

RESEARCH PAPER

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General characteristics of symbiotic relationships between bacteria belonging to the order rhizobiales and wild legumes**K. F. Bakhshaliyeva¹, N. D. İmamquliyev², M. İ. Qasımova², S. M. Muradova³**¹*Institute of Molecular Biology, PLE of the MSE RA, Baku city, Azerbaijan*²*Azerbaijan State Agrarian University, Ganja city, Azerbaijan*³*Azerbaijan State Pedagogical University, Baku, Azerbaijan***Key words:** *Rhizobium* genus, Legumes, Symbiotic tubers**Received:** June 20, 2026**Accepted:** June 30, 2026**Published:** July 07, 2026**DOI:** <https://dx.doi.org/10.12692/ijb/29.1.33-37>**ABSTRACT**

Over the different years, plants such as *Alhagi pseudalhagi* (M.Bieb.) Desv. ex B. Keller & Sha., *Cynodon dactylon* (L) Pers, *Glycyrrhiza glabra* L. and *Trifolium pratense* L., growing wild in ecologically diverse areas of the Republic of Azerbaijan, have been studied for their tuber bacteria and symbiotic tuber formation. It has become clear that the ecological differences in the environments in which plants grow determine both the number of bacteria and their ability to form symbiotic tubers. The relatively favorable environment does not have a clear negative impact on the processes studied. Understanding this may lead to the development of favorable approaches to prevent crop losses in the face of global climate change.

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INTRODUCTION

As is known, living things, which are one of the main components of the biosphere, acquire a number of adaptations in order to survive in the environment they inhabit, and sometimes these adaptations cannot be explained logically. This is primarily manifested in the mutual relationships between living things (Tu and Rappel, 2018). Thus, there are various mutual relationships between living things in nature, which are either beneficial or harmful to both or one of them (Hom and Penn, 2021).

Sometimes they can live together without benefiting or harming each other. These relationships occur between both eukaryotes and prokaryotes, as well as between eukaryotes and prokaryotes. Among these types of interactions, molecular nitrogen fixation, synthesis of biologically active substances, activation of nutrient uptake by roots, induction of systemic resistance of plants, biological control of phytopathogens, i.e., pathogens in plants, and others are of great importance.

Among these interactions, one of the most widespread and useful in terms of practical needs is the symbiotic relationship that serves to realize molecular nitrogen fixation (Vlk *et al.*, 2022), in the formation of which two components participate. One of these components is legumes, which belong to the Fabaceae family. They currently have 19,500 species in more than 750 genera, representing about 7% of all flowering plants (Maroyi, 2023), and are of significant economic importance.

The second component of the symbiosis is Gram(-) bacteria belonging to the group commonly known as Rhizobia, among which those belonging to the genus *Rhizobium*, which includes 112 species, are of greater importance (Lindström and Mousavi, 2020).

Interest in symbiotic relationships between legumes and bacteria began in the second half of the last century, more precisely in the 1970s (Arvin, 2025), which was due to the energy crisis that resulted from the increase in oil prices on the world market at that

time. This, in turn, has sparked renewed interest in the mechanisms of the process that uses solar energy to convert nitrogen from the atmosphere into compounds needed for plant growth (Bakhshaliyeva *et al.*, 2026; Herrero *et al.*, 2025). The study of factors that influence the efficiency or effectiveness of this process is relevant today, as the importance of sustainably feeding the world's population is as important today as it was in the last century.

The interest in this relationship between plants and microorganisms belonging to different taxonomic groups is also related to other issues, one of which is related to nitrates. Thus, nitrates occupy a special place among agrochemical pollutants of crop products, and excessive amounts of these compounds can lead to undesirable environmental consequences for human and animal health (Dodocioiu *et al.*, 2025). This is possible due to a disruption of the nitrogen cycle at some point in the soil-fertilizer-water-plant-human (animal) stages. For example, the misuse of fertilizers to increase the productivity of agricultural crops could be an example of this.

