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**THE GENETIC DIVERSITY AND SYMBIOTIC EFFICIENCY
OF THE NODULE MICROSymbionTS ISOLATED FROM
Oxytropis taimyrensis (Jurtz.) A. et D. Love, *Astragalus frigidus* (L.) A. Gray
AND *Astragalus tugarinovii* Basil. FROM ARCTIC YAKUTIA**

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Abstract

Legumes have significant potential for introduction into the Arctic regions of Russia. One of the key characteristics of legumes is the ability to form nitrogen-fixing symbioses with nodule bacteria (rhizobia). However, insufficient attention has been paid to the study of the biodiversity and symbiotic efficiency of Arctic rhizobia in Russia. The present work describes for the first time 13 strains of the order *Hyphomicrobiales* (formerly *Rhizobiales*) isolated from nodules of *Oxytropis taimyrensis*, *Astragalus frigidus* and *A. tugarinovii* growing in Arctic Yakutia. The ability of nine rhizobial strains *Rhizobium* sp. 7/1-1, 19-1/1, 20-1/1 and 33-1/1, *R. giardinii* 20/1-1, *M. norvegicum* 20/1-4 and *Mesorhizobium* sp. 9-4/1, 25-2/1 and 32-2/1 to nodulate the wild Arctic legumes *Oxytropis adamsiana* and *Astragalus frigidus* and the fodder legumes *Trifolium repens* and *Medicago sativa* was investigated under the conditions of a sterile test-tube experiment. The aim of the work was to isolate and study the genetic diversity of strains of the order *Hyphomicrobiales* isolated from nodules of the wild legumes *Oxytropis taimyrensis* (Jurtz.) A. et D. Love, *Astragalus frigidus* (L.) A. Gray and *Astragalus tugarinovii* Basil, collected in the Arctic zone of Yakutia, and to determine the ability of rhizobial strains to form nitrogen-fixing nodules on the roots of fodder and wild legumes *Trifolium repens* L., *Medicago sativa* L., *Oxytropis adamsiana* (Trautv.) Jurtzev and *Astragalus frigidus* (L.) A. Gray under the conditions of a sterile test-tube cross-nodulation experiment. Root nodules of wild populations of *O. taimyrensis*, *A. frigidus*, *A. tugarinovii* were collected in 2021 near the lake Sevastyan-Kyule and on Tit-Ary during the Russian-German expedition to the Lena River Delta. Rhizobial strains were isolated from legume nodules by standard methods using mannitol-yeast YMA nutrient medium. Genomic DNA was isolated from pure cultures using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) and Monarch® (New England Biolabs, USA). The primary identification of the strains was carried out by PCR followed by sequencing of the 16S rRNA marker gene (*rrs*) fragment (900-1400 bp). The ability of nine Arctic strains of the genera *Rhizobium* and *Mesorhizobium* to form nitrogen-fixing nodules on the roots of *Trifolium repens* L., *Medicago sativa* L., *O. adamsiana* and *A. frigidus* was studied under the conditions of sterile test-tube experiment. The strains studied were isolated in the present work and previously from nodules of the Arctic legumes *Lathyrus palustris* L., *Vicia cracca* L. and *Hedysarum arcticum* B. Fedtsch growing in the Lena River delta. Plants were grown in sterile 50 ml glass vessels containing

3 g vermiculite and 6 ml of Krasilnikov-Korenyako medium. Seedlings were inoculated with suspensions of individual strains at 10^6 c.ells/vessel. Commercial strains of *Rhizobium leguminosarum* RCAM1365 and *Sinorhizobium meliloti* RCAM1750 from the Russian Collection of Agricultural Microorganisms (RCAM, ARRIAM, St. Petersburg) were used as positive controls. Non-inoculated plants were used as negative controls. At the end of cultivation, nodules were counted and crude plant biomass was determined. Nitrogen fixation activity was determined by the acetylene method using a GC-2014 gas chromatograph (Shimadzu, Japan). The obtained isolates were assigned to the genera *Rhizobium* (family *Rhizobiaceae*), *Mesorhizobium* (family *Phyllobacteriaceae*), *Bosea* (family *Boseaceae*) and *Tardiphaga* (family *Bradyrhizobiaceae*). The strains *Rhizobium* sp. 7/1-1, *Tardiphaga robiniae* 7/2-2 and 7/4-2 were isolated from *A. tugarinovii*, the strains *Mesorhizobium* sp. 25-2/1, 25A/5-1, *Bosea* sp. 25A/1-3, *B. lathyri* 25A/2-1, *B. psychrotolerans* 25A/2-2 and 25A/4-1 were isolated from *A. frigidus*, whereas the strains *Mesorhizobium* sp. 9-4/1, *T. robiniae* 9/1-5, 9/3-1 and 9/5-1 were isolated from nodules of *O. taimyrensis*. Under sterile test-tube experiments the strain *R. giardinii* 20/1-1 did not form nodules in any of the inoculation variants, whereas the other eight strains were able to form both ineffective and nitrogen-fixing nodules depending on the legume inoculation variant. With respect to the native Arctic species *O. adamsiana* and *A. frigidus*, strains isolated from native legumes (*O. taimyrensis*, *A. frigidus*, *H. arcticum*) were more active, whereas the cultivated plants *M. sativa* and *T. repens* were more responsive to inoculation with strains isolated from introduced plants *L. palustris* and *V. cracca*. The ability of *O. adamsiana* to form an effective symbiosis with *M. norvegicum* 20/1-4, *Mesorhizobium* sp. 9-4/1 and 25-2/1, isolated from representatives of the legume genera *Hedysarum*, *Oxytropis* and *Astragalus*, respectively, indicates a low host specificity of this plant species, which may presumably allow it to use the resource potential of a wide range of symbiotic microorganisms inhabiting the soil of various Arctic regions. The *O. adamsiana* species is widespread in the Far North and is an important high-protein component of the diet of local animals, which makes it a promising species for the establishment of perennial pasture and hay phytocenoses in extreme Arctic conditions.

Keywords: Arctic Yakutia, legumes, pasture and hay agrophytocenoses, legume-rhizobial symbiosis, nitrogen-fixing nodule bacteria

Recently, more and more attention has been paid to the agriculture in the Arctic zone of the Russian Federation [1, 2]. Sustainable development of livestock farming and forage production based on local biological resources adapted to the complex soil and climatic conditions of the Arctic will create new jobs and provide the local population with high-quality and fresh food products, which will contribute to increasing the attractiveness of the northern Russia territories [3]. I.S. Khantimer [4] reported about high-productive perennial meadows in the Bolshzemelskaya tundra to provide forage for a livestock farm located in the vicinity of the city of Vorkuta.

