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STUDY OF THE GENETIC DIVERSITY AND SYMBIOTIC EFFICIENCY OF MICROSYMBIONTS ISOLATED FROM *Lathyrus palustris* L. AND *Vicia cracca* L. GROWING IN ARCTIC YAKUTIA

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Abstract

The formation of highly productive pasture phytocenoses, based on legumes that form nitrogen-fixing symbiosis with nodule bacteria, is a necessary condition for the spread and sustainable growth of herbivorous farm animals under climate change and radical restructuring of plant ecosystems in the Arctic. At the same time, the issues of biodiversity of nodule bacteria of Arctic territories and the efficiency of their symbiotic interaction with legumes are currently almost unstudied in Russia. In this work 12 strains isolated from *Lathyrus palustris* and *Vicia cracca* nodules growing in Arctic Yakutia were described for the first time. The taxonomic position of the strains was studied and their ability to form an effective symbiosis with both traditional legumes and wild plants, which are more adapted to the conditions of the Far North and can be used to create highly productive pasture phytocenoses, was shown. The aim of the work was to isolate and study the genetic diversity of nodule bacteria of various populations of wild legume plants of *Lathyrus palustris* L. and *Vicia cracca* L. growing in Arctic Yakutia. The ability of the obtained isolates to form nitrogen-fixing nodules on the roots of different species of forage legume crops was evaluated under the conditions of sterile test-tube experiments. Root nodules of *V. cracca* and *L. palustris* were collected on Samoilovsky Island and in the settlement of Tiksi during the Russian-German expedition to the Lena River Delta. Rhizobial strains from legume nodules were isolated according to the standard method using mannitol-yeast YMA nutrient media. The taxonomic position of 12 isolates was determined by 16S rDNA (*rrs*) sequencing. Seeds of *V. cracca*, *V. sativa*, *L. sativus*, and *L. pratensis* were used to set up of test-tube experiments. Plants were cultivated in sterile 300 ml glass vessels containing 50 ml of Krasilnikov-Korenyako agar medium. The seedlings were inoculated with suspensions of individual strains in the amount of 10^6 cells/vessel. Commercial strains of *Rhizobium leguminosarum* bv. *viciae* RCAM2802, RCAM2806 and RCAM0626 from the Russian Collection of Agricultural Microorganisms (RCAM, ARRIAM, St. Petersburg) were

used as a positive control. Uninoculated plants served as negative controls. The number of nodules formed on the plant roots was counted and described at the end of cultivation. The nitrogen-fixing activity of nodules was determined by the acetylene method using a GC-2014 gas chromatograph (Shimadzu, Japan). Seeds of *V. cracca* and *L. pratensis* were additionally inoculated under the conditions of a separate test-tube experiment with a soil extract from a sample taken from Kotelny Island (Novosibirsk Islands, Arctic Yakutia). A total of twelve rhizobial isolates assigned to the genera *Rhizobium*, *Mesorhizobium* and *Bosea* were isolated from root nodules of *L. palustris* and *V. cracca* populations. Strains of *Mesorhizobium* sp. 33-3/1 and 32-2/1 were isolated only from populations growing in Tiksi. *Rhizobium* sp. 32-5/1 strain showed a low similarity of the *rrs* gene with the closest type strain (less than 98.0 %), which suggests it belonging to the new species of microorganisms. As a result of test-tube experiments, nodules were formed only in the inoculation variants with strains of *Rhizobium* sp. 19-1/1, 20-1/1, 33-1/1 and *Mesorhizobium* sp. 32-2/1. *Rhizobium* sp. 19-1/1 strain formed inactive nodules on the roots of three legume species, except *V. cracca*. *Rhizobium* sp. 20-1/1 strain in the inoculation variant with *V. cracca* formed a greater number of nodules and showed a higher level of nitrogen-fixing activity compared with the commercial strain *Rhizobium leguminosarum* bv. *viciae* RCAM0626 for treatment of *V. sativa*, but the variants did not differ significantly from each other in the number of nodules.

