https://doi.org/10.17816/ecogen17143-51

THE GENETIC DIVERSITY OF MICROSYMBIONTS FROM *THERMOPSIS LANCEOLATA* GROWING IN MONGOLIA

© D.S. Karlov[1], A.L. Sazanova[1], I.G. Kuznetsova[1], V.I. Safronova[1], N.Y. Tikhomirova[1], Zh.P. Popova[1], Yu.S. Osledkin[1], A.V. Verkhozina[2], A.A. Belimov[1]

[1] All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg, Russia;
[2] Siberian Institute of Plant Physiology and Biochemistry (SIPPB), Irkutsk, Russia

For citation: Karlov DS, Sazanova AL, Kuznetsova IG, et al. The genetic diversity of microsymbionts from *Thermopsis lanceolata* growing in Mongolia. *Ecological genetics*. 2019;17(1):43-51. https://doi.org/10.17816/ecogen17143-51.

Received: *13.10.2018* Revised: *04.12.2018* Accepted: *25.03.2019*

❀ For the first time, bacteria were isolated and identified from the root nodules of a wild-growing medicinal legume plant *Thermopsis lanceolata*, originated from Mongolia. The taxonomic position of 14 isolates obtained was determined using of sequencing of the 16S rRNA (*rrs*) and *atpD* genes. It was shown a significant biodiversity of the isolates from *T. lanceolata*, which belonged to three genera of the order *Rhizobiales*: *Phyllobacterium* (family *Phyllobacteriaceae*), *Rhizobium* (family *Rhizobiaceae*) and *Bosea* (family *Bradyrhizobiaceae*). Six isolates belonged to the species *Phyllobacterium zundukense* and *Phyllobacterium trifolii* (100 и 99,9% *rrs* similarity with the type strains *P. zundukense* Tri-48^{T} and *P. trifolii* PETP02^{T}, respectivelly), three isolates were identified as *Rhizobium anhuiense* (99,8% *rrs* similarity with the type strain *R. anhuiense* CCBAU 23252^{T}). Two slow-growing isolates of the genus *Bosea* Tla-534 and Tla-545 may potentially belong to new species, since their *rrs*-similarity to the closest type strains *B. massiliensis* LMG 26221^{T}, *B. lathyri* LMG 26379^{T} and *B. vaviloviae* Vaf18T was 98,5-99,0%. Non-rhizobial strains were not isolated. The isolation and future investigation of the rhizobial microsymbionts of the valuable medicinal legume *Thermopsis lanceolata* is one of the necessary prerequisites for its industrial cultivation.

❀ **Keywords:** medicinal legume plants; *Thermopsis lanceolata*; root nodule bacteria; 16S rRNA and *atpD* genes.

ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ МИКРОСИМБИОНТОВ ТЕРМОПСИСА ЛАНЦЕТНОГО (*THERMOPSIS LANCEOLATA*), ПРОИЗРАСТАЮЩЕГО В МОНГОЛИИ

© Д.С. Карлов[1], А.Л. Сазанова[1], И.Г. Кузнецова[1], В.И. Сафронова[1], Н.Ю. Тихомирова[1], Ж.П. Попова[1], Ю.С. Оследкин[1], А.В. Верхозина[2], А.А. Белимов[1]

[1] ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Санкт-Петербург;
[2] ФГБУН «Сибирский институт физиологии и биохимии растений» СО РАН, Иркутск

Для цитирования: Карлов Д.С., Сазанова А.Л., Кузнецова И.Г., и др. Генетическое разнообразие микросимбионтов термопсиса ланцетного (*Thermopsis lanceolata*), произрастающего в Монголии // Экологическая генетика. – 2019. – Т. 17. – № 1. – С. 43–51. https://doi.org/10.17816/ecogen17143-51.

Поступила: *13.10.2018* Одобрена: *04.12.2018* Принята: *25.03.2019*

❀ Впервые были выделены и идентифицированы бактерии из клубеньков дикорастущего лекарственного бобового растения термопсиса ланцетного (*Thermopsis lanceolata*), произрастающего в Монголии. В результате секвенирования 16S рДНК (*rrs*) и гена «домашнего хозяйства» *atpD* определено таксономическое положение 14 изолятов. Показано значительное биоразнообразие микросимбионтов термопсиса, которые относились к трем родам порядка *Rhizobiales*: *Phyllobacterium* (сем. *Phyllobacteriaceae*), *Rhizobium* (сем. *Rhizobiaceae*) и *Bosea* (сем. *Bradyrhizobiaceae*). Видовая принадлежность была определена для девяти изолятов: шесть изолятов были идентифицированы как *Phyllobacterium zundukense* и *Phyllobacterium trifolii* (100 и 99,9 % *rrs* гомологии с типовыми штаммами *P. zundukense* Tri-48^{T} и *P. trifolii* PETP02^{T} соответственно), три изолята — как *Rhizobium anhuiense* (99,8 % *rrs* гомологии с типовым штаммом *R. anhuiense* CCBAU 23252^{T}). Два медленнорастущих изолята Tla-534 и Tla-545, отнесенных к роду *Bosea*, могут потенциально принадлежать к новым видам, поскольку их сходство по гену *rrs* с ближайшими типовыми штаммами *B. massiliensis* LMG 26221^{T}, *B. lathyri* LMG 26379^{T} и *B. vaviloviae* Vaf18^{T} составляло 98,5–99,0 %. Неризобиальных штаммов из клубеньков выделено не было. Создание коллекции микросимбионтов термопсиса ланцетного и их изучение является одной из необходимых предпосылок для промышленного возделывания этой ценной лекарственной культуры.

