

Bacteria associated with plant tissues infected by plant-parasitic nematodes from families Anguinidae Nicoll, 1935 and Aphelenchoididae Skarbilovich, 1947

Irina P. Starodumova¹, Lubov V. Dorofeeva¹, Vladimir N. Chizhov², Steven A. Nadler³, Sergei A. Subbotin^{2,3,4} and Lyudmila I. Evtushenko¹

¹All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino Scientific Centre for Biological Research, Russian Academy of Sciences, Pushchino, Russia

²A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

³Department of Entomology and Nematology, University of California, Davis, California, USA

⁴California Department of Food and Agriculture, Sacramento, California, USA

e-mail: iri-starodumova@yandex.ru

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Summary. A total of 38 bacterial strains were isolated from 23 samples of plants infected by 17 species of nematodes from the genera *Anguina*, *Heteroanguina*, *Mesoanguina*, *Ditylenchus* and *Aphelenchoides*. The 16S rRNA gene-based identification revealed that the strains belonged to 11 genera of the classes *Alphaproteobacteria* (*Sphingomonas*, *Caulobacter*, *Rhizobium*), *Bacilli* (*Bacillus*, *Paenibacillus*, *Lactococcus*, *Staphylococcus*) and *Actinobacteria* (*Rathayibacter*, *Brachybacterium*, *Mycolicibacterium*, *Plantibacter*). The isolated strains showed high 16S rRNA gene sequence identity (99.1-100%) to 19 type strains of validly described species, which indicates that they match known species or are closely related novel (not yet validly described) species. Among the species comprising the aforementioned bacterial genera, only several species of *Rathayibacter* and *Sphingomonas* are plant pathogens. Some of the known species of *Staphylococcus* and the other revealed genera that include human pathogens, were reported to show plant growth-promoting properties.

Key words: 16S rRNA gene, *Actinobacteria*, *Alphaproteobacteria*, *Anguina*, *Aphelenchoides*, *Bacilli*, *Ditylenchus*, *Heteroanguina*, *Mesoanguina*, plant pathogen.

It has been shown by several studies that parasitic nematodes inducing swellings and galls in plants are often associated with specific bacteria, and that such bacteria-nematode associations are widespread (Evtushenko *et al.*, 1994; Evtushenko & Takeuchi, 2006). Some seed gall and foliar nematodes act as specific vectors of bacterial plant pathogens and an obligate aetiological relationship is established between the two, resulting in manifestation of a new disease syndrome in host plants (Taylor, 1990). Plant pathogenic species of the genus *Rathayibacter* and other genera of the family *Microbacteriaceae* (*Actinobacteria*) or putative new species of the same family with unproven pathogenicity are often found in plants infected by plant-parasitic nematodes of the genera *Anguina*, *Mesoanguina* and *Aphelenchoides* (Evtushenko & Takeuchi, 2006; Murray *et al.*, 2017;

Starodumova *et al.*, 2017; Tarlachkov *et al.*, 2020). The goal of this study was to screen for presence of species of *Rathayibacter* and other bacterial genera in herbarium plant materials and in materials freshly collected in areas naturally infested by plant-parasitic nematodes of the genera *Anguina*, *Heteroanguina*, *Mesoanguina*, *Ditylenchus* and *Aphelenchoides*.

MATERIAL AND METHODS

Nematode sample collection. Plant nematode galls and other plant samples infected with nematodes were obtained from colleagues or collected from natural areas. Samples were kept in dry condition in paper envelopes. In total, twenty-three herbarium accessions were used for isolation of bacteria (Table 1).

Table 1. Identification of bacteria isolated from plants infected by nematodes of the families Anguinidae and Aphelenchoididae on the basis of 16S rRNA gene sequences.

