ISSN 1021-4437, Russian Journal of Plant Physiology, 2023, Vol. 70:185. © Pleiades Publishing, Ltd., 2023.

RESEARCH PAPERS

Genetic Diversity of Root Nodule Endophyte Strains Isolated from the Legumes *Astragalus umbellatus* and *A. inopinatus*, Growing on the Kamchatka Peninsula (Russian Federation)

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Received September 12, 2023; revised November 1, 2023; accepted November 2, 2023

Abstract—A collection of twenty-eight strains isolated from root nodules of *Astragalus inopinatus* Boriss. and *Astragalus umbellatus* Bunge originating from the Kamchatka Peninsula (Russian Federation) was obtained for the first time. Analysis of the 16S ribosomal RNA gene sequence revealed a significant diversity of isolates belonging to 4 genera of the order *Hyphomicrobiales*: *Mesorhizobium*, *Rhizobium*, *Bosea* and *Tardiphaga*. The presence of phenotypically and taxonomically different strains (*Bosea* + *Tardiphaga*, *Bosea* + *Mesorhizobium*, *Tardiphaga* + *Mesorhizobium*, *Bosea* + *Rhizobium*) in some nodules of both plants was also observed. The symbiotic efficiency of strains isolated from *A. inopinatus* plants in the nodulation assay showed that most isolates of the genus *Mesorhizobium* and *Rhizobium* can form a nitrogen-fixing symbiosis with *A. inopinatus*, leading to a significant increase in plant biomass compared to the non-inoculated control.

Keywords: *Astragalus inopinatus*, *Astragalus umbellatus*, plant-microbe interaction, symbiosis. *Astragalus* spp., Kamchatka region

DOI: 10.1134/S1021443723602100

INTRODUCTION

Root nodule bacteria are one of the most important groups of soil microorganisms that form nitrogen-fixing symbioses with legumes, allowing them to enrich nitrogen-deficient soils and make it available to other plants [1]. As a result of global climate change (global warming), Russia's northern territories will increasingly be used for agriculture. The northern zone of Russia has already experienced an expansion of areas occupied by more productive plant communities. This is mainly due to higher temperatures, longer growing seasons and changes in the thawing depth of permafrost soils. These processes also increase the activity of soil microorganisms. As a result, free-living bacteria are able to form highly adaptive legume-rhizobial symbioses and invade new landscapes, such as the Russian North. Despite the wealth of knowledge gained over the decades, there is still very little information available on nodulation, root nodule bacteria and nitrogen fixation by native legumes in northern habitats.

One of the agricultural problems in the Russian North Asia region is the limited species composition of cultivated crops, mainly perennial legumes. Currently, only alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) are grown in the region. As a result, the lack of perennial legumes in crop rotations, which play a supporting and ecological role, is one of the reasons for the decline in soil fertility and the lack of plant protein in livestock feed rations [2].

The complexity of increasing the diversity of perennial legume species is linked to the study and consideration of the abiotic conditions of the region: sharply continental climate, duration of the frost-free period, overwintering conditions for the plants. The introduction of plants involves the study of a complex of issues related to morphology, biology, ecology, zonal cultivation technology, as well as their biogeocenotic influence on the elements of soil fertility. Such introduced perennial legumes include plants of the genus *Astragalus*. The nitrogen-fixing symbiosis of this plant with root nodule bacteria develop a strong root system, which leads to the accumulation of root residues in the soil and the accumulation of labile organic matter and biological nitrogen [3]. *Astragalus* L. is one of the largest genus among seed plants with a very wide distribution [4]. The species of this genus are distributed unevenly from low to high altitudes with different numbers of endemisms in Asia, Europe, Africa and America. In Europe with 134 spp. (61 endemics), Southwest Asia with 1484 spp. (1024 endemics), Southeastern Asia with 430 spp. (257 endemics),

South Asiawith 172 spp. (50 endemics), the former Soviet Union with 835 spp. (512 endemics) and the African continent with 82 spp. (18 endemics) are the main and largest centres of endemism and diversity, while the Americas with 746 taxa (638 endemics) are the main and largest centre of endemism and diversity, despite its very large area and high number of endemisms, seems to be the secondary centre of diversity [5]. The main life forms of the genus *Astragalus* are herbs, shrubs and semi-shrubs.