The key high-protein component of natural hayfields and pastures are legumes widespread from the Arctic to the Antarctic islands [5]. For example, creeping clover (*Trifolium repens* L.) is one of the most productive pasture forage plants, significantly improving soil fertility and structure. Cold-resistant varieties of this species are bred specifically for cultivation in cold regions [6]. *Medicago sativa* L. possesses high ecological plasticity, resistance to adverse conditions and relatively high winter hardiness. It is used in grass mixtures with perennial cereal grasses to create cultivated pastures and restore degraded soils [7]. Plants of the genera *Oxytropis* DC. and *Astragalus* L. are widespread in the temperate and arctic zones of the Northern Hemisphere where they serve as feed for wild and farm animals and birds [8, 9]. Thus, in the tundra zone of Eastern Siberia, there are *Oxytropis taimyrensis* (Jurtz.) A. et D. Love and *Astragalus tugarinovii* Basil. which grow mainly on steppe slopes, screes and in dry sparse larch forests [10, 11]. The species *O. adamsiana* (Trautv.) Jurtzev is widespread in Taimyr and Northern Yakutia, grows in the tundra, sparse forests and in the highlands where it is an important component of the diet of herbivorous animals [8, 10-12]. The species *A. frigidus* (L.) A. Gray is a cold-resistant plant with a high protein content widespread beyond the Arctic Circle, it is recommended for use as a forage and medicinal crop. In pastures, it is well eaten by farm animals and reindeer [8]. It has been shown that legumes in the composition of grass mixtures of pastures and hayfields help to solve the problem of forage protein deficiency and ensures economical use of

resources in forage production and agriculture in the European North of Russia [13-15].

A key factor for the effective cultivation of legumes is their microsymbionts which can participate in the nutrition of host plants, protection from pathogens and phytophages, regulation of growth and adaptation to stress conditions. One of the main limiting factors for the distribution and productivity of plants in the Far North is the insufficient soil level of nitrogen compounds easily accessible to plants [16].

Symbiotic nodule bacteria (rhizobia), fixing atmospheric nitrogen, ensure a stable supply of plants with this essential biogenic element. Many legumes enter into symbiosis only with certain types of microorganisms. The main microsymbionts of plants of the genus *Trifolium* (tribe *Trifolieae* Endl.) are strains of the species *Rhizobium leguminosarum* bv. *Trifolii* [17]. However, bacteria atypical for this genus, related to the species *Rhizobium* and *Agrobacterium*, are also isolated from the root nodules of various *Trifolium* species [18, 19]. A cross-inoculation group of alfalfa legumes *Medicago* L. and members of the genera *Melilotus* (L.) Mill. and *Trigonella* L. form a nitrogen-fixing symbiosis with strains *Ensifer meliloti* (formerly *Sinorhizobium meliloti*), however, other genera, e.g., *Agrobacterium*, may also be present in their nodules [20]. It has been shown that microsymbionts of *Astragalus* plants may belong to the genera *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Bosea* [21-24]. For *Oxytropis* plants, typical microsymbionts the genus *Mesorhizobium* species, but *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Bosea*, and *Tardiphaga* are also found [21, 22, 25].

Legume-rhizobial interactions possess a complex multicomponent structure. Their ecological and practical role is due to the formation of mutually beneficial plant-microbe symbiosis which affects the crop quality and soil properties in any climatic zone. Therefore, the study of the biodiversity of Arctic rhizobia is important for the Far North agriculture.

In this paper, 13 strains of the order *Hyphomicrobiales* (formerly *Rhizobiales*) isolated from nodules of *Oxytropis taimyrensis*, *Astragalus frigidus* and *A. tugarinovii*, growing in Arctic Yakutia are described for the first time. The ability of nine rhizobial strains *Rhizobium* sp. 7/1-1, 19-1/1, 20-1/1 and 33-1/1, *R. giardinii* 20/1-1, *M. norvegicum* 20/1-4 and *Mesorhizobium* sp. 9-4/1, 25-2/1 and 32-2/1 to nodulate wild arctic legumes *Oxytropis adamsiana* and *Astragalus frigidus* and forage legumes *Trifolium repens* and *Medicago sativa* was studied in pot experiment.

The aim of the work was isolation of the order *Hyphomicrobiales* strains from nodules of wild legumes *Oxytropis taimyrensis* (Jurtz.) A. et D. Love, *Astragalus frigidus* (L.) A. Gray and *Astragalus tugarinovii* Basil. collected in the Arctic zone of Yakutia to study the genetic diversity of rhizobial strains and to identify their ability to form nitrogen-fixing nodules on the roots of forage and wild legumes *Trifolium repens* L., *Medicago sativa* L., *Oxytropis adamsiana* (Trautv.) Jurtzev and *Astragalus frigidus* (L.) A. Gray in the cross-nodulation test.

Materials and methods. During the Russian-German expedition to the Lena River delta in 2021, root nodules and seeds of wild populations of legumes *O. taimyrensis*, *A. frigidus* and *A. tugarinovii* were collected in the vicinity of the Lake Sevast'yan-Kyule (71°31'37.3" N 128°49'20.1" E) and on Tit-Ary Island (71°57'32.7" N 127°05'53.3" E).

Strains were isolated using the YMA mannitol-yeast nutrient medium (Novikova and Safronova, 1992) after sterilization of the nodules for 1 min in 96% ethanol. Pure cultures of isolates, after sequential double cloning, were deposited

for long-term storage in a low-temperature automated storage station for biological samples at $-80\text{ }^{\circ}\text{C}$ (Liconic Instruments, Liechtenstein). Information about strains has been entered into the Internet database of the Network Bioresource Collection for Genetic Technologies for Agriculture, ARRIAM (<https://arriam.ru/kollekciya-kultur1/>).