Keywords: Arctic Yakutia, Lena River Delta, legumes, *Lathyrus palustris*, *Vicia cracca*, nitrogen-fixing nodule bacteria

Global climate change causes a significant restructuring of the entire Arctic ecosystem with the active migration of plant communities northwards, the filling of new ecological niches and the displacement of native flora [1, 2]. In such areas, pasture phytocenoses can form a significant part of which are leguminous plants that enter into symbiosis with nitrogen-fixing nodule bacteria (rhizobia). This mutually beneficial strategy allows legumes to expand into new territories due to their broad ecological plasticity and tolerance to environmental stressors, including low soil nitrogen. Legumes are a major source of protein for both herbivorous farm animals and wild reindeer and musk oxen [3, 4].

The first and only description of nodule bacteria isolated from the root nodules of wild leguminous plants *Oxytropis nigrescens* (Pall.) Fisch., *O. maydelliana* Trautv., *Astragalus alpinus* L., *A. umbellatus* Bunge and *Hedysarum obscurum* L. which grow in the Asian part of the Arctic in the tundra of the Chukotka Peninsula, Kolyuchin and Wrangel Islands, is given in the work of A.E. Criss et al. [5]. The authors isolated eight bacterial strains that were not capable of forming nodules on the roots of agricultural legume plants clover, sweet clover, alfalfa, peas and vetch in pot trials. The ability of some strains to form nodules on legume plants *H. alpinum* L., *H. sibiricum* Poir. and *A. trautvetteri* Bunge. was observed but the nitrogen-fixing activity of nodules has not been studied.

The species *Lathyrus palustris* L. and *Vicia cracca* L. belong to the vetch tribe *Vicieae* (Adans.) Bronn of the *Fabaceae* family. Many mambers of the genera *Lathyrus* and *Vicia* are valuable forage pasture and hay crops in the diet of large and small ruminants, horses [6, 7], and wild herbivores of Central Yakutia [8]. In particular, the species *L. palustris* has high feeding qualities and is considered a particularly promising crop for introduction into pasture agrophytocenoses of Eastern Siberia [6, 9], while *V. cracca* is included in the State Register of Breeding Achievements approved for use and is actively cultivated as valuable forage grass in pasture and hay phytocenoses [10].

The main microsymbionts of leguminous plants of the genera *Vicia* and *Lathyrus* are strains of *Rhizobium leguminosarum* bv. *viciae* [11-13]. Symbionts that are not typical for these genera and related to both other *Rhizobium* species [14, 15] and the genus *Phyllobacterium* members [11] are also isolated from the root nodules of various *Vicia* and *Lathyrus* species. It was shown that *Mesorhizobium alhagi* CCNWXJ12-2T isolated from *Alhagi sparsifolia* native to northeastern China could form nodules on the *V. cracca* roots [16].

Arctic rhizobia are of interest for studying the evolutionary development

of nitrogen-fixing bacteria and their adaptation to low temperatures. Arctic rhizobia also provide an opportunity to analyze the functional relationships between rhizobia and leguminous plants in isolated aboriginal populations of the North [17]. It is promising to use Arctic strains of nodule bacteria in the production of microbial preparations to create highly productive pasture phytocenoses in the Arctic [18]. However, the issues of biodiversity of nodule bacteria in Arctic territories and the effectiveness of their symbiotic interaction with leguminous plants remain practically unstudied in Russia.

This paper is the first to report about the taxonomic position of 12 innovative strains from the nodules of the swamp lathyrus and mouse pea grown in Arctic Yakutia and their effective symbiotic nodulation of both traditional legumes and wild plants that are more adapted to the Far North conditions. These strains may be of interest for creating highly productive pasture phytocenoses.

