

## Variation of the bacterial community in the rhizosphere of the *Lotus corniculatus* L. in the four years of culture

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**Abstract** Rhizosphere is the area of the soil under direct influence of the root system. Plants remove metabolites in the soil with a positive or negative chemotactic effect on the microbiota. These root exudates bring changes in the structure of the microbial community, either by stimulation or by inhibition. For the study of rhizobacteria, soil samples were collected together with the adjacent plants of *Lotus corniculatus* L. from the crop years I-IV (R1, R2, R3, R4). The effect of the plants on the bacteria was reported in the control experimental samples (samples from uncultivated parcels - M1, M2, M3, M4). Isolation of bacteria from the eight experimental variants was performed on a specific environment. The incubation period was followed by colonial, cellular morphological studies, establishing tincture characters and identifying genus / bacterial species. The results have shown that in the 4 years of culture, the plants influence the bacterial community and its composition. The most abundant bacterial flora has been present in the 3-year rhizosphere culture (R3). Gram-positive bacteria also dominate, to the detriment of Gram negative, and the common species were *Bacillus*, *Pseudomonas* and *Arthrobacter*.

### Key words

bacterial community,  
*Bacillus* genus,  
*Pseudomonas* genus,  
*Arthrobacter* genus, *Lotus corniculatus* L.

As Nannipieri et al. (2003) considered [36] microbiological parameters are more sensitive to management strategies and the variation of environmental conditions, compared with the chemical and physical properties of the soil. Changes in soil microflora composition are essential to maintaining the integrity of soil functions [26].

There is a close connection between the soil and micro-organisms, because the soil's properties influence the microbial community, and this determines the soil functions. The presence of plant roots and root exudates have a strong impact on the structure of the microbial community in the soil.

The exudates released into the soil by the roots are composed of water, soluble sugars, organic acids, amino acids, hormones, vitamins, phenols, etc. [45].

The quality and quantity of the carbon and nitrogen compounds in rizodeposit varies according to the plant species [5; 47; 29; 31] and is reflected in the differences that occur in the microbial community of the rhizosphere [28; 43]. For this reason in this paper the root plant rhizosphere of *Lotus corniculatus* L. (common bird's-foot trefoil or bird's-foot trefoil,) was analyzed from the bacteriologically point of view, during four years of cultivation.

For us, bacteria have shown interest since many studies showed that they have the highest number in the soil and are essential for the nutrient cycle, plant

growth [7], organic contaminant degradation, phytotoxicity reduction, and phytopathogen control [39; 40; 46; 37; 20; 11]. Brimecombe et al., (2007), [12] stated that plant health and productivity depend on rhizobacteria. The benefits of the interaction between plants and rhizobacteria can reduce their dependence on synthetic chemicals that destabilize agro-ecosystems [35].

The diversity of microorganisms varies with environmental conditions [50; 17; 14], availability of nutrients [6], soil type, pH [3; 30; 49], the anthropogenic influence, materialized by the toxicity of organic or inorganic contaminants, but probably also because of differences in the quality or quantity of rizodeposit [1, 41]. and the type of vegetation.

Plant species, their development phase and soil type are considered major factors that determine the composition of microbial communities in the rhizosphere [13]. So, the diversity of micropopulation in the rhizosphere depends on the abiotic and biotic factors that predominate in the ecological microhabitat.

*Lotus corniculatus* is a leguminous plant included in the Leguminosae family, *Lotus* genus. This genus is spread in certain areas of Europe, North America, Africa, Asia, and includes annual and perennial plants that establish symbiosis with nitrogen fixation bacteria [2].

Through the symbiotic relationship they have with bacteria [27], *Lotus* species contribute to improving soil fertility by increasing nitrogen content and organic matter. In addition, they have high productivity on a wide range of soils [8]. Biologically estimated nitrogen is estimated to be about 200 million tonnes / year [21; 38].

