the biochemical tests showed similar results.

Table 3. Cultural characteristics of strain 4-3 (1) and closely related *Streptomyces globisporus* subsp. *globisporus* DSM 40199^T (2) and DSM 40136 (3)

Media	1	2	3
Yeast extract-malt extract (ISP 2)			
Growth	good	good	good
Aerial spore-mass colour	oyster white	oster white	cream
Substrate mycelial colour	beige	ochre yellow	beige
Soluble pigment	none	none	none
Oatmeal (ISP 3)			
Growth	good	good	good
Aerial spore-mass colour	oyster white	oster white	cream
Substrate mycelial colour	brown beige	ivory	beige
Soluble pigment	brown beige	none	none
Inorganic salts-starch (ISP 4)			
Growth	good	good	good

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Aerial spore-mass colour	white	light gray	sparse
Substrate mycelial colour	colorless	green brown	beige
Soluble pigment	none	none	none
Glycerol-asparagine (ISP 5)			
Growth	good	good	good
Aerial spore-mass colour	light olive	none	none
Substrate mycelial colour	sand yellow	ivory	beige
Soluble pigment	sand yellow	none	none
Peptone-yeast extract iron (ISP 6)			
Growth	good	good	good
Aerial spore-mass colour	white	none	none
Substrate mycelial colour	beige	sand yellow	beige
Soluble pigment	none	none	none
Tyrosine (ISP 7)			
Growth	weak	good	good
Aerial spore-mass colour	ivory	none	cream
Substrate mycelial colour	yellow-red	beige	beige
Soluble pigment	none	none	none

Data for *Streptomyces globisporus* subsp. *globisporus* DSM 40199^T and DSM 40136 are from DSMZ catalogue (https://www.dsmz.de/collection/catalogue/microorganisms/catalogue).

According to obtained data we may conclude that strain 4-3 isolated from *Messor structor* ants can be classified as *Streptomyces globisporus* subsp. *globisporus*.

3.4. Analysis of 4-3 bioactive compound biosynthetic gene clusters

The bioinformatics analysis of *Streptomyces globisporus* subsp. *globisporus 4-3* genome revealed biosynthetic gene cluster of albomycins, consisting of 18 genes from *abm*A to *abm*R, completely identical to that of *Streptomyces* sp. ATCC 700974 (Fig. 3), described in detail earlier [38]. The presence of *abm*K, participating directly in the formation of SB-217452 (the active seryl-tRNA synthetase inhibitor component of albomycin) [39] and also providing self-resistance to albomycins [40], indicates the ability of *Streptomyces globisporus subsp. globisporus* 4-3 to actively produce albomycin δ2.

Furthermore, 4-3 strain genome contains a number of second metabolic biosynthesis gene clusters (SMBGCs) with high identity to those, coded production of antimicrobial compounds (Streptophenazines B/C/E/H/G, mayamycins), odor substances (geosmin), pigments (melanin, isorenieratene), siderophores (streptobactin, coelichelin), cytoprotectants (ectoine) and others (Table S4).

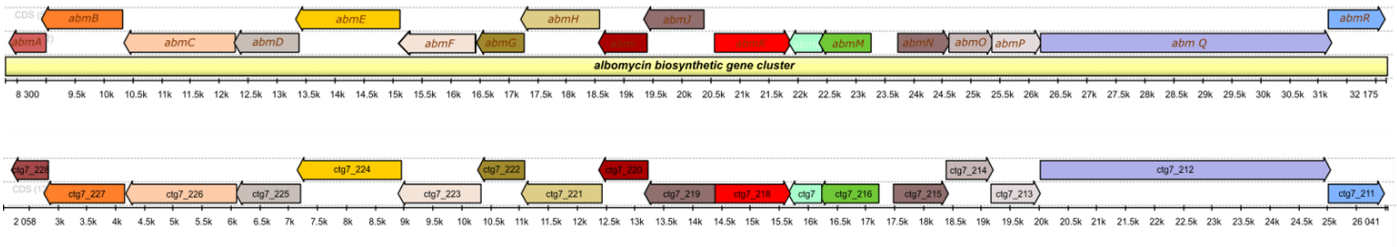

Figure 3. Biosynthetic gene clusters of albomycins: genetic organization of the albomycin (abm) gene cluster in *Streptomyces sp*. ATCC 700974 (top) and gene cluster in *Streptomyces*