Genomic DNA from pure cultures was isolated using the DNeasy Blood&Tissue kit (QIAGEN N.V., Germany) and Monarch® kit (New England Biolabs, USA). Primary identification of the strains was carried out by PCR followed by sequencing of the 16S rRNA marker gene fragment (900-1400 bp). The primers fD1 5'-AGAGTTTGATCCTGGCTCAG-3' and rD1 5'-AAGGAGGT-GATCCAGCC-3' [26] were used for amplification. The PCR protocols were as follows: 3 min 30 s at $95\text{ }^{\circ}\text{C}$ (primary denaturation); 1 min 10 s at $94\text{ }^{\circ}\text{C}$ (denaturation), 40 s at $56\text{ }^{\circ}\text{C}$ (primer annealing), 2 min 10 s at $72\text{ }^{\circ}\text{C}$ (elongation) (35 cycles); 6 min 10 s at $72\text{ }^{\circ}\text{C}$ (final elongation) (a T100 Thermal Cycler, Bio-Rad, USA). The PCR reaction mixture contained 38 μl milli-Q H₂O (ZAO Eurogen, Russia), 5 μl buffer (OOO Helikon Company, Russia), 5 μl dNTP kit (ZAO Eurogen, Russia), 0.5 μl primers (Eurogen, Russia), 0.5 μl Taq polymerase (OOO Helikon Company, Russia), and 1 μl (50-100 ng) mDNA. The amount of DNA was estimated visually by electrophoresis in 1.0% agarose gel in $0.5\times$ TAE buffer with the MassRuler molecular weight marker (Fermentas, Lithuania). The PCR product was purified from agarose gel using the Cleanup S-Cap kit (ZAO Eurogen, Russia).

The purified DNA was sequenced (an ABI PRISM 3500xl genetic analyzer, Life Technologies, USA) at the Center for Collective Use Genomic Technologies, Proteomics, and Cell Biology ARRIAM. The sequences were analyzed in the ChromasLite 2.6.4 program (<https://technelysium.com.au/wp/chromas/>). For multiple alignment and comparison of nucleotide sequences, the ClustalOmega program was used (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The sequences of closely related type strains were searched in the GenBank database (<https://www.ncbi.nlm.nih.gov/>). The nucleotide sequences were deposited in GenBank under the accession numbers PP512682-PP512693, PP578216.

In cross-nodulation test, seeds of wild Arctic (*A. frigidus* and *O. adamsiana*) and forage (*T. repens* and *M. sativa*) legumes were used. Nine bacterial strains isolated in this work and earlier from nodules of Arctic legumes *Lathyrus palustris* L., *Vicia cracca* L. and *Hedysarum arcticum* B. Fedtsch. [27] were tested.

The plant seeds were scarified and surface sterilized in 98% H₂SO₄ for 5 min, thoroughly washed with sterile tap water and germinated on filter paper in Petri dishes at $25\text{ }^{\circ}\text{C}$ in the dark for 3-5 days, depending on the plant species. The plants were grown in sterile 50 ml glass flasks with 3 g vermiculite, 6 ml Krasilnikov-Korenyako medium (K₂HPO₄ 1.0 g/l, MgSO₄·7H₂O 1.0 g/l, Ca₃(PO₄)₂ 0.2 g/l, FeSO₄ 0.02 g/l), a 1 ml mixture of microelements according to Fedorov containing (g/l) H₃BO₃ 0.05, (NH₄)₂MoO₄ 0.05, KCl 0.005, NaBr 0.005, ZnSO₄·7H₂O 0.003, MnSO₄ 0.002.

Test tubes with 2 seedlings were inoculated with suspension of individual strains, 10^6 cells per tube, four to five replicates depending on the quality and rate of seed germination in a particular plant species. Positive controls were the commercial strains *Rhizobium leguminosarum* RCAM1365 and *Sinorhizobium meliloti* RCAM1750 from the the Network Bioresource Collection for Genetic Technologies for Agriculture, ARRIAM (St. Petersburg), negative controls were non-inoculated plants.

The plants were grown for 30 days in a phytotron at $18-22\text{ }^{\circ}\text{C}$, 50% relative humidity and a four-level illumination/temperature regime: night ($18\text{ }^{\circ}\text{C}$, 8 h),

morning ($200 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, $20 \text{ }^\circ\text{C}$, 2 h), day ($400 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, $23 \text{ }^\circ\text{C}$, 12 h), evening ($200 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, $20 \text{ }^\circ\text{C}$, 2 h). Illumination was provided by L36W/77 FLUORA lamps (OSRAM Licht AG, Germany). After the end of cultivation, the raw biomass of the plants was weighed and the nodules were counted. The nitrogen-fixing activity of nodules was determined by the acetylene method using a GC-2014 gas chromatograph (Shimadzu, Japan).

The data were processed using the standard one-way analysis of variance (ANOVA) method in the R statistical environment (v. 4.2.3; R Core Team, 2023). The mean values (M) and standard deviations ($\pm\text{SD}$) were calculated for each of the measured symbiotic parameters in each inoculation variant. In case of statistically significant ANOVA results ($p < 0.05$), the Duncan's test for multiple comparisons of means was performed using the agricolae package.

Results. The Arctic Lake Sevastyan-Kyule (Fig. 1) is located in the subzone of typical tundra 10 km south of the Tiksi village among the low mountains of the Primorsky Ridge in the area of permafrost soils. The soils are low-power gravelly cryozems. The vegetation around the lake is tundra of various types, from dryad and fescue tundra in drained areas to marshy tundra in depressions near the lake with a predominance of dwarf shrub-green moss tundra. Over large areas, there are mobile fine-grained screes with original weakly closed plant communities. The climate is severe with only 2-month summer and an average July temperature of $11 \text{ }^\circ\text{C}$ [28].

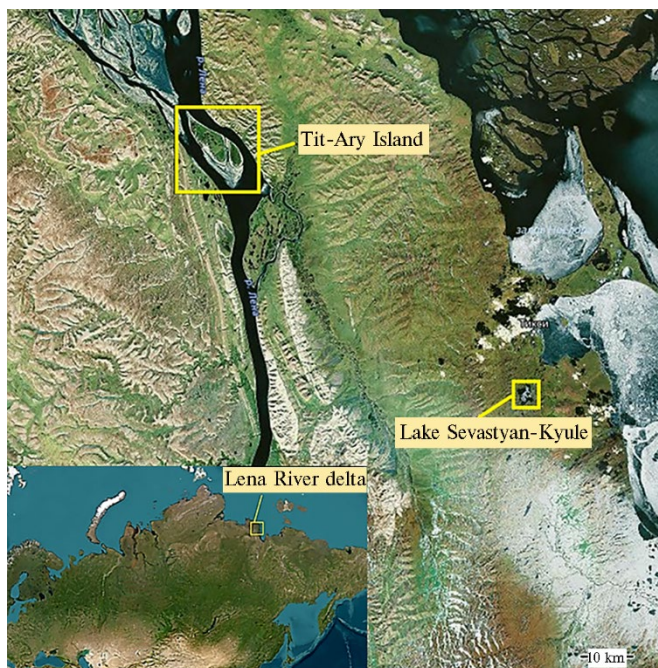


Fig. 1. Sites of sampling plants *Oxytropis taimyrensis* (Jurtz.) A. et D. Love, *Astragalus frigidus* (L.) A. Gray and *Astragalus tugarinovii* Basil. on Tit-Ary Island (Lena River delta) and in the area of Lake Sevastyan-Kyule (near Tiksi).

