



## ADAPTIVE ANTIBIOTIC RESISTANCE AND SYMBIOTIC PERFORMANCE IN RHIZOBIUM STRAINS

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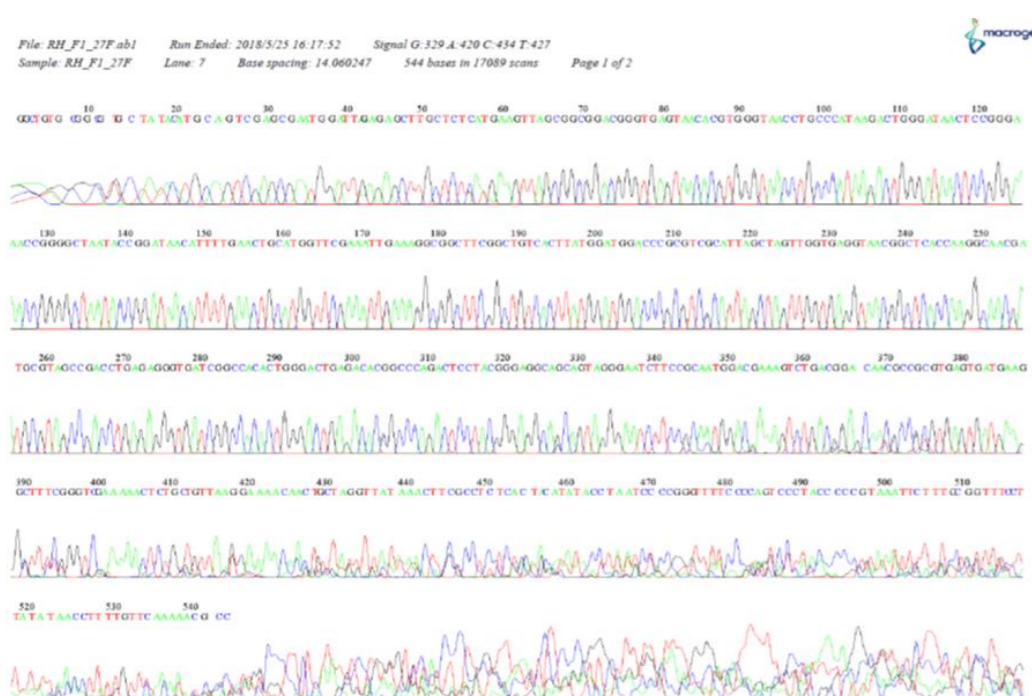
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The study of antibiotic resistance in nitrogen-fixing bacteria is important for agriculture, environmental protection, and public health. These microorganisms, including *Rhizobium*, *Azotobacter*, and *Frankia*, contribute to soil fertility by converting atmospheric nitrogen into forms available to plants, reducing the need for chemical fertilizers and supporting sustainable agriculture. Soil also acts as a reservoir of antibiotic resistance genes, as bacteria can exchange genetic material through horizontal gene transfer, potentially spreading resistance traits to pathogenic microorganisms. Studying the resistance profiles of nitrogen-fixing bacteria is essential for selecting safe and efficient biofertilizer strains and for monitoring environmental pollution caused by agricultural, livestock, or wastewater residues. Furthermore, resistance mechanisms are often linked to tolerance to heavy metals and other environmental stresses, providing insights into microbial adaptation in soil ecosystems. These studies are also relevant within the One Health framework, since resistance genes from agricultural environments may enter the food chain and affect human and animal health. Therefore, investigating antibiotic resistance in nitrogen-fixing bacteria has important ecological, agricultural, and medical implications.

