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Isolation of Soybean Nodule Bacteria and Nodule Formation in Uzbekistan Soybean Varieties

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Abstract

The main goal of our research work is to search for nodule bacteria of local soybean varieties and to identify and study their nodule-forming properties. In the present study, soil samples from fields in the Tashkent, Andijan, Bukhara, Jizzakh, Kashkadarya, Navoi, Namangan, Samarkand, Surkhandarya, Syrdarya, Fergana, and Khorezm regions of Uzbekistan were studied for the formation of symbiotic nodules in local soybean varieties. Nodules formed only in the soils of the Tashkent region in the root systems of local soybean varieties (Madad, Sevinch, Dostlik, Parvoz, Gavkhar, Khasildar, Baraka, Tashkent, Uzbekistan-6, Tumaris, Nafis, Orzu) were formed from 22 to 40 nodule. Forty-one bacterial species belonging to the genus *Bradyrhizobium* were isolated from the nodules of the different varieties. The specificity, virulence, and symbiotic efficacy of 12 active nodule bacteria were compared in the local Madad, Sevinch, Dostlik, and Parvoz varieties. The root systems of these varieties formed from 2 to 14 symbiotic pink nodules 0.5 - 10 mm in size. Inoculation of the Madad and Dostlik varieties with their specific M5-1 and D24-1 nodule bacteria resulted in a symbiotic efficiency 46.6% - 54.4% higher than in uninoculated control plants. Notably, the foreign inoculum "Rizovit" (Kazakhstan), created on the basis of *Bradyrhizobium japonicum*, did not form any nodules on the roots of the local Uzbekistan varieties. The main reason for this may be the difference in the genetic origin of foreign soybean varieties and domestic Uzbekistan varieties. The nucleotide sequences of 16S rRNA genes of nodule bacteria M5-1, S7-2, D24-1, and P12-1 showed

97.07% similarity with the 16S rRNA genes of *Bradyrhizobium japonicum* PRY65 (AF239848.2) and 98.98% similarity with *Bradyrhizobium japonicum* PRY62 (AF239847.2).

Keywords

Soybean, *Bradyrhizobium japonicum*, Nodulation, 16S rRNA

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is the most valuable protein-oil crop. In terms of quantity and quality of useful substances contained in soybean grain, it has no equal among all field crops. This crop is of particular importance in solving the protein problem due to the high protein content in the grain. Its protein contains all the essential amino acids and is easily digestible; in addition, soybean grain contains 20% - 25% oil with a favorable fatty acid composition, and a large set of minerals and vitamins. In the world production of vegetable oil, soybean ranks first among all oil-bearing plants, and in terms of protein collection, it is the leader among all grains and leguminous crops. Hundreds of food products, high-protein juicy, coarse and concentrated feed for all types of livestock and poultry, margarine, mayonnaise, various types of confectionery fats, medicines and cosmetics, and vitamin preparations are produced from it [1].

Obtaining high yields of soybeans depends on the activities by nodule bacteria of the species *Bradyrhizobium japonicum*, which enter into a symbiotic relationship with the soybean plant and provide it with biological nitrogen [2] [3] [4] [5]. In the absence of these specific microsymbionts, soybean crops cannot fix molecular nitrogen from the atmosphere. The cultivation of soybean in Central Asia is relatively recent. Therefore, symbiotic nodule bacteria of soybeans are completely absent from the soils of this region. Consequently, cultivating soybeans in this region requires seed inoculation (*i.e.*, artificial infection of their soybean seeds with specific and virulent races of nodule bacteria).

For successful inoculation, these bacteria must be capable of symbiosis with soybean and quickly multiply in the tissues of the soybean roots to form numerous large and pinkish nodules [6] [7] [8] [9] [10]. Many researchers have found that the use of biopreparations based on nodules from bacteria from regions of intensive soybean cultivation results in the formation of numerous populations of rhizobia in soils. However, numerous abiotic and biotic factors in the external environment can reduce the virulence and efficiency of inoculation of these root nodule bacterial strains. Aboriginal rhizobacteria are quantitatively and qualitatively heterogeneous, but they are ineffective and often show higher competitiveness compared to highly effective nodule bacterial strains [11]. Nodule bacterial inoculants must be able to survive and adapt to new habitats while also outnumbering and dominating the native bacteria present in the soil [12].

The aim of the present work was to isolate nodule bacteria from the nodules of local soybean varieties, obtain pure cultures of the strains, identify them, and study nodule formation in various Uzbekistan soybean varieties.

