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EXPERIMENTAL ARTICLES

Antimicrobial Activity of Bacteria Isolated from the Millipedes *Nedyopus dawydoffiae* and *Orthomorpha* sp.

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Abstract—From the intestines of millipedes of the species *Nedyopus dawydoffiae* and *Orthomorpha* sp. (class *Diplopoda*) and from their food substrates (plant residues), 72 bacterial strains belonging to 25 genera were isolated and identified. Among the studied strains, actinobacteria predominated, among which streptomycetes were the most numerous, although representatives of 14 other genera of actinobacteria were also present. High abundance of actinobacteria with antimicrobial activity was noted, including members of the "rare" genera *Actinoplanes*, *Amycolatopsis*, *Kitasatospora*, *Lechevalieria*, *Micromonospora*, *Nocardiopsis*, and *Saccharopolyspora*. This is the first report on antimicrobial activity in *Kitasatospora saccharophila* INA 01226 and *Nocardiopsis umidischolae* INA 01230. Heterogeneity in terms of antibiotic formation in the populations of *Streptomyces pratensis* and *S. termitum* was shown. The most promising bacterial strains chosen for the chemical study of antibiotics formed exhibited activity against methicillin-resistant *Staphylococcus aureus* INA 00761 (MRSA) and vancomycin-resistant strain *Leuconostoc mesenteroides* VKPM B-4177 (VRLM).

Keywords: bacteria, actinobacteria, streptomycetes, "rare" genera, *Diplopoda*, *Nedyopus dawydoffiae*, *Orthomorpha* sp., endobionts, antibiotic producers, antibiotic resistance

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The introduction of antibiotics into medical practice was a revolutionary event, but the selective pressure on pathogens caused a backlash: the emergence of antibiotic-resistant forms. The World Health Organization (WHO) predicts that this situation may lead to a decline in the effectiveness of antibiotics and make infectious diseases caused by pathogens one of the leading death causes worldwide by 2050 (O'Neill, 2016). One way to solve the problem of antibiotic resistance is to find new effective antimicrobial compounds to expand the arsenal of available drugs. The principal producers of antibiotics are actinobacteria and fungi isolated from complex multicomponent biocenoses, mainly from soils (Bérdy, 2005). This is due to the fact that, in the course of evolution, competition among microorganisms has been forcing them to produce various antimicrobial substances as a means of defense. Animal gut microbiota is another multicomponent biocenosis that can be viewed as a source of antibiotic producers. At the same time, poorly studied habitats are also promising in terms of finding producers of new antibiotics (Donadio et al., 2007). Considering both circumstances, it can be expected that the intestines of invertebrate animals, including millipedes, are a promising ecological system for the isolation of antibiotic-producing bacteria (Kaltenpoth, 2009).

Bacterial communities of millipede guts participate in digestion and methane biosynthesis, as well as maintain the microbiome stability. However, not all bacteria excreted from the gut of *Diplopoda* millipedes belong to the gut microbiome; many of them are transient species consumed with food. Using scanning electron microscopy, it was shown that the intestinal wall and cuticle, as well as the rest of the peritrophic membrane, were colonized by various bacteria, including actinobacteria, for which the intestinal environment appeared favorable (Szabo et al., 1992; Polyanskaya et al., 1996). These results suggest that part of the bacteria excreted from the intestines represent the microbiome of millipedes (Byzov et al., 1993; Byzov, 2005; König and Varma, 2006).

The present study was undertaken to continue our work on antimicrobial properties of bacteria isolated from the gut of the tropical millipede *Nedyopus dawydoffiae* (Glukhova et al., 2018).

The goal of this work was to investigate the antibiotic activity of bacterial strains isolated from the gut of the tropical diplopods *Nedyopus dawydoffiae* and *Orthomorpha* sp. and their feeding substrates.

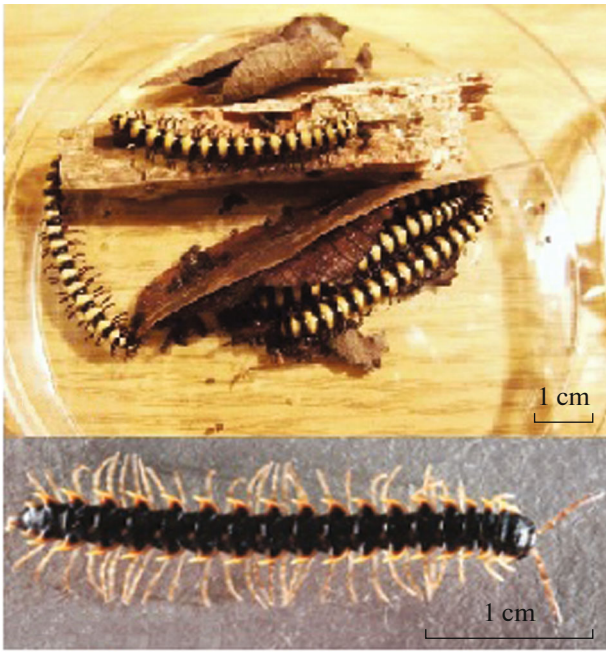


Fig. 1. Millipedes *Nedyopus dawydoffiae* (top) and *Orthomorpha* sp. (bottom).

MATERIALS AND METHODS

Objects. The study concerned bacteria isolated from the gut of millipedes *Nedyopus dawydoffiae* and *Orthomorpha* sp. (class *Diplopoda*) and their food substrates: rotten wood and leaf litter, respectively (Fig. 1). Millipedes were collected manually in the Cát Tiên National Park located in an indigenous monsoon rainforest in the south of Vietnam; they were kept in laboratory soil microcosms on natural substrates, which also served them as food. Bacteria were isolated in pure cultures by inoculating gut contents and food substrates on Gause agar medium no. 1.

Test strains. Antibiotic activity of bacterial isolates was assessed using the following test strains of gram-positive bacteria: methicillin-resistant *Staphylococcus aureus* INA 00761 (MRSA), methicillin-sensitive *Staphylococcus aureus* FDA 209P (MSSA), *Micrococcus luteus* NCTC 8340, *Bacillus subtilis* ATCC 6633, *B. pumilus* NCTC 8241, *B. mycoides* 537, and vancomycin-resistant *Leuconostoc mesenteroides* VKPM B-4177 (VRLM); gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with multiple drug resistance (MDR); fungi: *Aspergillus niger* INA 00760 and *Saccharomyces cerevisiae* RIA 259.

Nutrient media. The strains under study were cultured on the Gause media nos. 1 and 2, oat-, and soya-based agar media. The composition of the media was as follows (% in tap water):

(1) complete modified Gause medium no. 2: glucose, 1.0; peptone, 0.5; tryptone, 0.3; NaCl, 0.5; agar, 2.0; pH 7.2–7.4;

(2) Gause medium no. 1: starch, 2.0; KNO_3, 0.1; K_2HPO_4, 0.05; $MgSO_4$, 0.05; NaCl, 0.05; $FeSO_4$, 0.001; agar, 2.0; pH 7.2–7.4;

(3) oat medium: oat flour, 2.0; agar, 2.0; pH 7.2;

(4) soya medium: soybean flour, 2.0; glucose, 1.0; NaCl, 0.5; agar, 2.0; pH 7.2.