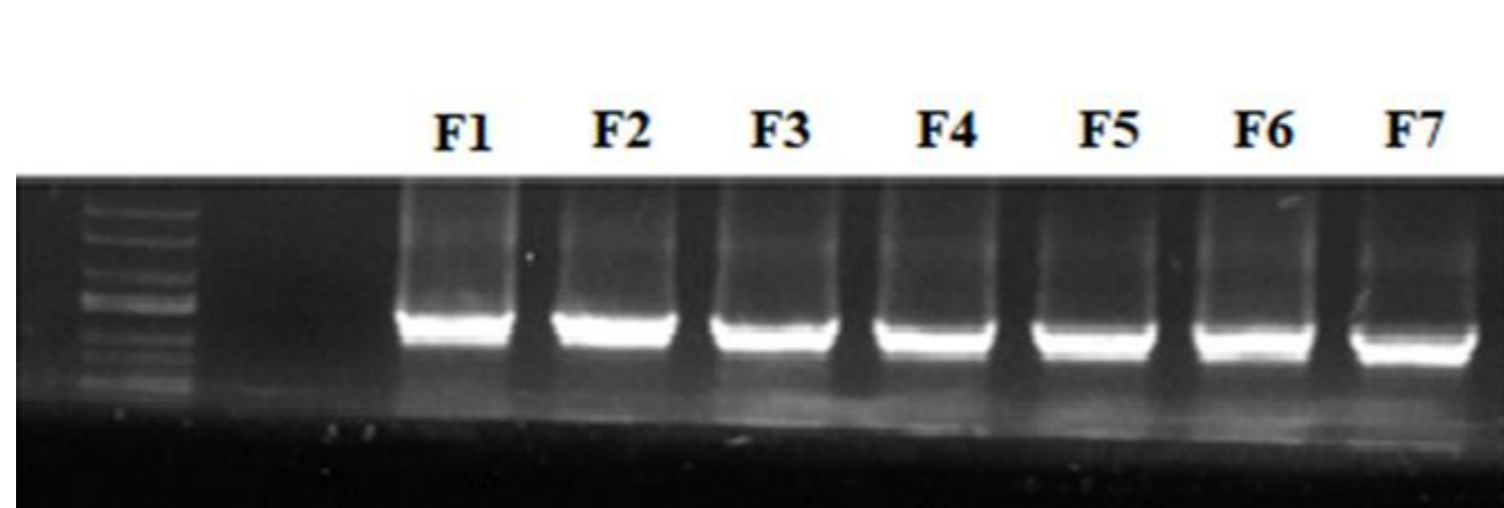
**AIM:** In this study it was investigated the antibiotic resistance profiles and symbiotic efficiency of seven rhizobial isolates (F1–F7), taxonomically assigned to *Rhizobium leguminosarum phaseoli* through DNA barcoding analysis, in order to evaluate potential correlations between resistance patterns and symbiotic performance, as well as their ecological implications.

### Identification of Bacterial Species by 16S rRNA Gene Sequencing

Identification of bacterial species through 16S rRNA gene sequencing is one of the most widely used molecular methods for bacterial taxonomic characterization. The 16S rRNA gene contains both conserved and variable regions, allowing the differentiation and identification of microorganisms at the genus and species levels. Comparison of the obtained sequences with genetic databases provides accurate information regarding the phylogenetic affiliation of the analyzed bacterial strains.



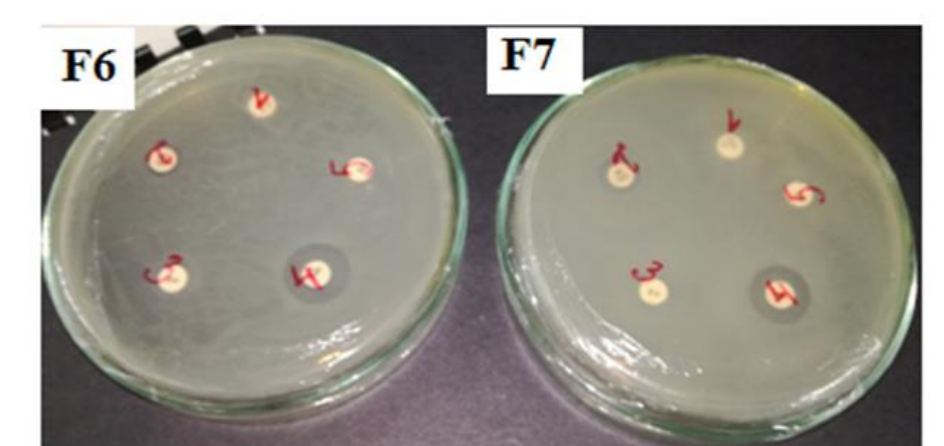
All seven previously isolated bacterial strains (F1-F7) were identified as *Rhizobium leguminosarum phaseoli*. The obtained sequences showed a high degree of similarity with reference sequences available in genetic databases, confirming the taxonomic affiliation of all isolates to this nitrogen-fixing bacterial species.