❀ **Ключевые слова:** лекарственные бобовые растения; термопсис ланцетный *Thermopsis lanceolata*; клубеньковые бактерии; гены 16S рРНК и *atpD*.

Legume-rhizobium symbiosis is a unique and widespread phenomenon among leguminous plants. Nodule bacteria (*Rhizobia*), which are the integral elements of such symbiotic activity, are used to explore such interactions and reveal the mechanisms of plant–microbe interactions. Leguminous plants have high morphophysiological and ecological diversity and contribute a great deal to nitrogen balance in numerous land ecosystems and agrocenosis. This is achieved by broadening the range of biochemical function in both symbiotic partners and obtaining novel adaptive features of the plants [1]. Therefore, studying legume-rhizobium symbiosis has significant ecological and practical value.

Thermopsis lanceolata, R. Br. is a perennial wild leguminous plant growing in West and East Siberia, Baikal region, Middle Asia, North of Mongolia, and China [2]. The plant contains numerous alkaloids and has various pharmacological applications. One of the major biologically active alkaloids is cytosine, which is mostly present in the seeds [3] and is used in nicotine addiction treatment (Tabex) [4] and as an expectorant drug (Thermopsol and Codelac Broncho). It is also widely applied in veterinary medicine (Cytitonum) [5]. Current literature does not report any studies on extraction of nodule bacteria from *T. lanceolata*. In addition, to effectively introduce the plants, it is necessary to collect its microsymbionts for the production of growth-promoting agents. The first stage of microsymbiont strain studying, which is their identification, requires the use of modern molecular genetic methods, such as sequencing of 16S rDNA and “housekeeping” genes.

Therefore, the goal of the present study is to prepare a collection of rhizobial microsymbionts of the wild-growing medical leguminous plant, *T. lanceolata*, growing in North Mongolia, and to determine the taxonomic status of the strains by sequencing 16S rDNA and the “housekeeping” gene *atpD*.

MATERIALS AND METHODS

The study materials were 14 bacterial isolates extracted from the root nodules of a leguminous plant, *T. lanceolata*, which were collected from the mountain-taiga region of Mongolia (vicinity of the lake Khubsugul, right bank of the river Eg-Gol, village Alag-Erdene), according to the standard method [6]. Bacteria were cultured on modified mannitol-yeast agar YMSA with 0.5% succinate [7]. One isolate was selected from each nodule. Species affiliation of the isolates was determined by amplifying and sequencing the 16S rRNA genes as previously described [7]. To specify the taxonomic status of the isolates, amplification and sequencing of *atpD*, which codes for β-subunit of the ATP-synthesis complex, were performed using atpD-273F/atpD-771R [8] and atpD352F/atpD871R [9] primers. The obtained PCR-product was extracted from gel and purified as previously described [10] for further sequencing on ABI PRISM 3500xl genetic analyzer (Applied Biosystems, USA). Search for homologous sequences was performed using the NCBI GenBank database (https://www.ncbi.nlm.nih.gov) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). MEGA7 (MEGA, PA, USA) and neighbor-joining method [11] were used for the construction of phylogenetic trees. Nucleic acid sequences obtained were deposited in the GenBank database under the accession numbers MH779890–MH779903 and MK135051–MK135064.

All obtained isolates were deposited in the Russian Collection of Agricultural Microorganisms (RCAM, WDCM 966) and placed at the Station for Low-Temperature Automated Storage of Biological Samples (Liconic Instruments, Liechtenstein) [12]. Information on isolates is available in the Internet database of RCAM (http://www.arriam.spb.ru.).

RESULTS AND DISCUSSION

The vicinity of Lake Khusbugul, where plants were collected for further extraction of bacteria from the root nodules, has a distinctly continental climate and the soils exist under aridization and cryodization conditions [13]. The soil at collection site can be characterized as gray humus lithozem [14], and the soil pH is acidic and neutral [15]. As a result of analysis of nodules collected from different *T. lanceolata* plants within a population, 14 bacterial isolates were obtained: two of them formed colonies on the 5–6th day; three of them on the 3rd day; and nine of them on the 4–5th day.

Analysis of 16S rRNA (*rrs*) gene sequences allowed to refer of obtained isolates to three genera in the order *Rhizobiales*: *Phyllobacterium* (fam. *Phyllobacteriaceae*), *Rhizobium* (fam. *Rhizobiaceae*), and *Bosea* (fam. *Bradyrhizobiaceae*). Figure 1 shows that the strains belonging to genera *Phyllobacterium* and *Rhizobium* form two separate groups. Strains of *Phyllobacterium*, however, are divided in three clusters. *Rrs*-cluster I included isolates Tla-531, Tla-536, Tla-538, Tla-546, and Tla-549, in addition to type strains *P. trifolii* PETP02^T and P. *loti* S658^T at 100% support level (see Fig. 1). Similarity of isolates based on gene *rrs* with specified type strains was 99.9% and 100% accordingly, whereas the gene sequences of the isolates were identical (Table 1). However, a phylogenetic tree constructed based on the analysis of gene *atpD* revealed that the isolates were split into two statistically reliable clusters (Fig. 2). Cluster Ia was formed at 100% support level and consisted of isolates Tla-531, Tla-546, Tla-549, and a type strain *P. trifolii* PETP02^T, whose similarity based on *atpD* gene was 100% (see Table 1). Therefore, based on the results of *atpD* sequencing, isolates Tla-531,