Tit-Ary Island (see Fig. 1) located in the southern part of the Lena Delta and is formed mainly by sandy alluvial deposits. It is one of the largest islands of the Lower Lena and the northernmost forest area in Eastern Siberia. The island's vegetation is sparse larch forests combined with shrub tundra communities, alder forests and hygrophilous grasslands. The territory is part of the Ust-Lensky State Nature Reserve and belongs to the Lena-Delta resource reserve [29, 30].

1. Isolates from nodules of *Astragalus tugarinovii* Basil., *Oxytropis taimyrensis* (Jurtz.) A. et D. Love and *Astragalus frigidus* (L.) A. Gray plants sampled in the area of Lake Sevastyan-Kyule and on Tit-Ary Island (2021)

Sites	Strain No.	Closest typical strain	The <i>rrs</i> gene similarity, %	Identification
<i>Astragalus tugarinovii</i>				
Lake Sevastyan-Kyule	7/1-1	<i>R. brockwellii</i> CC275e	100	<i>Rhizobium</i> sp.
		<i>R. sophorae</i> LMG 27901		
		<i>R. anhuiense</i> CCBAU 23252		
	7/2-2	<i>R. indicum</i> JKLM 12A2	99.93	<i>Tardiphaga robiniae</i>
	7/4-2	<i>T. robiniae</i> R-45977	99.93	<i>Tardiphaga robiniae</i>
<i>Oxytropis taimyrensis</i>				
Lake Sevastyan-Kyule	9/1-5	<i>T. robiniae</i> R-45977	99.93	<i>Tardiphaga robiniae</i>
	9/3-1	<i>T. robiniae</i> R-45977	99.93	<i>Tardiphaga robiniae</i>
	9-4/1	<i>M. jarvisii</i> ATCC 33669	99.93	<i>Mesorhizobium</i> sp.
		<i>M. carmichaelinearum</i> MonP1N1	99.92	
	9/5-1	<i>T. robiniae</i> R-45977	99.93	<i>Tardiphaga robiniae</i>
<i>Astragalus frigidus</i>				
Tit-Ary Island	25A/1-3	<i>B. lathyri</i> R-46060	99.13	<i>Bosea</i> sp.
	25-2/1	<i>M. shangrilense</i> CCBAU 65327	99.85	<i>Mesorhizobium</i> sp.
		<i>M. qingshengii</i> CCBAU 33460		
	25A/2-1	<i>B. lathyri</i> R-46060	100	<i>Bosea lathyri</i>
	25A/2-2	<i>B. psychrotolerans</i> 1131	99.93	<i>Bosea</i> sp.
		<i>B. vaviloviae</i> Vaf18	99.86	
	25A/4-1	<i>B. psychrotolerans</i> 1131	100	<i>Bosea</i> sp.
		<i>B. vaviloviae</i> Vaf18	99.78	
	25A/5-1	<i>M. shangrilense</i> CCBAU 65327	99.92	<i>Mesorhizobium</i> sp.
		<i>M. qingshengii</i> CCBAU 33460		

Four, three and six bacterial strains were isolated from the nodules of *O. taimyrensis*, *A. tugarinovii* and *A. frigidus* collected in the area of Lake Sevastyan-Kyule and on Tit-Ary Island. Strains 7/1-1, 9-4/1, 25-2/1 and 25A/5-1 formed colonies on days 3-4, the remaining strains on days 5-6. Based on the *rrs* gene analysis, the isolates were assigned to the genera *Rhizobium* (*Rhizobiaceae*), *Mesorhizobium* (*Phyllobacteriaceae*), *Bosea* (*Boseaceae*) and *Tardiphaga* (*Bradyrhizobiaceae*) of the order *Hypomicrobiales* (Table 1).

Strain 7/1-1 from the nodule of *A. tugarinovii* showed 100% similarity in the *rrs* gene with nine typical strains *R. brockwellii* CC275e, *R. sophorae* LMG 27901, *R. indigoferae* CIP 108029, *R. anhuiense* CCBAU 23252, *R. indicum* JKLM 12A2, *R. ruizarguesonis* UPM1133, *R. laguerreae* FB206, *R. leguminosarum* LMG 14904, *R. leguminosarum* bv. *viciae* USDA 2370 at once. The species affiliation of strain 7/1-1 will be determined by sequencing and analysis of its complete genome.

Strain 9-4/1 from a nodule of *O. taimyrensis* showed more than 99.9% similarity in the *rrs* gene to the type strains *M. jarvisii* ATCC 33669 and *M. carmichaelinearum* MonP1N1. Strain *M. jarvisii* ATCC 33669 was first isolated from nodules of *Lotus corniculatus* L. [31], while strain *M. carmichaelinearum* MonP1N1 from nodules of *Montigena novae-zelandiae* (Hook.f.) Heenan, growing on the South Island of New Zealand [32].

Strains 25-2/1 and 25A/5-1 from *A. frigidus* nodules exerted the highest similarity in the *rrs* gene of more than 99.85% to the type strains *M. shangrilense* CCBAU 65327 and *M. qingshengii* CCBAU 33460 from the nodule of *Caragana bicolor* Kom. and *A. sinicus* L., respectively [33, 34].