For submerged cultivation of actinobacteria, the following eight liquid growth media developed for actinobacterial antibiotic producers in the Gause Institute were used (% in tap water):

(1) STR: glucose, 1.0; peptone, 0.5, tryptone, 0.3; NaCl, 0.5; pH 7.2–7.4;

(2) A4: glucose, 1; soybean flour, 1; NaCl, 0.5; $CaCO_3$, 0.25; pH 6.8;

(3) 6613: starch, 2; maize extract, 0.3; KNO_3, 0.4; NaCl, 0.5; $CaCO_3$, 0.5; pH 7.0–7.2;

(4) sucrose: sucrose, 2; soybean flour, 1; NaCl, 0.3; $CaCO_3$, 0.3; pH 6.8–7.0;

(5) 2663: glycerol, 3; soybean flour, 1.5; NaCl, 0.3; $CaCO_3$, 0.3; pH 7.0;

(6) 330: sucrose, 2.1; starch, 0.85; pea flour, 1.5; $CaCO_3$, 0.5; NaCl, 0.5; $NaNO_3$, 0.5; pH 7.0;

(7) 5539: glycerol, 2; soybean flour, 0.5; $(NH_4)_2SO_4$, 0.15; NaCl, 0.3; $CaCO_3$, 0.3; pH 6.8;

(8) Am: sucrose, 4; K_2HPO_4, 0.1; Na_2SO_4, 0.1; NaCl, 0.1; $(NH_4)_2SO_4$, 0.2; dry yeast extract, 0.25, $FeSO_4{\cdot}7H_2O$, 0.0001; $MnCl_2{\cdot}4H_2O$, 0.0001; NaI, 0.00005; $CaCO_3$, 0.2; pH 6.5–6.7.

Cultivation conditions. Test strain cultures were grown for 24 h at 28°C for fungi and *L. mesenteroides* and at 37°C for all other bacterial strains. Fermentation of actinobacterial cultures was carried out as submerged cultivation in 750-mL Erlenmeyer flasks containing 150 mL of medium on a rotary shaker at 220 rpm and 28°C in two stages. At the first stage, to obtain material for inoculation, a 1-cm^2 fragment of oat agar with the bacteria under study was excised and transferred into STR medium; these cultures were grown for 4 days. At the second stage, 5-mL aliquots of the culture liquid were used to inoculate flasks with seven different media.

Species identification. Actinobacterial species were identified based on morphological traits and on analysis of 16S rRNA gene sequences. Morphological description included the structure of sporophores, pigmentation of aerial and substrate mycelium, and pigment secretion into the medium (Gause et al., 1983; Bergey, 1984). DNA was isolated from the biomass of 3-day-old cultures grown in liquid STR medium. Bacterial genomic DNA was isolated using a PowerSoil DNA Kit (MO BIO, United States). Fragments of 16S rRNA genes were amplified by PCR

using a PCR Master Mix kit (Thermo Scientific, United States) with the universal bacterial primers 27f (aga gtt tga tcc tgg ctcag) and 1492r (tac ggy tac ctt gtt acg act t). The reaction was conducted in an Applied Biosystems 2720 Thermal Cycler (United States) according to the following protocol: (1) 94°C, 5 min; (2) 30 cycles of 1 min at 94°C, 1 min at 51°C, and 2 min at 72°C; (3) 7 min at 72°C. The PCR products were analyzed by electrophoresis in a 1% agarose gel (using Tris-borate buffer, TBE) at electric field strength of 7.6 V/cm. The products were purified by DNA precipitation under mild conditions using 0.125 M ammonium acetate in 70% ethanol. Nucleotide sequences were determined using the Sanger's method with the universal bacterial primers 27f, 341f (cct acg gga ggc agc ag), 519r (gta tta ccg cgg ctg ctg), 785f (ggm tta gat acc tgg tag tcc), 907r (ccg tca att cct ttg agt tt), 1100r (ggg ttg cgc tcg ttg), 1114f (gca acg agc gca acc c), 1392r (acg ggc ggt gtg trc), and 1492r on an automated Applied Biosystems 3500 analyzer. Sequences were assembled using the Mega 7 software. The obtained sequences were compared to 16S rRNA gene sequences of bacterial type strains available in GenBank (blast.ncbi.nlm.nih.gov/Blast.cgi) and Ribosomal Database Project (RDP; http://rdp.cme.msu.edu/).

Assessment of antibiotic activity. To evaluate antimicrobial activity of the strains under study, 0.1-mL aliquots of the culture liquid were loaded into wells 9 mm in diameter made in Gause agar no. 2 inoculated with test microorganisms. Antibiotic activity of the culture liquid was assessed on days 4 and 7 of submerged cultivation for actinobacteria and on days 2 and 4 for bacteria of other taxonomic groups. The result was presented as the diameter (mm) of the zone where the growth of the test strain was suppressed. Statistical analysis of the data was performed with Excel 2016; antibiotic activity in the culture liquid was analyzed in five replicates.

RESULTS AND DISCUSSION

Altogether, we studied 72 bacterial strains presented in Table 1. The dominant group among the actinobacteria studied were streptomycetes. The obtained sequences of 16S rRNA genes were aligned with the sequences of type strains from the RDP database. Sequences that exhibited at least 98% identity were deposited into the GenBank database. Species identification of the isolated bacteria revealed great taxonomic diversity: the isolates represented 25 genera, and 16 of them belonged to the class *Actinobacteria*.

Actinobacteria dominate in the gut bacterial communities of millipedes and represent the second most abundant group after γ-proteobacteria (König and Varma, 2006; Knapp et al., 2010). According to the previous publications, the most abundant genus of actinobacteria in the gut of *Diplopoda* is *Streptomyces* (Byzov et al., 1993). Actinobacteria of millipede guts participate in hydrolysis of the gut content; their abundance is the greatest in the posterior section (Byzov et al., 1993; Zvyagintsev et al., 1996; Polyanskaya et al., 1996). Subsequent studies revealed a great diversity of actinobacterial genera. For instance, König and Varma (2006) isolated members of the genera *Micromonospora*, *Actinomadura*, and *Streptosporangium*, as well as nocardioide-like forms. Data obtained in our work agree with these results.

Evaluation of antibiotic activity revealed a considerable share of active strains among the isolates listed in Table 1. The spectra of their antimicrobial activity against the collection test strains are presented in Table 2.