Sample number	Nematode	Host plant name	Host plant family	Plant organ	Country	Bacterium strain number	GenBank accession number	Closest type strain	16S rRNA gene sequence identity (%) and size (bp)	Class
Family Anguinidae										
34	<i>Afrina sporoboliae</i>	<i>Sporobolus cryptandrus</i>	Poaceae	Seed gall	USA, Idaho	CA-741	MT853077	<i>Rathayibacter agropyri</i> CA-4 ^T	99.1% (1418)	Actinobacteria
39	<i>Afrina wevelli</i>	<i>Eragrostis curvula</i>	Poaceae	Seed gall	South Africa	CA-833	MT853111	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (1431)	Alphaproteobacteria
60	<i>Anguina agropyri</i>	<i>Elytrigia repens</i>	Poaceae	Stem gall	Russia	CA-764	MT853096	<i>Sphingomonas zeae</i> JM-791 ^T	100% (1367)	Alphaproteobacteria
60	<i>A. agropyri</i>	<i>Elytrigia repens</i>	Poaceae	Stem gall	Russia	CA-765	MT853097	<i>Caulobacter vibrioides</i> CBS1 ^T	99.49% (1407)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-762	MT853094	<i>Sphingomonas zeae</i> JM-791 ^T	99.9% (1341)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-763	MT853095	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (1442)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-775	MT853081	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (618)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-776	MT853082	<i>Sphingomonas zeae</i> JM-791 ^T	99.7% (1341)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-779	MT853090	<i>Sphingomonas melonis</i> DAPP-PG 224 ^T	99.49% (1206)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-817	MT853078	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (649)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-818	MT853080	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (656)	Alphaproteobacteria
38	<i>A. agrostis</i>	<i>Agrostis</i> sp.	Poaceae	Seed gall	Brazil	CA-840	MT853079	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (1383)	Alphaproteobacteria
33	<i>A. finesta</i>	<i>Lolium rigidum</i>	Poaceae	Seed gall	Australia	CA-778	MT853089	<i>Staphylococcus pasteurii</i> ATCC 51129 ^T	99.9% (1437)	Bacilli
31	<i>Anguina</i> sp.	<i>Astrebla pectinata</i>	Poaceae	Seed gall	Australia	CA-766	MT853098	<i>Sphingomonas zeae</i> JM-791 ^T	100% (1250)	Alphaproteobacteria
31	<i>Anguina</i> sp.	<i>A. pectinata</i>	Poaceae	Seed gall	Australia	CA-774	MT853106	<i>Rhizobium metallidurans</i> ChimEc512 ^T	99.9% (1408)	Alphaproteobacteria
58	<i>Anguina</i> sp.	<i>Dactylis glomerata</i>	Poaceae	Seed gall	USA, Oregon	CA-770	MT853102	<i>Sphingomonas zeae</i> JM-791 ^T	100% (1367)	Alphaproteobacteria
58	<i>Anguina</i> sp.	<i>D. glomerata</i>	Poaceae	Seed gall	USA, Oregon	CA-835	MT853112	<i>Caulobacter vibrioides</i> CBS1 ^T	99.5% (1439)	Alphaproteobacteria
63	<i>Anguina</i> sp.	<i>Acrocladium cuspidatum</i>	Amblystegiaceae	Leaf gall	Estonia	CA-771	MT853103	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> NCIB 3610 ^T	99.9% (1420)	Bacilli

Table 1 (continued). Identification of bacteria isolated from plants infected by nematodes of the families Anguinidae and Aphelenchoiidae on the basis of 16S rRNA gene sequences.