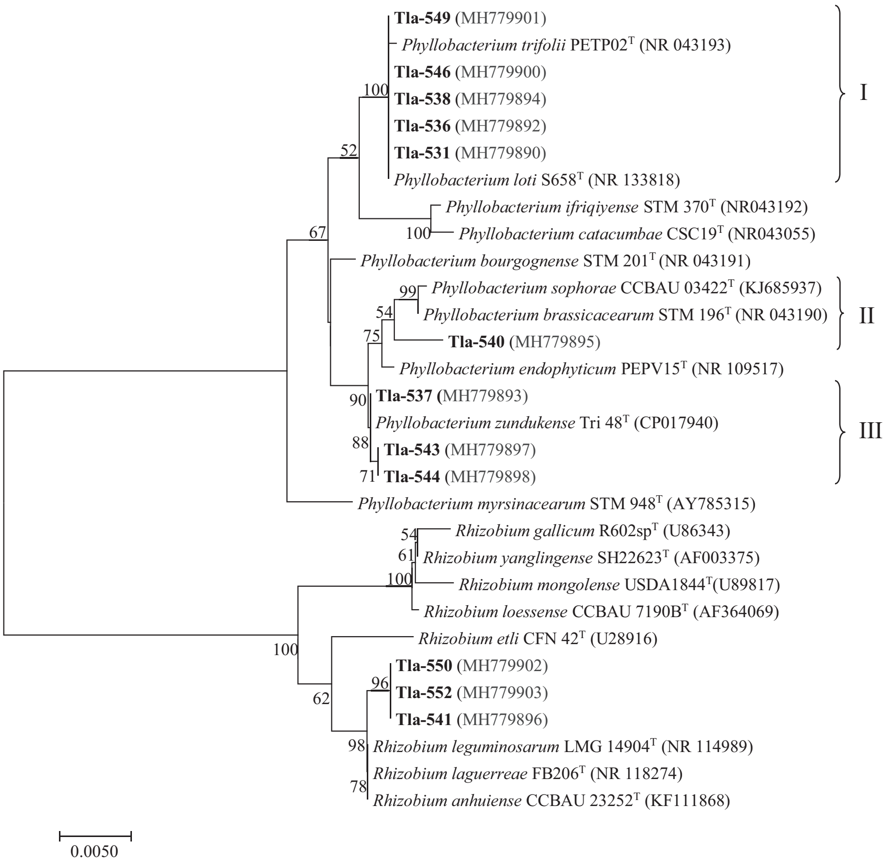

Fig. 1. Phylogenetic tree generated by the neighbour-joining method using partial 16S rRNA gene sequences of the isolated strains from *Thermopsis lanceolata* nodules and representatives of closely related to *Phyllobacterium* and *Rhizobium* species. The isolated strains in bold. Type species are indicated by the letter T. I–III clusters that formed by *Phyllobacterium* isolates obtained in the work. Bootstrap values more than 50% are given

Table 1

The similarity of 16S rRNA and *atpD* genes between the isolated strains from *Thermopsis lanceolata* nodules and the type strains of closely related to the *Bosea* and *Rhizobium species*

Type strains	Loci	Isolates similarity (%)								
		Cluster I					Cluster II	Cluster III		
		Tla-531	Tla-536	Tla-538	Tla-546	Tla-549	Tla-540	Tla-537	Tla-543	Tla-544
P. trifolii PETP02^{T}	16S рРНК	99.9	99.9	99.9	99.9	99.9	98.3	98.9	98.9	98.9
	atpD	100	93.3	93.4	100	100	89.5	89.0	89.0	89.0
P. loti S658^{T}	16S рРНК	100	100	100	100	100	98.4	99.0	99.0	99.0
	atpD	96.7	92.6	92.6	96.7	96.7	89.3	87.7	87.7	87.7
P. bourgognense STM 201^{T}	16S рРНК	99.1	99.1	99.2	99.2	99.2	98.8	99.4	99.3	99.3
	atpD	88.9	89.6	89.6	88.9	88.9	89.6	91.8	91.8	91.8
P. brassicacearum STM 196^{T}	16S рРНК	98.7	98.7	98.7	98.7	98.7	99.3	99.3	99.3	99.3
	atpD	96.9	94.2	94.2	96.9	96.9	88.9	87.9	87.9	87.9
P. endophyticum PEPV15^{T}	16S рРНК	98.1	98.1	98.7	98.7	98.7	99.2	99.5	99.4	99.4
	atpD	90.8	90.8	90.1	90.8	90.8	89.8	89.6	89.6	89.6
P. zundukense Tri-48^{T}	16S рРНК	99.0	99.0	99.0	99.0	99.0	99.3	100	99.9	99.9
	atpD	89.0	89.8	89.8	89.0	89.0	89.3	100	100	100
P. sophorae CCBAU03422^{T}	16S рРНК	98.3	98.3	98.5	98.5	98.5	99.1	99.0	99.2	99.2
	atpD	88.9	89.4	89.4	88.9	88.9	87.3	91.5	91.5	91.5