As it follows from the data presented in the tables, all isolated streptomycetes exhibited antibiotic activity, as it could be expected from decades of the experience accumulated during the golden era of antibiotics. According to the MIBiG database (Minimum Information about a Biosynthetic Gene cluster), members of the genus *Streptomyces* have an average total genome length of 9 Mb; at the same time, this genus is a clear leader in the number of detected biosynthetic gene clusters (BGCs): to date, there are 637 of them (NCBI (Assembly), www.ncbi.nlm.nih.gov/assembly; MIBiG, http://mibig.secondarymetabolites.org). It is known that members of the genus *Streptomyces* produce 70–80% of known secondary metabolites, but after them, important producers of antibiotics are members of "rare" genera of actinobacteria (Bérdy, 2005; Ventura et al., 2007; Raja and Prabakarana, 2011). In this work, in addition to streptomycetes, we isolated members of rare actinobacterial genera: *Actinoplanes*, *Amycolatopsis*, *Kitasatospora*, *Lechevalieria*, *Micromonospora*, *Nocardiopsis*, and *Saccharopolyspora*, including some strains with antibiotic activity. Detection of antimicrobial activity in actinobacteria of rare genera, which are considerably less studied than streptomycetes, increases the probability of finding new antibiotics (Fig. 2).

Since antibiotics are secondary metabolites, their biosynthesis is a trait that can exhibit intraspecies variation. For instance, Table 2 includes two strains of *S. pratensis* that differed in their antimicrobial activity against three strains of bacilli and the fungus *Aspergillus niger*: strain INA 01179 was active against *Bacillus subtilis* ATCC 6633, *B. pumilus* NCTC 8241, and *B. mycoides* 537, but not against the fungus; in contrast, strain INA 01182 was not active against these bacilli but exhibited antimycotic activity. Another example is two isolated strains of *S. termitum*: strain INA 01244 was active against the gram-positive bacterium *Leuconostoc mesenteroides* VKPM B-4177 and the gram-negative bacterium *E. coli* ATCC 25922, whereas strain INA 01245 did not possess such activity; in other tests, both strains exhibited identical activity (which indicates that members of this species synthesize more than one antibiotic). In a previous

Table 1. Taxonomic affiliation of the isolated bacterial strains based on the analysis of 16S rRNA gene sequences

Genus, species, strain	Fragment length, bp	Identity, %	GenBank
Bacteria isolated from the gut of *Nedyopus dawydoffiae*			
Actinobacteria			
Streptomyces griseoplanus INA 01177	1485	99.2	MH635265
S. hydrogenans INA 01173	1116	100	ON231534
S. hydrogenans INA 01175	1387	100	ON231535
S. pratensis INA 01179	1436	99.4	MH635263
S. pratensis INA 01182	1424	98.4	MH635266
S. setonii INA 01178	1473	98.7	ON231536
S. setonii INA 01181	1493	100	MH635267
S. spororaveus INA 01183	1451	100	MH635268
S. turgidiscabies INA 01184	1309	96.5	–
Streptomyces sp. INA 01174	551	100	ON231537
Streptomyces sp. INA 01176	569	100	ON231538
Streptomyces sp. INA 01180	568	99.3	MH635264
Streptomyces sp. INA 01185	631	100	ON231539
Bacteria isolated from the food substrate of *Nedyopus dawydoffiae* (rotting wood)			
Actinobacteria			
Kitasatospora saccharophila INA 01226	1338	96.6	–
Nocardiopsis umidischolae INA 01230	1371	100	ON231540
S. gelaticus INA 01231	1379	94.2	–
S. gelaticus INA 01233	1361	94.1	–
S. hydrogenans INA 01235	1015	100	ON231541
S. olivochromogenes INA 01229	1376	96.0	–
S. parvulus INA 01300	1373	100	ON231544
S. scopuliridis INA 01227	1003	95.7	–
S. scopuliridis INA 01232	1320	96.2	–
S. seoulensis INA 01234	985	100	ON231542
S. seoulensis INA 01228	1026	100	ON231543
Members of other taxonomic groups			
Stenotrophomonas rhizophila INA 01299	1409	97.7	–
Bacteria isolated from the gut of *Orthomorpha* sp.			
Actinobacteria			
Arthrobacter sp. INA 01380	1257	98.3	ON231545
Micrococcus aloeverae INA 01381	1260	98.8	ON231546
Micromonospora aurantiaca INA 01238	1381	98.0	ON231547
Micromonospora tulbaghiae INA 01239	1350	98.4	ON231548
Mycobacterium hodleri INA 01382	1269	98.3	ON231549
Plantactinospora mayteni INA 01241	1412	95.2	–
Saccharopolyspora dendranthemae INA 01240	1373	98.3	ON231550
S. coelicoflavus INA 01237	1372	100	ON231551
S. prunicolor INA 01236	1391	99.3	ON231552

Table 1. (Contd.)

Genus, species, strain	Fragment length, bp	Identity, %	GenBank
Members of other taxonomic groups			
Afipia birgiae INA 01383	1234	99.5	ON231553
Afipia birgiae INA 01384	1237	100	ON231554
Blastomonas sp. INA 01385	1217	99.4	ON231555
Peribacillus simplex INA 01386	1452	98.5	ON231556
Sphingopyxis panaciterrae INA 01387	1232	96.7	–
Bacteria isolated from the food substrate of *Orthomorpha* sp. (leaf litter)			
Actinobacteria			
Actinoplanes capillaceus INA 01251	1406	97.1	–
Agrococcus citreus INA 01388	1367	98.5	ON231572
Amycolatopsis bullii INA 01250	1368	99.2	ON231557
Arthrobacter pascens INA 01389	714	98.1	ON231573
Arthrobacter pascens INA 01390	641	100	ON231574
Herbiconiux flava INA 01391	1183	96.6	–
Lechevalieria fradiae INA 01246	1349	95.6	–
Microbacterium oxydans INA 01392	1372	97.8	–
Micrococcus aloeverae INA 01393	1279	98.7	ON231575
Mycobacterium chubuense INA 01394	779	92.5	–
Nocardiopsis sp. INA 01296	656	93.4	–
S. albolongus INA 01247	1370	99.0	ON231558
S. atratus INA 01297	1292	98.7	ON231559
S. cinereoruber INA 01242	1360	98.0	ON231560
S. lavendulae INA 01243	1359	98.5	ON231561
S. microflavus INA 01294	1383	100	ON231562
S. omiyaensis INA 01252	1388	99.1	ON231563
S. seoulensis INA 01291	1374	100	ON231564
S. seoulensis INA 01292	1415	99.5	ON231565
S. seoulensis INA 01295	1385	99.5	ON231566
S. termitum INA 01244	1375	99.7	ON231567
S. termitum INA 01245	1374	99.7	ON231568
S. zaomyceticus INA 01248	1373	98.9	ON231569
Streptomyces sp. INA 01249	1432	99.5	ON231570
Streptomyces sp. INA 01293	1387	100	ON231571
Members of other taxonomic groups			
Acinetobacter venetianus INA 01395	610	98.8	ON231576
Bacillus mycoides INA 01396	1301	99.4	ON231577
Bacillus toyonensis INA 01397	1409	100	ON231578
Brucella pseudogrignonensis INA 01398	1342	99.0	ON231579
Methylobacterium variabile INA 01399	1244	100	ON231580
Pseudomonas taiwanensis INA 01400	1400	98.5	ON231581
Stenotrophomonas bentonitica INA 01401	1411	96.6	–
Stenotrophomonas rhizophila INA 01298	1410	98.1	ON231582

The results of actinomycete species identification based on the analysis of 16S rRNA gene sequences are consistent with the morphological traits of the strains.