Most studies aimed at identifying or characterizing root nodule bacteria associated with *Astragalus* spp. have been carried out mainly in North America, China and rarely in the western part of northern Europe. *Mesorhizobium* has been identified as the predominant genus nodulating *Astragalu*s plants, while the genera *Rhizobium*, *Ensifer* and *Bradyrhizobium* have been found as minor symbionts of this plant genus [6–8]. The aim of our work was to create and study a collection of strains isolated from nodules of *A. inopinatus* and *A. umbellatus* plants, two species whose microsymbionts have not been described previously. *A. umbellatus* Bunge 1868 is a circumpolar species found in the tundra and mountains of the taiga zone of the northern hemisphere: on steep stony slopes, scree slopes, is a mesophilous species. Stems are few in number, erect, pubescent with soft white hairs, leafless at the base, covered with brownish webbed, highly interlaced bracts. There is an assumption that *A. umbellatus* is an alpine relict in Kamchatka peninsula, preserved in the high mountains since the cooling of the Lower and Middle Pleistocene [9]. *A. umbellatus* plays an important role in the diet of musk oxen (*Ovibos moschatus*), reindeer (*Rangifer tarandus*) and snow goose (*Anser caerulescens*). The feed value of this species is high in ascorbic acid and available protein (24.1–32.1%) [10, 11].

A. inopinatus Boriss.is a valuable perennial plant of the legume family, taken from the local flora and introduced to the Siberian region of Russia [12]. *A. inopinatus* has a complex of valuable ecological and biological properties and multifunctional economic use as a fodder, sideral, meliorative plant that has a biocenotic effect on the accumulation of organic compounds and biological nitrogen. One of the valuable morphological characteristics of *A. inopinatus* is the speed of formation and strength of the root system, which penetrates deeply into the subsoil horizons. The deep penetration of the root system into the soil makes it possible to effectively use the moisture, which ensures a high yield of biomass already in the first year of *A. inopinatus* plants vegetation [12]. In addition, already in the first year of life, symbiotrophic nodule bacteria colonize the root system. In the second year of vegetation, the root system of *A. inopinatus* penetrates the soil to a depth of about one meter.

Thus, the present study reports for the first time the genetic diversity of microsymbionts of *A. umbellatus* and *A. inopinatus* plants collected in the Kamchatka region. The symbiotic efficiency of strains isolated from *A. inopinatus* plants has also been studied in the nodulation assay, taking into account that this plant species is a very promising crop for cultivation in the northern regions.

MATERIALS AND METHODS

Root nodule strain isolation. The nodules of two narrowly endemic species of the legume genus *Astragalus* (*A. umbellatus* and *A. inopinatus*) were collected from the Kamchatka Peninsula (Russian Federation, N = 55°57.201′; E = 159°45.310′). Individual root nodules were surface sterilized with 70% ethanol for 1 min, washed thoroughly with sterile tap water, homogenized and 100 μL of the homogenized nodules were plated on modified yeast extract mannitol agar plates (YMA) supplemented with 0.5% succinate. Further streaking of the individual colony was used to isolate the pure culture. Isolates were grown at 28°C for 3–7 days. All isolates are deposited in the Russian Collection of Agricultural Microorganisms (RCAM, http://62.152.67.70/cryobank/login.jsp) and are stored at 80°C in the automated Tube Store (Liconic Instruments, Liechtenstein).

Identification of isolates by the sequencing of the 16S rRNA gene. The following PCR primers were used to identify all isolates: fD1 (59-AGAGTTT-GATCCTGGCTCAG-39) and rD1 (59-CTTAAG-GAGGTGATCCAGCC-39) for an approximately 1400 bp segment of the 16S rRNA (*rrs*) gene (Weisburg et al., 1991). PCR was performed in 25 μL reaction mixtures containing 150 μM dNTPs (Promega, United States), 5 pmol of each primer, 1 U Taq polymerase (Helicon, Russia) and 50 to 100 ng purified template DNA. PCR conditions for amplification of the 16S rRNA gene were as follows: initial denaturation at 95°C for 3 min 30 s; 35 cycles of denaturation at 94°C for 1 min 10 s, annealing at 56°C for 40 s and extension at 72°C for 2 min 10 s; final extension at 72°C for 6 min 10 s. Electrophoresis was performed on 1% agarose gel (Invitrogen, United States) in Tris-acetate-EDTA. A 100 bp GeneRuler and Lambda DNA/HindIII markers (Fermentas, United States) were used for size determination and approximate quantification of DNA fragments. Purification of PCR products was normally performed using the PureLink Quick Kit (Invitrogen, United States) according to the manufacturer's instructions. Direct sequencing of the PCR products was performed on an ABI PRISM 3500xl Genetic Analyser (Applied Biosystems, United States). Sequences were compared with related sequences of the type strains available in the GenBank database using BLAST analysis (basic logical alignment search tool) at the National Center for Biotechnology Information (NCBI). Evolutionary distances were calculated using the maximum composite likelihood method in the MEGA X software