## Materials and Methods

The experimental plots (*Lotus corniculatus* L.), the years of culture I-IV (Table 1), were located in the western part of Romania (Arad county). Both the rhizospheric microbiota (R1-R4) in the area under the *Lotus corniculatus* L. roots and the control variants (C1-C4) was studied during the same calendar year.

Table 1

Experimental variants	
Experimental variant /year	
C1-	control, uncultivated soil with <i>Lotus corniculatus</i> L. and R1- rhizosphere of <i>Lotus corniculatus</i> L. / year I of culture
C2-	control, uncultivated soil with <i>Lotus corniculatus</i> L. and R2- rhizosphere of <i>Lotus corniculatus</i> L. / year II of culture
C3-	control, uncultivated soil with <i>Lotus corniculatus</i> L. and R3- rhizosphere of <i>Lotus corniculatus</i> L. / year III of culture
C4-	control, uncultivated soil with <i>Lotus corniculatus</i> L. and R4- rhizosphere of <i>Lotus corniculatus</i> L. / year IV

Soil harvesting was performed when *Lotus corniculatus* L. was bloomed in the summer season from the root zone (rhizosphere), along with the adjacent plants, and from the non-influenced areas of the leguminous crop (control). The depth of soil harvest was 0-20 cm. The processing and study of bacteria took place in the laboratory of Microbiology, University of Agricultural Sciences and Veterinary Medicine of Banat "King Mihai I of Romania" in Timisoara

Isolation of soil microorganisms was performed on Topping nutritional medium, specific to soil bacteria, using the incorporation technique [9; 44; 48]. The growth temperature of the bacteria was 28°C and incubation time of 48 h. The growth of bacteria was followed by pure cultures obtaining, the study of colonial and cellular morphology, Gram technique and the determination of dominant genus or species.

### Results and discussions

Through the root exudates, plants have an impact on the microbial community and select a specific

microbiota. The quantity and quality of rhizodeposition depends, however, largely on the growth phase and the physiological state of the plant [22] and the root system (Di Battista, 2002, quoted by Oger, 2004), [33] which shows the great importance of monitoring the number and composition of rhizospheric bacteria during the years of culture in the case of perennial plants.

Estimating the abundance of bacteria in the soil is an important condition for the complex analysis of the structure and function of the microbial community in the natural environments [16; 18; 19].

The soil microbiological analysis was performed after 48 hours, and was reported on observations made after 24 hours, data that were published in 2014 [10]. Additionally, the morphology of bacterial colonies was studied and the Gram character was established to follow the prevailing of Gram negative bacteria compared with the Gram positives, specifying the frequent species in the analyzed soil samples.