Strains 25A/1-3, 25A/2-1, 25A/2-2, and 25A/4-1 from *A. frigidus* nodules were assigned to the genus *Bosea* based on the *rrs* gene analysis. Strains 25A/1-3 and 25A/2-1 showed 99.13 and 100% similarity, respectively, to the closest type strain *B. lathyri* R-46060 isolated from a nodule of broadleaf vetch (*L. latifolius* L.) growing in Belgium [35]. Strains 25A/2-2 and 25A/4-1 were related at the 99.86-100% similarity level to the type strains *B. psychrotolerans* 1131 and *B. vaviloviae* Vaf18. Strain *B. psychrotolerans* 1131 was isolated from the water of Lake Michigan (USA) and is described as a psychrotrophic representative of the *Prote-*

obacteria, while *B. vaviloviae* Vaf18 was isolated from a nodule of *Vavilovia formosa* (Steven) Fed., growing in the North Caucasus (the Russian Federation) [36, 37]. It should be noted that bacteria of the genus *Bosea* are found in the nodules of various legume species, e.g., *Lupinus*, *Lathyrus*, *Robinia*, *Vavilovia*, *Caragana*, *Spartocytisus*, *Vicia*, *Astragalus*, *Oxytropis*, *Hedysarum* [24, 26, 35-40]. However, the ability of *Bosea* strains to independently form nodules has not yet been established.

Isolates 7/2-2, 7/4-2 and 9/1-5, 9/3-1, 9/5-1 from *A. tugarinovii* and *O. taimyrensis*, respectively, showed 99.93% similarity in the *rrs* gene to the closest type strain *T. robiniae* R-45977. Members of the species *T. robiniae* are found in nodules of local endemics of the genus *Oxytropis*, the relict plant *Vavilovia formosa* (Steven) Fed., *Robinia pseudoacacia* L. [25, 41], and in nodules of other legume species [24, 26, 40]. It has been shown that the genomes of some *T. robiniae* strains may contain individual symbiotic genes (*nod*, *nif* and *fix*) [25].

The ability of nine rhizobial strains *Rhizobium* sp. 7/1-1, 19-1/1, 20-1/1 and 33-1/1, *R. giardinii* 20/1-1, *M. norvegicum* 20/1-4 and *Mesorhizobium* sp. 9-4/1, 25-2/1 and 32-2/1 to nodulate wild arctic legumes *O. adamsiana* and *A. frigidus* (Table 2) and forage legumes *T. repens* and *M. sativa* (Table 3) was studied in sterile pot experiments.

2. Effect of *Oxytropis adamsiana* (Trautv.) Jurtzev and *Astragalus frigidus* (L.) A. Gray inoculation with Arctic rhizobial isolates (sterile pot test, $M \pm SD$)

Inoculation	Number of nodules per plant	Raw biomass, mg/plant			Acetylene reductase activity, $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{day}^{-1}$
		shoot	root	total mass	
<i>Oxytropis adamsiana</i> (n = 4)					
Not inoculated	0	33.2±8.6	17.7±4.4 ^{abcd}	50.8±10.6	0
<i>Rhizobium</i> sp. 19-1/1*	0	42.4±11.4	26.2±10.9 ^{ab}	68.6±13.5	n/d
<i>Rhizobium</i> sp. 33-1/1*	0	45.9±7.7	27.9±5.9 ^a	73.8±11.3	n/d
<i>Rhizobium</i> sp. 20-1/1*	0	40.2±12.6	22.0±7.4 ^{abcd}	62.2±19.4	n/d
<i>Mesorhizobium</i> sp. 32-2/1*	0	28.5±7.8	14.8±2.0 ^d	43.3±7.9	n/d
<i>R. giardinii</i> 20/1-1**	0	40.6±6.8	15.5±4.5 ^{cd}	56.1±10.4	n/d
<i>Mesorhizobium</i> sp. 9-4/1***	2.0±0.0 ^b	43.7±19.3	17.9±5.3 ^{abcd}	62.4±21.4	0.6±0.12 ^a
<i>Mesorhizobium</i> sp. 25-2/1***	4.8±0.8 ^a	44.7±5.2	24.5±6.8 ^{abc}	69.2±6.3	0.6±0.24 ^a
<i>Rhizobium</i> sp. 7/1-1***	0	44.7±13.4	15.2±4.0 ^{cd}	59.8±12.3	n/d
<i>M. norvegicum</i> 20/1-4**	4.0±1.4 ^a	41.0±4.0	16.7±3.9 ^{bcd}	57.7±8.2	0.6±0.18 ^a
<i>Astragalus frigidus</i> (n = 5)					
Not inoculated	0	51.2±2.2 ^{ab}	14.2±4.9 ^c	65.4±5.8 ^{bc}	0
<i>Rhizobium</i> sp. 19-1/1*	0	41.0±7.4 ^{bc}	21.8±4.9 ^{bc}	62.8±8.0 ^{bc}	n/d
<i>Rhizobium</i> sp. 33-1/1*	0	26.0±8.9 ^e	36.0±15.2 ^a	62.0±21.7 ^{bc}	n/d
<i>Rhizobium</i> sp. 20-1/1*	0	54.6±7.1 ^a	33.2±9.3 ^{ab}	87.8±4.4 ^a	n/d
<i>Mesorhizobium</i> sp. 32-2/1*	0	42.0±8.4 ^{bc}	28.0±8.4 ^{ab}	70.0±15.8 ^b	n/d
<i>R. giardinii</i> 20/1-1**	0	24.0±5.5 ^e	22.2±7.4 ^{bc}	46.2±10.6 ^c	n/d
<i>Mesorhizobium</i> sp. 9-4/1***	1.8±0.4 ^a	30.0±10.0 ^{de}	23.0±6.7 ^{bc}	53.0±13.0 ^{bc}	0.1±0.02 ^b
<i>Mesorhizobium</i> sp. 25-2/1***	1.8±0.4 ^a	30.0±7.1 ^{de}	22.0±4.5 ^{bc}	52.0±4.5 ^{bc}	0.2±0.02 ^a
<i>Rhizobium</i> sp. 7/1-1***	0	20.0±7.1 ^e	26.0±5.5 ^{abc}	46.0±5.5 ^c	n/d
<i>M. norvegicum</i> 20/1-4**	0.8±0.4 ^b	38.0±10.4 ^{cd}	23.0±9.7 ^{bc}	61.0±18.8 ^{bc}	0

Note. n/d — not determined; * — strains previously studied for the ability to form nitrogen-fixing nodules on forage legumes *V. cracca*, *V. sativa*, *L. sativus* and *L. pratensis* [22]; ** — strains previously isolated from nodules of the Arctic wild legume *H. arcticum* [42]; *** — strains isolated in the present work.

a, b, c, d, e Different Latin letters mark the variants, the differences between which are statistically significant (Duncan's test, $p < 0.05$). Without letter ranking, statistically significant group differences were not revealed ($p > 0.05$) and Duncan's test was not performed.