package [13]. Bootstrap analysis with 500 replicates was used to estimate cluster support.

Accession numbers. The nucleotide sequences obtained in this study were deposited in the Genbank under accession numbers from OR366566 to OR366593 for the 16S rRNA fragments (~1400 bp).

Plant nodulation assay. Seeds of *A. inopinatus* plants were surface sterilized with H_2SO_4 for 15 min, washed with sterile tap water and germinated on filter paper in Petri dishes at 25°C in the dark for 4 days. The *A. umbellatus* species was not used due to lack of seeds. Germinated seedlings were transferred to 50 mL glass test tubes (two seedlings per tube) containing 3 g of sterile vermiculite. To each tube 6 mL of nutrient solution [grams per liter: 1.0 K_2HPO_4, 0.25 KH_2PO_4, 1.0 $MgSO_4$, $Ca_3(PO_4)_2$ 0.2, 0.02 $FeSO_4$, 0.005 H_3BO_3, 0.005 $(NH_4)_2MoO_4$, 0.005 $ZnSO_4 \cdot 7H_2O$, 0.002 $MnSO_4$] was added. Seedlings were inoculated with single strains or a pair of strains isolated from the same nodule at 10^{-6} cells of each strain per test tube. Non-inoculated plants were used as negative controls. Plants were grown for 5 weeks in a growth chamber with 50% relative humidity and a four-stage illumination/temperature mode: night (dark, 18°C, 8 h), morning (200 μmol/m^2 s, 20°C, 2 h), day (400 μmol/m^2 s, 23°C, 12 h), evening (200 μmol/m^2 s, 20°C, 2 h). Illumination was performed with L 36W/77 Fluora lamps (Osram, Germany). The experiment was carried out with three replicates. At the end of the experiment, the nodules were counted and the fresh biomass of the plants (shoots plus roots) was determined. Nodule nitrogen fixation was measured by the acetylene reduction method (Turner and Gibson, 1980) using a GC-2014 gas chromatograph (Shimadzu, Japan). The data were processed in the R statistical environment (v. 4.2.3) [14]. One-way analysis of variance (ANOVA) was used to test for differences in root, shoot and whole plant biomass weight, number of nodules and nitrogenase activity. Tukey's post-hoc test was performed between all groups to determine the differences within groups.

RESULTS

Root Nodule Strain Isolation and Identification

A total of 28 isolates belonging to 4 genera and families of the order *Hyphomicrobiales* (*Alphaproteobacteria*) were obtained (Table 1). Thirteen and fifteen strains were isolated from nodules of *A. umbellatus* and *A. inopinatus*, respectively. Two isolates formed colonies on day 3 (fast-growing), twenty-five on days 4–5 (meso-growing) and one on day 7 (slow-growing). Analysis of the *rrs* gene sequences made it possible to attribute the isolates to four genera of the order *Hyphomicrobiales*. Thus, 6 isolates from *A. umbellatus* belonged to the genus *Mesorhizobium* (*Phyllobacteriaceae* family), 6 isolates—to the genus *Bosea* (*Boseaceae* family), and 1 isolate—to the genus *Rhizobium* (*Rhizobiaceae* family). *A. inopinatus* isolates were assigned to the genera *Mesorhizobium* (9 isolates), *Bosea* (3 isolates) and *Rhizobium* (1 isolate), while 2 isolates belonged to the genus *Tardiphaga* (*Nitrobacteraceae* family).