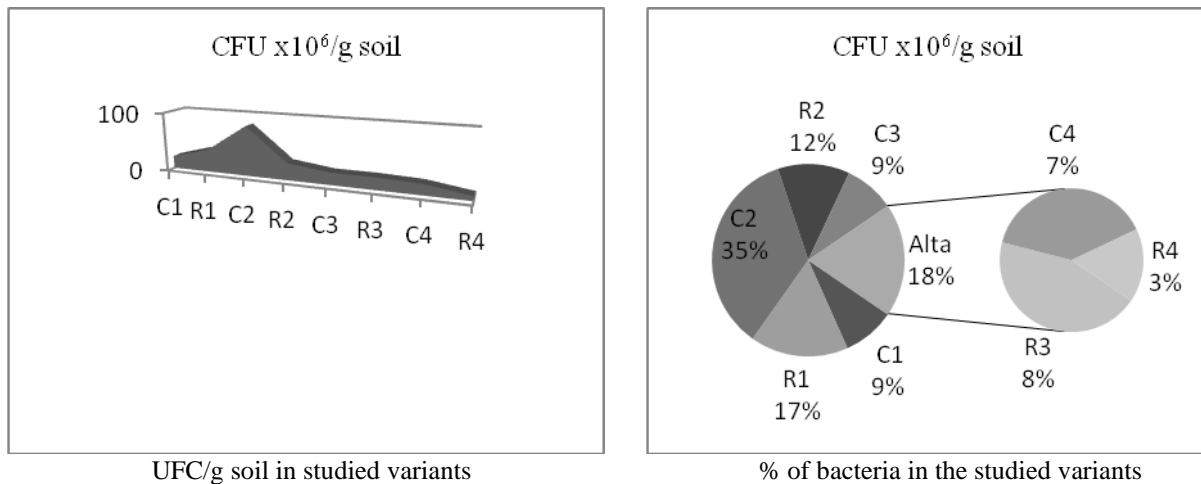


Fig. 1. Evolution of bacteria in soil cultivated with *Lotus corniculatus* L. after 24 h of incubation [10].

Legend: C1-control, R1-rhizosphere, 1st year of culture of *Lotus corniculatus* L.; C2-control, R2-rhizosphere, 2nd year of culture of *Lotus corniculatus* L.; C3-control, R3-rhizosphere, 3rd year of culture of *Lotus corniculatus* L.; C4-control, R4-rhizosphere, fourth year of culture of *Lotus corniculatus* L.

The studies shown in Fig. 1, shows that the highest percentage is held by the control variant (C2), compared to all the variants analyzed. In the rhizosphere area, the bacteria experienced a regression over the 4 years ( $R1 < R2 < R3 < R4$ ), although in the first year there was an increase in the number of bacteria in the rhizosphere (R1) compared to the control (C1) but in fourth year of culture (R4) there are values below the control limit (C4) [10].

Referring to Figure 1, after 48 hours of incubation (Figure 2), a quantitative increase in bacteria was observed in all analyzed, but especially in the 3-year culture rhizosphere (R3). In this variant, the increase is 30% compared to the values recorded after 24 hours of incubation. Except for variant R2, in all other rhizospheric zones (R1, R3, R4), there was an increase compared to control variants. The analysis of the bacteria in the areas influenced by the plant roots of *Lotus corniculatus* L. in the four years of culture shows that both after 24 hours and after 48 hours a difference in value between variants C2 and R2 is preserved but the values of the controls are exceeded in the R1, R3 and R4 variants. The same situation was observed after 24 hours, when the highest percentage was obtained by control variant (C2) versus all the variants analyzed.

Depending on the incubation period (in this case 48 h) and the year of culture, the pattern of evolution of the bacterial community is the following:  $R3 > C2 > R1 > R2 > R4$ ,  $C1 > C4 > C3$ . The most abundant bacterial community is in R3, followed by control variant C2.

As can be seen in other studies, plants can change the soil microbiota by secreting bioactive compounds in the rhizosphere [32; 2; 3; 15].

Also, carbon and nitrogen (the result of root exudates) are essential macronutrients for microbial growth and have a multitude of effects on microorganisms in the rhizosphere (or rhizobacteria) by acting as signaling molecules, attractants (chemotactism), stimulants and inhibitors or repellents [4].

Therefore in our studies, in some variants we also observed an increase in the bacteria in the area of influence of the roots of *Lotus corniculatus* L., but also a regression in others.

According to research done by Hu et al. (2018), [25]. plants condition the microbiota from the rhizosphere by means of root exudates and implicitly affect the growth and the way of defending of successive cultures