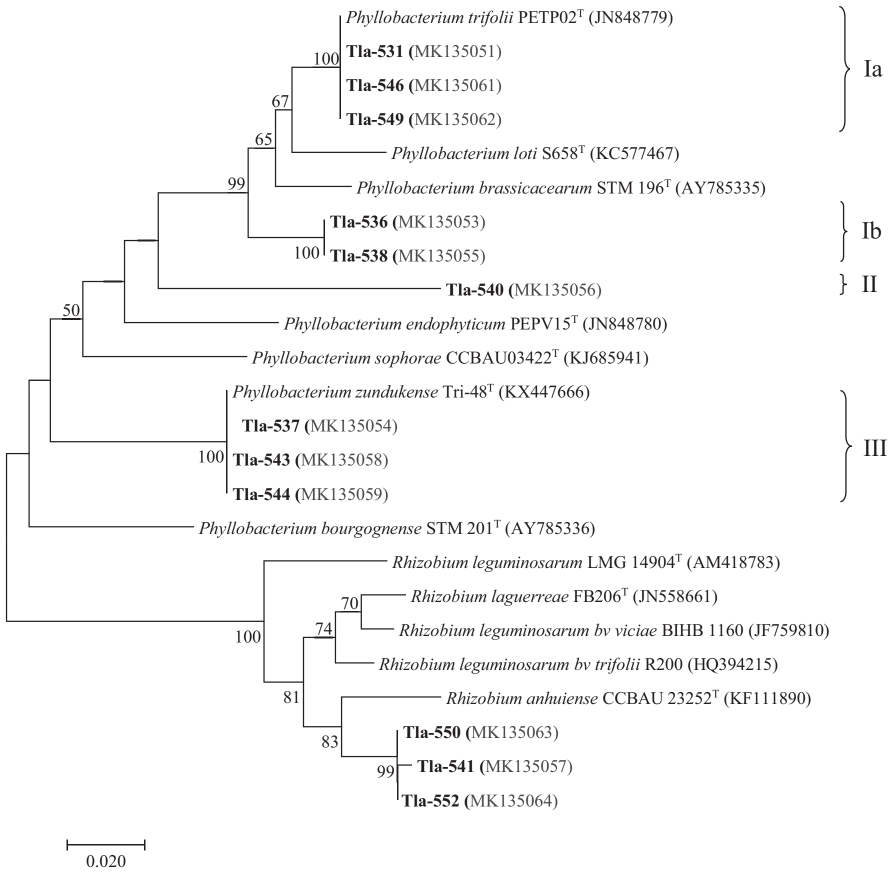

Fig. 2. Phylogenetic tree generated by the neighbour-joining method using *atpD* gene sequences of the isolated strains from *Thermopsis lanceolata* nodules and representatives of closely related to *Phyllobacterium* and *Rhizobium* species. The isolated strains in bold. Type species are indicated by the letter T. Ia, Ib, II и III — clusters that formed by *Phyllobacterium* isolates obtained in the work. Bootstrap values more than 50% are given

Tla-546, and Tla-549 were identified as *P. trifolii*. It should be noted that bacteria of this type were first isolated from the nodules of *Trifolium pretense* [16]. Previously, it was reported that the strain *P. trifolii* PETP02$^{\text{T}}$ could establish symbiotic interactions with *Trifolium repens* and *Lupinus albus* [16, 17]. In addition, bacteria that are closely related to species *P. trifolii*, with 99.9% similarity based on *rrs* were isolated from nodules of *Onobrychis viciifolia* [18], which indicates that there is a wide range of host plants for the group of nodule bacteria under study. Cluster Ib was formed by isolates Tla-536 and Tla-538, identified as *Phyllobacterium* sp., and were most similar to *P. loti* and *P. trifolii* (see Fig. 1, 2; Table 1).

Rrs-cluster II was formed at a low support level of 54% by strains *P. sophorae* CCBAU03422$^{\text{T}}$, *P. brassicacearum* STM 196$^{\text{T}}$, and isolate Tla-540, which, based on an *atpD*-dendrogram, was not grouped with any other strains (see Fig. 1, 2). Similarity of the isolate with the closest species, *P. brassicacearum* and *P. zundukense*, was 99.3%, based on *rrs* (see Table 1). Based on the analysis of the data obtained, isolate Tla-540 was identified as *Phyllobacterium* sp.

Rrs-cluster III combined isolates Tla-537, Tla-543, Tla-544, and type strain *P. zundukense* Tri-48T at a support level of 88% (see Fig. 1). On the *atpD*-dendrogram (see Fig. 2), the strains also formed a statistically reliable cluster (support level 100%). With regard to the high degree of homology based on *rrs* (99.9%–100%) and *atpD* (100%), isolates Tla-537, Tla-543, and Tla-544 were identified as *P. zundukense* (see Table 1), and have recently been described as microsymbionts of a relict leguminous plant, *Oxytropis triphylla*, growing in the Baikal region [19]. In addition, it has been demonstrated that strains of *P. zundukense* isolated from nodules of *O. triphylla* do not potentially carry out individual symbiosis as they do not have common *nod*ABC genes required

Table 2

The similarity of 16S rRNA and *atpD* genes between the isolated strains from *Thermopsis lanceolata* nodules and the type strains of closely related to the *Bosea* and *Rhizobium species*