Table 2. Range of antimicrobial activity of the isolated bacterial strains

Strain	Optimal medium (days)*	Test cultures										
		Staphylococcus aureus INA 00761 (MRSA)	*St. aureus* FDA 209P	*Bacillus subtilis* ATCC 6633	*B. mycoides* 537	*B. pumilus* NCTC 8241	*Leuconostoc mesenteroides* VKPM B-4177 (VRLM)	*Micrococcus luteus* NCTC 8340	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853 (MDR)	*Saccharomyces cerevisiae* RIA 259	*Aspergillus niger* INA 00760
Bacteria isolated from the gut of *Nedyopus dawydoffiae*												
Actinobacteria												
Streptomyces griseoplanus INA 01177	Sucrose (7)	–	–	–	–	–	+++	+++	–	–	–	+++
S. hydrogenans INA 01173	Am (4), 5339 (7)	++++	++++	++++	++	++++	+++	++	–	–	++++	++++
S. hydrogenans INA 01175	Am (4)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++++
S. pratensis INA 01179	Sucrose (7), 2663 (7)	–	++	++	++	++	+++	+++	–	–	–	–
S. pratensis INA 01182	Sucrose (4)	–	++	–	–	–	++++	++++	–	–	–	++
S. setonii INA 01178	Sucrose (7)	–	–	–	–	–	+++	+++	–	–	–	++
S. setonii INA 01181	Am (4), Sucrose (4)	–	–	++	–	–	++++	++++	–	–	–	–
S. spororaveus INA 01183	Am (4), 5339 (7)	+++	+++	+++	++++	++++	++++	+++	–	–	++++	++++
S. turgidiscabies INA 01184	Sucrose (4)	–	–	–	–	–	++++	–	–	–	–	–
Streptomyces sp. INA 01174	5539 (4)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++++
Streptomyces sp. INA 01176	Sucrose (7)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++++
Streptomyces sp. INA 01180	330 (7)	++	+	–	–	++	++++	++++	–	–	++	++
Streptomyces sp. INA 01185	330 (4), Am (7)	–	–	–	–	++++	++++	+++	–	–	–	–
Bacteria isolated from the food substrate of *Nedyopus dawydoffiae* (rotten wood)												
Actinobacteria												
Kitasatospora saccharophila INA 01226	A4 (4), 330 (4)	–	–	–	–	–	++++	+++	–	–	–	+++
Nocardiopsis umidischolae INA 01230	Am (7), Sucrose (7)	++	++	++	++	+++	+++	++++	–	–	++	–
S. gelaticus INA 01231	A4 (7)	++++	+++	+++	++++	++++	++++	++++	–	–	++++	++++
S. gelaticus INA 01233	6613 (7)	++	++	+++	+++	+	+++	++++	–	–	+++	++++
S. hydrogenans INA 01235	Am (4)	++++	++++	++++	++++	++++	+++	++++	–	–	++++	++++
S. olivochromogenes INA 01229	330 (4), Am (7)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++++
S. parvulus INA 01300	330 (4, 7)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++

Table 2. (Contd.)

Strain	Optimal medium (days)*	Test cultures										
		Staphylococcus aureus INA 00761 (MRSA)	*St. aureus* FDA 209P	*Bacillus subtilis* ATCC 6633	*B. mycoides* 537	*B. pumilus* NCTC 8241	*Leuconostoc mesenteroides* VKPM B-4177 (VRLM)	*Micrococcus luteus* NCTC 8340	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853 (MDR)	*Saccharomyces cerevisiae* RIA 259	*Aspergillus niger* INA 00760
S. scopuliridis INA 01227	330 (4), A4 (7)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	++++
S. scopuliridis INA 01232	Am (4), 330 (4, 7)	++	–	+++	+++	+	+++	++++	–	–	+++	++++
S. seoulensis INA 01228	5339(4), Am (7)	++++	++++	++++	++++	++++	++++	++++	++++	–	++++	++
S. seoulensis INA 01234	330 (7), 2663 (7)	++++	++++	++++	++++	++++	+++	++++	++++	–	+++	+++
Members of other taxonomic groups												
Stenotrophomonas rhizophila INA 01299	A4 (4), Am (7)	–	–	–	++	–	+++	+++	–	–	–	+
Bacteria isolated from the gut of *Orthomorpha* sp.												
Actinobacteria												
Micromonospora aurantiaca INA 01238	Am (4)	+++	+++	+++	+++	+++	+++	++++	–	–	++++	–
Micromonospora tulbaghiae INA 01239	Am (4)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	–
Saccharopolyspora dendranthemae INA 01240	Am (4), Sucrose (4)	–	–	–	–	–	++++	–	–	–	++++	+
S. coelicoflavus INA 01237	330 (4, 7)	–	–	++++	++++	+++	++++	+++	–	–	–	+
S. prunicolor INA 01236	330 (4, 7)	+++	+++	++++	++++	+++	+++	++++	+++	–	–	–
Members of other taxonomic groups												
Peribacillus simplex INA 01386	STR (4)	+++	–	–	–	–	–	–	–	–	–	–
Bacteria isolated from the food substrate of *Orthomorpha* sp. (leaf litter)												
Actinobacteria												
Actinoplanes capillaceus INA 01251	A4 (4), Am (7)	–	–	–	–	–	+++	–	–	–	–	++++
Amycolatopsis bullii INA 01250	Am (7)	++++	++++	++++	++++	++++	++++	++++	–	–	++++	–
Arthrobacter pascens INA 01389	STR (4)	–	–	–	–	–	–	(++)	–	–	–	–
Lechevalieria fradiae INA 01246	5339 (4), 330 (7)	–	–	+++	+++	–	–	++	–	–	–	++++
Nocardiopsis sp. INA 01296	A4 (4)	–	–	–	–	–	–	–	–	–	–	+

Table 2. (Contd.)