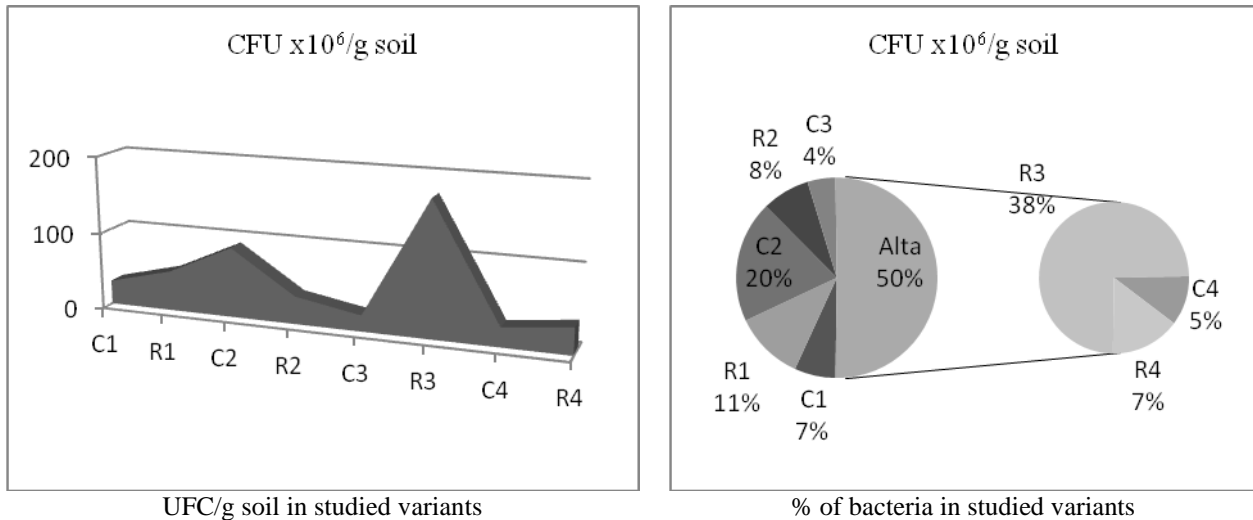


Fig. 2. Evolution of bacteria in soil cultivated with *Lotus corniculatus* L. after 48 h of incubation  
 Legend: C1-control, R1-rhizosphere, 1st year of culture of *Lotus corniculatus* L.; C2-control, R2-rhizosphere, 2nd year of culture of *Lotus corniculatus* L.; C3-control, R3-rhizosphere, 3rd year of culture of *Lotus corniculatus* L.; C4-control, R4-rhizosphere, fourth year of culture of *Lotus corniculatus* L.

The quantification of the number of bacteria in the soil gives us insights on soil health. According to some studies, a healthy soil has between 10<sup>6</sup> and 10<sup>8</sup> bacteria per gram of soil. In the soil studied by us, UFC / g soil reach the minimum limit of 10<sup>6</sup> [51].

Some recent studies show that plants select and promote the growth of microorganisms by producing hormones that stimulate the microbial flora or eliminate pathogens from the soil, or help them to survive under stress [6; 24; 34; 42].

According to Table 1, six types of colonies with different morphology were analyzed. The number of colonies varies in the 8 analyzed variants (Figure 3.4). M2 and M3 colonies are present both in the control variant (M1) and in the R1 variant.

The M1 colonies dominate the control variant from the first year of culture (C1), but their place is taken over by M4 colonies in the same year of culture of *Lotus corniculatus* L. (R1). In control variant C2, only the M1 and M2 colony types remain and the composition of the bacteria in the 2nd crop rhizosphere (R2) changes due to the influence of the crop culture. As a result, in this variant there is a small number of M1 colonies, but there are bacterial species that are present in control variants (C1) and rhizosphere (R1) of culture year I, ie M4 colonies (which dominate, like the variant R1 ) followed by M3 colonies, but there are two new species (M5, M6).