Therefore, special attention is paid to the symbiotic fixation of molecular nitrogen, not least because it is the only process that ensures the satisfaction of plants' nitrogen needs without damaging the ecological environment, and replacing fertilizers with it would allow for a significant reduction in the costs of crop production (Goyal *et al.*, 2021). For example, according to expert calculations, depending on the variety and yield, 50-70 kg/ha of nitrogen remains in the soil after harvest in a field planted with peas, which is equivalent to applying 10 t/ha of manure or 1.5 quintals of nitrogen fertilizer (saltpeter) to the soil. In addition, the remaining part of this plant in the soil is also rich in nitrogen and easily degrades in the soil.

The formation of the symbiotic relationship between legumes and Root nodule bacteria in legumes (those belonging to the genus *Rhizobium*) is a complex, multi-stage process that is influenced by various

abiotic and biotic factors that regulate the formation of this system (Yeremko *et al.*, 2025). The influence of these factors is not always universal, and the effectiveness of the symbiotic process occurring in specific ecological conditions differs from that occurring in other conditions.

Taking this into account, the aim of the presented study was to isolate bacteria belonging to the genus *Rhizobium*, which are in a symbiotic relationship with the same type of legume plants growing wild in different ecological conditions, and to study some of their morpho-culturological characteristics.

MATERIALS AND METHODS

Samples for the studies were taken from the territories of the Central Aran, Ganja-Dashkasan, and Lankaran-Astara economic regions. These regions differ from each other in terms of soil-climate, flora-fauna, as well as anthropogenic impact (<https://www.eco.gov.az/>).

Samples were taken from both the rhizosphere and roots (tubers) of legumes (*Alhagi pseudalhagi* (M.Bieb.) Desv. ex B. Keller and Sha., *Cynodon dactylon* (L) Pers, *Glycyrrhiza glabra* L., *Trifolium pratense* L.). Plant seeds were also collected for laboratory experiments.

Isolation of microorganisms from soil and tubers and obtaining pure cultures were carried out according to standard methods (Bergin, 2005). Meat peptone agar (MPA) and mannitol-yeast agar (MYA) were used to isolate pure cultures, as well as to study the morpho-culturological characteristics of the isolated cultures. The composition of the MYA was as follows (g/l): mannitol (lactose) – 10,0; yeast extract – 2,0; K₂PO₄; MgSO₄ – 0,2; CaCO₃ – 0,1; NaCl– 0,1.

The nutrient medium was sterilized at 1 atmosphere for 30 minutes. The incubation process was carried out at 28°C for 7-14 days. The growth of the separated cultures was visually assessed on a 5-point scale: 0 – no growth; 1 – weak growth; 2 – moderate growth; 3 – good growth and 4 – strong growth.

To identify symbiotic rhizobia in the soil, the seeds of the plants sampled are sterilized with 98% H₂SO₄, then washed thoroughly with sterile water, placed on filter paper in a Petri dish, and germinated at 25-26°C for at least 30 days. The seedlings are transferred to a plastic container containing 200-250 g of soil taken from the place where the plant grew in 4 replicates and cultivated in a climate chamber for 2 months. After the period has expired, bacteria are isolated from the roots of the plant removed from the soil according to the standard microbiological method mentioned above.

All experiments conducted in the studies to obtain quantitative data were performed in at least 4 replicates and the results were statistically analyzed.

RESULTS AND DISCUSSION

In order to study plant-microorganism relationships, 107 bacterial strains were isolated as a result of the analysis of 81 samples taken from plants grown in the study areas and germinated and cultivated in laboratory conditions during 2024-2026, of which 83 were determined to belong to the genus *Rhizobium* (Table 1). As seen, a total of 19 samples were taken from the *Alhagi pseudalhagi* plant, 21 from the *Cynodon dactylon* plant, 19 from the *Glycyrrhiza glabra* plant, and 21 from the *Trifolium pratense* plant, from which 20, 18, 17, and 28 strains were isolated, respectively. More specifically, there are more strains isolated from samples taken from the *Trifolium pratense* plant, and all of the bacteria in symbiotic relationships with it belong to the genus *Rhizobium*. This also allows us to note the higher symbiotic potential of the plant in question. Thus, a number of studies have shown that the symbiotic potential of bacteria belonging to the genus *Rhizobium* is stronger (Angus and Hirsch, 2010).