2. Materials and Methods

2.1. Studying the Formation of Symbiotic Nodules of Local Soybean Varieties in Soil Samples of Different Zones of Uzbekistan in Vegetative Experiments

Soil samples were collected from different zones of Uzbekistan (the Tashkent, Andijan, Bukhara, Jizzakh, Kashkadarya, Navoi, Namangan, Samarkand, Surkhandarya, Syrdarya, Ferghana, and Khorezm regions) to study the nodulation of local soybean varieties. The nodule formation of the Madad, Sevinch, Dostlik, Parvoz, Gavhar, Hasildar, Baraka, Tashkent, Uzbekistan-6, Tumaris, Nafis, and Orzu soybean varieties in various soils was determined using a vegetation experiment. Soybean plants were grown for 30 days at 24˚C temperature and 10,000 lux light. Soybean plants were watered every 3 days with Hoagland's nutrient medium [13].

2.2. Growing Local Varieties of Soybeans in the Field

Soil samples brought from the fields of the Rice Research Institute of the Tashkent region were spread over a 36 m^2 area. The 12 soybean varieties were then planted in separate 3 m^2 plots. After growing for 1 month under natural lighting, the plants of all varieties had strong stems and large and healthy leaves, and a large number of symbiotic nodules formed in the root systems.

2.3. Isolation of Bacterial Isolates from Soybean Nodules and Characterization of Their Morphological and Cultural Properties

Well-developed plants of each soybean cultivar were selected for isolation of bacteria from the nodules. Pink nodules larger than 5 mm in size were collected from the plant root systems. The nodules were washed several times with sterile water and then placed in ethanol for 2 min. The nodules were then held with tweezers and passed over a gas burner to burn off the alcohol. The fully sterilized nodules were then placed in Eppendorf tubes containing 0.5 mL of sterile water, crushed, and mixed. The resulting suspension of nodules (bacteroids) was diluted and sown on a medium of the following composition (g/L): glucose - 5, sucrose—5, K_2HPO_4—0.5, KH_2PO_4—0.5, $MgSO_4 \cdot 7H_2O$—0.5, $CaSO_4$—0.2, peas—50, agar—20, distilled water-up to 1 L, pH 7.0 (the peas were boiled for 1 h, and the medium was prepared with a base of pea broth) [14]. The nodule bacteria isolates were sown on agar nutrient medium and incubated at 28˚C for 7 days. Nodule bacteria colonies formed on the surface of the agar nutrient medium already the 3rd day of incubation. Their morphological and cultural characteristics were established according to Berger's definitions [15].

2.4. PCR Amplification of the Bacterial 16S rRNA Gene

Genomic DNA was isolated from rhizobacteria using a modified Marmur method [16]. The following universal oligonucleotide primers were used in PCR amplification: 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) [17]. DNA samples isolated from the bacterial strains were amplified using PCR and the GenPak® PCR MasterMix kit. The reaction mixture, in a total volume of 20 μL, consisted of 10 μL of Dilution solution, containing Master Mix, 8.2 μL of double distilled water, 0.4 μL (27F and 1492R) primers, and 1 μL of DNA sample. PCR amplification was conducted, with an initial denaturation at 94˚C for 3 min, followed by subsequent denaturation at 94˚C for 40 s, primer annealing at 55˚C for 40 s, elongation at 70˚C for 90 s, final elongation at 70˚C for 7 min, and the reaction was repeated for 35 cycles. Amplicons were detected by electrophoresis in a 2% agarose gel stained with ethidium bromide.

2.5. Phylogenetic Analysis

The partial 312 - 340-bp 16S rRNA gene sequences were compared with the sequences available in the GenBank database using the standard Basic Local Alignment Search Tool, BLASTn [18], at the National Center for Biotechnology Information (NCBI). From the aligned sequences, neighbor-joining dendrograms [19] were constructed with MEGA version 5 software [20]. The robustness of the inferred trees was evaluated by 1000 bootstrap resamplings.

3. Results and Discussion

3.1. Symbiotic Nodule Formation of Local Soybean Varieties

Vegetative experiments showed that symbiotic nodulation of local soybean varieties (Madad, Sevinch, Dostlik, Parvoz, Gavhar, Hasildar, Baraka, Tashkent, Uzbekistan-6, Tumaris, Nafis, and Orzu) was unsuccessful in soil samples collected from various zones of Uzbekistan (the Tashkent, Andijan, Bukhara, Jizzakh, Kashkadarya, Navoi, Namangan, Samarkand, Surkhandarya, Syrdarya, Fergana, and Khorezm regions). Symbiotic nodules formed only in the root systems of all soybean varieties in soil samples taken from the fields of the Research Institute of Rice in the Tashkent region. However, the plants grew very weakly (**Figure 1**). The nodules were whitish in color and ranged in diameter from 0.5 mm to 1.0 mm. Because nodules did not fully form in the root systems of any of the soybean varieties, isolation of nodule bacteria from those nodules was deemed inappropriate for further research.

Experiments were instead carried out on nodulation in the root systems of local soybean varieties in April in the field following inoculation with soil samples from the Rice Research Institute of the Tashkent region. Large numbers of symbiotic nodules formed in the root systems of all soybean varieties during flowering (**Table 1** and **Figure 2**). The Sevinch variety formed a maximum of 40

Figure 1. Vegetative studies of symbiotic nodule formation of 12 local soybean varieties in soil samples from different regions of Uzbekistan.