57	<i>Heteroanguina graminophila</i>	<i>Calamagrostis</i> sp.	Poaceae	Leaf gall	Russia, Moscow	CA-768	MT853100	<i>Bacillus megaterium</i> NBRC 15308 ^T	100% (1408)	<i>Bacilli</i>
57	<i>H. graminophila</i>	<i>Calamagrostis</i> sp.	Poaceae	Leaf gall	Russia, Moscow	CA-769	MT853101	<i>Staphylococcus warneri</i> ATCC 27836 ^T	100% (1447)	<i>Bacilli</i>
43	<i>H. graminophila</i>	<i>Calamagrostis</i> sp.	Poaceae	Leaf gall	Russia, Far East	CA-828	MT853110	<i>Mycobacterium aurum</i> NCTC 10437 ^T	100% (1475)	<i>Actinobacteria</i>
32	<i>Mesoanguina chilensis</i>	<i>Nothofagus pumilio</i>	Nothofagaceae	Leaf gall	Chile	CA-791	MT853085	<i>Lactococcus lactis</i> subsp. <i>lactis</i> JCM 5805 ^T	100% (1434)	<i>Bacilli</i>
37	<i>M. mobilis</i>	<i>Arctioheca calendula</i>	Asteraceae	Leaf gall	Australia	CA-780	MT853088	<i>Sphingomonas zeae</i> JM-791 ^T	100% (1367)	<i>Alphaproteobacteria</i>
61	<i>M. moxae</i>	<i>Artemisia rubripes</i>	Asteraceae	Leaf gall	Russia	CA-767	MT853099	<i>Caulobacter vibrioides</i> CB51 ^T	99.5% (1439)	<i>Alphaproteobacteria</i>
35	<i>M. picridis</i>	<i>Cousinia onopordoides</i>	Asteraceae	Leaf gall	Turkmenistan	CA-788	MT853083	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (511)	<i>Alphaproteobacteria</i>
35	<i>M. picridis</i>	<i>C. onopordoides</i>	Asteraceae	Leaf gall	Turkmenistan	CA-789	MT853084	<i>Sphingomonas leidyi</i> ATCC 15260 ^T	100% (1442)	<i>Alphaproteobacteria</i>
35	<i>M. picridis</i>	<i>C. onopordoides</i>	Asteraceae	Leaf gall	Turkmenistan	CA-841	MT853091	<i>Caulobacter vibrioides</i> CB51 ^T	99.5% (1316)	<i>Alphaproteobacteria</i>
53	<i>M. picridis</i>	<i>Serratula latifolia</i>	Asteraceae	Leaf gall	Iran	CA-837	MT853114	<i>Plantibacter flavus</i> VKM Ac-2504 ^T	100% (1420)	<i>Actinobacteria</i>
14	<i>Ditylenchus gigas</i>	<i>Vicia faba</i>	Fabaceae	Stem gall	Germany	CA-772	MT853104	<i>Bacillus aryabhatai</i> B8W22 ^T	100% (1438)	<i>Bacilli</i>
14	<i>D. gigas</i>	<i>V. faba</i>	Fabaceae	Stem gall	Germany	CA-773	MT853105	<i>Bacillus megaterium</i> NBRC 15308 ^T	100% (1440)	<i>Bacilli</i>
13	<i>D. dipsaci</i>	<i>V. faba</i>	Fabaceae	Seed	UK	CA-758	MT853093	<i>Paenibacillus tundrae</i> A106 ^T	99.5% (1487)	<i>Bacilli</i>
12	<i>D. dipsaci</i>	<i>Phlox</i> sp.	Polemoniaceae	Stem gall	Canada	CA-792	MT853086	<i>Bacillus megaterium</i> NBRC 15308 ^T	100% (1423)	<i>Bacilli</i>
12	<i>D. dipsaci</i>	<i>Phlox</i> sp.	Polemoniaceae	Stem gall	Canada	CA-793	MT853087	<i>Bacillus megaterium</i> NBRC 15308 ^T	100% (868)	<i>Bacilli</i>
Family Aphelenchoiidae										
20	<i>Aphelenchoides fragariae</i>	<i>Boykinia aconitifolia</i>	Saxifragaceae	Leaf	Germany	CA-825	MT853108	<i>Bacillus megaterium</i> NBRC 15308 ^T	100% (1450)	<i>Bacilli</i>
23	<i>A. ritzemobosi</i>	<i>Doronium orientale</i>	Asteraceae	Leaf	Germany	CA-781	MT853092	<i>Staphylococcus epidermidis</i> NCTC 11047 ^T	100% (1491)	<i>Bacilli</i>
16	<i>Aphelenchoides</i> sp.	Fern	No data	Leaf	USA	CA-839	MT853107	<i>Brachy bacterium rhamnosum</i> LMG 19848 ^T	99.8% (1474)	<i>Actinobacteria</i>
71	<i>Aphelenchoides</i> sp.	<i>Tellima grandiflora</i>	Saxifragaceae	Leaf	USA	CA-827	MT853109	<i>Staphylococcus argenteus</i> MSHR1132 ^T	99.9% (1378)	<i>Bacilli</i>
42	<i>Aphelenchoides</i> sp.	<i>Tanacetum</i> sp.	Asteraceae	Leaf	Russia	CA-836	MT853113	<i>Rathayibacter rathayi</i> VKM Ac-1601 ^T	99.8% (1299)	<i>Actinobacteria</i>

Isolation of bacteria. The air-dried plant samples infected by nematodes were soaked in sterile distilled water for 1 h, macerated, and washed twice in sterile distilled water. The washed samples were placed in 1 ml of 0.85% NaCl solution and milled. One drop (50 µl) of the obtained suspension was plated on R2A medium (Fluka Analytical, USA) and incubated for up to 3 weeks at room temperature (18-24°C). Bacteria from representative colonies were isolated after 1, 2 and 3 weeks and cultured on R2A medium.