Considering the different numbers of strains isolated in the studies, they were evaluated according to the growth they showed during isolation, and 3 strains were selected for each plant and economic region, and their growth ability was evaluated in different nutrient media.

Table 1. General characteristics of bacterial strains isolated from samples taken in studies

Name of the plants sampled	Number of samples taken		Number of strains isolated	Number of species belonging to the genus <i>Rhizobium</i>
	From field conditions	From the experience of vegetation		
Central Aran economic region				
<i>Alhagi pseudalhagi</i>	5	2	10	7
<i>Cynodon dactylon</i>	4	2	7	5
<i>Glycyrrhiza glabra</i>	5	3	9	6
<i>Trifolium pratense</i>	4	3	11	10
Ganja-Dashkasan economic region				
<i>Alhagi pseudalhagi</i>	4	1	8	6
<i>Cynodon dactylon</i>	5	1	9	7
<i>Glycyrrhiza glabra</i>	4	2	7	5
<i>Trifolium pratense</i>	4	3	10	9
Lankaran-Astara economic region				
<i>Alhagi pseudalhagi</i>	5	2	9	7
<i>Cynodon dactylon</i>	5	1	8	6
<i>Glycyrrhiza glabra</i>	4	2	9	6
<i>Trifolium pratense</i>	6	3	9	9
Total	55	26	107	83
81				

Table 2. Characterization of bacterial counts in samples taken from the studied plants in the field and laboratory conditions

Name of the plants sampled	MYA		MPA
	Mannitol	Lactose	
Central Aran economic region			
<i>Alhagi pseudalhagi</i>	3; 3; 3	1; 2; 2	0; 0; 0
<i>Cynodeta dactylonae</i>	3; 3; 2	1; 2; 1	0; 0; 1
<i>Glycyrrhiza glabra</i>	3; 4; 3	1; 2; 2	0; 1; 0
<i>Trifolium pratense</i>	4; 4; 4	2; 2; 3	1; 0; 1
Ganja-Dashkasan economic region			
<i>Alhagi pseudalhagi</i>	3; 4; 3	1; 2; 1	0; 1; 0
<i>Cynodeta dactylonae</i>	4; 3; 3	1; 1; 1	0; 0; 0
<i>Glycyrrhiza glabra</i>	4; 3; 3	1; 2; 2	0; 0; 1
<i>Trifolium pratense</i>	3; 4; 4	2; 1; 2	0; 1; 0
Lankaran-Astara economic region			
<i>Alhagi pseudalhagi</i>	3; 2; 3	2; 1; 1	0; 0; 0
<i>Cynodeta dactylonae</i>	2; 2; 2	1; 2; 1	0; 0; 0
<i>Glycyrrhiza glabra</i>	2; 3; 3;	1; 2; 2	0; 0; 1
<i>Trifolium pratense</i>	3; 3; 3	2; 1; 2	1; 0; 0

From the obtained results became clear that most of the strains belonging to the genus *Rhizobium* have better growth ability in the MYA containing mannitol. A similar situation was not observed with the use of lactose, as well as MPA. More specifically, the growth of the separated cultures varied depending on both the carbon source and the plant from which it was separated (Table 2). As can be seen, in this case, the growth ability of the cultures separated from the *T. pratense* plant was better, and this was evident in the cultures separated from all economic regions. Interestingly, the use of MPA was observed to result in a lack of growth in most cases (except for 9 out of 36 options, i.e. 75%). The inability of bacteria of the genus *Rhizobium* to grow on MPA has been

confirmed in other studies (Vlk *et al.*, 2022; Yakimenki *et al.*, 2024).

It would be appropriate to touch on one more point regarding the data provided in Table 2, which is the relationship between the soil-climatic conditions of the economic regions and the metabolic activity of tubers. So, if we analyze these economic regions in general according to soil and climatic conditions, the ecological conditions in the Central Aran economic region are not as favorable as in others. However, the bacteria that are released from it absorb both mannitol and lactose to a higher degree. This also allows us to note that the negative impact of unfavorable conditions on symbiotic processes is not clearly observed.