The work aimed at studying the genetic diversity of rhizobial isolates from populations of arctic wild legumes *Lathyrus palustris* L. and *Vicia cracca* L. and to assess their ability to form nitrogen-fixing nodules on the roots of forage pasture legumes in micro-pot trials.

Materials and methods. Root nodules of wild populations of leguminous plants *V. cracca* and *L. palustris* were collected in 2021 on Samoilovsky Island and in the village of Tiksi during a Russian-German expedition to the Lena River delta (Arctic Yakutia).

Rhizobial strains were isolated according to a standard procedure using YMA mannitol-yeast nutrient medium [19] after sterilizing the nodules for 1 min in 96% ethanol. rDNA isolation from pure cultures was performed using the DNeasy Blood & Tissue kit (QIAGEN, Germany) and Monarch® (New England Biolabs, USA). Primary identification of strains was carried out by PCR followed by the 16S rRNA marker gene sequencing. Primer pairs fD1 5'-AGAGTTTGATCCTGGCTCAG-3' and rD1 5'-AAGGAGGTGATCCAGCC-3' [20] were used for amplification. The 16S rRNA gene amplification protocol was 3 min 30 s at 95 °C (primary denaturation); 1 min 10 s at 94 °C (denaturation), 40 s at 56 °C (primer annealing), 2 min 10 s at 72 °C (elongation) (35 cycles); 6 min 10 s at 72 °C (final elongation) (a T100 Thermal Cycler, Bio-Rad, USA). The reaction mixture was 38 μl milli-Q H_2O (Evrogen, Russia), 5 μl buffer (Helikon, Russia), 5 μl dNTP kit (Promega, USA), 0.5 μl primers (Evrogen, Russia), 0.5 μl Taq polymerase (Helicon, Russia) and 1 μl (50-100 ng) mDNA. The amount of DNA was assessed visually using electrophoresis in a 1.0% agarose gel in 0.5× TAE buffer with a MassRuler molecular weight marker (Fermentas, Lithuania). The PCR product was purified from an agarose gel using the Cleanup S-Cap kit (Evrogen, 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” of the All-Russian Research Institute of Agricultural Microbiology (CCU GTPCB ARRIAM).

The obtained DNA sequences were analyzed using the Chromas Lite 2.6.4 program (https://technelysium.com.au/wp/chromas/). For multiple alignment and comparison of nucleotide sequences, the ClustalOmega program (https://www.ebi.ac.uk/Tools/msa/clu-stalo/) was used. Sequences of closely related type strains were searched in the GenBank database (https://www.ncbi.nlm.nih.gov/). Nucleotide sequences have been deposited in the GenBank database (ac. nos OQ685989-OQ686000.

For micro-pot experiments (MPEs), seeds of *V. cracca*, *V. sativa*, *L. sativus* and *L. pratensis* were scarified and surface sterilized in 98% H_2SO_4 for 10 minutes, thoroughly washed with sterile tap water and germinated on filter paper in Petri dishes at 25 °C in the dark for 3-5 days (depending on the plant species). Seeds of *V. sativa* and *L. sativus* were kindly provided by employees of the Vavilov

All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg). *L. pratensis* was used instead of *L. palustris* as the inoculation target due to the lack of seeds.

Plants were grown in 300-ml sterile glass vessels with 50 ml of Krasilnikov-Korenyako agar medium (K_2HPO_4 1.0 g/l, $MgSO_4 \cdot 7H_2O$ 1.0 g/l, $Ca_3(PO_4)_2$ 0.2 g/l, $FeSO_4$ 0.02 g/l, Fedorov's microelement mixture of H_3BO_3_0.05 g/l, $(NH_4)_2MoO_4$ 0.05 g/l, KCl 0.005 g/l, NaBr 0.005 g/l, $ZnSO_4 \cdot 7H_2O$ 0.003 g/l, $MnSO_4$ 0.002 g/l). Each MPE was performed in triplicate (3 glass vessels with 1 seedling for each inoculation treatment).