Strain	Optimal medium (days)*	Test cultures										
		Staphylococcus aureus INA 00761 (MRSA)	*St. aureus* FDA 209P	*Bacillus subtilis* ATCC 6633	*B. mycoides* 537	*B. pumilus* NCTC 8241	*Leuconostoc mesenteroides* VKPM B-4177 (VRLM)	*Micrococcus luteus* NCTC 8340	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853 (MDR)	*Saccharomyces cerevisiae* RIA 259	*Aspergillus niger* INA 00760
S. albolongus INA 01247	330 (7)	++	++	++++	++++	+++	++	+++	++	–	–	+++
S. atratus INA 01297	330 (7)	–	–	–	–	–	–	–	–	–	–	–
S. cinereoruber INA 01242	330 (4)	–	+++	++++	++++	++++	+++	++++	++++	++++	+++	–
S. lavendulae INA 01243	330 (4, 7)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
S. microflavus INA 01294	5339 (7)	–	–	–	–	–	–	–	–	–	–	++
S. omiyaensis INA 01252	330 (4)	++	+++	++	–	++	+	+++	–	–	–	–
S. seoulensis INA 01291	6613 (7)	++++	++++	++++	++++	++++	–	++++	–	–	–	+
S. seoulensis INA 01292	330 (7)	++++	++++	++++	++++	++++	+++	++++	–	–	–	+
S. seoulensis INA 01295	330 (4), 5339 (7)	++++	++++	++++	+++	++++	–	++++	–	–	–	+
S. termitum INA 01244	330 (7)	++	++++	++++	++++	++++	++	+++	++	–	–	++
S. termitum INA 01245	6613 (4), 5339 (7)	++++	++++	++++	++++	++++	–	+++	–	–	–	++
S. zaomyceticus INA 01248	Am (7)	++	++	++++	++++	++++	–	++++	–	–	–	–
Streptomyces sp. INA 01249	330 (4)	–	++	++	++	++	–	+++	–	–	++++	–
Streptomyces sp. INA 01293	6613 (7)	–	–	–	++	++	–	–	–	–	–	++
Members of other taxonomic groups												
Acinetobacter venetianus INA 01395	STR (4)	–	–	–	–	–	–	(++)	–	–	–	–
Bacillus toyonensis INA 01397	STR (4)	+++	+++	–	–	–	–	++	–	–	–	–
Stenotrophomonas rhizophila INA 01298	2663 (4)	–	++++	–	++	–	–	++++	–	–	–	–

* A medium was considered optimal if the strain exhibited the highest antibiotic activity when growing on this medium. Diameter of growth suppression zones: –, no suppression; +, ≤10 mm; ++, 11–15 mm; +++, 16–20 mm; ++++, >20 mm. Zones of incomplete growth suppression are indicated in parentheses.

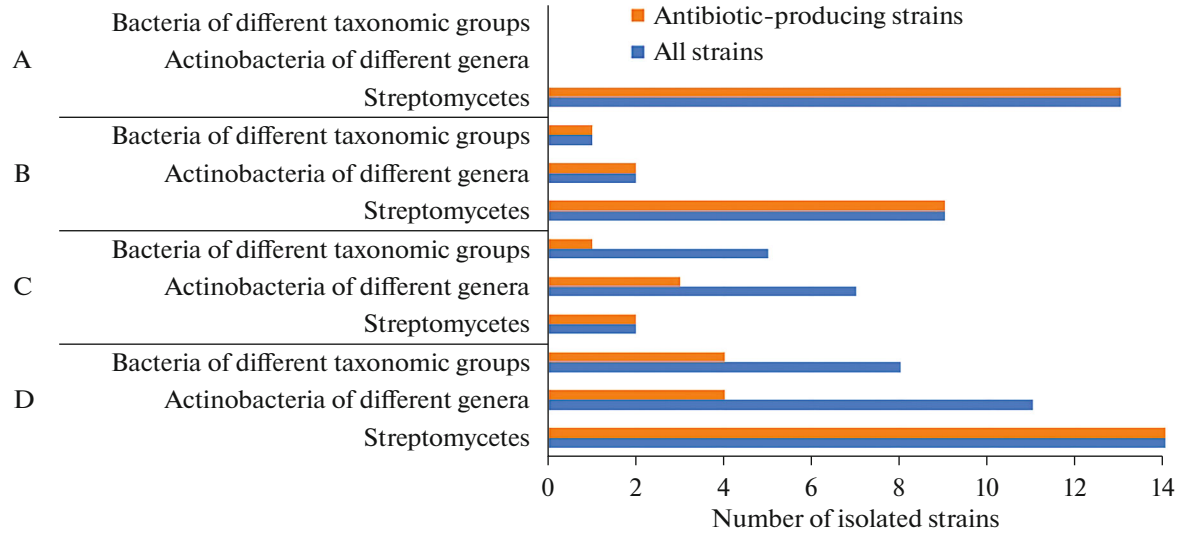


Fig. 2. Distribution of the strains studied by bacterial groups: A, gut of *Nedyopus dawydoffiae*; B, food substrate of *Nedyopus dawydoffiae* (rotten wood); C, gut of *Orthomorpha* sp.; D, food substrate of *Orthomorpha* sp. (leaf litter).

study, we found that *S. antibioticus* strains isolated from a nest of the black garden ant *Lasius niger* were heterogeneous in respect of production of actinomycin antibiotics (Efimenko et al., 2020). Heterogeneity by this trait is determined by the presence of competition in the biocenosis on the one hand and by available nutrient sources on the other hand. In the present work, we observed that two isolates of *S. termitum* obtained from the same population (leaf litter) and grown as submerged cultures in two media exhibited differences both in the level of antibiotic synthesis and in the spectra of antimicrobial activity (Fig. 3).

When assessing the antimicrobial spectra of the identified producers, we paid special attention to the test strains *Staphylococcus aureus* INA 00761 (MRSA), *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, which are included in the WHO list of species of antibiotic resistance concern (O'Neill, 2016; Tacconelli, 2017; Efimenko, 2019). In this work, among the isolates representing rare genera of actinobacteria, there were strains producing antibiotics active against the above test strains, and their antimicrobial activity had not been described previously. For instance, *Kitasatospora saccharophila* was described as a new species in 2009 (Li et al., 2009). However, available publications do not mention any antibiotic production by this species. In our study, the strain *Kitasatospora saccharophila* INA 01226 produced antimicrobial compounds active against *Leuconostoc mesenteroides* VKPM B-4177 (VRLM), *Micrococcus luteus* NCTC 8340, and *Aspergillus niger* INA 00760. The species *Nocardiopsis umidischolae* was first isolated in 2001 from dust in a water-damaged school building, and the type strain *Nocardiopsis umidischolae* 66/93 (=DSM 44362 = NRRL B-24122) was described (Peltola et al., 2001). Available publications report no data on antibiotic production in this species. However, *Nocardiopsis umidischolae* strain INA 01230 isolated in our work from rotten wood, food substrate of *Nedyopus dawydoffiae*, exhibited activity against all test strains of gram-positive bacteria used, including methicillin-resistant *Staphylococcus aureus* INA 00761 (MRSA), and against the yeast *Saccharomyces cerevisiae* RIA 259. That is, the strains *Kitasatospora saccharophila* INA 01226 and *Nocardiopsis umidischolae* INA 01230 have for the first time been described to possess antimicrobial activity, in particular, against resistant forms of pathogenic microorganisms.