Previously, strains *Rhizobium* sp. 19-1/1 and 33-1/1, *Rhizobium* sp. 20-1/1 and *Mesorhizobium* sp. 32-2/1, *R. giardinii* 20/1-1 and *M. norvegicum* 20/1-4 were isolated from nodules of arctic wild legumes, the marsh pea (*L. palustris*), mouse vetch (*V. cracca*) and arctic sweetvetch (*H. arcticum*), respectively, collected in the Lena River delta and the vicinity of the Tiksi village in the Arctic Yakutia [27, 42]. Strains *Mesorhizobium* sp. 32-2/1 and *Rhizobium* sp. 19-1/1, 20-1/1 and 33-1/1 can form predominantly non-nitrogen-fixing nodules on the roots of agricultural legumes *V. cracca*, *V. sativa*, *L. sativus* and *L. pratensis* [27]. For two strains *R. giardinii* 20/1-1 and *M. norvegicum* 20/1-4, the species affiliation was established by molecular phylogenetic methods [42].

3. Effect of *Trifolium repens* L. and *Medicago sativa* L. inoculation with Arctic rhizobial isolates and commercial strains *Rhizobium leguminosarum* bv. *viciae* RCAM1365 and *Sinorhizobium meliloti* RCAM1750 (sterile pot test, $M \pm SD$)

Inoculation	Number of nodules per plant	Raw biomass, mg/plant			Acetylene reductase activity, $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{day}^{-1}$
		shoot	root	total mass	
<i>Trifolium repens</i> (n = 5)					
Not inoculated	0	47.6 \pm 4.3 ^{ab}	46.0 \pm 19.5 ^{bc}	93.6 \pm 23.1	0
<i>R. leguminosarum</i> bv. <i>viciae</i> RCAM1365	6.6 \pm 1.8 ^b	58.0 \pm 8.4 ^a	29.0 \pm 8.9 ^c	87.0 \pm 16.4	0.9 \pm 0.5
<i>Rhizobium</i> sp. 19-1/1*	5.4 \pm 1.5 ^b	53.0 \pm 14.8 ^a	60.0 \pm 12.2 ^{abc}	113.0 \pm 24.4	0
<i>Rhizobium</i> sp. 33-1/1*	5.4 \pm 1.1 ^b	43.6 \pm 8.6 ^{ab}	50.0 \pm 18.7 ^{ab}	93.6 \pm 24.0	0
<i>Rhizobium</i> sp. 20-1/1*	2.2 \pm 0.8 ^c	44.0 \pm 7.1 ^{ab}	45.0 \pm 11.2 ^{bc}	89.0 \pm 13.4	0
<i>Mesorhizobium</i> sp. 32-2/1*	3.0 \pm 1.0 ^c	50.0 \pm 15.2 ^a	46.0 \pm 18.2 ^{bc}	96.0 \pm 20.7	0
<i>R. giardinii</i> 20/1-1	0	54.0 \pm 20.7 ^a	52.0 \pm 25.9 ^{abc}	106.0 \pm 42.2	n/d
<i>Mesorhizobium</i> sp. 9-4/1	0	44.0 \pm 5.5 ^{ab}	64.0 \pm 23.0 ^{ab}	108.0 \pm 22.8	n/d
<i>Mesorhizobium</i> sp. 25-2/1	0	32.0 \pm 13.0 ^b	70.0 \pm 12.2 ^{ab}	102.0 \pm 21.7	n/d
<i>Rhizobium</i> sp. 7/1-1	0	44.0 \pm 8.9 ^{ab}	76.0 \pm 18.2 ^a	120.0 \pm 22.4	n/d
<i>M. norvegicum</i> 20/1-4**	0	44.0 \pm 11.4 ^{ab}	58.0 \pm 21.7 ^{ab}	102.0 \pm 26.8	n/d
<i>Medicago sativa</i> (n = 5)					
Not inoculated	0	42.0 \pm 8.4 ^{ab}	57.6 \pm 18.4	99.6 \pm 16.5	0
<i>S. meliloti</i> RCAM1750	9.0 \pm 3.4 ^a	48.8 \pm 19.6 ^a	40.4 \pm 8.0	89.2 \pm 25.2	0.9 \pm 0.4
<i>Rhizobium</i> sp. 19-1/1*	2.0 \pm 0.7 ^c	38.0 \pm 11.0 ^{abc}	66.4 \pm 20.3	104.4 \pm 26.7	0
<i>Rhizobium</i> sp. 33-1/1*	2.4 \pm 0.5 ^c	36.0 \pm 11.9 ^{abc}	48.6 \pm 17.5	84.6 \pm 22.6	0
<i>Rhizobium</i> sp. 20-1/1*	2.4 \pm 0.4 ^c	26.0 \pm 15.2 ^{bc}	46.4 \pm 20.1	72.4 \pm 33.6	0
<i>Mesorhizobium</i> sp. 32-2/1*	0	26.0 \pm 5.5 ^{bc}	44.0 \pm 13.4	70.0 \pm 18.7	n/d
<i>R. giardinii</i> 20/1-1**	0	24.0 \pm 4.2 ^c	31.8 \pm 16.2	55.8 \pm 14.8	n/d
<i>Mesorhizobium</i> sp. 9-4/1***	0	33.0 \pm 14.8 ^{abc}	46.0 \pm 17.1	79.0 \pm 26.6	n/d
<i>Mesorhizobium</i> sp. 25-2/1***	5.2 \pm 1.3 ^b	38.0 \pm 11.0 ^{abc}	50.0 \pm 15.8	88.0 \pm 25.9	0
<i>Rhizobium</i> sp. 7/1-1***	3.2 \pm 0.4 ^c	36.0 \pm 11.4 ^{abc}	52.0 \pm 19.2	88.0 \pm 17.9	0
<i>M. norvegicum</i> 20/1-4**	0	28.0 \pm 4.5 ^{bc}	52.0 \pm 19.2	80.0 \pm 17.3	n/d

Note. n/d — not determined. * — strains previously studied for the ability to form nitrogen-fixing nodules on forage legumes *V. cracca*, *V. sativa*, *L. sativus* and *L. pratensis* [22]; ** — strains previously isolated from nodules of the Arctic wild legume *H. arcticum* [42]; *** — strains isolated in this study.
a, b, c, d, e Different Latin letters indicate variants whose differences are statistically significant (Duncan's test, $p < 0.05$). Without letter ranking, statistically significant group differences were not detected ($p > 0.05$) and Duncan's test was not performed.