Table 2

**Morphology of colonies from uncultivated soil and cultivated with *Lotus corniculatus* L..**

Variant	The morphological characters of the colonies	
C1	M1, M2, M3	Legend (M1) - large, thin, transparent colonies (M2)- filamentous colonies (M3) - large, crater-shaped, glossy colonies (M4) - glossy spotted colonies (M5)- large, flat, wrinkled colonies (M6)- large colonies, toothed edges, matte, shielded
R1	M3, M2, M4	
C2	M2, M1	
R2	M5, M3, M4, M1	
C3	M4, M1	
R3	M2, M3, M4	
C4	M3, M6, M4	
R4	M1, M3	

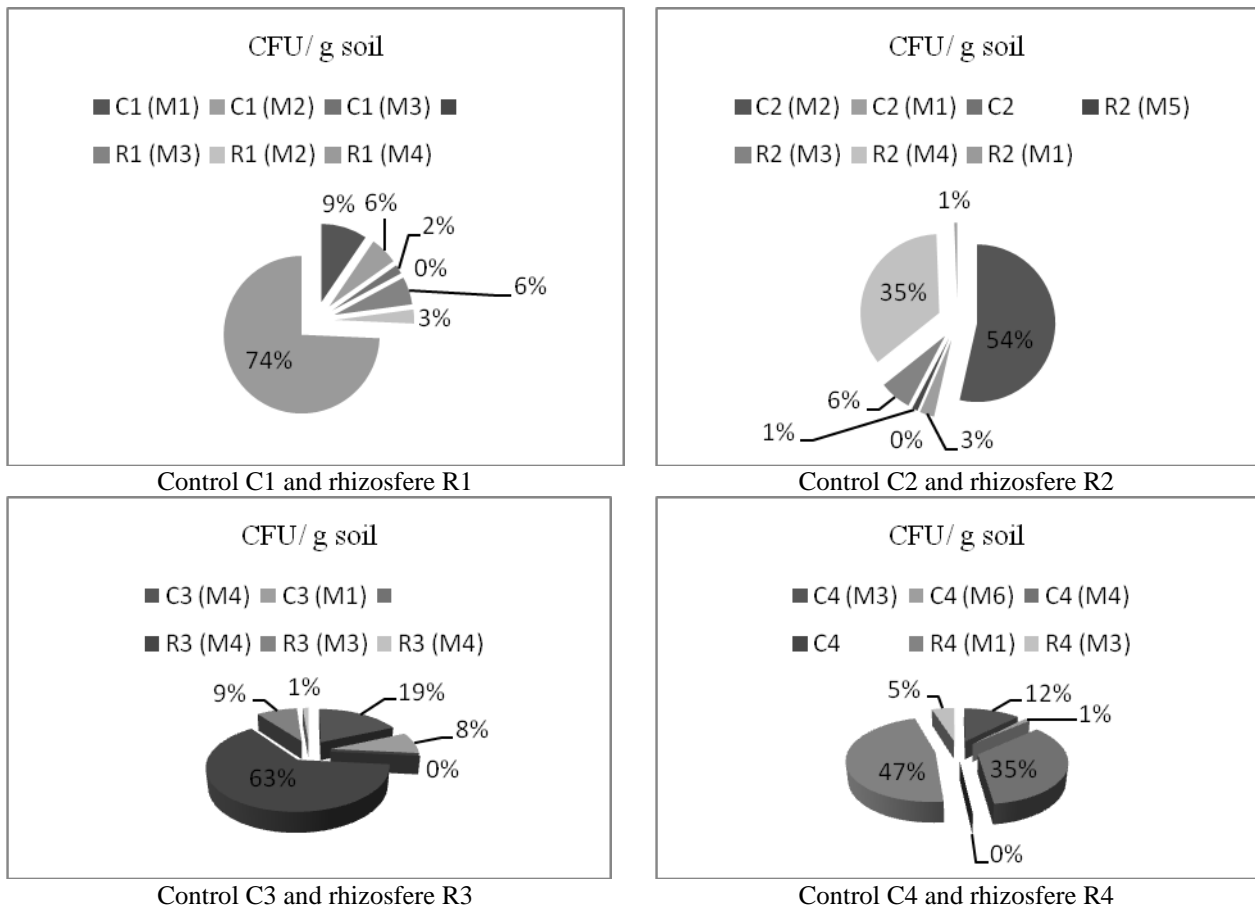
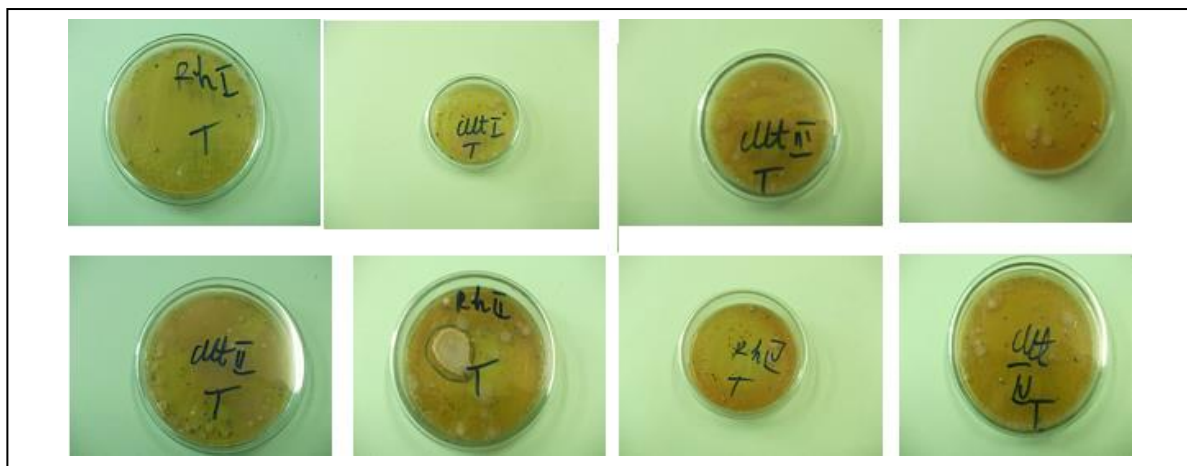