(a)

(b)

(c)

(d)

Figure 2. Formation of symbiotic nodules by local soybean varieties growing in soil samples from the Tashkent region: (a) Madad variety, (b) Sevinch variety, (c) Dostlik n variety, (d) Parvoz variety.

Table 1. Formation of symbiotic nodules of local soybean varieties in soil samples from the Tashkent region the average biomass of the other soybean varieties was 0.85 - 1.674 g.

Soybean variety	Average nodule number per 1 plant	Nodule size, mm	Average Dry biomass of plant, g	Stem length, cm
Madad	35 ± 1.89	1 - 5	1.67 ± 0.115	45
Sevinch	40 ± 6.45	0.5 - 9	1.90 ± 0.064	45
Dustlik	33 ± 8.42	1 - 10	1.63 ± 0.041	48
Parvoz	31 ± 1.82	1 - 5	1.41 ± 0.048	45
Gavkhar	27 ± 7.88	2 - 5	0.9 ± 0.035	39
Hosildor	28 ± 4.34	1 - 7	1.57 ± 0.078	41
Baraka	30 ± 6,65	1 - 5	1.43 ± 0.1	43
Tashkent	32 ± 4.96	1 - 3	1.45 ± 0.073	49
Uzbekistan-6	24 ± 8.30	0.5 - 5	1.32 ± 0.86	39
Tumaris	31 ± 5.37	1 - 5	0.85 ± 0.059	33
Nafis	22 ± 2.44	1 - 6	1.33 ± 0.095	40
Orzu	18 ± 4.61	2 - 5	1.19 ± 0.036	27

symbiotic nodules. The Baraka, Parvoz, Tomaris, Tashkent, Dostlik, and Madad varieties formed an average of 30 - 35 nodules. The Orzu, Nafis, Gavkhar, Hasildar, and Uzbekistan-6 varieties formed 18 - 28 nodules. The nodules were pink in color, with diameters of 1 mm to 10 mm. The symbiotic efficiency of nodules was high in the Sevinch variety, which had an average plant biomass of 1.9 g.

3.2. Isolation of Bacterial Isolates from Nodules of Local Soybean Varieties and Characterization of Their Morphological and Cultural Properties

The nodules were crushed, and the nodule bacterial suspensions were sown on pea medium and incubated at 28˚C for 7 days. Colonies of fast-growing bacteria appeared after 2 - 3 days, and colonies of slow-growing bacteria appeared after 3 - 7 days (**Figure 3(a)**). Bacteria cultured on bean agar formed colonies that were colorless, whitish, grayish, pale gray and yellowish-reddish, transparent, translucent, and cloudy, with varying degrees of sliminess and convex, conical, and spherical in shape, in addition to rough colonies. The colony diameters were 1 - 5 mm. More than 150 rhizobial isolates were isolated from these colonies. Notably, a study of the endophytes of soybean nodules showed the presence of various rhizobial endophytes, as well as nonrhizobial endophytes, in the nodules [21]. Mayhood and Mirzaa (2022) found that the most dominant bacteria (93% to 99%) in soybean root nodules belonged to the genus *Bradyrhizobium*. Regardless