Type strains	Loci	Isolates similarity (%)		Type strains	Loci	Isolates similarity (%)		
		Tla-534	Tla-545			Tla-541	Tla-550	Tla-552
B. massiliensis LMG 26221^{T}	16S rRNA	99.0	98.6	*R. leguminosarum* LMG 14904^{T}	16S rRNA	99.8	99.8	99.8
	atpD	92.4	95.6		*atpD*	93.5	93.7	93.7
B. lathyri LMG 26379^{T}	16S rRNA	99.0	98.5	*R. anhuiense* CCBAU23252^{T}	16S rRNA	99.8	99.8	99.8
	atpD	91.9	94.0		*atpD*	96.8	97.0	97.0
B. vaviloviae Vaf18^{T}	16S rRNA	98.9	98.5	*R. laguerreae* FB206^{T}	16S rRNA	99.8	99.8	99.8
	atpD	91.0	92.2		*atpD*	95.7	95.9	95.9

for plants nodulation [19]. Currently, genus *Phyllobacterium* is represented by only 11 species, most of which are isolated from the root nodules of leguminous plants [16, 19–23]. However, *nodACD* and *nifH* genes, which are required for effective symbiosis with host plants, have been found only in two species (*P. trifolii* and *P. sophorae*). *Phyllobacterium trifolii* and *P. sophorae* are able to form nodules independently on host plants [16, 23, and 24].

Fast-growing isolates, Tla-541, Tla-550, and Tla-552, belonged to genus *Rhizobium* and demonstrated similar levels of *rrs*-homology (99.8%) with three type strains, *R. leguminosarum* LMG 14904^{T}, *R. anhuiense* CCBAU23252^{T}, and *R. laguerreae* FB206^{T}, with which they formed a common cluster with a support level of 98% (see Fig. 1, Table 2). On *atpD*-dendrogram, isolates Tla-541, Tla-550, and Tla-552 were clustered only with type strain *R. anhuiense* CCBAU23252^{T} at a relatively high support level of 83% (see Fig. 2). Considering the significant similarity in *atpD* gene between strain *R. anhuiense* CCBAU23252^{T} and isolates Tla-541, Tla-550, and Tla-552 (96.8%–97.0%), the isolates were identified as *Rhizobium anhuiense* (see Table 2). Strains of the species were isolated from nodules of *Vicia faba* and *Pisum sativum* growing in China [25]. *R. anhuiense* are also microsymbionts of *Lathyrus japonicus* [26]. It has been demonstrated that *L. japonicus* has a high capacity for nitrogen fixation, particularly under low temperature conditions associated with arctic and subarctic regions, where the plant is considered a prospective forage crop [27]. In general, genus *Rhizobium* represents the most representative group in the family *Rhizobiaceae*, with all species being able to fix atmospheric nitrogen and form stable symbioses with leguminous plants [28].

Slow-growing isolates, Tla-534 and Tla-545, did not form statistically reliable groups with any other strains on either *rrs*- or *atpD*-dendrograms (Fig. 3, 4). Based on the homology levels of *rrs* (see Table 2), isolates Tla-534 and Tla-545 demonstrated maximum similarity with type strains of genus *Bosea*: *B. massiliensis* LMG 26221^{T}, *B. lathyri* LMG 26379^{T}, and *B. vaviloviae* Vaf18^{T} (similarity 98.5–99.0%). Based on the obtained data, both isolates were identified as *Bosea* sp. With regard to the obtained results, one could assume that isolates Tla-534 and Tla-545 are novel species in genus *Bosea*. The genus consists of nine species, among which four species, *B. lupini*, *B. lathyri*, *B. robiniae*, and *B. vaviloviae*, were isolated from nodules of leguminous plants of genus *Lupinus*, *Lathyrus*, *Robinia*, and *Vavilovia*, respectively. However, the capacity of the strains to independently form symbioses has not yet been studied [7, 29, 30].

We obtained bacterial isolates from the root nodules of a leguminous plant, *T. lanceolata*, for the first time. Nodule bacteria, *P. trifolii* and и *R. anhuiense*, were identified, which form nodules on the plant, as well as bacteria *P. zundukense*, with the capacity to independently carry out symbiosis, which has not been previously reported. Isolates belonged to genus *Bosea* could be representatives of novel species of slow-growing nodule bacteria. It should be noted that to clarify the taxonomic status of nine out of 14 obtained isolates, analysis of gene *atpD* was used, which facilitated the description of novel species, *P. zundukense* and *B. vaviloviae* [7, 19]. Formation and study of collections of microsymbionts of *Thermopsis lanceolata* could facilitate the enhanced and effective industrial production of this valuable medical plant.