Seedlings were inoculated with suspensions of individual strains (10^6 cells per vessel). Commercial strains of *Rhizobium leguminosarum* bv. *viciae* RCAM2802, RCAM2806 and RCAM0626 from the Network Bioresource Collection for agriculture genetic technologies (ARRIAM, St. Petersburg) served as a positive control, uninoculated plants were negative controls. Plants were grown in a phytotron at 18-22 °C for 30 days at 50% relative humidity and a four-level lighting/temperature regime: night (18 °C, 8 hours), morning (200 μmol · $m^{-2} \cdot s^{-1}$, 20 °C, 2 hours), day (400 μmol · $m^{-2} \cdot s^{-1}$, 23 °C, 12 hours), evening (200 μmol · $m^{-2} \cdot s^{-1}$, 20 °C, 2 hours). Lighting was provided by L36W/77 FLUORA lamps (Osram, Germany).

At the end of cultivation, the nodules formed on the roots were counted and described. The nitrogen-fixing activity of nodules was determined by the acetylene method using a gas chromatograph GC-2014 (Shimadzu, Japan).

In a separate MPE, the seeds of *V. cracca* and *L. pratensis* were inoculated with a soil extract from a sample taken from Kotelny Island (New Siberian Islands, Arctic Yakutia). To prepare the soil extract, 1 g of sample was added to a sterile flask with 50 ml of liquid Krasilnikov-Korenyako medium. The flask was placed on a shaker and incubated for 1 hour at room temperature. The seedlings were inoculated with 1 ml soil extract per vessel.

Statistical analysis was performed using the STATISTICA 10 program (StatSoft, Inc., USA). For each inoculation treatment, means (*M*) and standard deviations (±SD) were calculated. To assess the significance of differences between mean values, one-way analysis of variance and Fisher's LSD test were used.

Results. Samoilovsky Island (72°22′00″N, 126°30′01″E) is located in the southern part of the Lena River delta in the subzone of typical tundras (Fig. 1).

Fig. 1. Sites for *Vicia cracca* L. and *Lathyrus palustris* L. plants collection on the Samoilovsky Island (the Lena River delta) and the village Tiksi (marked with white dots).

Geomorphologically, the island is clearly divided into two parts, different in age and genesis. Two-thirds of the island is occupied by the surface of the first terrace, and one-third in the western part of the island is the surface of a high floodplain, subject to seasonal flooding by river waters (21). The village of Tiksi (71°38′12″N, 128v52′04″ E) is located on the shore of the bay of the same name, confined to the southern part of the Laptev Sea (see Fig. 1). The predominant elements of the relief in the vicinity of the village are low hills and intermountain swampy saddles. The basis of the vegetation cover contains various variants of tundra and low-lying shallow swamps. The soils are frozen,

gravelly-stony. Flora is mountainous, temperate arctic [22].

The boreal species *Lathyrus palustris* L. and mouse pea *Vicia cracca* L. were found on the territory of the village Tiksi relatively recently [23]. The species *V. cracca* (Fig. 2, B) on Samoilovsky Island was first discovered by N.N. Lashchinsky (personal communication) in 2017, and *L. palustris* plants (Fig. 2, A) by him during the Russian-German expedition to the Lena River delta in 2021. Populations of *V. cracca* and *L. palustris* grow mainly in the northwestern part of the island which is sandy and subject to seasonal flooding by Lena River [24].

FIg. 2. Plants *Lathyrus palustris* L. (a) **and *Vicia cracca* L.** (b) **on the Samoilovsky Island (the Lena River delta)** (photo by I.A. Alyokhina).