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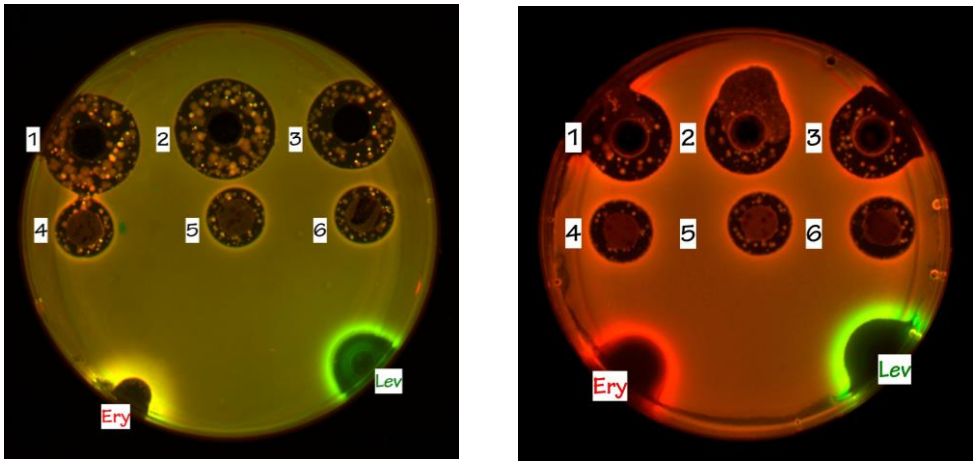
globisporus subsp. globisporus 4-3 (bottom). The homologous abm and ctg genes are filled with the same colors.

3.5. Screening antimicrobial activity

All isolated strains were initially tested for the ability to secrete antimicrobial substances. It was noticed that all phenotypically similar mycelial strains showed the same activity pattern, so we focused on the study of strain 4-3. Analysis of antimicrobial activity demonstrated, that 4-3 strain noticeably inhibited the growth of various pathogenic microorganisms (Table S5): bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and fungi (*Aspergillus niger*), but it is especially active on entomopathogenic microorganisms (*Bacillus thuringiensis*, *Paenibacillus alvei*, *Beauveria bassiana*, *Entomophthora coronata*).

The agar plugs and cultural broth aliquots of 4-3 demonstrated prominent antibiotic activity in tests on the reporter strains (Fig. 4): exhibited strong Katushka2S reporter induction, indicating that the active compound produced by the isolate functions as an inhibitor of protein biosynthesis. To further evaluate the possible mechanism of action the *in vitro* translation analysis was performed.



(**a**) (**b**)

Figure 4. In vitro testing of ant-associated strains activity using: (**a**) *E. coli* BW25113 wild-type pDualRep2 reporter strain; (**b**) *E. coli* JW5503 ΔtolC pDualRep2 reporter strain. 1, 2, 3 -- crude broth aliquots and 4, 5, 6 -- agar plugs of X2, X1 and 4-3, accordingly.

3.6. Analysis of bioactive compounds: in vitro translation

It was decided to test HPLC-purified sample 4-3 in in vitro translation procedure to completely evaluate its translation inhibitory activity. Despite the induction of Katushka2S reporter system strain 4-3 did not suppress *in vitro* translation (Fig. 5). One biological replication was carried out, the error was calculated from three independent points.

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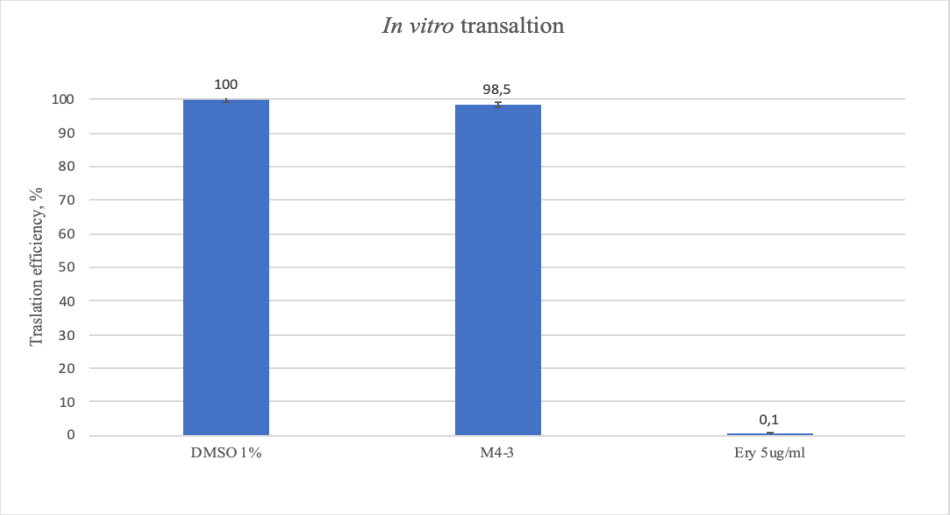