The greatest genetic diversity was observed for *Mesorhizobium*-related isolates. Twelve strains were identified as *Mesorhizobium* sp.: 11 isolates with the same level of *rrs* similarity (99.7–100%) to two type strains *M. norvegicum* 10.2.2T and *M. loti* NZP2213T, and 1 isolate with 99.86% *rrs* similarity with the type strains *M. jarvisii* ATCC 33669T and *M. erdmanii* USDA 3471T. Two strains were classified as *M. erdmanii* (100% *rrs* similarity with the type strain *M. erdmanii* USDA 3471T) and *M. tianshanense* (99.86% *rrs* similarity with the type strain *M. tianshanense* A-1BST). These results are consistent with previous studies that found *Mesorhizobium* to be the predominant genus nodulating *Astragalus* plants [15]. Twelve *Mesorhizobium*-related isolates (712, 713, 700, 691, 689, 715, 693, 703, 699, 708, 702 and 707) formed a significant cluster with a statistical support more than 95% (Fig. 1).

Of the nine strains related to the genus *Bosea,* 8 were identified as *B. vaviloviae* (99.6–100% *rrs* similarity with the type strain *B. vaviloviae* Vaf18T and one isolate without species affiliation had 98.93% *rrs* similarity with the type strain *B. lathyri* R-46060T (Fig. 2).

Two isolates (698 and 709) were identified as *Tardiphaga robiniae* at the level of *rrs* similarity with the type strain *T. robiniae* R-45977T 99.7 and 99.6%, respectively (Fig. 2). Similarly, only two isolates (687 and 705) were related to the genus *Rhizobium* and were identified as *Rhizobium* sp. (*rrs* similarity with both type strains *R. lusitanum* P1-7T and *R. rhizogenes* NBRC 13257T was 99.93% for the isolate 687 and 99.89% for the isolate 705) (Fig. S1).

It was also observed that pairs of phenotypically and taxonomically different strains were isolated from some nodules of both plants studied, *A. umbellatus* and *A. inopinatus* (Table 1). The combination of strains in the pairs was varied: *Bosea* + *Tardiphaga*, *Bosea* + *Mesorhizobium*, *Tardiphaga* + *Mesorhizobium*, *Bosea* + *Rhizobium*.

Plant Nodulation Assay

The plant nodulation test was carried out on *A. inopinatus* plants with the participation of some isolated strains representing different genera of order *Hyphomicrobiales* (*Bosea*, *Tardiphaga*, *Mesorhizobium* and *Rhizobium* (Table 2). Three variants of co-inoculation with pairs of strains isolated from the same nodule of *A. inopinatus* were also studied (*B. vaviloviae* 704 + *Rhizobium* sp. 705, *Mesorhizobium* sp. 708 + *T. robiniae* 709, *Bosea* sp. 714 + *Mesorhizobium*sp. 715).

No nodules were formed on plants inoculated with all isolates of the genera *Bosea* and *Tardiphaga,* as well as with one isolate of the genus *Mesorhizobium* (712). At the same time, most isolates of the genus *Mesorhi-*

Table 1. Identification of strains isolated from *A. umbellatus* and *A. inopinatus* plants by the 16S rRNA gene sequencing