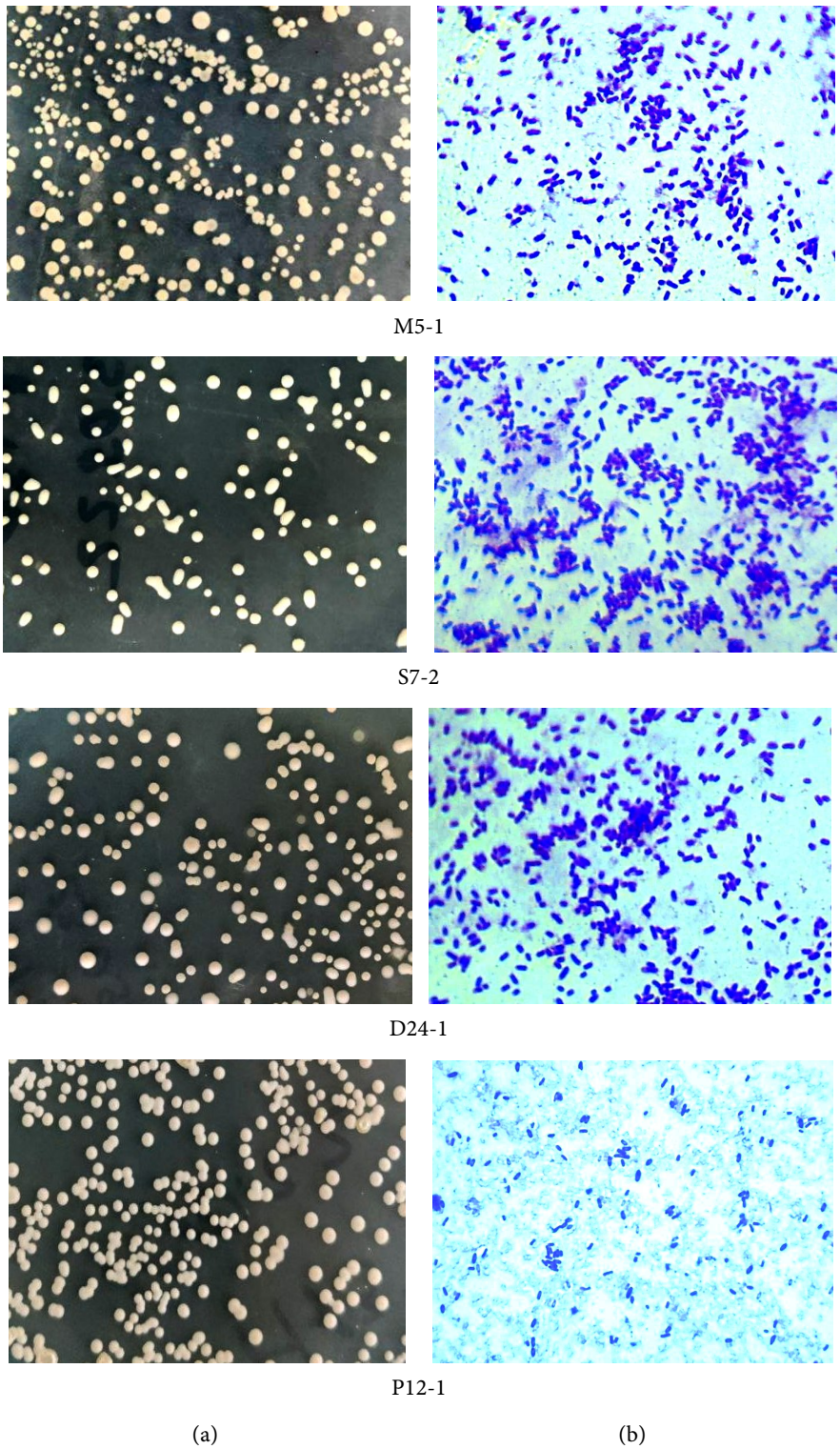


Figure 3. Colonies (a) and cells (b) of nodule bacteria from local soybeans.

of the location of the nodules (tap or lateral roots) or the size of the root nodules, the relative abundance of *Bradyrhizobium* was consistently high (90%) in all nodules. The non-rhizobial endophytes found in the nodules included the

genera *Nitrobacter*, *Tardiphaga*, *Novosphingobium*, *Pseudomonas*, *Variovorax*, *Paenibacillus*, *Flavobacterium*, *Stenotrophomonas Nitrospira*, and *Arthrobacter* [22]. Many researchers have successfully isolated and identified several non-rhizobial endophytes from legume root nodules and have reported their potential beneficial effects on host plant growth [23] [24] [25] [26].

In our studies, taking into account the presence of nonrhizobial bacteria in nodules isolates, the main focus was on slow-growing bacteria. The purity of the nodule bacteria was determined by microscopy of live and stained cell preparations (**Figure 3(b)**). The nodule bacteria from all isolated cultures were gram-negative. Observation of living bacterial cells by microscopy revealed rapid movement. The nodule bacteria cells had a regular rod shape with rounded ends and a polar flagellum. The cells were very small in size $(0.6 - 0.9) \times (1.4 - 2.9)$ μm. The bacteria were polymorphic and assumed a rounded shape over time. Fixed stained preparations showed clear granularity of the internal cell contents. The optimum conditions for bacterial growth were 28˚C and pH 7.2. The isolated bacteria were aerobes and did not grow on meat-peptone media or other protein substrates of animal origin. The strains were able to use glucose, mannitol, arabinose, xylose, galactose, and fructose as the sole carbon source. The bacteria did not liquefy gelatin and did not decompose starch. The present research work resulted in the isolation of 41 strains of bacteriologically pure nodule bacteria from the nodules of the 12 different soybean varieties. The main morpho-cultural properties resulted in the assignment of the selected bacteria to the genus *Bradyrhizobium*.

3.3. Nodulation of Local Uzbekistan Soybean Varieties

The symbiotic properties of macro- and microsymbionts were tested by searching for optimal conditions for seed germination, plant growth and development, and nodule formation following inoculation with selected nodule bacterial isolates.

For a vegetative experiment, seeds of the Madad, Sevinch, Dostlik, and Parvoz varieties were sterilized in H_2O_2 for 2 min, washed several times in sterile water, and grown in Petri dishes at 24˚C. After 3 days, when the roots had reached a length of about 3 - 5 cm, the seedlings were inoculated with 4-day-old local nodule bacterial strains (M5-1, S7-2, D24-1, P12-1, G9-1, H7-2, B16-1, T28-3, Uz5-1, Ty9-3, N18-2, and O4-1) with titers of 2.5×10^9 cells/mL. Optimal plant growth and development and formation of symbiotic nodules were achieved in a mixed substrate of vermiculite, sand, and soil (1:1:1, v/v).