The genus *Micromonospora* is the second richest source of isolation of natural antibiotic producers after *Streptomyces* (Wagman and Weinstein, 1980). First reports on antibacterial activity of *Micromonospora* members appeared in 1942, and gentamicin, an aminoglycoside isolated from *Micromonospora purpurea* (the species was reclassified as *Micromonospora echinospora* Luedemann, Brodsky, 1964), was described in 1963, which subsequently inspired broad-scale screening of this rare genus actinobacteria for the presence of antibiotics (Wagman and Weinstein, 1980). In 2019, three novel isoflavonoid glycosides: daidzein-4'-(2-deoxy-α-l-fucopyranoside), daidzein-7-(2-deoxy-α-l-fucopyranoside), and daidzein-4',7-di-(2-deoxy-α-l-fucopyranoside), were isolated and identified from the fermentation broth of *Micromonospora aurantiaca* 110B. It was shown that all three compounds possessed cytotoxic activity, but did not exhibit activity against *Candida albicans*, MRSA, and *E. coli* (Wang et al., 2019). In 2020, the complete genome of *M. aurantiaca* sp. 01 was obtained; based on the 16S rRNA gene sequence, it had 99.28% identity with the type strain *M. aurantiaca* ATCC 27029^T. Genome sequence analysis showed that strain *M. aurantiaca* sp.

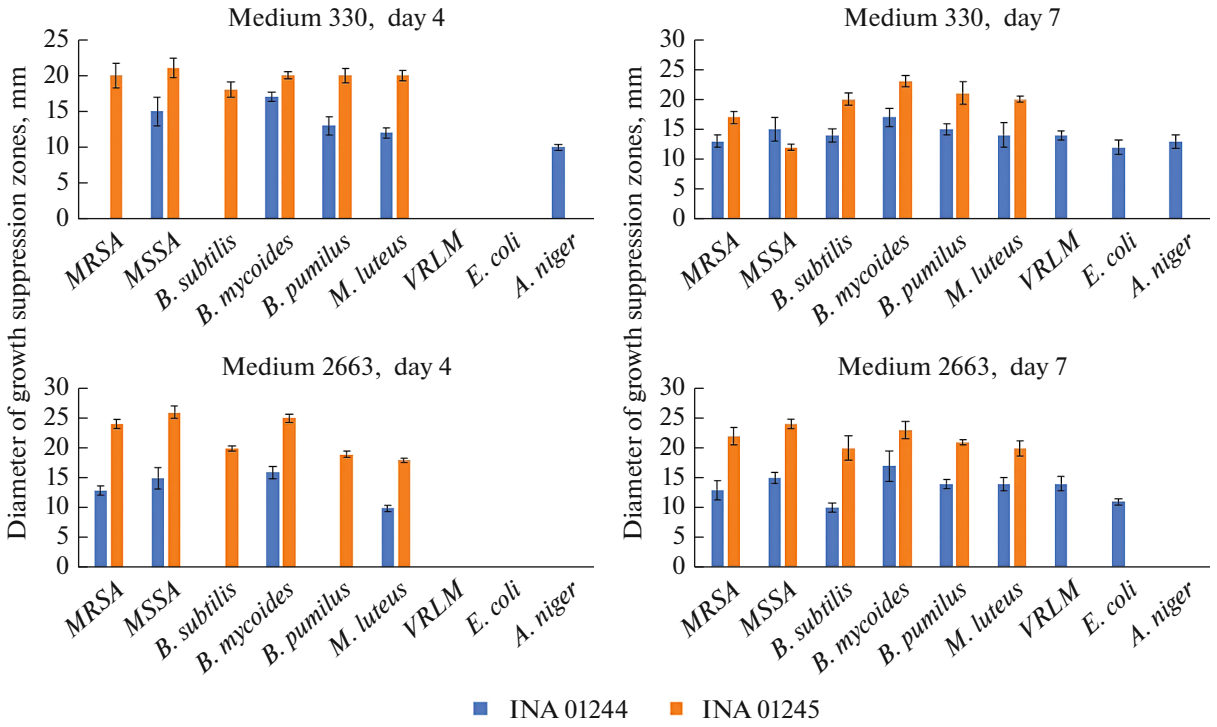


Fig. 3. Spectra of antibiotic activity of *S. termitum* strains grown on media 330 and 2663 after 4 and 7 days of cultivation.

01 possessed 77 clusters of genes involved in biosynthesis of secondary metabolites. It was predicted that they participate in production of polyketides, nonribosomal peptides, bacteriocins, and many other compounds. Among them, 42 genes clusters were annotated as associated with 37 known secondary metabolites, including antibiotics. Among antibiotics predicted based on genome analysis, targeted MS/MS analysis detected kanamycin, which was first obtained from a strain of *M. aurantiaca*. Furthermore, a study of organization of biosynthetic gene clusters revealed 21 clusters of antibiotic determinants, which is more than in other species of *Micromonospora* (Hu et al., 2020). In our study, the strain *Micromonospora aurantiaca* INA 01238 isolated from the gut of *Orthomorpha* sp. exhibited high levels of activity against all gram-positive test bacteria, including MRSA, as well as against *Saccharomyces cerevisiae* RIA 259. That is, antibiotic activity observed in this strain was different from the activity of antibiotics isolated previously from the same species, and we are planning further investigation of its the chemical nature.

The species *Micromonospora tulbaghiae* was first described in 2010; the type strain TVU1^T (=DSM 45142^T = NRRL B-24576^T) was isolated from leaves of wild garlic (*Tulbaghia violacea*). This strain was shown to exhibit moderate activity against *Mycobacterium aurum* A+ and no activity against *Enterococcus faecium* (VanA), *E. coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923 (Kirby and Meyers, 2010). In our study, the strain *Micromonospora tulbaghiae* INA 01239 was found to be active against all gram-positive bacteria tested, including methicillin-resistant *Staphylococcus aureus* INA 00761 (MRSA), as well as against the yeast strain *Saccharomyces cerevisiae* RIA 259, which was different from the activity described previously.

In 2000, Fukami et al. described an antibiotic isolated from *Actinoplanes capillaceus* and active against *B. subtilis*, *Sac. cerevisiae*, and *E. coli* (Fukami et al., 2000). The strain *Ac. capillaceus* INA 01251 isolated in our work had a different spectrum of antimicrobial activity and was active against *Leuconostoc mesenteroides* VKPM B-4177 (VRLM) and *Aspergillus niger* INA 00760. Accordingly, the compound produced by strain INA 01251 can be considered promising and is worth further chemical investigation.

Previously, no information was available on antibiotic activity of two species belonging to the rare genera of actinobacteria, *Amycolatopsis bullii* and *Saccharopolyspora dendranthemae*, described in the recent decade (Zucchi et al., 2012; Zhang et al., 2013). In our work, we found that *Amycolatopsis bullii* INA 01250 was highly active against all gram-positive bacteria tested and against *S. cerevisiae* RIA 259, whereas *Saccharopolyspora dendranthemae* INA 01240 was active against *Leuconostoc mesenteroides* VKPM B-4177, which is naturally resistant to glycopeptide antibiotics of the vancomycin group, as well as against *S. cerevisiae* RIA 259.