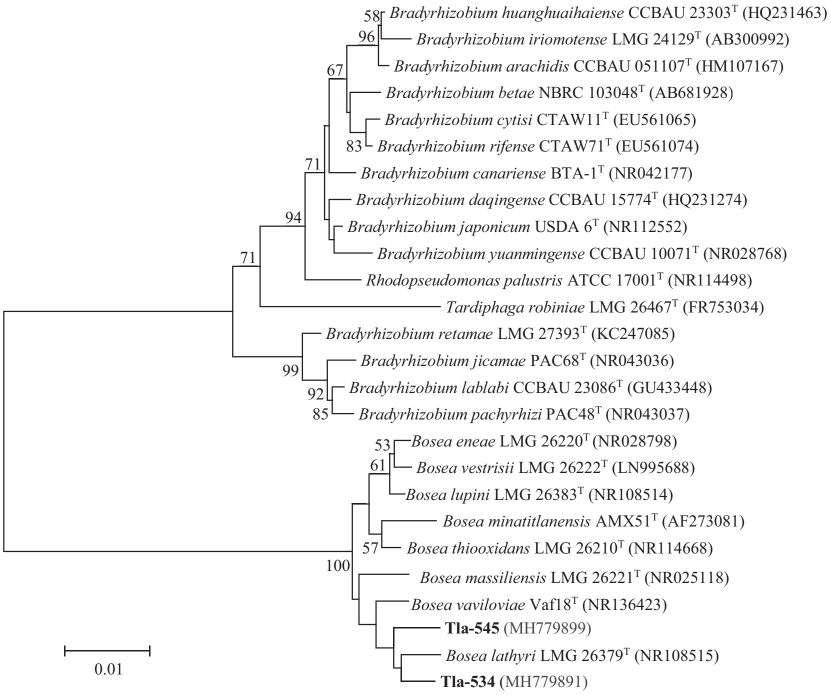

Fig. 3. Phylogenetic tree generated by the neighbour-joining method using partial 16S rRNA gene sequences of the isolated strains from *Thermopsis lanceolata* nodules and representatives of closely related to *Bosea* species. The isolated strains in bold. Type species are indicated by the letter T. Bootstrap values more than 50% are given

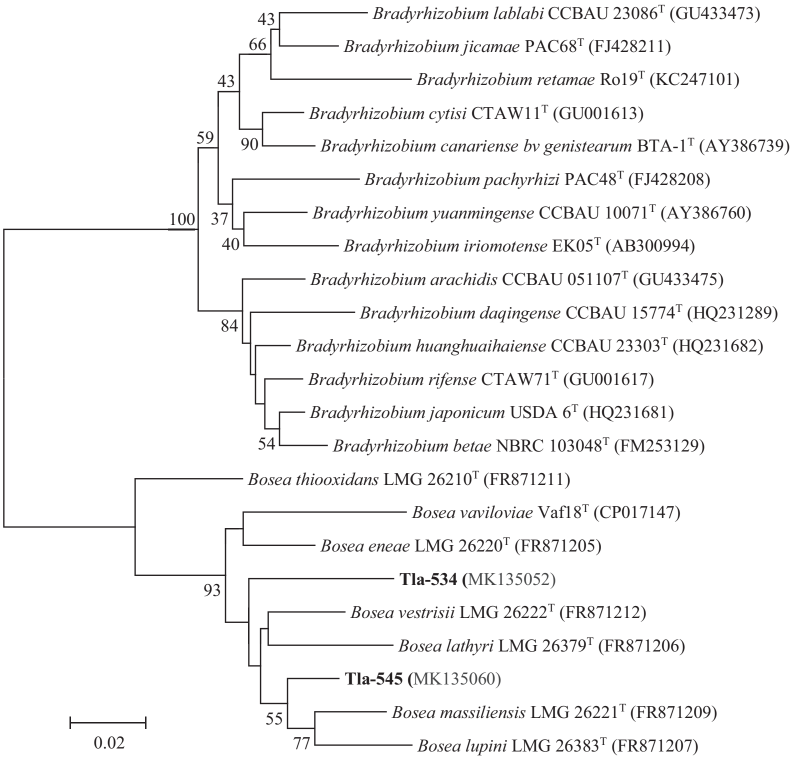

Fig. 4. Phylogenetic tree generated by the neighbour-joining method using *atpD* gene sequences of the isolated strains from *Thermopsis lanceolata* nodules and representatives of closely related to *Bosea* species. The isolated strains in bold. Type species are indicated by the letter T. Bootstrap values more than 30% are given

The work was performed as part of scientific project of FASO of Russia (Title No. 0664-2018-0001). Sequencing of *atpD* gene was conducted with RSF support (grant No. 16-16-00080). The long-term storage of strains was supported by the Program for the Development and Inventory of Bioresource Collections.