Figure 5. In vitro translation of sample 4-3: 1% DMSO, as negative control and erythromycin (Ery), as positive control.

3.7. Purification and identification of Albomycin

The culture liquid of *Streptomyces globisporus* subsp. *globisporus* 4-3 was fractionated by solid-phase extraction on LPS500H sorbent by aqueous solution of acetonitrile (MeCN) as eluent with MeCN concentration gradient, and active fractions were determined by subsequent activity validation. The next step fractionation of the active fractions eluted at 10% MeCN was carried out by RP HPLC on a C18 column with aqueous solution of ammonium acetate - MeCN as eluent. Thus, a pure compound was isolated with a UV maxima at 283 and 424 nm, and the activity testing confirmed that it corresponded to an active metabolite (Fig. S6).

The metabolite identification was carried out using LC-HRMS/MS analysis (Fig. S7). The observed exact masses 1046.3102 of molecular ion $[M+H]^+$ of the compound and characteristic isotope distribution corresponded to the composition $C_{37}H_{57}FeN_{12}O_{18}S$ (calculated exact mass 1046.3057). The main fragmentary ion in the MS2 spectrum due to the loss of cytosine part was observed at m/z 878. MS1-MS2 raw data were analyzed in Compound Discoverer 3.2 software (Thermo Fisher Scientific). Peak annotation was performed with ChemSpider, Natural Product Atlas 2020, and COCONUT databases using the mass spectra information with 5 ppm mass accuracy, isotopic distribution ≥ 50%, and match score ≥ 85%. The result of the analysis allowed us to conclude that the active compound is a known inhibitor of bacterial seryl-tRNA synthetase, albomycin δ2 (Fig. 6) [41].

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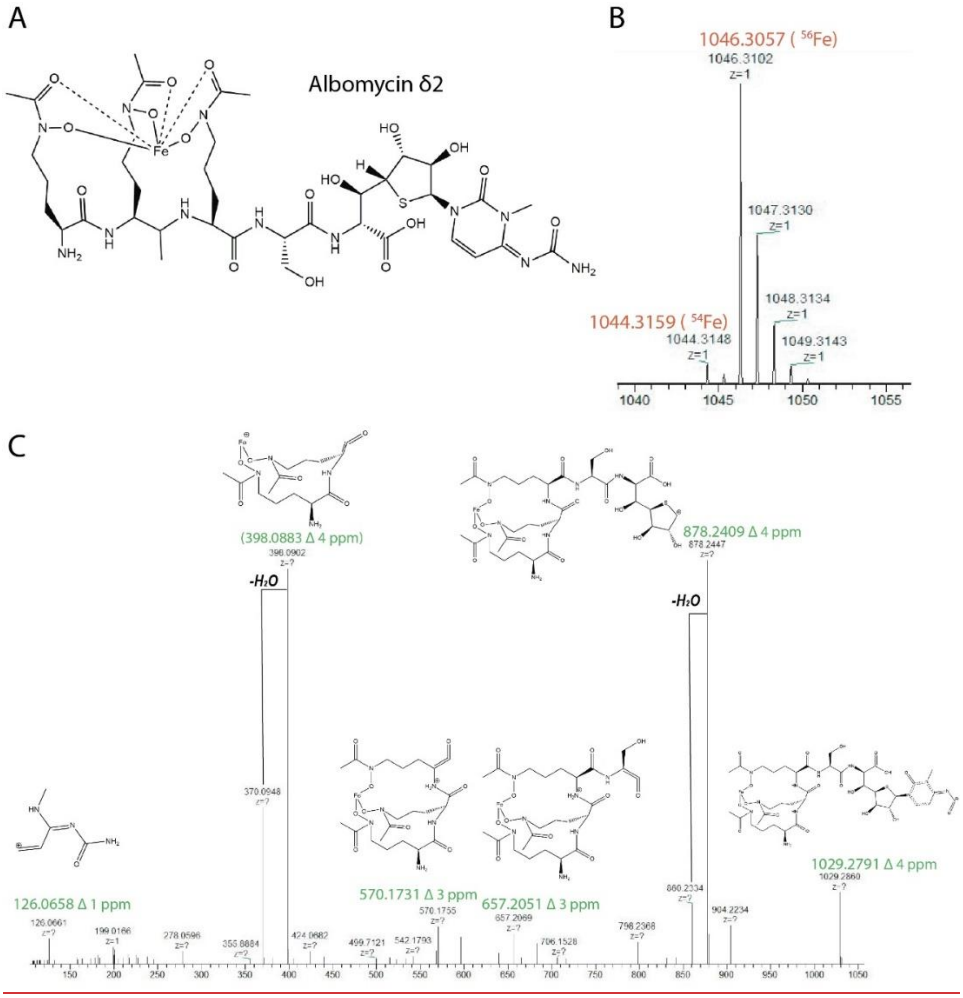