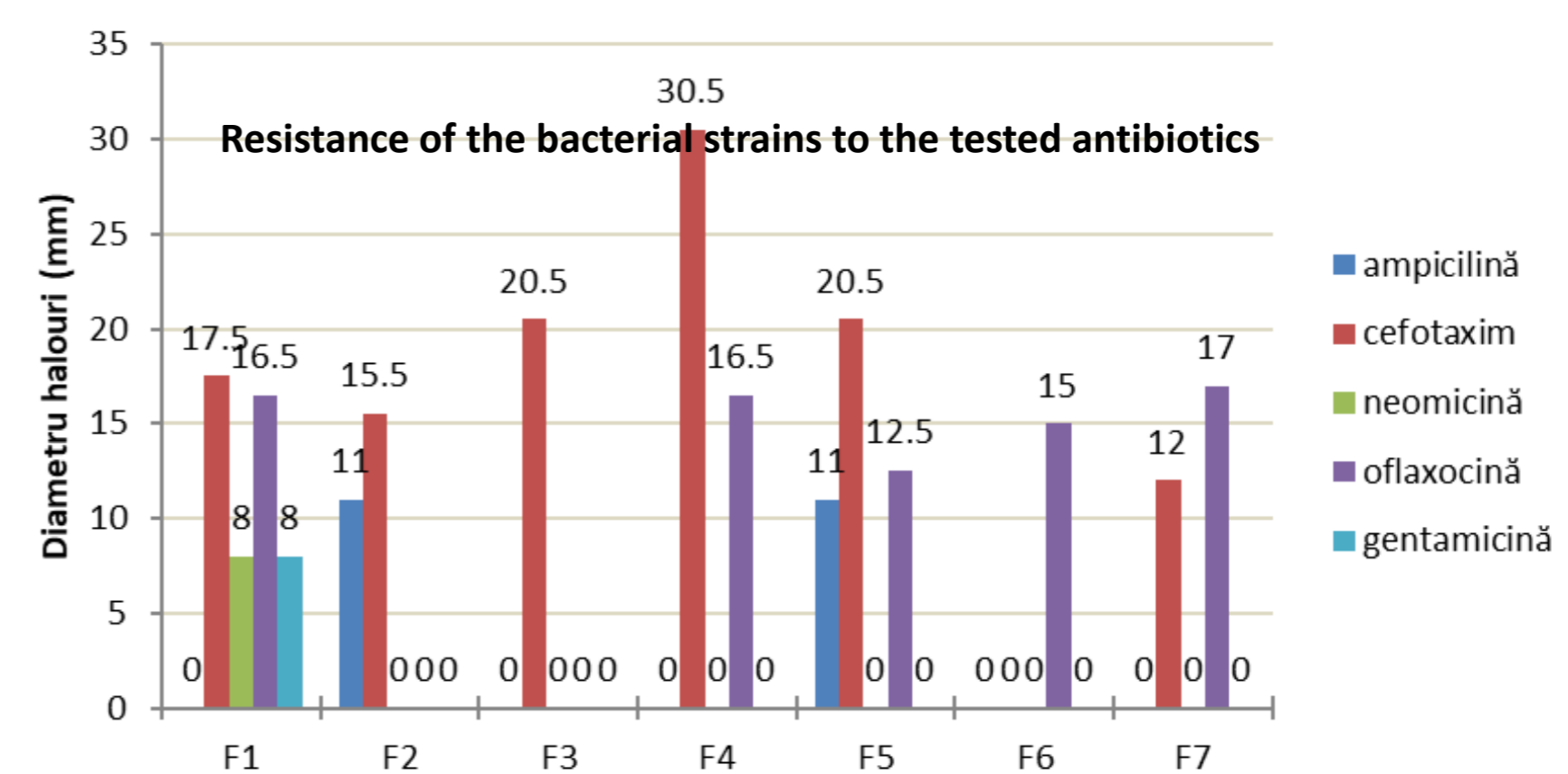
### Evaluation of Antibiotic Resistance

To assess antibiotic resistance, the isolated bacterial strains were inoculated onto solid culture medium in Petri dishes. Shortly after inoculation, discs containing the tested antibiotics were placed on the medium, and the Petri dishes were incubated at 27 °C.

Five antibiotics were tested for each isolated bacterial strain: ampicillin, cefotaxime, neomycin, ofloxacin, and gentamicin.