From the root nodules of *L. palustris* and *V. cracca*, we cultured 8 and 4 rhizobial isolates, respectively (Table 1), 4 isolates from each of two *L. palustris* populations, and 2 isolates from each of *V. cracca* populations. Most isolates formed colonies on days 3 and 4, with the exception of strain 33-5/1, which formed colonies on day 5. Based on analysis of the *rrs* gene, the isolates were assigned to the genera *Rhizobium* (family *Rhizobiaceae*), *Mesorhizobium* (family *Phyllobacteriaceae*), and *Bosea* (family *Boseaceae*) of the order *Rhizobiales* (*Hyphomicrobiales*). Note that from the nodules of the *L. palustris* population growing on the island. Samoilovsky, only representatives of *Rhizobium* were isolated while the population from the village Tiksi turned out to be represented by a wider composition of microsymbionts (*Rhizobium*, *Mesorhizobium* and *Bosea*), which is apparently associated with different soil and climatic features of these areas (see Table 1).

1. Isolates from nodules of *Lathyrus palustris* L. and *Vicia cracca* L. collected on the Samoilovsky Island (the Lena River delta) and in the village Tiksi (2021)

Site of plant collection	Strain number	Similarity of the *rrs* gene, %	Closest type strain	Identification
		Lathyrus palustris L.		
Samoilovsky islanf	19-1/1	100	*Rhizobium leguminosarum* bv. *viceae* LMG 14904, *R. sophorae* LMG 27901, *R. anhuiense* CCBAU 23252, *R. laguerreae* FB206	*Rhizobium* sp.
	19-3/1			
	19-4/1			
	19-5/1			
Tiksi village	33-1/1			
	33-3/1	100	*Mesorhizobium norvegicum* 10.2.2, *M. loti* LMG 6125	*Mesorhizobium* sp.
	33-4/1	99,72	*Bosea lathyri* R-46060	*Bosea lathyri*
	33-5/1	99,21	*B. lathyri* R-46060	*Bosea* sp.
		Vicia cracca L.		
Samoilovsky islanf	20-1/1	100	*R. leguminosarum* bv. *viceae* LMG 14904, *R. sophorae* LMG 27901, *R. anhuiense* CCBAU 23252, *R. laguerreae* FB206	*Rhizobium* sp.
	20-5/1	99,21	*B. lathyri* R-46060	*Bosea* sp.
пос. Тикси	32-2/1	100	*M. norvegicum* 10.2.2, *M. loti* LMG 6125	*Mesorhizobium* sp.
	32-5/1	97,85	*R. giardinii* H152	*Rhizobium* sp.

Isolates from nodules of *L. palustris* (19-1/1, 19-3/1, 19-4/1, 19-5/1, 33-1/1) and V. *cracca* (20-1/1) showed 100% similarity of the *rrs* gene to four type strains at once, the *R. leguminosarum* bv. *viciae* LMG 14904T, *R. anhuiense* CCBAU 23252T, *R. sophorae* LMG 27901T and *R. laguerreae* FB206T (see Table 1). Therefore, the resulting isolates were not identified to species. To clarify their species identity, it is necessary to sequence and analyze the housekeeping genes

(*recA*, *atpD*, *dnaK*, *gyrB* and *rpoB*).

It is known that the strain *R. leguminosarum* bv. *viciae* LMG 14904T is able to form an effective symbiosis with leguminous plants from the genera *Pisum*, *Vicia*, *Lathyrus*, *Lens* and *Vavilovia* from the tribe *Fabeae* [25, 26]. *R. anhuiense* strain CCBAU 23252T was isolated from the legume plant *V. faba*, native to China. It formed nitrogen-fixing nodules on the roots of *V. faba* and P*isum sativum* [27]. In cross-nodulation experiments, this strain formed an ineffective symbiosis with *Phaseolus vulgaris* and was unable to form nodules on food and forage legumes *Glycine max*, *Arachis hypogaea*, *Medicago sativa*, *Trifolium repens*, *Lablab purpureu*s [27].