Plant species	Strain	Similarity with the closest species		
		Type strain	Similarity, %	Identification result
A. umbellatus	682[a]	*Boseavaviloviae* Vaf18T	99.77	*Bosea vaviloviae*
	683[a]	*Mesorhizobiumtianshanense* A-1BST	99.86	*Mesorhizobium tianshanense*
	684	*B. vaviloviae* Vaf18T	99.64	*Bosea vaviloviae*
	685	*B. vaviloviae* Vaf18T	99.57	*Bosea vaviloviae*
	686	*B. vaviloviae* Vaf18T	99.8	*Bosea vaviloviae*
	687	*Rhizobium lusitanum* P1-7T/*R. rhizogenes* NBRC 13257T	99.93	*Rhizobium* sp.
	689	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.86	*Mesorhizobium* sp.
	691	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.86	*Mesorhizobium* sp.
	692[b]	*B. vaviloviae* Vaf18T	99.64	*Bosea vaviloviae*
	693[b]	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.76	*Mesorhizobium* sp.
	694	*M. erdmanii* USDA 3471T	100	*Mesorhizobium erdmanii*
	695[c]	*M. jarvisii* ATCC 33669T/*M. erdmanii* USDA 3471T	99.86	*Mesorhizobium* sp.
	696[c]	*B. vaviloviae*Vaf18T	100	*Bosea vaviloviae*
A. inopinatus	697[c]	*B. vaviloviae* Vaf18*T*	99.64	*Bosea vaviloviae*
	698[c]	*Tardiphagarobiniae* R-45977T	99.72	*Tardiphaga robiniae*
	699	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.78	*Mesorhizobium* sp.
	700	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.86	*Mesorhizobium* sp.
	702	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.71	*Mesorhizobium* sp.
	703	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.49	*Mesorhizobium* sp.
	704[d]	*B. vaviloviae* Vaf18T	99.53	*Bosea vaviloviae*
	705[d]	*R. lusitanum* P1-7T/*R. rhizogenes* NBRC 13257T	99.89	*Rhizobium* sp.
	707	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.86	*Mesorhizobium* sp.
	708[e]	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.86	*Mesorhizobium* sp.
	709[e]	*T. robiniae* R-45977T	99.57	*Tardiphaga robiniae*
	712	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.27	*Mesorhizobium* sp.
	713	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	100	*Mesorhizobium* sp.
	714[f]	*B. lathyri* R-46060T	98.93	*Bosea* sp.
	715[f]	*M. norvegicum* 10.2.2 *T*/*M. loti* NZP2213T	99.78	*Mesorhizobium* sp.

[a–f] Matching letters indicate pairs of strains isolated from the same nodule.

zobium (703, 708 and 715), *Rhizobium* (705) and all co-inoculation pairs formed a nitrogen-fixing symbiosis. The highest nitrogenase activity, root and total plant biomass was observed after inoculation with the isolate *Mesorhizobium* sp. 708 (Table 2). The co-inoculation pair *Mesorhizobium* sp. 708 + *T. robiniae* 709 was able to produce the maximum number of nodules and also had one of the highest values for total plant and shoot biomass (Table 2, Supplementary Fig. S2). Co-inoculation with the isolates *Bosea* sp. 714 + *Mesorhizobium* sp. 715 significantly reduced the nitrogenase activity, while the pair of *Bosea* sp. 704 + *Rhizobium* sp. 705 negatively affected 3 parameters of symbiosis (total plant and shoot biomass) compared to mono-inoculations with the corresponding nodule forming isolates 715 and 705. Of the four strains (704 *Bosea* sp., 709 *Tardiphaga* sp., 712 *Mesorhizobium* sp., 714 *Bosea* sp.) that did not form nodules, two strains, 712 and 714, were growth stimulating in three parameters—total plant biomass, root and shoot weight (Table 2). The plant hormone ethylene is well known for its inhibitory effects on various aspects of nodule formation and development in many different legumes [16]. Much of the growth inhibition that occurs as a result of environmental stress is the result of the plant's response to increased levels of stress ethylene, which exacerbates the response to the

