



The efficiency of nitrogen fixation through the prism of the interaction of rhizobia with antibiotics



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MATERIAL AND METHODS

INTRODUCTION

The symbiosis of bacteria with leguminous plants is very common and quite profitable from an economic and ecological point of view reminds the importance of symbiotic bacteria in promoting plant growth. However, there are countless environmental factors that influence the symbiosis of bacteria with plants. Among these factors are mentioned: antibiotics, metals, pH, humidity, temperature. There are studies that show that the bacteria involved in symbiosis can show resistance to antibiotics. Overcoming the antagonism exerted by antibiotic-producing microorganisms is a way of survival of bacterial inoculants and a way of increasing the amount of biologically fixed nitrogen, which leads to efficiency and benefits for agriculture. All this information proves that the presence of rhizobia resistance to abiotic and biotic factors is essential, which is why the selection of bacterial strains that show resistance to antibiotics is also the goal of our work.

The pea (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) plants were harvested from family gardens, located in the western part of the country, in the localities: Timișoara (P1, P4) and Topolovăț (P2), (Timiș county) and Lipova (P3), (Arad county). Instead, all alfalfa plants (*Medicago sativa* L) (A1, A2, A3, A4) come from Timisoara (Timis county). The root nodules were subjected to a sterilization operation. Isolation of bacteria from nodules was carried out under sterile conditions, on Yest-Manitol-Agar (YMA) culture medium. After purifying the cultures, the sensitivity of the bacteria to antibiotics (ampicillin, cefatixime, neomycin, ofloxacin and gentamicin) was tested. The sensitivity of rhizobia to antibiotics was determined by the diameter of the halo (D) around the antibiotic biodiscs, expressed in mm.

RESULTS AND DISCUSSIONS

The results demonstrated that there is a variation in the degree of sensitivity of the rhizobia isolated from the nodules of the two legumes depending on the antibiotic, as evidenced by the presence of halos with diameters between 7-30.5 mm in the case of bacteria associated with peas, respectively 8-26.5 mm in the case of bacteria associated with alfalfa (figures 1,2). Our results are in agreement with other studies. There were also situations where the inhibition zones were absent in the case of some antibiotics. This was observed both in peas for the plants from the localities of Timisoara (P1,P4), and Lipova (P3), (Arad county), as well as in alfalfa, for the plants harvested from the locality of Timisoara (A1-A3) (figures 1,4). The symbiotic bacteria isolated from the roots of pea plants from the Topolovăț locality (P2), respectively those isolated from the roots of alfalfa plants (A4) were sensitive to the antibiotics tested. The rhizobia from the symbiosis with the pea plants harvested from Lipova (P3) were the most sensitive compared to the other rhizobia associated with peas. Their sensitivity was high to cefotaxime (10 μg). The high sensitivity to this antibiotic was also observed in the case of rhizobia on the roots of alfalfa A4 (D 26.5 mm) (figure 2). The cluster analysis was performed using Paired Group Algorithm and Euclidian Distance as Similarity measure (figures 3a,b).

Symbiotic bacteria for peas

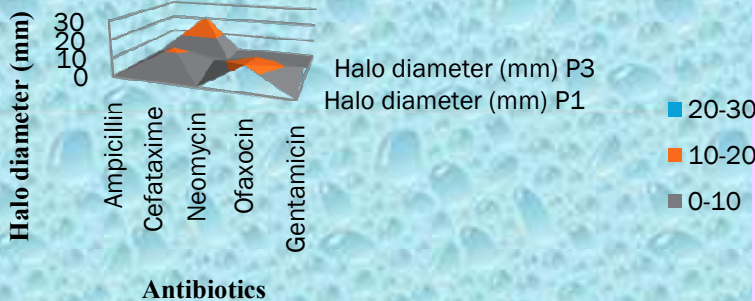


Figure 1 The diameter of the inhibition zones through the interaction of *Rhizobium leguminosarum* species with antibiotics

Symbiotic bacteria for alfalfa

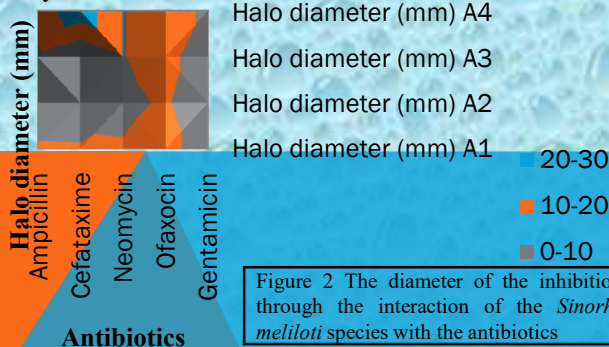


Figure 2 The diameter of the inhibition zones through the interaction of the *Sinorhizobium meliloti* species with the antibiotics

Legend: 0-30 range of inhibition zones (mm) for rhizobia isolated from the roots of pea and alfalfa plants from four different zones ; P1 and P4 – pea plants from the town of Timisoara; P2 – pea plants from Topolovăț locality; P3 – pea plants from the town of Lipova; A1-A4-alfalfa plants from four different locations in Timisoara.

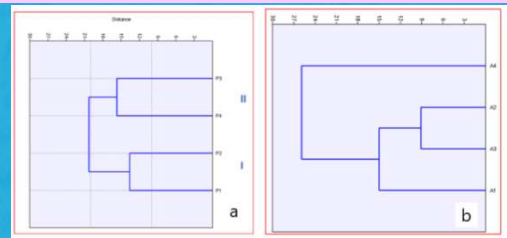


Figure 3 Cluster Analysis representation Data for peas (a) and alfalfa (b)

CONCLUSIONS

Attention should be paid to cefotaxime, which had an inhibitory effect on all bacteria isolated from the roots of pea plants, regardless of location, and in the case of alfalfa, neomycin negatively influenced the growth of rhizobia. But there have also been situations in which symbiotic bacteria have shown resistance to antibiotics. The presence of this trait supports the multiplication, persistence, infectious capacity of rhizobia for the host plant and their efficiency in fixing molecular nitrogen.

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