Members of the species *R. anhuiense* are known to serve as the main microsymbents of the species *Lathyrus maritimus*, which grows along the marine coastline in China [15]. The *R. sophorae* LMG 27901T was isolated from the nodule of the medicinal legume *Sophora flavescens*, also collected in China [28]. Strain LMG 27901T was shown to be able to form active nodules on the roots of *S. flavescens* and *P. vulgaris*, while other *R. sophorae* strains could form effective symbioses with *V. sativa* and *P. sativum* plants growing in Northern China [29]. *R. laguerreae* FB206T was isolated from the effective nodule of *V. faba* in Tunisia [30], and members of this rhizobial species serve as effective symbionts of many *Pisum*, *Vicia*, *Lens*, and *Phaseolus* species found in various regions of the world [31-34], and have growth-promoting properties, improving crop productivity [35, 36].

Isolates 33-3/1 and 32-2/1 were found in populations of *L. palustris* and *V. cracca* from the village Tiksi, respectively. The isolates showed 100% similarity of the *rrs* gene with the type strains *Mesorhizobium norvegicum* 10.2.2T and *M. loti* LMG 6125T isolated from root nodules of the legume plant *Lotus corniculatus* in New Zealand [37] and Norway [38], respectively. Note that we failed to isolate bacteria of the genus *Mesorhizobium* from the populations of *L. palustris* and *V. cracca* of the Samoilovsky Island.

Isolates 33-4/1, 33-5/1 and 20-5/1 showed the highest similarity of the *rrs* gene (33-4/1 - 99.72%; 33-5/1 and 20-5/1 - 99.21%) with the type strain *B. lathyri* R-46060T from *Lathyrus latifolius* native to Belgium [39]. Isolates related to *Bosea* had different origins: strains 33-4/1 and 33-5/1 were isolated from *L. palustris* plants in the village Tiksi, while 20-5/1 was isolated from *V. cracca* on the Samoilovsky Island. It should be noted that the genus *Bosea* is currently represented by 12 species, of which only 6 species (*B. lupini*, *B. lathyri*, *B. robiniae*, *B. caraganae*, *B. vaviloviae* and *B. spartocytisi*) were isolated from the nodules of leguminous plants of the genus *Lupinus*, *Lathyrus*, *Robinia*, *Caragana*, *Vavilovia* and *Spartocytisus* [39-42]). However, the ability of these strains to independently form nodules has not yet been described. Strain *Rhizobium* sp. 32-5/1 showed low similarity in the *rrs* gene with the nearest type strain (less than 98.0%), which suggests that it belongs to new types of microorganisms.

The ability of isolates to nodulate legumes was studied under two sterile MPE conditions using 9 rhizobial strains of different taxonomic positions (three genera *Rhizobium*, *Mesorhizobium* and *Bosea*) and 4 species of agricultural forage legumes (*V. cracca*, *V. sativa*, *L. sativus* and *L pratensis*) (Tables 2, 3). In the first MPE, nodules on *L. pratensis* were formed only upon inoculation with strains of Rhizobium sp. 19-1/1 and 33-1/1, although the symbiosis in both cases was not nitrogen-fixing (see Table 2). Nodules on *V. cracca* were formed only in the variant with inoculation with a strain of *Rhizobium* sp. 20-1/1, which formed an effective symbiosis with higher nitrogen-fixing activity than when inoculated with a commercial strain of *R. leguminosarum* bv. *viciae* RCAM0626 (differences between treatments in the nitrogen fixation parameter were statistically significant at $p < 0.05$). In inoculation of *V. cracca* with strains of *Mesorhizobium* sp. 32-2/1 and *Rhizobium*

sp. 33-1/1, tumor-like nodule-like formations occurred on the roots (see Table 2).