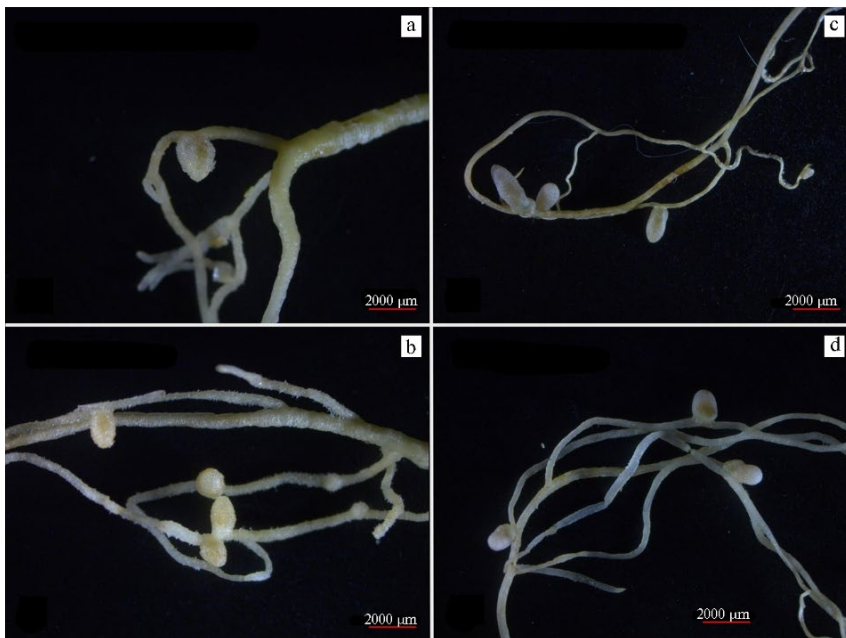


Fig. 2. Nodules on the roots of *Astragalus frigidus* (L.) A. Gray (a, b) and *Oxytropis adamsiana* (Trautv.) Jurtzev (c, d) upon plant inoculation with strains *Mesorhizobium* sp. 9-4/1 (b, d) and 25-2/1 (a, c) (pot test).

Effective nodules (Fig. 2, c, d) were formed on *O. adamsiana* plants upon inoculation with *Mesorhizobium* sp. strains 9-4/1, 25-2/1 and *M. norvegicum* 20/1-4.

Despite the same nitrogen-fixing activity, the number of nodules varied in the variants. The average shoot weight and total fresh weight of most inoculated plants were higher compared to not inoculated, however, we did not identify statistically significant group differences between the variants (see Table 2). It should be noted that *Mesorhizobium* sp. strains 9-4/1, 25-2/1 and *M. norvegicum* 20/1-4 were isolated from nodules of *O. taimyrensis*, *A. frigidus* and *H. arcticum*, respectively, indicating a broad specificity of *O. adamsiana* plants which presumably allows this species to better adapt to various Arctic soil and climatic conditions. Interestingly, the inoculation with *Rhizobium* sp. 33-1/1 provides the highest symbiotic parameters, e.g., total raw biomass, shoot and root mass, although this strain did not form nodules on *O. adamsiana*. For explanation, it is necessary to assess the ability of *Rhizobium* sp. 33-1/1 to produce auxin and cytokinin phytohormones which affect the susceptibility of the plant root system.

On *A. frigidus*, nodules (see Fig. 2, a, b) were formed upon inoculation with *M. norvegicum* 20/1-4, *Mesorhizobium* sp. 25-2/1 and 9-4/1, whereas symbiosis was effective only in the last two cases (see Table 2). In most variants, the average shoot mass was significantly ($p < 0.05$) lower compared to the control without inoculation. However, with *Rhizobium* sp. 20-1/1, 33-1/1 and *Mesorhizobium* sp. 32-2/1, the average root mass was higher ($p < 0.05$) compared to the uninoculated control. With the strain *Rhizobium* sp. 20-1/1, the average total plant weight was also significantly ($p < 0.05$) higher compared to other variants of inoculation.

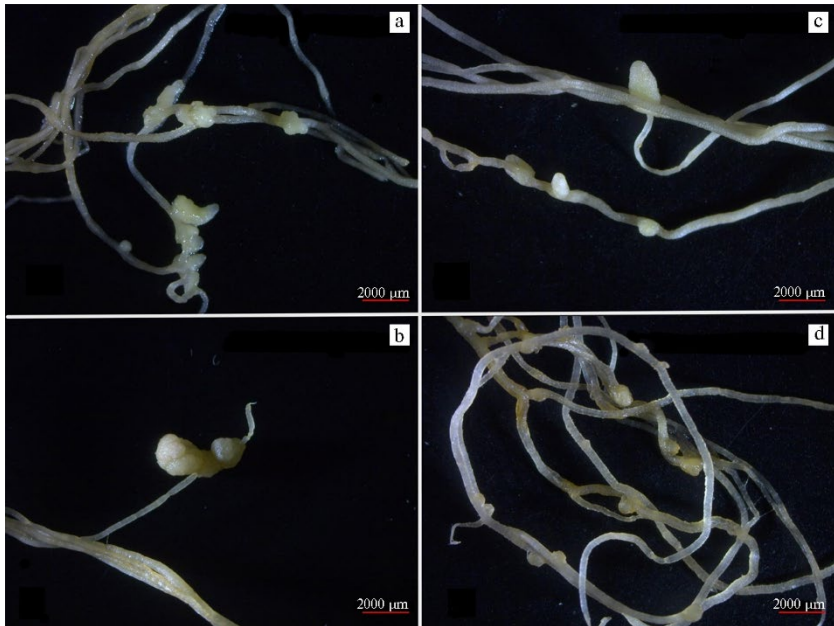


Fig. 3. Nodules and nodule-like formations on the roots of *Medicago sativa* L. (a, b) and *Trifolium repens* L. (c, d) upon inoculation with *Rhizobium* sp. strains 20-1/1 (a), 19-1/1 (c), and 33-1/1 (b, d) (pot test).