Figure 6. Identification of albomycin δ2. **A.** Molecular structure of albomycin δ2. **B.** Albomycin δ2 molecular ion [M+H]+ observed at 1046.3102 and its isotopic distribution (calculated masses are signed as red). **C.** The positive mode HCD mass spectra of the parent ion at m/z 1046.3102 with the molecular fragments structures (calculated masses for the fragments are signed as green).

4. Discussion

Albomycin δ2 belongs to the group of sideromycins, antibiotics covalently bound to siderophore fragments and penetrating into the cell through siderophore absorption pathways, implementing the so-called "Trojan horse" strategy [42].

Albomycin, originally reported as grisein, were first isolated from soil from the soil-dwelling *Streptomyces griseus* in 1947 by S. Waksman and colleagues. It was also identified in *Streptomyces subtropicus* (previously known as *Actinomyces subtropicus*) by Gause and Brazhnikova in 1951, and their identity was confirmed later [43]. It is noteworthy that albomycin was also known as alveomycin, antibiotics A 1787, LA 5352 and LA 5937, and Ro 5-2667 in the literature [44].

Albomycins have attracted significant attention due to their potent antibacterial activities against both Gram-negative and Gram-positive bacteria, including multi-drug resistant strains [45]. Moreover, no toxicity was observed during *in vivo* studies of albomycins, and it was well tolerated and safe up to a maximum dose evaluated in mice [41].

The structure of albomycin and related compounds (δ1, δ2, and ε) was fully established more than 30 years after the initial discovery [46]. Albomycins has a thioribosyl nucleoside moiety linked to an iron-chelating a ferrichrome-type siderophore

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through a serine residue. The L-serine-thioheptose dipeptide partial structure, known as SB-217452, has been found to be the active seryl-tRNA synthetase inhibitor [39].

The iron-chelator portion serves as a vehicle for active delivery of albomycin warhead inside both Gram-positive and Gram-negative bacterial cells through the ferrichrome-specific transporter system. The formation of the active inhibitory form of albomycin, SB-217452, occurs intracellularly under the action of PepN peptidase (*E. coli*), which cleaves off the siderophore part. As a result, the toxic nucleoside part is accumulated in the cytoplasm of *E. coli* in ∽500-fold excess over the concentration of antibiotic in the medium [42]. This explains the fact that albomycin does not act on translation in a cell-free extract -- in such a system there are no enzymes that cleave off the siderophore (Fig. 4). Likewise added directly to bacterial culture, the nucleoside portion of albomycin does not inhibit cell growth [42] since it cannot get inside the cells without the siderophore part.

In the crops of *Messor structor* ants contained in laboratory conditions, we constantly observed mycelial bacteria of a certain phenotype, most closely associated with individuals from the soldier caste. A representative of this phenotype, strain 4-3, was identified using a polyphase approach as *Streptomyces globisporus* subsp. *globisporus*.