Among prokaryotes of other taxonomic groups, the best-known producers are members of the class *Bacilli* (Bérdy, 2005). In our study, the most promising potential producers were *Peribacillus simplex* INA 01386 and *Bacillus toyonensis* INA 01397, which were active against MRSA. While *Bacilli* are a well-known source of antibiotic producers, descriptions of new antibiotic compounds of this origin keep appearing (Wang et al., 2020a, 2020b). Two strains of *Stenotrophomonas rhizophila* (INA 01299 and INA 01298) were isolated from leaf litter and rotten wood. These strains differed in their antibiotic activity: strain INA 01298 was active against MSSA, and strain INA 01299 was active against VRLM and mildly active against *Aspergillus niger* INA 00760. Previously, we showed that *Stenotrophomonas rhizophila* INA 01137 (endobiont of the mushroom-forming fungus *Coprinellus micaceus*) was active against *St. aureus* INA 0076 and *E. coli* ATCC 25922, as well as against *Aspergillus niger* INA 00760 (Efimenko et al., 2016). Previous publications indicate that *Stenotrophomonas rhizophila* can produce compounds active against a number of phytopathogenic fungi and the human pathogen *Candida albicans* (Wolf et al., 2002).

Bacteria isolated from the guts of millipedes and from their food substrates exhibited great diversity, both in terms of species affiliation and in spectra of antibiotic activity. The isolated strains were dominated by actinobacteria, which represented not only the genus *Streptomyces*, but also 14 other genera: *Actinoplanes*, *Agrococcus*, *Amycolatopsis*, *Arthrobacter*, *Herbiconiux*, *Kitasatospora*, *Lechevalieria*, *Microbacterium*, *Micrococcus*, *Micromonospora*, *Mycobacterium*, *Nocardiopsis*, *Plantactinospora*, and *Saccharopolyspora*. Among them, there were species that had not been previously described as antibiotic producers or that exhibited a different range of antimicrobial activity. For instance, it is for the first time that we report antimicrobial activity, in particular, against antibiotic-resistant forms of pathogenic microorganisms, for *Amycolatopsis bullii*, *Kitasatospora saccharophila*, *Nocardiopsis umidischolae*, and *Saccharopolyspora dendranthemae*. Two species of the genus *Micromonospora* (*Micromonospora aurantiaca* and *M. tulbaghiae*) were for the first time shown to exhibit activity against the methicillin-resistant strain *Staphylococcus aureus* INA 00761 (MRSA), and *Actinoplanes capillaceus* was found to be active against the vancomycin-resistant *Leuconostoc mesenteroides* VKPM B-4177 (VRLM). Activity against the resistant test strains *St. aureus* INA 00761 (MRSA) and *L. mesenteroides* VKPM B-4177 (VRLM) makes these isolates promising candidates for isolation of antibiotics that can overcome drug resistance.

The number of strains with antimicrobial activity isolated from millipede guts was as great as the number of active strains isolated from decaying plant debris and apparently was not affected by the gut environments. Presumably, this is related to the climate conditions in this habitat (Deshcherevskaya et al., 2013; Chernov et al., 2019). Previous studies showed that, judging by the numbers of ribosomal gene copies, tropical soil samples from largely intact monsoon forests of the Cát Tiên National Park are characterized by high levels of microbial abundance comparable to those of the richest soils of temperate latitudes, e.g., chernozems (Semenov et al., 2018; Chernov et al., 2019). The abundance of microorganisms promotes their intense competition in which biosynthesis of antibiotics represents an important evolutionary adaptation (Gause, 1934).

The great diversity of actinobacterial species isolated from the guts of tropical diplopods and their food substrates, as well as the observed high levels of their antibiotic activity, justify the need for further investigation of bacteria associated with this natural ecosystem. The isolated and selected actinobacteria will subsequently be subject to chemical studies as potential producers of new antibiotics.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interests.

Statement on the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

Bérdy, J., Bioactive microbial metabolites, *J. Antibiot.*, 2005, vol. 58, pp. 1–26.

Bergey, D.H., *Bergey’s Manual of Systematic Bacteriology*, Holt, J., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and William, S.T., Eds., Baltimore: Williams & Wilkins, 1984.

Byzov, B.A., Zenova, G.M., Babkina, N.I., Dobrovol’skaya, T.G., Tret’yakova, E.B., and Zvyagintsev, D.G., Actinomycetes in the food, gut, and feces of soil multipedes *Pahyiulis flavipes* C.L. Koch., *Mikrobiology* (Moscow), 1993, vol. 62, pp. 916–927.

Byzov, B.A., *Zoomikrobnye vzaimodeistviya v pochve* (Zoomicrobial Interactions in Soil), Moscow: GEOS, 2005.

Chernov, T.I., Zhelezova, A.D., Tkhakakhova, A.K., Bgazhba, N.A., and Zverev, A.O., Microbiomes of virgin soils of Southern Vietnam tropical forests, *Microbiology* (Moscow), 2019, vol. 88, pp. 489–498.

Deshcherevskaya, O.A., Avilov, V.K., Dinh, B.D., Tran, C.H., Kurbatova, J.A., Modern climate of the Cát Tiên National Park (Southern Vietnam): climatological data for ecological studies, *Izv. Atmos. Ocean. Phys.*, 2013, vol. 49, no. 8, pp. 819–838.

Donadio, S., Brandi, L., Monciardini, P., Sosio, M., and Gualerzi, C.O., Novel assays and novel strains – promising routes to new antibiotics?, *Expert Opin. Drug Discov.*, 2007, vol. 2, pp. 789–798.

Efimenko, T.A., Malanicheva, I.A., Vasil'eva, B.F., Glukhova, A.A., Sumarukova, I.G., Boikova, Y.V., Malkina, N.D., Terekhova, L.P., and Efremenkova, O.V., Antibiotic activity of bacterial endobionts of basidiomycete fruit bodies, *Microbiology* (Moscow), 2016, vol. 85, pp. 752–758.

Efimenko, T.A., Terekhova, L.P., and Efremenkova, O.V., Current state the problem of antibiotic resistance of pathogens, *Antibiot. Chemother.*, 2019, vol. 64, nos. 5–6, pp. 64–68.

Efimenko, T.A., Glukhova, A.A., Demiankova, M.V., Boykova, Y.V., Malkina, N.D., Sumarukova, I.G., Vasilieva, B.F., Rogozhin, E.A., Ivanov, I.A., Krassilnikov, V.A., and Efremenkova, O.V., Antimicrobial activity of microorganisms isolated from ant nests of *Lasius niger, Life*, 2020, vol. 10, p. 91.

Fukami, A., Nakamura, T., Kawaguchi, K., Rho, M.C., Matsumoto, A., Takahashi, Y., Shiomi, K., Hayashi, M., Komiyama, K., and Omura, S., A new antimicrobial antibiotic from *Actinoplanes capillaceus* sp. K95-5561T, *J. Antibiot.* (Tokyo), 2000, vol. 53, pp. 1212–1214.