REFERENCES

1. Проворов Н.А., Воробьев Н.И. Генетические основы эволюции растительно-микробного симбиоза. – СПб.: Информ-Навигатор, 2012. [Provorov NA, Vorob'ev NI. Geneticheskie osnovy evolyutsii rastitel'no-mikrobnogo simbioza. Saint Petersburg: Inform-Navigator; 2012. (In Russ.)]
2. Мальцев А.И. Атлас важнейших видов сорных растений СССР. – М.; Ленинград: ОГИЗ Сельхозгиз, 1939. [Mal'tsev AI. Atlas vazhneyshikh vidov sornykh rasteniy SSSR. Moscow, Leningrad: OGIZ Sel'khozgiz; 1939. (In Russ.)]
3. Shakirov TT, Sabirov KA. The production of cysine from the seeds of *Thermopsis lanceolata*. *Chem Nat Compd*. 1970;6(6):733-734. https://doi.org/10.1007/BF00565346.
4. Tutka P, Zatonski W. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. *Pharmacol Rep*. 2006;58(6):777-798.
5. Рабинович М.И. Лекарственные растения в ветеринарной практике. – М.: Агропромиздат, 1987. [Rabinovich MI. Lekarstvennye rasteniya v veterinarnoy praktike. Moscow: Agropromizdat; 1987. (In Russ.)]
6. Novikova N, Safronova V. Transconjugants of *Agrobacterium radiobacter harbouring sym* genes of *Rhizobium galegae* can form an effective symbiosis with *Medicago sativa*. *FEMS Microbiol Lett*. 1992;72(3):261-268. https://doi.org/10.1111/j.1574-6968.1992.tb05107.x.
7. Safronova VI, Kuznetsova IG, Sazanova AL, et al. *Bosea vaviloviae sp. nov.*, a new species of slow-growing rhizobia isolated from nodules of the relict species *Vavilovia formosa (Stev.) Fed. Antonie Van Leeuwenhoek*. 2015;107(4):911-920. https://doi.org/10.1007/s10482-015-0383-9.
8. Gaunt MW, Turner SL, Rigottier-Gois L, et al. Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. *Int J Syst Evol Microbiol*. 2001;51(Pt 6):2037-2048. https://doi.org/10.1099/00207713-51-6-2037.
9. Martens M, Dawyndt P, Coopman R, et al. Advantages of multilocus sequence analysis for taxonomic studies: a case study using 10 housekeeping genes in the genus Ensifer (including former *Sinorhizobium*). *Int J Syst Evol Microbiol*. 2008;58(Pt 1):200-214. https://doi.org/10.1099/ijs.0.65392-0.
10. Stepkowski T, Zak M, Moulin L, et al. *Bradyrhizobium canariense* and *Bradyrhizobium japonicum* are the two dominant rhizobium species in root nodules of lupin and serradella plants growing in Europe. *Syst Appl Microbiol*. 2011;34(5):368-375. https://doi.org/10.1016/j.syapm.2011.03.002.
11. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28(10):2731-9. https://doi.org/10.1093/molbev/msr121.
12. Safronova V, Tikhonovich I. Automated cryobank of microorganisms: Unique possibilities for long-term authorized depositing of commercial microbial strains. In: Microbes in applied research: current advances and challenges. Ed. by A. Mendez-Vilas. Hackensack: World Scientific Publishing Co; 2012. P. 331-334. https://doi.org/10.1142/9789814405041_0066.
13. Убугунова В.И. Экологические условия формирования почв речных пойм Монголии и их свойства: Автореф. дис. ... д-ра биол. наук. – Иркутск, 1999. [Ubugunova VI. Ekologicheskie usloviya formirovaniya pochv rechnykh poym Mongolii i ikh svoystva. [dissertation] Irkutsk; 1999. (In Russ.)]
14. Белозерцева И.А., Сороковой А.А., Доржготов Д., и др. Почвы бассейна озера Байкал и их картографирование на территории России и Монголии // Международный журнал прикладных и фундаментальных исследований. – 2014. – № 5–2. – С. 114–120. [Belozertseva IA, Sorokovoy AA, Dorzhgotov D, et al. Ground of pool of lake baikal and their mapping in territory of Russia and Mongolia. *Mezhdunarodnyy zhurnal prikladnykh i fundamental'nykh issledovaniy*. 2014;(5-2):114-120. (In Russ.)]
15. Шишов Л.Л., Тонконогов В.Д., Лебедева И.И., Герасимова М.И. Классификация и диагностика почв России. – Смоленск: Ойкумена, 2004. [Shishov LL, Tonkonogov VD, Lebedeva II, Gerasimova MI. Klassifikatsiya i diagnostika pochv Rossii. Smolensk: Oykumena; 2004. (In Russ.)]
16. Valverde A, Velazquez E, Fernandez-Santos F, et al. *Phyllobacterium trifolii sp. nov.*, nodulating *Trifolium* and *Lupinus* in Spanish soils. *Int J Syst Evol Microbiol*. 2005;55(Pt 5):1985-1989. https://doi.org/10.1099/ijs.0.63551-0.
17. Zamlynska K, Komaniecka I, Zebracki K, et al. Studies on lipid A isolated from *Phyllobacterium*

trifolii PETP02(T) lipopolysaccharide. *Antonie Van Leeuwenhoek*. 2017;110(11):1413-1433. https://doi.org/10.1007/s10482-017-0872-0.