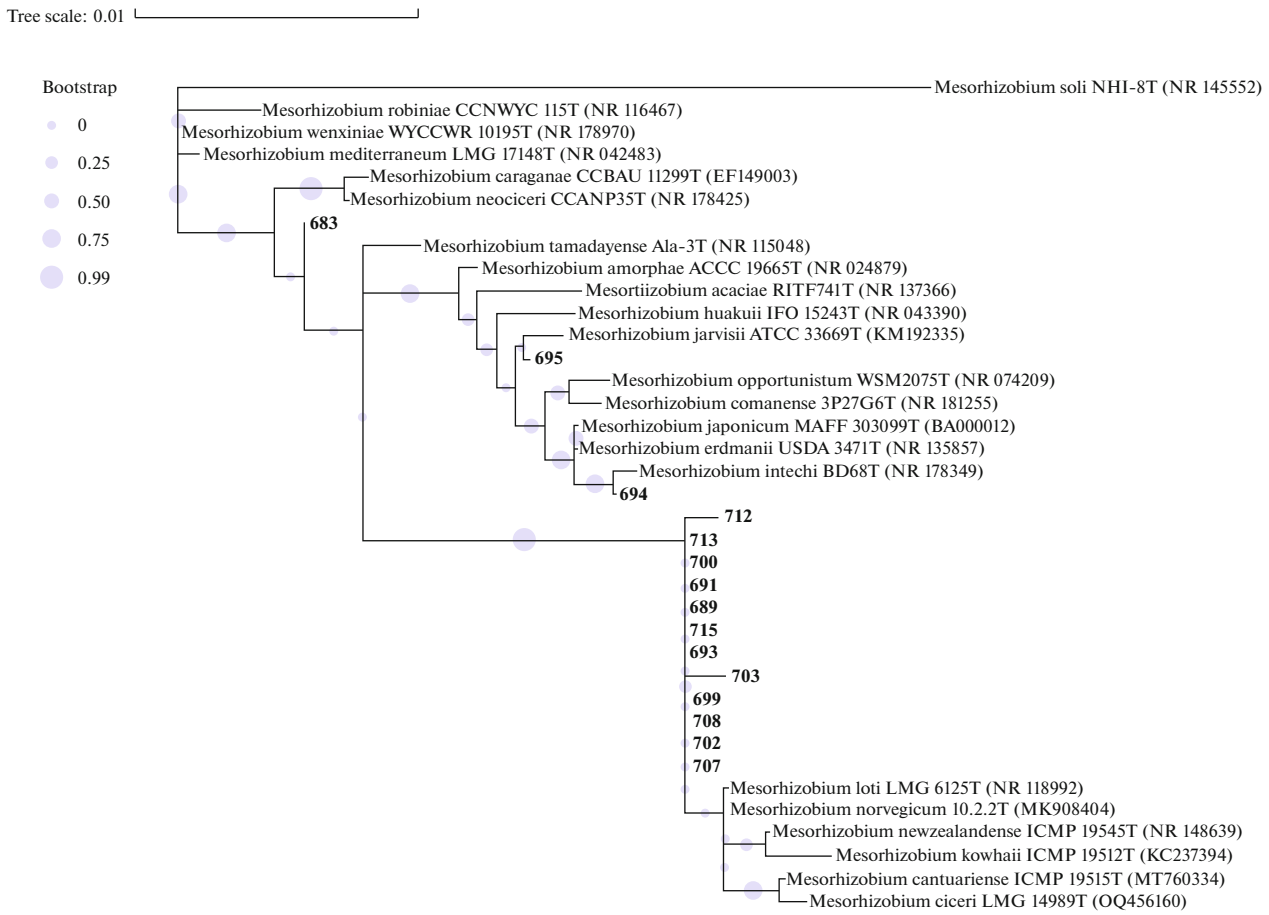

Fig. 1. Phylogenetic tree generated by the maximum composite likelihood method using 16S rRNA gene sequences of the *Mesorhizobium*-related isolates from *A. umbellatus* and *A. inopinatus* as well as type strains of closely related species.