DNA extraction. DNA was extracted from pure bacterial cultures grown on R2A agar for 2-3 days. One colony was put into 100 µl ddH₂O using a loop, vortexed, and incubated at 95°C for 15 min. After incubation, the tubes kept at -20°C until use.

PCR amplification, sequencing and molecular identification. For the 16S rRNA gene sequence study, 2 µl of extracted DNA was transferred into a 0.2 ml Eppendorf tube containing 2.5 µl 10× PCR buffer, 5 µl Q solution (Qiagen), 0.5 µl of dNTPs mixture (*Taq* PCR Core Kit, Qiagen), 0.15 µl of each primer (1.0 µg µl⁻¹), 0.1 µl *Taq* polymerase, and 12.6 µl distilled water. The PCR amplification profile consisted of 4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min 30 s at 55°C, and 2 min at 72°C, followed by a final step of 10 min at 72°C. The 16S rRNA gene was amplified with the universal eubacterial 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1525R (5'-AAG GAG GTG ATC CAG CC-3') primers and sequenced using universal 785F (5'-GGM TTA GAT ACC TGG TAG TCC-3') and 907R (5'-CCG TCA ATT CCT TTG AGT TT-3') primers (Weisburg *et al.*, 1991). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and directly sequenced. Sequencing was performed by Quintara Biosciences (CA, USA). The closest relatives and pairwise similarity of the 16S rRNA gene sequences was determined using EzBioCloud (Yoon *et al.*, 2017). All new sequences obtained in this study were deposited in the GenBank database (Table 1).

RESULTS AND DISCUSSION

A total of 38 bacterial strains were isolated from 23 samples of plants infected by 17 nematode species belonging to the families Anguinidae and Aphelenchoididae (Table 1). The 16S rRNA gene-based identification using the EzBioCloud database (Yoon *et al.*, 2017) revealed that the strains belonged to 11 genera of the classes *Alphaproteobacteria* (20 strains), *Bacilli* (13 strains), and *Actinobacteria* (5 strains). Most of the isolates were affiliated with the genus *Sphingomonas* (15 strains), followed by *Bacillus* (7 strains), *Staphylococcus* (4 strains),

Caulobacter (4 strains) and *Rathayibacter* (2 strains). Six genera were represented by a single isolate: *Brachybacterium*, *Mycolicibacterium*, *Plantibacter*, *Rhizobium*, *Paenibacillus* and *Lactococcus*. Members of the aforementioned 11 genera showed highest 16S rRNA gene sequence identity (99.1-100%) to 19 type strains of validly described species (Table 1), which indicates that they match known species or are closely related novel (not yet validly described) species.

Strains of the genus *Sphingomonas* (*Alphaproteobacteria*) were most frequently isolated from galls induced by *Anguina* spp. and *Afrina* sp. on plants of the family Poaceae (*Agrostis* sp., *Astrebla pectinata*, *Elytrigia repens*, *Eragrostis curvula*, *Dactylis glomerata*) and from galls of *Mesoanguina* spp. on Asteraceae (*Arctotheca calendula*, *Artemisia rubripes* and *Cousinia onopordioides*). Four *Caulobacter* strains, also belonging to *Alphaproteobacteria* were found exclusively in plants of Poaceae and Asteraceae infested by *Anguina* spp. and *Mesoanguina* spp. By contrast, the endospore-forming bacteria of the genera *Bacillus* and *Paenibacillus* (*Bacilli*) tended to be associated mostly with plants infested by nematodes of the genera *Ditylenchus* and *Aphelenchoides* (6 out of 8 strains isolated) (Table 1).

These bacterial genera and many species comprising these genera are mostly known as biotechnologically and agriculturally important taxa (*Sphingomonas* spp., *Bacillus* spp., *Paenibacillus* spp.) or documented as human pathogens (*Staphylococcus* spp.) or commensals (*Lactococcus* spp., *Staphylococcus* spp.) (Yabuuchi & Kosako, 2015; Pérez-García *et al.*, 2011; Grady *et al.*, 2016; Olishevskaya *et al.*, 2019). Several species of *Rathayibacter* and *Sphingomonas* are known as plant pathogens (Buonaurio *et al.*, 2002; Evtushenko & Dorofeeva, 2012; Deldavleh *et al.*, 2013; Kini *et al.*, 2017; Murray *et al.*, 2020). Some *Caulobacter* strains were reported to inhibit plant growth (Berrios & Ely, 2020).