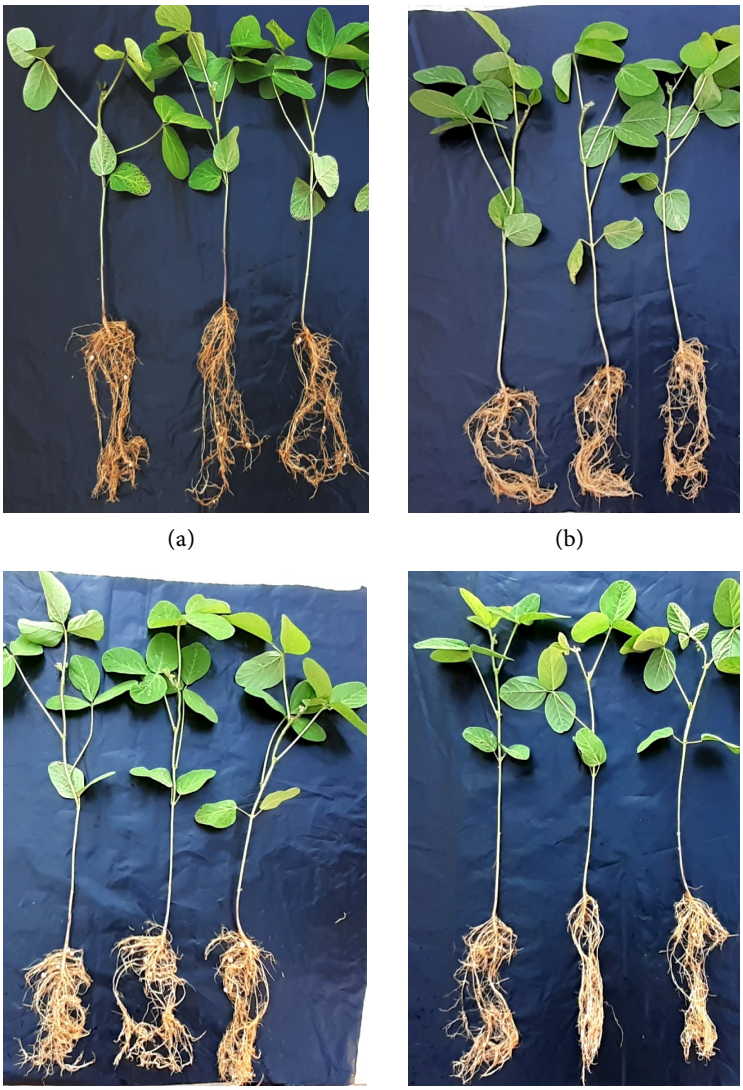
Studying nodule formation required the determination of 2 features:

1) The affiliation (biological test) of the isolated nodule bacteria with their mother plant hosts, thereby confirming their origin (“direct inoculation”);

2) The host specificity of the nodule bacteria in relation to another (non-mother) host plant; that is, their ability to form nodules on “foreign” plant hosts (“cross-inoculation”).

The Madad, Sevinch, Dostlik, and Parvoz varieties, when inoculated with bacteria isolated from the nodules of the 12 local soybean varieties, were healthy and grew vigorously. The root systems of each variety showed the formation of symbiotic nodules after one month of growth.

Inoculation of the Madad (host plant) variety with nodule bacterium M5-1 resulted in the formation of an average of 8 pink nodules with a diameter of 1 - 9 mm and a symbiosis efficiency of 46.6%. Inoculation of the Madad variety with B16-1, S7-2, G9-1, Tu9-3, P12-1, T28-3, H18-2, D24-1, Uz5-1, O4-1, and H7-2 bacteria (cross-inoculation) resulted in the formation of 2 to 10 nodules (**Figure 4(a)** and **Table 2**). Among the tested bacteria, the H18-2, TU9-3, and S7-2 strains were the most highly virulent and resulted in an increase in biomass formation of 41.3% - 45.3% compared with controls.



(a) (b) (c) (d)

Figure 4. Nodulation of plants of the Madad (a), Sevinch (b), Dustlik (c) and Parvoz (d) soybean varieties.

Table 2. Nodulation of plants of the Madad and Sevinch soybean varieties following inoculation with local strains of bacteria isolated from nodules of different soybean varieties.

