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CHARACTERIZATION OF TOXIN-ANTITOXIN MECHANISMS IN CRYPTIC PLASMIDS OF *Sinorhizobium meliloti* LPU88

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Rhizobia are soil bacteria capable of establishing a symbiotic interaction with leguminous plants. Their genome is composed of at least three replicons: a chromosome and two symbiotic plasmids. Furthermore, some strains can harbor accessory plasmids. Plasmids are prokaryotic DNA molecules capable of auto-replicating and stably coexisting with the chromosome. Additionally, some plasmids may exhibit mechanisms that ensure their inheritance through cellular division. One such mechanism is the toxin-antitoxin (TA) systems. Generally, these systems consist of one gene encoding the toxin and another one that encodes its corresponding antitoxin. In the genomic study of the strain isolated in our laboratory, *Sinorhizobium meliloti* LPU88, we were able to identify two TA systems present in the accessory plasmids p88a and p88b. For that, we decided to characterize both stability systems. In order to determine the functionality of these systems, we performed stability assays. For that purpose, the TA system from the p88b accessory plasmid was cloned in a broad host range plasmid, pBBR1MCS-2 (Km), using primers flanking the entire region. Then, we conducted daily dilutions over 5-10 days in a medium without selective pressure, measuring the percentage of antibiotic resistant bacteria, meaning those that carry the plasmid. This value was compared with the percentage of bacteria that retained the empty plasmid. We observed slight differences between the stability of plasmid with toxin with respect to its control. It will perform such an analysis in the strain *S. meliloti* LPU88. Additionally, we employed bioinformatics techniques to compare the TA systems of both accessory plasmids. As a result, we observed a non-existent sequence identity that suggests they belong to different groups. Furthermore, the three dimensional structure of proteins was modeled, revealing notable structural differences, consistent with sequence analysis. Ultimately, we built phylogenetic trees in order to compare our toxin sequences with those of previously classified type II toxins. In the near future, we plan to conduct stability assays of the TA system present in the accessory plasmid p88a in order to compare it with the results obtained for the TA system in the accessory plasmid p88b.

Palabras clave: *Sinorhizobium meliloti* - Toxin - Antitoxin