Members of the aforementioned genera, including *Staphylococcus*, were also reported within plant tissues without causing any evident damage to the host, and showed plant growth-promoting properties. They play crucial roles in plant growth, development, fitness and protection using different mechanisms. Different plant growth-promoting properties of such endophytic bacteria have been described in many studies (Ulrich *et al.*, 2008; Evtushenko & Dorofeeva, 2012; Phukon *et al.*, 2013; De Meyer *et al.*, 2015; Zhao *et al.*, 2015; de Lacerda *et al.*, 2016; Midha *et al.*, 2016; Thomas & Sekhar, 2017; Gao *et al.*, 2018; Marag & Suman, 2018; Tuo *et al.*, 2018;

Berrios & Ely, 2020). Plant endophytic bacteria ubiquitously colonise a variety of internal plant tissues (intracellular or intercellular spaces) and are found in nearly every plant worldwide (Hallmann *et al.*, 1997; Santoyo *et al.*, 2016). Most species to which our isolates can be assigned (or are closely related to) occur, along with their usual habitat, in tissues of various plants, and some exhibited direct or indirect plant growth promoting properties as exemplified by *Bacillus megaterium*, *B. subtilis*, *Lactococcus lactis*, *Plantibacter flavus*, *Staphylococcus epidermidis*, *S. pasteurii*, *Caulobacter vibrioides*, and some others (Innerebner *et al.*, 2011; Wang *et al.*, 2013; de Lacerda *et al.*, 2016; Zhao *et al.*, 2015; Alibrandi *et al.*, 2018; Marag & Suman, 2018; Gupta *et al.*, 2019, Mayer *et al.*, 2019; Ullah *et al.*, 2019).

Three isolated strains showed closest 16S rRNA gene sequence identity to the plant pathogens in the genera *Rathayibacter* and *Sphingomonas* (Buonauro *et al.*, 2002; Evtushenko & Dorofeeva, 2012). These include *Rathayibacter* sp. CA-836 (99.8% identity to *Rathayibacter rathayi*), *Rathayibacter* sp. CA-741 (99.1% identity to *Rathayibacter agropyri*), and *Sphingomonas* sp. CA-779 (99.5% identity to *Sphingomonas melonis*). *Rathayibacter rathayi* is known to be nematode-associated and transmitted to host plants (wheat) by a seed gall nematode of the genus *Anguina* (Evtushenko & Dorofeeva, 2012). No precise data are available on the association of the two other plant pathogenic species, *Rathayibacter agropyri* (Murray *et al.*, 2020) and *Sphingomonas melonis* (Buonauro *et al.*, 2002) with nematodes.

It is also worth noting that the bacteria identified here were recovered from plant samples that were stored at room temperatures for a long time, up to 67 years (seed galls on *Elytrigia repens*). The bacteria being in a hypobiotic state in dry plant samples are probably protected by the surrounding masses of dead bacterial and plant cells and some chemicals produced by bacteria and plants that assist bacteria to withstand drought stress (Lata *et al.*, 2018; Ullah *et al.*, 2019).

Further study of these and other bacteria from the nematode-infested plant tissues, including whole-genome sequencing, will reveal the nematode-associated bacterial diversity in plant microbiomes and specific bacterial-nematode plant pathogenic complexes. Such research also has the potential to provide insight into molecular mechanisms involved in interactions of bacteria, nematodes and plants.