Nodule bacterium	Madad variety				Sevinch variety			
	Nodule number per 1 plant	Nodule size, mm	Average dry biomass of 1 plant, g	Efficiency symbiosis, %	Nodule number per 1 plant	Nodule size, mm	Average dry biomass of 1 plant, g	Efficiency symbiosis, %
Control	-	-	0.75 ± 0.091	-	-	-	0.81 ± 0.07	-
B16-1	2 ± 1.0	2 - 9	0.94 ± 0.085	25.3	2 ± 0.57	0.5 - 7	0.95 ± 0.05	17.2
S7-2	8 ± 2.64	0.5 - 10	1.09 ± 0.052	45.3	9 ± 3.64	1 - 7	1.03 ± 0.19	27.1
G9-1	6 ± 2.0	1 - 8	0.95 ± 0.04	26.6	4 ± 2.0	0.5 - 8	0.77 ± 0.11	−5
TU9-3	9 ± 2.0	1 - 8	1.07 ± 0.41	42.6	5 ± 2.0	0.5 - 8	0.88 ± 0.25	8.6
M5-1	8 ± 2.64	1 - 9	1.1 ± 0.10	46.6	4 ± 2.30	1 - 7	0.91 ± 0.2	12.3
P12-1	10 ± 2.64	0.5 - 8	0.83 ± 0.05	10.6	8 ± 2.0	0.5 - 10	0.91 ± 0.08	12.3
T28-3	3 ± 1.0	0.5 - 7	0.55 ± 0.043	−26.6	7 ± 2.64	1 - 8	0.95 ± 0.13	17.2
N18-2	8 ± 2.64	1 - 9	1.06 ± 0.098	41.3	8 ± 2.0	0.5 - 9	0.96 ± 0.26	18.5
D24-1	5 ± 2.0	0.5 - 7	0.9 ± 0.08	20.0	12 ± 3.21	1 - 9	0.99 ± 0.17	22.2
Uz5-1	9 ± 2.64	1 - 8	0.91 ± 0.13	21.3	14 ± 7.0	1 - 7	1.08 ± 0.19	33.3
O4-1	8 ± 2.0	0.5 - 7	0.83 ± 0.052	10.6	11 ± 4.0	0.5 - 10	0.89 ± 0.08	9.8
H7-2	7 ± 3.0	0.5 - 10	0.76 ± 0.055	1.3	10 ± 3.0	1 - 6	0.94 ± 0.15	16.0
Rizovit-AKS	-	-	0.73 ± 0.06	−2.6	-	-	0.84 ± 0.1	9.8

Note: Values are the ±SE, n = 3.

Inoculation of the Sevinch (host plant) variety with its nodule bacterium (S7-2; direct inoculation) resulted in the formation of an average of 9 nodules 1 - 7 mm in size and a symbiotic efficiency of 27.1% (**Figure 4(b)** and **Table 2**). Inoculation of the Sevinch variety with bacteria D24-1 and U5-1 (cross-inoculation) resulted in the formation of 12 - 14 nodules and a symbiotic efficiency of 22.2% - 33.3%.

Inoculation of the Dustlik variety with its nodule bacteria D24-1 resulted in the formation of 8 nodules 4 - 8 mm in size and a symbiosis efficiency of 54.4% (**Figure 4(c)** and **Table 3**). Notably, cross-inoculation of this variety with the alien M5-1 nodule bacterium resulted in the formation of 4 nodules 5 - 7 mm in size, but the productivity of the plant biomass was very high and was 44.3% higher than in the control.

Nodule bacteria P12-1 had a high virulence in relation to its host plant, the Parvoz variety, with a formation of 11 nodules 1 - 6 mm in size and a plant biomass efficiency of 24.6% (**Table 3** and **Figure 4(d)**). Cross-inoculation of the Parvoz variety with foreign bacteria N18-2 and S7-2 resulted in the formation of 8 nodules with a size of 1 - 8 mm. However, the symbiotic efficiency was 4.5 times higher with the N18-2 nodule bacteria than with the S7-2 nodule bacteria.

Table 3. Nodulation of plants of the Dustlik and Parvoz soybean varieties following inoculation with local strains of bacteria isolated from nodules of different soybean varieties.