The members of *Streptomyces* are widely known for their ability to produce various antibiotic compounds and often in association with insects: ants, wasps, beetles [7]. According to the literature *Streptomyces globisporus* was most often isolated from soils, plants, and so on [47], but rarely from insects. There is an example of the *Streptomyces globisporus* SP6C4, which plays a significant role in the mutualistic relationship between pineapple strawberries (*Fragaria ananassa*) and bees (Apidae), protecting both the plant and the insect from pathogenic microorganisms, including the phytopathogenic fungus *Botrytis cinerea* [48]. In addition, it is reported about *Streptomyces globisporus* WA5-2-37, isolated from intestinal tract of american cockroach (*Periplaneta americana*), produced actinomycin X2 and collismycin A which showed great activity against MRSA ATCC 43300 [49]. This is the first reported naturally occurring strain of *S. globisporus subsp. globisporus* isolated from Formicidae.

The genus *Messor* Forel, 1890 is a moderately large genus, more than 126 species in the worldwide were recognized, which are mainly distributed in the Palearctic, Afrotropical, and Oriental regions. Messor species are granivorous and play an important role in ecosystem maintenance, and plant seeds dispersal. The harvesting ant, *Messor structor* (Latreille, 1798) is ecosystem engineer in many dry biocenosis [50].

The main food resource for harvester ants are grains of cereals and oilseed plants. Such seeds have a solid endosperm, and require considerable effort to grind them. Representatives of the soldier caste have a large head — the result of the development of massive occipital muscles responsible for the work of the lower jaw, and powerful mandibles. They initially grind the seeds, and then smaller worker ants process the prepared pieces of seeds, since it requires less effort, turn them into flour, moisten with saliva and are used as food for the colony. Their saliva is dominated by amylase enzymes that break down starch [51].

The greatest abundance of actinobacteria, isolated from soldiers and workers, which represent a conveyor for the production of food for the colony, may indicate a possible symbiotic relationship between ants and streptomycetes. Actinobacteria receive food and shelter, and in return produce a substance with a wide spectrum of action that protects the food resource of ants from spoilage. The ability of associated streptomycete to synthesize Albomycin can be extremely useful for hosts -- since harvester ants contact with soils and plants and encounter a large number of microorganisms, Gram-positive and Gram-negative, as well as fungi.

As is known, Albomycin δ2 is characterized by surprisingly low inhibitory concentrations for many pathogenic microorganisms: minimum inhibitory concentrations (MICs) as low as 5 ng/mL against *Escherichia coli* and 10 ng/mL against *Streptococcus*

pneumoniae [52]. In our *in vitro* experiments *Streptomyces globisporus subsp. globisporus* 4-3 very actively suppressed the growth of various entomopathogens: *Paenibacillus alvei* VKM B-502, *Bacillus thuringiensis* VKM B-6650, *Beauveria bassiana* VKM F-1357, *Entomophthora coronata* VKM F-1359 (Table S5), which can provide one of the forms of protecting the of the ant family health.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1: Table S1: The isolation of Gram-positive bacteria strains from *Messor structor* individuals; Table S2: General features of the genome of strain 4-3 and its closely related strains of *Streptomyces globisporus*; Figure S1: Neighbour-joining phylogenetic tree of strain 4-3; Figure S2: Maximum-parsimony phylogenetic tree of strain 4-3; Figure S3: Maximum-likelihood phylogenetic tree of strain 4-3; Table S3: Morphological, physiological and biochemical characteristics of strain 4-3 and closest *Streptomyces globisporus subsp. globisporus* strains; Figure S4: Cultural properties of 4-3 on ISP media after 14 days at 28°C; Figure S5: Utilization of different sugars (1.0%, w/v) as sole carbon source by strain 4-3; Table S4: Some of the secondary metabolite gene clusters in *Streptomyces globisporus subsp. globisporus* 4-3; Table S5: Antimicrobial activity of *Streptomyces globisporus subsp. globisporus* 4-3, isolated from *Messor structor* ants.

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Data Availability Statement: Genomic data of *Streptomyces globisporus* subsp. *globisporus* 4-3 can be found at https://www.ebi.ac.uk/ena/browser/home with project accession PRJEB51905.

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