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REFERENCES

- ANDRÁSSY ALIBRANDI, P., CARDINALE, M., RAHMAN, M.M., STRATI, F., CINÁ, P., DE VIANA, M.L., GIAMMINOLA, E.M., GALLO, G., SCHNELL, S. & DE FILIPPO, C. 2018. The seed endosphere of *Anadenanthera colubrina* is inhabited by a complex microbiota, including *Methylobacterium* spp. and *Staphylococcus* spp. with potential plant-growth promoting activities. *Plant and Soil* 422: 81-99. DOI: 10.1007/s11104-017-3182-4
- BERRIOS, L. & ELY, B. 2020. Plant growth enhancement is not a conserved feature in the *Caulobacter* genus. *Plant and Soil* 1: 15. DOI: 10.1007/s11104-020-04472-w
- BUONAURIO, R., STRAVATO, V.M., KOSAKO, Y., FUJIWARA, N., NAKA, T., KOBAYASHI, K., CURGONIO CAPPELLI, C. & YABUUCHI, E. 2002. *Sphingomonas melonis* sp. nov., a novel pathogen that causes brown spots on yellow Spanish melon fruits. *International Journal of Systematic and Evolutionary Microbiology* 52: 2081-2087. DOI: 10.1099/00207713-52-6-2081
- DELDAVLEH, M., BAHMANI, K. & HARIGHI, B. 2013. Bacterial leaf blight of Christ’s thorn in Iran: a new disease caused by *Sphingomonas* sp. *Journal of Plant Pathology* 95: 75-78. DOI: 10.4454/JPP.V95I1.018
- DE LACERDA, J.R.M., DA SILVA, T.F., VOLLÚ, R.E., MARQUES, J.M. & SELDIN, L. 2016. Generally recognized as safe (GRAS) *Lactococcus lactis* strains associated with *Lippia sidoides* Cham. are able to solubilize/mineralize phosphate. *SpringerPlus* 5: 1-7. DOI: 10.1186/s40064-016-2596-4
- DE MEYER, S.E., DE BEUF, K., VEKEMAN, B. & WILLEMS, A. 2015. A large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium). *Soil Biology and Biochemistry* 83: 1-11. DOI: 10.1016/j.soilbio.2015.01.002
- EVTUSHENKO, L.I. & DOROFEEVA, L.V. 2012. Genus XXII. *Rathayibacter* Zgurskaya, Evtushenko, Akimov and Kalakoutskii, 1993, 147^{VP}. In: *Bergey’s Manual of Systematic Bacteriology, Volume 5* (W.B. Whitman, M. Goodfellow, P. Kämpfer, H.-J. Busse, M.E. Trujillo, W. Ludwig, K.-I. Suzuki & A. Parte Eds). pp. 953-964. New York, USA, Springer-Verlag GmbH. DOI: 10.1007/978-0-387-68233-4