Nodule bacterium	Dustlik soybean variety				Parvoz soybean variety			
	Nodule number per 1 plant	Nodule size, mm	Average dry biomass of 1 plant, g	Efficiency symbiosis, %	Nodule number per 1 plant	Nodule size, mm	Average dry biomass of 1 plant, g	Efficiency symbiosis, %
Control	-	-	0.79 ± 0.057	-	-	-	0.73 ± 0.052	
B16-1	5 ± 1.73	1 - 10	0.99 ± 0.087	25.3	8 ± 3.0	1 - 8	1.0 ± 0.199	36.9
S7-2	9 ± 3.05	1 - 6	0.88 ± 0.078	11.3	8 ± 2.0	0.5 - 7	0.79 ± 0.113	8.2
G9-1	4 ± 2.64	0.5 - 9	0.97 ± 0.13	22.7	4 ± 3.46	0.5 - 5	0.73 ± 0.085	-
TU9-3	7 ± 2.08	0.5 - 4	0.79 ± 0.062	-	5 ± 3.46	0.5 - 10	0.84 ± 0.088	15.0
M5-1	4 ± 2.64	5 - 7	1.14 ± 0.164	44.3	5 ± 2.64	0.5 - 7	0.87 ± 0.072	19.1
P12-1	6 ± 4.58	0.5 - 6	0.81 ± 0.04	2.5	11 ± 3.6	1 - 6	0.91 ± 0.081	24.6
T28-3	7 ± 2.0	1 - 6	0.97 ± 0.175	22.7	5 ± 1.0	0.5 - 6	0.72 ± 0.078	−1.3
N18-2	6 ± 2.64	0.5 - 9	0.92 ± 0.078	16.4	8 ± 3.0	1 - 8	1.0 ± 0.286	36.9
D24-1	8 ± 2.64	4 - 8	1.22 ± 0.236	54.4	4 ± 2.64	0.5 - 6	0.69 ± 0.141	−5.4
Uz5-1	8 ± 2.0	1 - 7	0.98 ± 0.095	24.0	8 ± 2.0	0.5 - 6	0.71 ± 0.072	−2.7
O4-1	9 ± 3.6	0.5 - 8	0.89 ± 0.07	12.6	7 ± 3.0	1 - 7	0.84 ± 0.045	15.0
H7-2	5 ± 2.64	1 - 7	0.92 ± 0.121	16.4	8 ± 3.46	1 - 7	0.90 ± 0.036	23.2
Rizovit-AKS	-	-	0.81 ± 0.079	2.5	-	-	0.78 ± 0.088	6.8

Note: Values are the ±SE, n = 3.

Inoculation of the soybean varieties with the "Rizovit-AKS" commercial product, which includes the bacteria *Bradyrizobium japonicum* (Kazakhstan), did not lead to nodule formation on any of the 12 tested soybean varieties.

A similar phenomenon was observed for the Viliana (Russia) soybean variety, which did not form root nodules when inoculated with local soybean nodule bacteria. The main reason for the lack of formation of symbiotic nodules in soybean plants may be the absence of specific flavonoids that induce the nod gene of the nodule bacteria. For leguminous plants, bacteria of the genus *Rhizobium* have a known and highly specific route of entry into plant tissues through the root hairs, due to a whole cascade of molecular signals of mutual recognition that result in the formation of a channel infection thread, followed by nodule organogenesis [27]. Plant flavonoids secreted by plant roots attract rhizobia and induce the expression of genes for the synthesis of Nod factors, which are molecular signals specific to each species of leguminous plants. Evolutionary processes leading to the formation of rhizobia species and supraspecific taxa can be induced by joint promotion of bacteria and plants into new ecological zones, Adaptation of hosts to "unaccustomed" conditions is most effective if accompanied by a symbiont-mutualist [28] [29] [30]. The joint migration of partners in a N_2-fixing symbiosis in new habitats has repeatedly occurred during the evolution of legumes and arose and spread to all climatic zones of the Earth [31].

3.4. Phylogenetic Analysis of Nodule Bacteria Strains

The molecular taxonomy and creation of a phylogenetic tree of local nodule bacteria were evaluated by selecting active specific nodule bacteria of the local M5-1 (Madad), S7-2 (Sevinch), D24-1 (Dostlik), and P12-1 (Parvoz) soybean varieties. The nucleotide sequences of the 16S rRNA genes showed that strains of nodule bacteria M5-1, S7-2, D24-1, and P12-1 shared 97.07% similarity with the 16S rRNA genes of *Bradyrhizobium japonicum* PRY65 (AF239848.2) and 98.98% similarity with *Bradyrhizobium japonicum* PRY62 (AF239847.2). BLAST (NCBI) analysis of the 16S rRNA gene sequence of nodule bacteria revealed that the studied bacteria belong to the classes *Alphaproteobacteria*, the order *Rhizobiales*, the family *Rhizobiaceae*, and the genus *Bradyrhizobium*. The phylogenetic tree of the root nodule bacteria of local soybean varieties (Figure 5), built on