On *T. repens*, ineffective nodules (Fig. 3, c, d) appeared upon inoculation with the strains *Mesorhizobium* sp. 32-2/1, *Rhizobium* sp. 19-1/1, 33-1/1 and 20-1/1 (see Table 3). Upon inoculation with *Mesorhizobium* sp. 32-2/1 and *Rhizobium* sp. 20-1/1, the average number of nodules was significantly ($p < 0.05$) less vs. the commercial strain *R. leguminosarum* RCAM1365. Note that with *Rhizobium* sp. 7/1-1, the average root weight was significantly higher, whereas the average value of total plant biomass was insignificantly greater compared to the positive control. With *Mesorhizobium* sp. 25-2/1 the average shoot weight was also greater than with

R. leguminosarum RCAM1365 ($p < 0.05$).

In *M. sativa*, ineffective nodules were formed with the strains *Mesorhizobium* sp. 25-2/1 and *Rhizobium* sp. 19-1/1, 33-1/1, 20-1/1 and 7/1-1. In addition to typical nodules, nodule-like root formations appeared in some variants of inoculation (see Fig. 3, a, b). With the strain *Rhizobium* sp. 19-1/1, the average root weight and total biomass was greater compared to the other isolates and the commercial strain *S. meliloti* RCAM1750, but the differences were statistically insignificant. When inoculated with *R. giardinii* 20/1-1, all symbiotic parameters decreased compared to *S. meliloti* RCAM1750, however, only average shoot weight differed reliably ($p < 0.05$).

Earlier, A.E. Kriss et al. [43] isolated eight bacterial strains from nodules of wild legumes *Oxytropis nigrescens* (Pall.) Fisch., *O. maydelliana* Trautv., *Astragalus alpinus* L., *A. umbellatus* Bunge and *Hedysarum obscurum* L. growing in the tundra of the Chukotka Peninsula, Kolyuchin and Wrangel Islands. The strains were unable to form nodules on the roots of forage legumes clover, sweet clover, alfalfa, pea and vetch in pot experiments. However, some strains can nodulate wild legumes *H. alpinum* L., *H. sibiricum* Poir. and *A. trautvetteri* Bunge. while the nitrogen-fixing activity of the nodules was not studied [43]. Representatives of the genera *Rhizobium* (family *Rhizobiaceae*), *Bosea* (family *Boseaceae*) and *Tardiphaga* (family *Bradyrhizobiaceae*) were detected in root nodules of wild legume populations of *Lathyrus pratensis* L., *Vicia cracca* L., *Trifolium repens* L. and *Astragalus schelichowii* Turcz. grown in the vicinity of the city of Norilsk, However, the authors did not assess symbiotic parameters (total raw biomass of plants, nitrogen-fixing activity, etc.) of legume-rhizobial symbiosis [40].

D. Prévost et al. [44, 45] revealed the ability of isolates from nodules of Arctic legumes of the genera *Astragalus* and *Oxytropis* growing in Canada to effectively nodulate the forage legume plant *Onobrychis viciifolia* growing in temperate latitudes. It was shown that Arctic rhizobia were more effective than commercial rhizobia strains of temperate latitudes in improving the growth and productivity of sainfoin under low-temperature lab and field experiments. According to some reports [46, 47], ATPase transporting β -glucan which is involved in the attachment of bacterial cells to plant roots to initiate symbiotic interaction, may play a decisive role in the initiation of symbiosis at low temperatures. The increased cold shock protein synthesis in Arctic rhizobia may also promote their adaptation to extreme conditions of the North [47]. Studying the efficiency of symbiosis of legumes with Arctic rhizobia allows selecting the most effective nitrogen-fixing strains adapted to local extreme soil and climatic conditions. Such rhizobia may also be a basis to produce strains for the Far North agriculture by targeted genetic engineering [27].

Thus, we have isolated 13 strains of the genera *Rhizobium*, *Mesorhizobium*, *Bosea* and *Tardiphaga*, the order *Hyphomicrobiales* (α -Proteobacteria) from nodules of Arctic wild legumes *Oxytropis taimyrensis*, *Astragalus frigidus* and *Astragalus tugarinovii*. Strains *Rhizobium* sp. 7/1-1, *Tardiphaga robiniae* 7/2-2 and 7/4-2 were isolated from *A. tugarinovii*, strains *Mesorhizobium* sp. 25-2/1, 25A/5-1, *Bosea* sp. 25A/1-3, *B. lathyri* 25A/2-1, *B. psychrotolerans* 25A/2-2 and 25A/4-1 from *A. frigidus*, whereas *Mesorhizobium* sp. 9-4/1, *T. robiniae* 9/1-5, 9/3-1 and 9/5-1 from *O. taimyrensis*. In pot trials with wild Arctic (*Oxytropis adamsiana* and *Astragalus frigidus*) and forage (*Trifolium repens* and *Medicago sativa*) legumes, only the strain *R. giardinii* 20/1-1 did not form nodules in any inoculations. In general, strains isolated from local legume species *O. taimyrensis*, *A. frigidus* and *H. arcticum* better interact with the local arctic species *O. adamsiana* and *A. frigidus*, while cultivated plants *M. sativa* and *T. repens* were more responsive to inoculation with strains *Rhizobium* sp. 19-1/1 and 33-1/1, *Rhizobium* sp. 20-1/1 and *Mesorhizobium* sp.

32-2/1, isolated from the alien boreal plants *L. palustris* and *V. cracca*, respectively. In symbiosis formed the nitrogen-fixing activity was relatively low and did not lead to a statistically significant increase in shoot biomass and total plant weight, despite a 34-36% increase vs. not inoculated control in some cases. However, it is necessary to note a significant positive effect on root biomass that we revealed in *M. norvegicum* 20/1-4, *Mesorhizobium* sp. 9-4/1, 25-2/1 and 32-2/1, *Rhizobium* sp. 7/1-1 and 20-1/1, 33-1/1 strains incapable of symbiosis or forming inactive nodules. Whole genome sequencing coupled with search for and functional study of the growth-stimulating genes involved in the synthesis of auxins, gibberellins and cytokinins will elucidate the mechanism of this effect. We consider that the *O. adamsiana* species which is common in Taimyr and Yakutia and can form an effective symbiosis with three Arctic rhizobial strains is promising to create highly productive legume-cereal agrophytocenoses in various soil and climatic conditions of the Arctic.

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