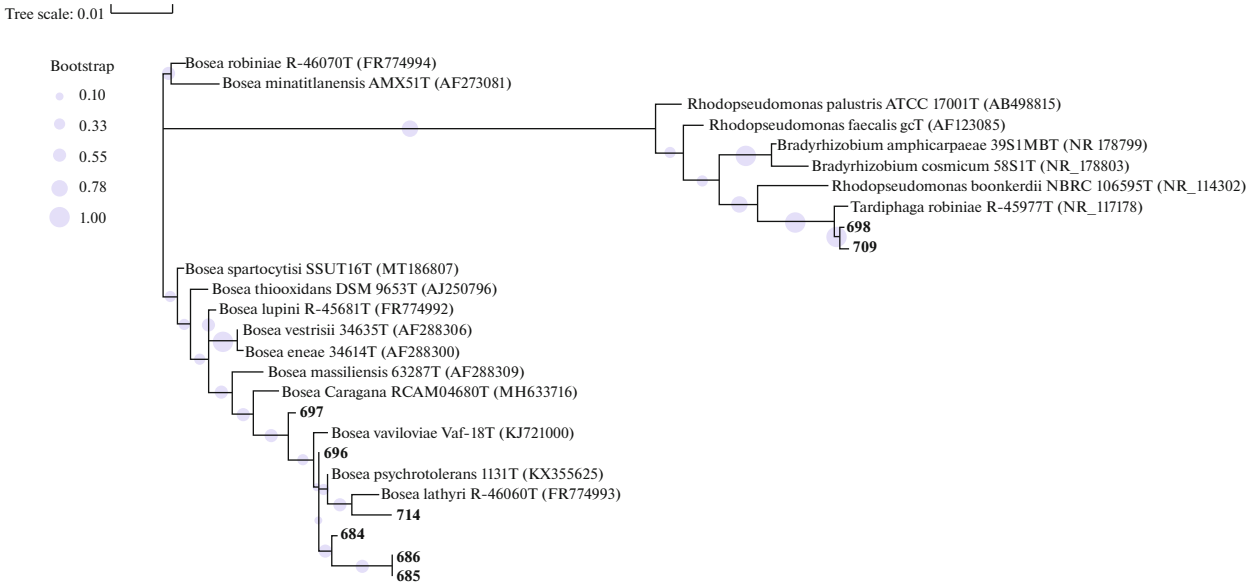

Fig. 2. Phylogenetic tree generated by the maximum composite likelihood method, using 16S rRNA gene sequences of the *Bosea*- and *Tardiphaga*-related isolates from *A. umbellatu*s and *A. inopinatus,* as well as type strains of closely related species.

Table 2. Effects of mono- and co-inoculation of *A. inopinatus* plants with the isolated strains representing different genera of the order *Hyphomicrobiales* in the gnotobiotic plant nodulation assay

Treatment		Number of nodules	Plant biomass, mg			Nitrogenase activity*
bacterial species	strain		shoot	root	totalplant	
Mesorhizobium sp.	703	2.33 ± 0.51abc	32 ± 11.18ab	18.33 ± 5.64ab	50.33 ± 15.31ab	0.77 ± 0.16b
Bosea sp.	704	0c	22.67 ± 8.01b	20.33 ± 6.28ab	43 ± 10.21ab	0.02 ± 0d
Rhizobium sp.	705	3.17 ± 2.13ab	52.17 ± 12.92a	17 ± 2.68ab	69.17 ± 13.6a	0.82 ± 0.16b
Bosea sp. + *Rhizobium* sp.	704 + 705	2.33 ± 1.03abc	38.83 ± 12.02ab	12 ± 0.89b	50.83 ± 12.78ab	0.73 ± 0.16b
Mesorhizobium sp.	708	1.5 ± 0.54bc	45.17 ± 7.1ab	27.5 ± 6.25a	72.67 ± 5.85a	1.1 ± 0.15a
T. robiniae	709	0c	34.83 ± 8.06ab	22 ± 6.16ab	56.83 ± 12.20ab	0.02 ± 0d
Mesorhizobium sp. + *T. robiniae*	708 + 709	4.67 ± 2.33a	45.83 ± 15.6ab	22.17 ± 2.31ab	68 ± 14.43ab	0.86 ± 0.13b
Mesorhizobium sp.	712	0c	38.17 ± 7.46ab	26.17 ± 6.76a	64.33 ± 13.53ab	0.02 ± 0d
Bosea sp.	714	0c	40.67 ± 4.54ab	23.67 ± 3.50ab	64.33 ± 5.20ab	0.02 ± 0d
Mesorhizobium sp.	715	1.17 ± 0.40bc	36.17 ± 2.48ab	15.33 ± 1.03ab	51.5 ± 2.42ab	0.42 ± 0.06c
Bosea sp. + *Mesorhizobium* sp.	714 + 715	1bc	30.17 ± 3.97ab	13.67 ± 3.20ab	43.83 ± 3.18ab	0.23 ± 0.05cd
Control without inoculation	Cont-	0c	19.5 ± 2.42b	15.33 ± 1.96ab	34.83 ± 3.54b	0.02 ± 0d

Data are presented as mean ± standard deviation of a representative experiment ($n = 6$). Different letters indicate significant differences between treatments ($P < 0.001$).
* Shown in nanomoles C_2H_4 per plant per hour

stressor. In addition, inhibitors of ethylene synthesis can significantly reduce the severity of some environmental stresses. To overcome some of the inhibitory effects of ethylene, some rhizobial strains use different mechanisms to reduce ethylene levels, such as the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase; this enzyme is responsible for the cleavage of ACC (the immediate precursor of ethylene in plants) to ammonia and α-ketobutyrate [16]. Many *Rhizobium* spp. and *Mesorhizobium* spp. have been found to possess an *acdSR* gene and produce ACC deaminase under free-living or symbiotic conditions [17–19]. An endophytic plant growth promoting bacterial strain *Bosea minatitlanensis* SSB-9 has also been reported to produce the phytohormone indole-3-acetic acid (IAA), which can stimulate both cell elongation and cell division in host plants [20]. Therefore, it can be assumed that strains 712 *Mesorhizobium* sp. and 714 *Bosea* sp., which did not form nodules under the experimental conditions but increased plant biomass, may have growth stimulating properties. However, further studies are required.