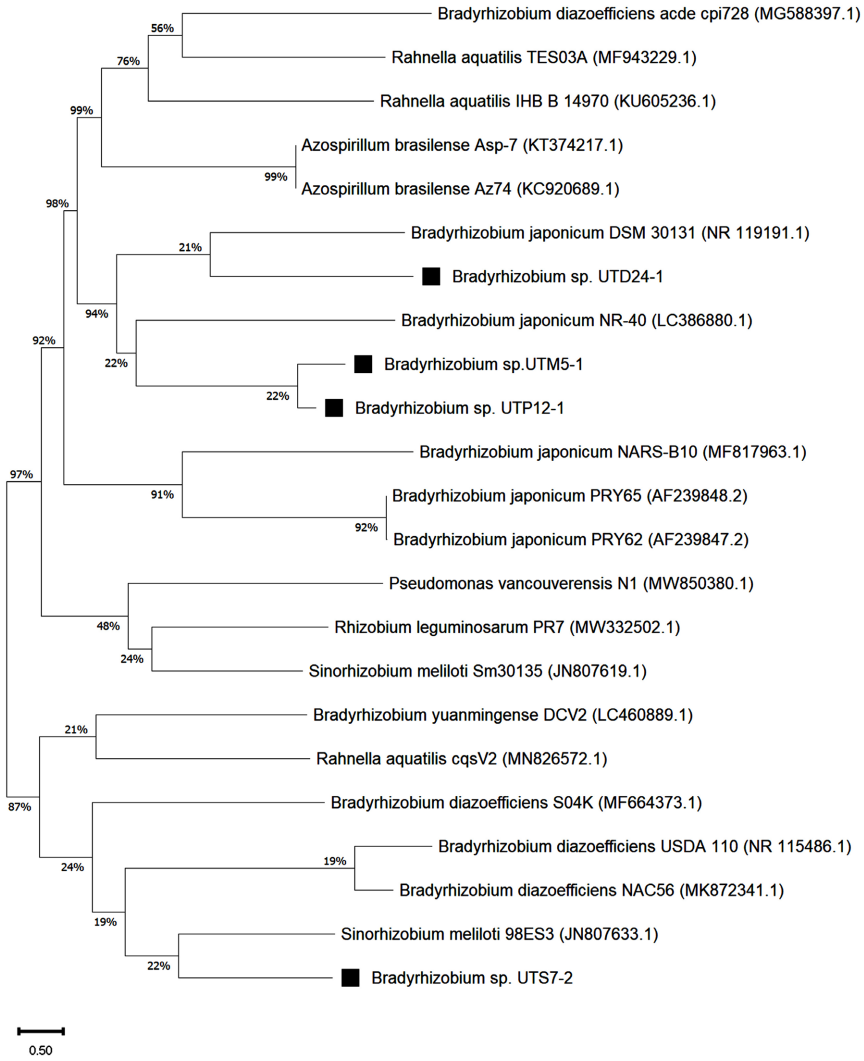


Figure 5. A phylogenetic tree based on the 16S rRNA gene of the *Bradyrhizobium* sp. UTD24-1, *Bradyrhizobium* sp. UTM5-1, *Bradyrhizobium* sp. UTP12-1, and *Bradyrhizobium* sp. UTS7-2 strains. The networked sample was obtained using the neighbor-joining method. GenBank sample numbers for applicable sequences are shown in parentheses.

the basis of the nucleotide sequence of the 16S rRNA gene of the studied bacteria, formed three clusters. The first cluster included the bacteria *Bradyrhizobium* sp. UTD24-1, the second included *Bradyrhizobium* sp. UTM5-1 and *Bradyrhizobium* sp. UTP12-1, and the third included *Bradyrhizobium* sp. UTS7-2. Notably, the *Bradyrhizobium* sp. UTM5-1 and *Bradyrhizobium* sp. UTP12-1 strains are very closely related in origin.

4. Conclusions

Nodule bacteria, which are known to play an important role in the process of fixing molecular nitrogen from the atmosphere, are capable of initiating the formation of nitrogen-fixing nodules on the roots of leguminous plants. Legume crops, such as alfalfa, peas, mung beans, beans, and peanuts, have been grown in Uzbekistan since ancient times; therefore, populations of nodule bacteria in these plants are found in the soils of all regions of Uzbekistan. By contrast, soybeans have not been cultivated in Uzbekistan until recently, and soybean nodule bacteria are absent from Uzbekistan soils. The failure to form symbiotic nodules in soybean roots leads to significantly low productivity. The main goal of our research is therefore to populate the agricultural fields of Uzbekistan with specific nodule bacteria of local soybean varieties to optimize the growth of local soybean plants in an ecologically clean and high-yield cultivation system. In this study, only the soil samples collected from agricultural fields in the Tashkent region of Uzbekistan resulted in the formation of symbiotic nodules in local soybean varieties. Nodule bacteria were isolated from healthy and vigorous soybean plants of 12 local soybean varieties with well-developed root systems and large numbers of nodules on the roots. Culture of the isolated nodule bacteria from the different soybean varieties on a bean medium under laboratory conditions led to the isolation of 41 pure cultures of nodule bacteria isolates.

The morphological-cultural properties and 16S rRNA analysis of the bacterial gene confirmed that the 41 isolates belonged to the genus *Bradyrhizobium*. Vegetative experiments conducted on 12 nodulation bacteria from the soybean varieties to determine their nodulation properties revealed that the nodule bacteria produced large numbers of pink-colored nodules in all the local soybean varieties, regardless of the soybean variety from which they were isolated. Notably, the local strains of nodule bacteria were specific microsymbionts only for the local soybean varieties, and they did not form nodules in foreign soybean varieties. The main reason for this may be differences in the genetic origins of foreign soybean varieties compared to domestic Uzbekistan soybean varieties. The use of these local nodule bacteria in the cultivation of local soybean varieties is predicted to lead to the emergence of populations of nodule bacteria adapted to the various soil and climatic conditions found in Uzbekistan.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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