CONCLUSION

In the conducted studies, 107 bacterial strains, 83 of which belong to the genus *Rhizobium*, were isolated from legumes such as *Alhagi pseudalhagi*, *Cynodon dactylon*, *Glycyrrhiza glabra* and *Trifolium pratense*, which grow wild in some economic regions of the Republic of Azerbaijan, as well as from plants obtained during the cultivation of their seeds in laboratory conditions. Although the isolated strains differed in their ability to absorb mannitol and lactose, the bacteria isolated from the *Trifolium pratense* plant were more active than the others.

REFERENCES

- Angus AA, Hirsch AM.** 2010. Insights into the history of the legume-betaproteobacterial symbiosis. *Molecular Ecology* **19**(1), 28–30.
<https://doi.org/10.1111/j.1365-294X.2009.04459.x>
- Arvin M.** 2025. Rhizobial symbiosis and nitrogen fixation: A key biological partnership for sustainable plant growth. *International Research Journal of Plant Science* **16**(12), 1–2.
<http://dx.doi.org/10.14303/irjps.2025.12>
- Bakhshaliyeva KF, İmamquliyev ND, Gasimova MB, Muradova SM, Muradov PZ.** 2026. Characteristics of symbiotic relationships between plants and bacteria and the influence of stress factors on them. *International Journal of Biosciences* **28**(2), 75–90.
- Bergin SA.** 2005. Methods and Techniques for Studying and Selecting Effective Strains of Soybean Nodule Bacteria: Analytical Selection Methods. Blagoveshchensk: PKİ «ZEY». 70 p.
- Dodocioiu AM, Buzatu GD, Botu M.** 2025. Nitrates and nitrites in vegetables and the health risk. *Foods* **14**, 3037. <https://doi.org/10.3390/foods14173037>
- Goyal RK, Mattoo AK, Schmidt MA.** 2021. Rhizobial-host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. *Frontiers in Microbiology* **12**, 669404.
<https://doi.org/10.3389/fmicb.2021.669404>
- Herrero J, Ramírez-Santos A, Díaz-Santos E, Torres-Cortés G.** 2025. Biofertilizers for enhanced nitrogen use efficiency: Mechanisms, innovations, and challenges. *Nitrogen* **6**, 111.
<https://doi.org/10.3390/nitrogen6040111>
- Hom EFY, Penn AS.** 2021. Symbiosis and the Anthropocene. *Symbiosis* **84**(3), 239–270.
<https://doi.org/10.1007/s13199-021-00794-0>
- Lindström K, Mousavi SA.** 2020. Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology* **13**(5), 1314–1335.
<https://doi.org/10.1111/1751-7915.13517>
- Maroyi A.** 2023. Medicinal uses of the Fabaceae family in Zimbabwe: A review. *Plants* **12**(6), 1255.
<https://doi.org/10.3390/plants12061255>
- Ministry of Ecology and Natural Resources of the Republic of Azerbaijan.** Official website.
<https://www.eco.gov.az/>
- Tu Y, Rappel WJ.** 2018. Adaptation of living systems. *Annual Review of Condensed Matter Physics* **9**, 183–205.
<https://doi.org/10.1146/annurev-conmatphys-033117-054046>
- Vlk D, Trněný O, Řepková J.** 2022. Genes associated with biological nitrogen fixation efficiency identified using RNA sequencing in red clover (*Trifolium pratense* L.). *Life* **12**, 1975.
<https://doi.org/10.3390/life12121975>
- Yakimenki MV, Tatarenko İY, Sorokina AI.** 2024. Isolation of pure culture of rhizobia strains from cowpea and soybean nodules. *Agroscience* **2**(1), 61–69.
<https://doi.org/10.24412/2949-2211-2024-2-1-61-69>
- Yeremko L, Czopek K, Staniak M, Marenych M, Hanhur V.** 2025. Role of environmental factors in legume-rhizobium symbiosis: A review. *Biomolecules* **15**, 118.
<https://doi.org/10.3390/biom15010118>