2. The effect of *Lathyrus palustris* L. and *Vicia cracca* L. plant inoculation with commercial strains of *Rhizobium leguminosarum* bv. *viciae* RCAM2806, RCAM0626 and arctic isolates (sterile micro-pot tests, $n = 3$, $M \pm SD$)

Inoculation	Number of nodules per pot	Acetylene reductase activity, μmol $C_2H_4 \cdot pot^{-1} \cdot day^{-1}$
	Lathyrus palustris L.	
R. leguminosarum RCAM2806	5.5±2.1[a]	0.37±0.08
Без инокуляции	0	0
Rhizobium sp. 19-1/1*	2.6±1.1[a]	0
Rhizobium sp. 20-1/1	0	Not detected
Bosea sp. 20-5/1	0	Not detected
Mesorhizobium sp. 32-2/1	0	Not detected
Rhizobium sp. 32-5/1	0	Not detected
Rhizobium sp. 33-1/1*	4.0±2.0[a]	0
Mesorhizobium sp. 33-3/1*	0	Not detected
Bosea lathyri 33-4/1*	0	Not detected
Bosea sp. 33-5/1*	0	Not detected
	Vicia cracca L.	
R. leguminosarum RCAM0626	5.3±1.5[a]	0.05±0.01[a]
Без инокуляции	0	0
Rhizobium sp. 19-1/1*	0	Not detected
Rhizobium sp. 20-1/1	7.3±0.5[a]	0.68±0.35[b]
Bosea sp. 20-5/1	0	Not detected
Mesorhizobium sp. 32-2/1	NS	0
Rhizobium sp. 32-5/1	0	Not detected
Rhizobium sp. 33-1/1*	NS	0
Mesorhizobium sp. 33-3/1*	0	Not detected
Bosea lathyri 33-4/1*	0	Not detected
Bosea sp. 33-5/1*	0	Not detected

N o t e. NS — nodule-like srucures. Seeds of *L. pratensis* were collected in the Irkutsk Province, seeds of *V. cracca* on the island Samoilovsky (the Lena River delta). An asterisk (*) indicates strains isolated from *L. palustris* nodules; other strains were isolated from *V. cracca*.

[a, b] Variants are marked with different Latin letters, the differences between which are statistically significant (Fisher's LSD test, $p < 0.05$).

3. The effect of *Lathyrus palustris* L. and *Vicia cracca* L. plant inoculation with commercial strains of *Rhizobium leguminosarum* bv. *viciae* RCAM2806, RCAM0626 and arctic isolates (sterile micro-pot tests, $n = 3$, $M \pm SD$)

Inoculation	Number of nodules per pot	Acetylene reductase activity, μmol $C_2H_4 \cdot pot^{-1} \cdot day^{-1}$
	Lathyrus sativus L.	
R. leguminosarum RCAM2802	21.0±2.8[a]	5.20±0.03
Без инокуляции	0	0
Rhizobium sp. 19-1/1*	29.3±9.5[a]	0
Rhizobium sp. 20-1/1	0	Not detected
Bosea sp. 20-5/1	0	Not detected
Mesorhizobium sp. 32-2/1	0	Not detected
Rhizobium sp. 32-5/1	0	Not detected
Rhizobium sp. 33-1/1*	0	Not detected
Mesorhizobium sp. 33-3/1*	0	Not detected
Bosea lathyri 33-4/1*	0	Not detected
Bosea sp. 33-5/1*	0	Not detected
	Vicia sativa L.	
R. leguminosarum RCAM0626	110.0±41.0[ab]	3.70±2.00[a]
Без инокуляции	0	0
Rhizobium sp. 19-1/1*	122.0±13.0[b]	0
Rhizobium sp. 20-1/1	0	0
Bosea sp. 20-5/1	0	н/о
Mesorhizobium sp. 32-2/1	68.0±30.0[a]	0
Rhizobium sp. 32-5/1	0	Not detected
Rhizobium sp. 33-1/1*	107.0±8.0[ab]	0.20±0.10[b]
Mesorhizobium sp. 33-3/1*	0	Not detected
Bosea lathyri 33-4/1*	0	Not detected
Bosea sp. 33-5/1*	0	Not detected

N o t e. Seeds of *L. pratensis* were collected in the Irkutsk Province, seeds of *V. cracca* on the island Samoilovsky (the Lena River delta). An asterisk (*) indicates strains isolated from *L. palustris* nodules; other strains were isolated from *V. cracca*.