- EVTUSHENKO, L.I. & TAKEUCHI, M. 2006. The family *Microbacteriaceae*. In: *The Prokaryotes: a Handbook on the Biology of Bacteria. Volume 3: Archaea. Bacteria: Firmicutes, Actinomycetes* (M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt Eds). pp. 1020-1098. New York, USA, Springer-Verlag GmbH.
- EVTUSHENKO, L.I., DOROFEEVA, L.V., DOBROVOLSKAYA, T.G. & SUBBOTIN, S.A. 1994. Coryneform bacteria from plant galls induced by nematodes of the subfamily Anguininae. *Russian Journal of Nematology* 2: 99-104.
- GAO, J.L., SUN, P., SUN, X.H., TONG, S., YAN, H., HAN, M.L., MAO X.G. & SUN, J.G. 2018. *Caulobacter zae* sp. nov. and *Caulobacter radidis* sp. nov., novel endophytic bacteria isolated from maize root (*Zea mays* L.). *Systematic and Applied Microbiology* 41: 604-610. DOI: 10.1016/j.syapm.2018.08.010
- GRADY, E.N., MACDONALD, J., LIU, L., RICHMAN, A. & YUAN, Z.C. 2016. Current knowledge and perspectives of *Paenibacillus*: a review. *Microbial Cell Factories* 15: 203. DOI: 10.1186/s12934-016-0603-7
- GUPTA, A., VERMA, H., SINGH, P.P., SINGH, P., SINGH, M., MISHRA, V. & KUMAR, A. 2019. Rhizome endophytes: roles and applications in sustainable agriculture. In: *Seed Endophytes: Biology and Biotechnology* (S.K. Verma & J.F. White Eds). pp. 405-421. Cham, Switzerland, Springer. DOI: 10.1007/978-3-030-10504-4
- HALLMANN, J., QUADT-HALLMANN, A., MAHAFFEE, W.F. & KLOPPER, J.W. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43: 895-917.
- INNEREBNER, G., KNIEF, C. & VORHOLT, J.A. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied and Environmental Microbiology* 77: 3202-3210. DOI: 10.1128/AEM.00133-11
- KINI, K., AGNIMONHAN, R., DOSSA, R., SOGLONOU, B., GBOGBO, V., OUEDRAOGO, I., KPÉMOUA, K., TRAORÉ, M. & SILUE, D. 2017. First report of *Sphingomonas* sp. causing bacterial leaf blight of rice in Benin, Burkina Faso, The Gambia, Ivory Coast, Mali, Nigeria, Tanzania and Togo. *New Disease Reports* 35: 32-32. DOI: 10.5197/j.2044-0588.2017.035.032
- LATA, R., CHOWDHURY, S., GOND, S.K. & WHITE JR., J.F. 2018. Induction of abiotic stress tolerance in plants by endophytic microbes. *Letters in Applied Microbiology* 66: 268-276. DOI: 10.1111/lam.12855
- MARAG, P.S. & SUMAN, A. 2018. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). *Microbiological Research* 214: 101-113. DOI: 10.1016/j.micres.2018.05.016
- MAYER, E., DE QUADROS, P.D. & FULTHORPE, R. 2019. *Plantibacter flavus*, *Curtobacterium herbarum*, *Paenibacillus taichungensis*, and *Rhizobium selenitireducens* endophytes provide host-specific growth promotion of *Arabidopsis thaliana*, basil, lettuce, and bok choy plants. *Applied and Environmental Microbiology* 85: e00383-19. DOI: 10.1128/AEM.00383-19
- MIDHA, S., BANSAL, K., SHARMA, S., KUMAR, N., PATIL, P.P., CHAUDHRY, V. & PATIL, P.B. 2016. Genomic resource of rice seed associated bacteria. *Frontiers in Microbiology* 6: 1551. DOI: 10.3389/fmicb.2015.01551
- MURRAY, T.D., SCHROEDER, B.K., SCHNEIDER, W.L., LUSTER, D.G., SECHLER, A., ROGERS, E.E. & SUBBOTIN, S.A. 2017. *Rathayibacter toxicus*, other *Rathayibacter* species inducing bacterial head blight of grasses, and the potential for livestock poisonings. *Phytopathology* 107: 804-815. DOI: 10.1094/PDIS-06-19-1233-PDN
- MURRAY, T.D., BARRANTES-INFANTE, B. & SCHROEDER, B.K. 2020. First report of bacterial head blight of *Pseudoroegneria spicata* subsp. *spicata* caused by *Rathayibacter agropyri* in Idaho. *Plant Disease* 104: 1534. DOI: 10.1094/PDIS-06-19-1233-PDN
- OLISHEVSKA, S., NICKZAD, A. & DÉZIEL, E. 2019. *Bacillus* and *Paenibacillus* secreted polyketides and peptides involved in controlling human and plant pathogens. *Applied Microbiology and Biotechnology* 103: 1189-1215. DOI: 10.1007/s00253-018-9541-0
- PÉREZ-GARCÍA, A., ROMERO, D. & DE VICENTE, A. 2011. Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in agriculture. *Current Opinion in Biotechnology* 22: 187-193. DOI: 10.1016/j.copbio.2010.12.003
- PHUKON, M., SAHU, P., SRINATH, R., NITHYA, A. & BABU, S. 2013. Unusual occurrence of *Staphylococcus warneri* as endophyte in fresh fruits along with usual *Bacillus* spp. *Journal of Food Safety* 33: 102-106. DOI: 10.1111/jfs.12028
- SANTOYO, G., MORENO-HAGELSIEB, G., MDEL, C.O. & GLICK, B.R. 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research* 183: 92-99. DOI: 10.1016/j.micres.2015.11.008
- STARODUMOVA, I.P., TARLACHKOV, S.V., PRISYAZHNAYA, N.V., DOROFEEVA, L.V., ARISKINA, E.V., CHIZHOV, V.N., SUBBOTIN, S.A., EVTUSHENKO, L.I. & VASILENKO, O.V. 2017. Draft genome sequence of *Rathayibacter* sp. VKM Ac-2630 isolated from the leaf gall induced by the knapweed nematode *Mesoanguina picridis* on *Acroptilon repens*. *Genome Announcements* 5: e00650-17. DOI: 10.1128/genomeA.00650-17
- TARLACHKOV, S.V., STARODUMOVA, I.P., DOROFEEVA, L.V., PRISYAZHNAYA, N.V., LEYN, S.A., ZLAMAL, J.E.,