Among the five antibiotics tested, cefotaxime exhibited the broadest spectrum of activity, showing both the largest inhibition zone diameter (30.4 mm in strain F4) and the highest number of bacterial strains sensitive to its action. The only bacterial strain resistant to cefotaxime was strain F6. In contrast, neomycin and gentamicin showed the narrowest spectrum of activity, with only one bacterial strain being sensitive to each antibiotic, namely strain F1 in both cases.



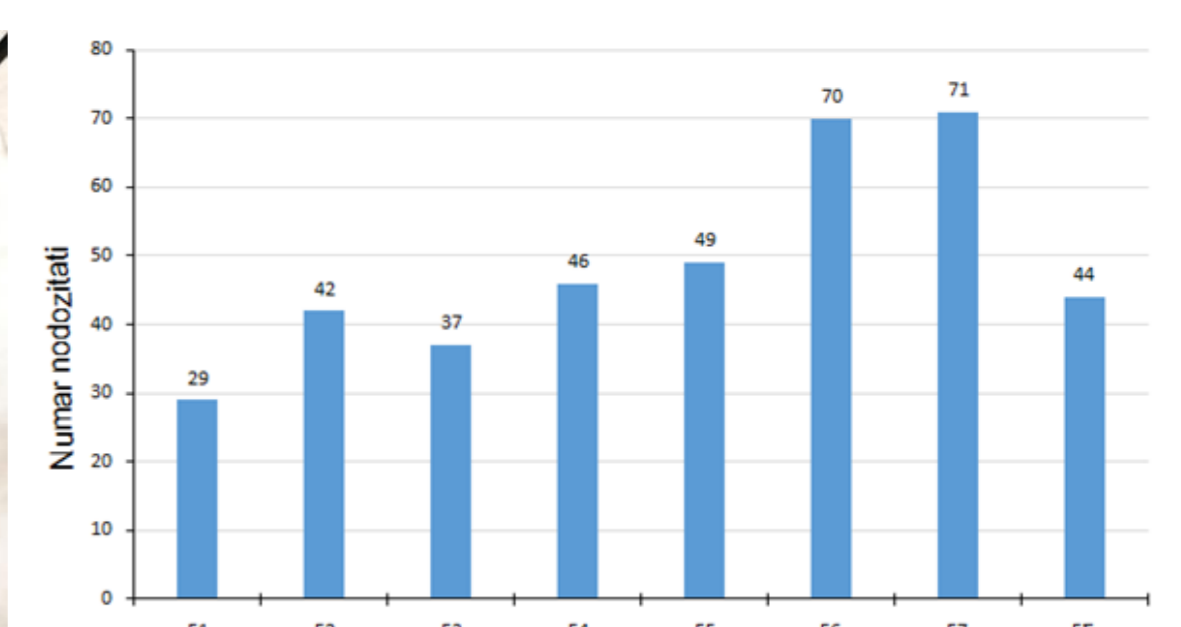
### Influence of Rhizobial Inoculation on Nodulation Capacity in Plants

Among the inoculated plants, the best results were observed in plants inoculated with bacterial strain F7, which developed an average of 71 nodules, followed closely by plants inoculated with strain F6, with an average of 70 nodules.

The lowest number of nodules was recorded in plants inoculated with bacterial strain F1, which developed an average of 29 nodules.

Nevertheless, even this minimum number of nodules was higher than that observed in most of the non-inoculated plants.

Plants inoculated with a mixture containing all seven isolated bacterial strains showed intermediate results, developing an average of 44 nodules.



### Conclusions

The study concludes that *Rhizobium* strains display variable antibiotic resistance profiles, reflecting notable genetic and metabolic diversity. Cefotaxime proved to be the most effective antibiotic, while ampicillin, neomycin, and gentamicin showed limited activity, suggesting the presence of  $\beta$ -lactamases and intrinsic resistance mechanisms. Despite these differences, no negative correlation was observed between antibiotic resistance and symbiotic efficiency; in fact, the most resistant strains (F6 and F7) also exhibited the highest nodulation capacity. These findings indicate that antibiotic resistance in *Rhizobium* is primarily an adaptive ecological trait linked to microbial competition and environmental selective pressures rather than a direct consequence of anthropogenic contamination. However, since resistance genes may be transferable, antibiotic susceptibility testing should be included in the biosecurity evaluation of microbial inoculants. Overall, the strains analyzed demonstrate strong ecological adaptability and symbiotic potential, making them promising candidates for use in sustainable agriculture, provided their genetic safety is confirmed.