[a, b] Variants are marked with different Latin letters, the differences between which are statistically significant (Fisher's LSD test, $p < 0.05$).

In the second MPE, when *L. sativus* was inoculated with Rhizobium sp.

19-1/1, round inactive nodules were formed on the roots of plants, as in the case of *L. pratensis* (see Tables 2, 3). Nodules on *V. sativa* were formed in three inoculation variants, but only with *Rhizobium* sp. 33-1/1 symbiosis was effective: insignificant nitrogen-fixing activity was shown in comparison with the commercial strain *R. leguminosarum* bv. *viciae* RCAM0626 (see Table 3). Interestingly, strains *Mesorhizobium* sp. 32-2/1 and *Rhizobium* sp. 33-1/1 from the nodules of *V. cracca* and *L. palustris*, respectively, formed only nodule-like structures on the roots of *V. cracca*, while on the roots of *V. sativa* both strains formed a determinate type of rounded nodules. The reason for this is probably due to the fairly high degree of specificity of the host plant to the microsymbiont. The resulting nodules were mostly round, white, ineffective, and determinate, with the exception of inoculation of *V. cracca* with *Rhizobium* sp. 20-1/1 and *V. sativa* with *Rhizobium* sp. 33-1/1, where some of the formed nodules were distinguished by their oblong shape and pinkish tint, indicating their nitrogen-fixing activity.

As a result of inoculation of *V. cracca* and *L. pratensis* plants with a soil extract from a sample taken on the island Kotelny, nodules on the roots were not found, which is most likely due to the absence of the corresponding microsymbionts in the soil, since at present the presence of legumes on the island. Boiler room not identified.

Thus, 12 rhizobial strians belonging to the genera *Rhizobium*, *Mesorhizobium*, and *Bosea* were isolated from the nodules of Arctic wild leguminous plants *Lathyrus palustris* and *Vicia cracca*. Most isolates belonged to the genus *Rhizobium*. Strains *Mesorhizobium* sp. 33-3/1 and 32-2/1 were isolated from the village Tiksi populations of *L. palustris* and *V. cracca*, respectively, but in the Samoilovsky Island populations bacteria of this genus were not found. The *L. palustris* on the Samoilovsky Island has been described for the first time. Strain *Rhizobium* sp. 32-5/1 showed low similarity in the *rrs* gene with the nearest type strain (less than 98.0%) which suggests that it belongs to new species of microorganisms. In micro-pot experiments to study cross-nodulation for agricultural forage legumes *Vicia cracca*, *Vicia sativa*, *Lathyrus sativus* and *Lathyrus pratensis*, nodules were formed only upon inoculation with *Rhizobium* sp. 19-1/1, 20-1/1, 33-1/1 and *Mesorhizobium* sp. 32-2/1. Strain *Rhizobium* sp. 19-1/1 was the only one that formed ineffective nodules on the roots of three legume species at once, except for *V. cracca*, which may indicate its wide specificity to various genera and species of host plants. Note that *Rhizobium* sp. 20-1/1 in inoculation of *V. cracca* formed a larger number of nodules (the differences between the experimental variants were not significant due to significant variation in this parameter) and showed higher nitrogen-fixing activity compared to the commercial strain *R. leguminosarum* bv. *viciae* RCAM0626, which makes it promising for the development of new highly effective microbial preparations with the aim of creating productive pasture phytocenoses in the Arctic territories of Russia. To further study the host specificity and symbiotic efficiency of the obtained strains, micro-pot experiments will be carried out with an expanded selection of forage agricultural and wild Arctic legumes.

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