- ELANE, M.L., OSTERMAN, A.L., NADLER, S.A., SUBBOTIN, S.A. & EVTUSHENKO, L.I. 2020. Complete and draft genome sequences of 12 plant-associated *Rathayibacter* strains of known and putative new species. *Microbiology Resource Announcements* 9: e00316-20. DOI: 10.1128/MRA.00316-20
- TAYLOR, C.E. 1990. Nematode interactions with other pathogens. *Annals of Applied Biology* 116: 405-416. DOI: 10.1111/j.1744-7348.1990.tb06622.x
- THOMAS, P. & SEKHAR, A.C. 2017. Cultivation versus molecular analysis of banana (*Musa* sp.) shoot-tip tissue reveals enormous diversity of normally uncultivable endophytic bacteria. *Microbial Ecology* 73: 885-899. DOI: 10.1007/s00248-016-0877-7
- TUO, L., YAN, X.R., LI, F.N., BAO, Y.X., SHI, H.C., LI, H.Y. & SUN, C.H. 2018. *Brachybacterium endophyticum* sp. nov., a novel endophytic actinobacterium isolated from bark of *Scutellaria baicalensis* Georgi. *International Journal of Systematic and Evolutionary Microbiology* 68: 3563-3568. DOI: 10.1099/ijsem.0.003032
- ULLAH, A., NISAR, M., ALI, H., HAZRAT, A., HAYAT, K., KEERIO, A.A., IHSAN, M., LAIQ, M., ULLAH, S., FAHAD, S., KHAN, A., KHAN, A.H., ADNAN AKBAR, A. & YANG, X. 2019. Drought tolerance improvement in plants: an endophytic bacterial approach. *Applied Microbiology and Biotechnology* 103: 7385-7397. DOI: 10.1007/s00253-019-10045-4
- ULRICH, K., STAUBER, T. & EWALD, D. 2008. *Paenibacillus* – a predominant endophytic bacterium colonising tissue cultures of woody plants. *Plant Cell, Tissue and Organ Culture* 93: 347-351. DOI: 10.1007/s11240-008-9367-z
- WANG, S., WANG, W., JIN, Z., DU, B., DING, Y., NI, T. & JIAO, F. 2013. Screening and diversity of plant growth promoting endophytic bacteria from peanut. *African Journal of Microbiology Research* 7: 875-884. DOI: 10.5897/AJMR12.1500
- WEISBURG, W.G., BARNS, S.M., PELLETIER, D.A. & LANE, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173: 697-703. DOI: 10.1128/jb.173.2.697-703.1991
- YABUUCHI, E. & KOSAKO, Y. 2015. Sphingomonas. In: *Bergey's Manual of Systematics of Archaea and Bacteria* (W.B. Whitman, F. Rainey, P. Kämpfer, M. Trujillo, J. Chun, P. DeVos, B. Hedlund & S. Dedysh Eds). pp. 1-39. Hoboken (NJ), USA, John Wiley & Sons, Inc. DOI: 10.1002/9781118960608.gbm00470
- YOON, S.H., HA, S.M., KWON, S., LIM, J., KIM, Y., SEO, H. & CHUN, J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology* 67: 1613-1617. DOI: 10.1099/ijsem.0.001755
- ZHAO, L., XU, Y., LAI, X.H., SHAN, C., DENG, Z., & JI, Y. 2015. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. *Brazilian Journal of Microbiology* 46: 977-989. DOI: 10.1590/S1517-838246420140024

I.P. Starodumova, L.V. Dorofeeva, V.N. Chizhov, S.A. Nadler, S.A. Subbotin and L.I. Evtushenko.

Бактерии, ассоциированные с тканями растений, инфицированных фитопаразитическими нематодами из семейств Anguinidae Nicoll, 1935 и Aphelenchoididae Skarbilovich, 1947.

Резюме. Из 23 изученных образцов растений, инфицированных нематодами 17 видов из родов *Anguina*, *Heteroanguina*, *Mesoanguina*, *Ditylenchus* и *Aphelenchoides*, было выделено 38 штаммов бактерий. Идентификация на основе гена 16S рПНК показала, что эти штаммы принадлежали к 11 родам классов *Alphaproteobacteria* (*Sphingomonas*, *Caulobacter*, *Rhizobium*), *Bacilli* (*Bacillus*, *Paenibacillus*, *Lactococcus*, *Staphylococcus*) и *Actinobacteria* (*Rathayibacter*, *Brachybacterium*, *Mycolicibacterium*, *Plantibacter*). Выделенные штаммы показали высокий уровень сходства с 19-ю типовыми штаммами валидно описанных видов по последовательностям генов 16S рПНК (99.1-100%). Эти данные указывают на то, что изученные штаммы являются предшественниками известных или еще не описанных новых видов. Среди видов, составляющих вышеупомянутые роды, только несколько видов *Rathayibacter* и *Sphingomonas* известны как патогены растений. Некоторые виды из выявленных родов, содержащих патогенов человека, включая род *Staphylococcus*, обладают свойствами, способствующими росту растений.