Fig. 3 The proportion of colonies in the experimental analyzed variants

Legend: C1-control, R1-rhizosphere, 1st year of culture of *Lotus corniculatus* L.; C2-control, R2-rhizosphere, 2nd year of culture of *Lotus corniculatus* L.; C3-control, R3-rhizosphere, 3rd year of culture of *Lotus corniculatus* L.; C4-control, R4-rhizosphere, fourth year of culture of *Lotus corniculatus* L.



Following the microscopic study, most bacteria were found to be Gram positive (G +), with the exception of the M4 and R4 variants, where Gram negative (G-) bacteria dominated.

Bacterial genus and species present include *Bacillus sphaericus*, *Pseudomonas* spp, *Arthrobacter* spp, *Bacillus cereus* and *Bacillus cereus* var. *mycoides*.

Morgan et al., (2005) asserted that rhizobacteria are frequently studied and the genus *Pseudomonas* and *Bacillus* are often the dominant bacterial groups in the rhizosphere.

## Conclusions

Plants contribute to the modification of the soil microbiota through the secretion of bioactive exudates. Phase and crop year of the plant has an effect on soil bacteria.

After 48 hours of incubation, the most abundant bacterial community was identified in the 3-year-old *Lotus corniculatus* L. (R3) rhizosphere, followed by the second crop control (C2). Instead, after 24 hours of incubation, the largest number of bacteria was observed in control variant C2, followed by the same year's rhizosphere (R1).

Among the colonies that dominate either the control variants or the variants cultivated with *Lotus corniculatus* L. are colonies M1 (large, transparent, transparent colonies) and M4 (punctate, glossy colonies). Among the frequent but less numerical colonies are M2 and M3 respectively. M5 and M6 colonies were evident in a small number in the rhizosphere of the 2nd year of culture (R2, respectively) in the control variant of the fourth year of culture (C4).

Regarding the tincture characters, Gram positive bacteria dominate and among the listed species we mention: *Bacillus sphaericus*, *Bacillus cereus* and *Bacillus cereus* var. *mycoides* *Pseudomonas* spp., *Arthrobacter* spp.

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