Gause, G.F., *The Struggle for Existence*, Baltimore: Williams & Wilkins, 1934.

Gause, G.F., Preobrazhenskaya, T.P., Sveshnikova, M.L., Terekhova, L.P., and Maksimova, T.S., *Opredelitel' aktinomycetov* (Manual on Identification of Actinomycetes). Moscow: Nauka, 1983.

Glukhova, A.A., Karabanova, A.A., Yakushev, A.V., Semenyuk, I.I., Boykova, Yu.V., Malkina, N.D., Efimenko, T.A., Ivankova, T.D., Terekhova, L.P., and Efremenkova, O.V., Antibiotic activity of Actinobacteria from the digestive tract of millipede *Nedyopus dawydoffiae* (Diplopoda), *Antibiotics* (Basel), 2018, vol. 7, no. 4, p. 94.

Hu, D., Sun, C., Jin, T., Fan, G., Mok, K.M., Li, K., and Lee, S.M., Exploring the potential of antibiotic production from rare *Actinobacteria* by whole-genome sequencing and guided MS/MS analysis, *Front. Microbiol.*, 2020, vol. 11, p. 1540.

O'Neill, J., The Review on Antimicrobial Resistance. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, 2016. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.

Kaltenpoth, M., *Actinobacteria* as mutualists: general healthcare for insects?, *Trends Microbiol.*, 2009, vol. 17, pp. 529–535.

Kirby, B.M. and Meyers, P.R., *Micromonospora tulbaghiae* sp. nov., isolated from the leaves of wild garlic, *Tulbaghia violacea, Int. J. Syst. Evol. Microbiol.*, 2010, vol. 60, pp. 1328–1333.

Knapp, B.A., Seeber, J., Rief, A., Meyer, E., and Insam, H., Bacterial community composition of the gut microbiota of *Cylindroiulus fulviceps* (*Diplopoda*) as revealed by molecular fingerprinting and cloning, *Folia Microbiol.*, 2010, vol. 55, pp. 489–496.

Intestinal Microorganisms of Termites and Other Invertebrates, König, H. and Varma, A., Eds., Berlin Heidelberg: Springer-Verlag, 2006.

Li, B., Furihata, K., Kudo, T., and Yokota, A., *Kitasatospora saccharophila* sp. nov. and *Kitasatospora kazusanensis* sp. nov., isolated from soil and transfer of *Streptomyces atroaurantiacus* to the genus *Kitasatospora* as *Kitasatospora atroaurantiaca* comb. nov., *J. Gen. Appl. Microbiol.*, 2009, vol. 55, pp. 19–26.

Peltola, J.S., Andersson, M.A., Kämpfer, P., Auling, G., Kroppenstedt, R.M., Busse, H.J., Salkinoja-Salonen, M.S., and Rainey, F.A., Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov., *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 4293–4304.

Polyanskaya, L.M., Babkina, N.I., Zenova, G.M., and Zvyagintsev, D.G., Fate of actinomycetes in the intestinal tract of soil invertebrates fed on streptomycete spores, *Microbiology* (Moscow), 1996, vol. 65, pp. 493–498.

Raja, A. and Prabakarana, P., Actinomycetes and drug—an overview, *Am. J. Drug Discov. Dev.*, 2001, vol. 1, pp. 75–84.

Semenov, M.V., Chernov, T.I., Tkhakakhova, A.K., Zhelezova, A.D., Ivanova, E.A., Kolganova, T.V., and Kutovaya, O.V., Distribution of prokaryotic communities throughout the Chernozem profiles under different land uses for over a century, *Appl. Soil Ecol.*, 2018, vol. 127, pp. 8–18.

Szabo, I.M., Nasser, El-G. A., Striganova, B., Rakhmo, Y.R., Jager, K., and Heydrich, M., Interactions among Millipedes (*Diplopoda*) and their Intestinal Bacteria, *Berichte des Naturwissenschaftlich-medizinischen Vereins in Innsbruck*, 1990, vol. S10, pp. 289–296.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., et al., WHO Pathogens Priority List Working Group, Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis, *Lancet Infect. Dis.*, 2018, vol. 18, pp. 318–327.

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F., and van Sinderen, D., Genomics of *Actinobacteria*: tracing the evolutionary history of an ancient phylum, *Microbiol. Mol. Biol. Rev.*, 2007, vol. 71, pp. 495–548.

Wagman, G.H. and Weinstein, M.J., Antibiotic from *Micromonospora, Annu. Rev. Microbiol.*, 1980, vol. 34, pp. 537–557.

Wang, R.J., Zhang, S.Y., Ye, Y.H., Yu, Z., Qi, H., Zhang, H., Xue, Z.L., Wang, J.D., and Wu, M., Three new isoflavonoid glycosides from the mangrove-derived actinomycete *Micromonospora aurantiaca* 110B, *Mar. Drugs*, 2019, vol. 17, p. 294.

Wang, W., Park, K.H., Lee, J., Oh, E., Park, C., Kang, E., Lee, J., and Kang, H., A new thiopeptide antibiotic, micrococcin p3, from a marine-derived strain of the bacterium *Bacillus stratosphericus, Molecules* (Basel), 2020a, vol. 25, p. 4383.

Wang, T., Lu, Q., Sun, C., Lukianov, D., Osterman, I.A., Sergiev, P.V., Dontsova, O.A., Hu, X., You, X., Liu, S., and Wu, G., Hetiamacin E and F, new amicoumacin antibiotics from *Bacillus subtilis* PJS using MS/MS-based molecular networking, *Molecules*, 2020b, vol. 25, p. 4446.

Wolf, A., Fritze, A., Hagemann, M., and Berg, G., *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties, *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 1937–1944.

Zhang, Y.J., Zhang, W.D., Qin, S., Bian, G.K., Xing, K., Li, Y.F., Cao, C.L., and Jiang, J.H., *Saccharopolyspora dendranthemae* sp. nov., a halotolerant endophytic actinomycete isolated from a coastal salt marsh plant in Jiangsu, China, *Antonie van Leeuwenhoek*, 2013, vol. 103, pp. 1369–1376.

Zucchi, T.D., Bonda, A.N., Frank, S., Kim, B.Y., Kshetrimayum, J.D., and Goodfellow, M., *Amycolatopsis bartoniae* sp. nov. and *Amycolatopsis bullii* sp. nov., mesophilic actinomycetes isolated from arid Australian soils, *Antonie van Leeuwenhoek*, 2012, vol. 102, pp. 91–98.

Zvyagintsev, D.G., Polyanskaya, L.M., Zenova, G.M., and Babkina, N.I., Dynamics of the length of actinomycete mycelium and prokaryotic cell number in the invertebrate intestinal tract, *Microbiology* (Moscow), 1996, vol. 65, pp. 238–245.

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