DISCUSSION

As a result, strains of *Mesorhizobium* and *Rhizobium* were isolated, which form a nitrogen-fixing symbiosis with *A. inopinatus*, leading to a significant increase in plant biomass compared to the non-inoculated control. Compared to the control without inoculation, the maximum increase was 79, 167 and 108% for the parameters of root, shoot and total plant biomass, respectively. Besides two non-symbiotic strains, 712 *Mesorhizobium* sp. and 714 *Bosea* sp. may have growth-stimulating properties that require further investigation. These strains may be promising for practical use in cultivation of *A. inopinatus* plants in the northern territories of Siberia. The inoculation of *A. inopinatus* with three pairs of taxonomically different isolates had a multidirectional influence on the symbiosis. The reasons for the observed effects, as well as the possibility of their agricultural application, require further study. Additional phenotypic and genetic analyses, such as housekeeping gene sequencing and multisubstrate analysis using MicroPlate GENIII BioLog, will be required to clarify the taxonomic position of isolates of uncertain species affiliation. The results of the study of symbioses formed by endemic, as well as currently existing relict leguminous plants can be used to elucidate the evolutionary paths of specific plant-microbial relationships. Since the studied *Astragalus* species are valuable fodder plants with high productivity potential in Siberia region, the results obtained may be of great practical importance. An increase in the shoot biomass of inoculated *A. inopinatus* plants in the gnotobiotic nodulation assay by more than 2 times demonstrates the promise of creating biopreparations based on isolated

microsymbionts. Thus, for the first time a collection of *A. inopinatus* and *A. umbellatus* microsymbionts was created and a significant taxonomic diversity of the isolated strains was demonstrated. A total of 28 isolates belonging to 4 genera from order *Hyphomicrobiales* were identified: *Mesorhizobium*, *Rhizobium*, *Bosea* and *Tardiphaga*. The strains isolated from *A. umbellatus* nodules belonged to the genera *Mesorhizobium, Bosea* and *Rhizobium* (*Rhizobiaceae*, *Phyllobacteriaceae* and *Boseaceae* families). Nodules of *A. inopinatus* plants were characterized by a higher taxonomic diversity of microsymbionts and, in addition to strains of the above genera, contained strains of the genus *Tardiphaga*. It is noteworthy that two taxonomically distinct strains of the order *Hyphomicrobiales* are often present in the same nodule of relict and endemic legume species. Typically, one of them is nodule-forming (e.g. *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium*) and the other is not able to form nodules (e.g. *Phyllobacterium*, *Bosea* and *Tardiphaga*). Previously, several pairs of strains (*Bosea* + *Mesorhizobium*, *Phyllobacterium* + *Rhizobiu*m, and *Mesorhizobium* + *Tardiphaga*) were isolated from the root nodules of the far-eastern endemic legumes *Oxytropis erecta* Kom., *O. kamtschatica* Hultén and *O. pumilio* (Pall.) Ledeb. also from the Kamchatka Peninsula region [21]. Pairs of strains belonging to different taxonomic groups (*Bosea* + *Phyllobacterium* and *Mesorhizobium* + *Bradyrhizobium*) were also previously described for nodules of the Miocene-Pliocene relict legume *A. chorinensis* Bunge and the narrowly endemic *A. mongholicus* Bunge from the Lake Baikal region [22, 23]. Subsequent studies of such pairs suggested that taxonomically different strains can be co-microsymbionts infecting the same nodule and promoting the development of symbiosis due to complementary sets of symbiotic genes [24, 25]. Among the isolated strains, both typical and non-typical microsymbionts of legumes were present.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1021443723602100.

FUNDING

This research was funded by the Ministry of Science and Higher Education of the Russian Federation within project "Mobilization of the genetic resources of microorganisms on the basis of the RCAM at the ARRIAM according to the network principle of organization" (agreement nos. 075-15-2021-1055, 28.09.2021).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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