18. Baimiev AK, Baimiev AK, Gubaidullin II, et al. Bacteria closely related to *Phyllobacterium trifolii* according to their 16S rRNA gene are discovered in the nodules of *Hungarian sainfoin*. *Russ J Genet*. 2007;43(5):587-90. https://doi.org/10.1134/S1022795407050146.
19. Safronova VI, Sazanova AL, Kuznetsova IG, et al. *Phyllobacterium zundukense* sp. nov., a novel species of rhizobia isolated from root nodules of the legume species *Oxytropis triphylla (Pall.) Pers*. *Int J Syst Evol Microbiol*. 2018;68(5):1644-1651. https://doi.org/10.1099/ijsem.0.002722.
20. Mantelin S, Saux MF, Zakhia F, et al. Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense sp. nov.*, *Phyllobacterium ifriqiyense sp. nov.*, *Phyllobacterium leguminum sp. nov.* and *Phyllobacterium brassicacearum sp. nov*. *Int J Syst Evol Microbiol*. 2006;56(Pt 4):827-839. https://doi.org/10.1099/ijs.0.63911-0.
21. Flores-Felix JD, Carro L, Velazquez E, et al. *Phyllobacterium endophyticum sp.* nov., isolated from nodules of *Phaseolus vulgaris*. *Int J Syst Evol Microbiol*. 2013;63(Pt 3):821-6. https://doi.org/10.1099/ijs.0.038497-0.
22. Sanchez M, Ramirez-Bahena MH, Peix A, et al. *Phyllobacterium loti sp. nov*. isolated from nodules of *Lotus corniculatus*. *Int J Syst Evol Microbiol*. 2014;64(Pt 3):781-6. https://doi.org/10.1099/ijs.0.052993-0.
23. Jiao YS, Yan H, Ji ZJ, et al. *Phyllobacterium sophorae sp. nov.*, a symbiotic bacterium isolated from root nodules of *Sophora flavescens*. *Int J Syst Evol Microbiol*. 2015;65(Pt 2):399-406. https://doi.org/10.1099/ijs.0.067017-0.
24. Zhao L, Deng Z, Yang W, et al. Diverse rhizobia associated with *Sophora alopecuroides* grown in different regions of Loess Plateau in China. *Syst Appl Microbiol*. 2010;33(8):468-477. https://doi.org/10.1016/j.syapm.2010.08.004.
25. Zhang YJ, Zheng WT, Everall I, et al. *Rhizobium anhuiense sp. nov.*, isolated from effective nodules of *Vicia faba* and *Pisum sativum*. *Int J Syst Evol Microbiol*. 2015;65(9):2960-7. https://doi.org/10.1099/ijs.0.000365.
26. Li Y, Wang ET, Liu Y, et al. *Rhizobium anhuiense* as the predominant microsymbionts of *Lathyrus maritimus* along the Shandong Peninsula seashore line. *Syst Appl Microbiol*. 2016;39(6):384-390. https://doi.org/10.1016/j.syapm.2016.07.001.
27. Gurusamy C, Bal A, McKenzie D. Nodulation of beach pea (*Lathyrus maritimus* [L.] Bigel.) induced by different strains of rhizobia. *Can J Plant Sci*. 1999;79(2):239-42. https://doi.org/10.4141/P98-039.
28. Alves LM, de Souza JAM, Varani AM, Lemos EGM. The family *Rhizobiaceae*. In: The Prokaryotes. Ed. by E. Rosenberg, E.F. DeLong, S. Lory, et al. Berlin, Heidelberg: Springer; 2014.P. 419-437. https://doi.org/10.1007/978-3-642-30197-1_297.
29. De Meyer SE, Willems A. Multilocus sequence analysis of *Bosea species* and description of *Bosea lupini sp. nov.*, *Bosea lathyri sp. nov.* and *Bosea robiniae sp. nov.*, isolated from legumes. *Int J Syst Evol Microbiol*. 2012;62(Pt 10):2505-10. https://doi.org/10.1099/ijs.0.035477-0.
30. Сазанова А.Л., Кузнецова И.Г., Сафронова В.И., и др. Изучение генетического разнообразия микросимбионтов копеечника щетинистого *Hedysarum gmelinii subsp. setigerum*, произрастающего в Прибайкалье // Сельскохозяйственная биология. – 2017. – Т. 52. – № 5. – С. 1004–1011. [Sazanova AL, Kuznetsova IG, Safronova VI, et al. Study of the genetic diversity of microsymbionts isolated from *Hedysarum gmelinii subsp. setigerum*, growing in the Baikal lake region. *Selskokhoziaistvennaia Biol*. 2017;52(5):1004-1011. (In Russ.)]. https://doi.org/10.15389/agrobiology.2017.5.1004rus.

❀ Information about the authors

Denis S. Karlov – PhD, Junior Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. SPIN: 8355-8091. E-mail: makondo07@gmail.com.

Anna L. Sazanova – PhD, Senior Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: anna_sazanova@mail.ru.

❀ Информация об авторах

Денис Сергеевич Карлов — канд. биол. наук, младший научный сотрудник ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. SPIN: 8355-8091. E-mail: makondo07@gmail.com.

Анна Львовна Сазанова — канд. биол. наук, старший научный сотрудник ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: anna_sazanova@mail.ru.

❀ Information about the authors	❀ Информация об авторах
Irina G. Kuznetsova — Engineer-Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: kuznetsova_rina@mail.ru.	**Ирина Геннадьевна Кузнецова** — инженер-исследователь ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: kuznetsova_rina@mail.ru.
Vera I. Safronova — PhD, Head, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: v.safronova@rambler.ru.	**Вера Игоревна Сафронова** — канд. биол. наук, заведующая ведомственной коллекцией полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: v.safronova@rambler.ru.
Nina Y. Tikhomirova — Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: arriam2008@yandex.ru.	**Нина Юрьевна Тихомирова** — научный сотрудник ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: arriam2008@yandex.ru.
Zhanna P. Popova — PhD, Senior Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: elestd@yandex.ru.	**Жанна Павловна Попова** — канд. биол. наук, старший научный сотрудник ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: elestd@yandex.ru.
Yuriy S. Osledkin — PhD, Leading Researcher, Russian Collection of Agricultural Microorganisms. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: arriam2008@yandex.ru.	**Юрий Сергеевич Оследкин** — канд. биол. наук, ведущий научный сотрудник ведомственной коллекции полезных микроорганизмов сельскохозяйственного назначения. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: arriam2008@yandex.ru.
Alla V. Verkhozina — PhD, Head of The Herbarium Group, Department of Terrestrial Ecosystems Resistance. Siberian Institute of Plant Physiology and Biochemistry (SIPPB), Irkutsk, Russia. E-mail: allaverh@list.ru.	**Алла Васильевна Верхозина** — канд. биол. наук, руководитель группы «Гербарий» отдела устойчивости наземных экоситем. ФГБУН «Сибирский институт физиологии и биохимии растений» СО РАН, Иркутск. E-mail: allaverh@list.ru.
Andrey A. Belimov — DrSci, Head of Laboratory of Rhizosphere Microflora. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: belimov@rambler.ru.	**Андрей Алексеевич Белимов** — д-р биол. наук, заведующий лабораторией ризосферной микрофлоры. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: belimov@rambler.ru.