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**SEQUENTIAL COCULTIVATION EXPERIMENTS TO EVALUATE
EARLY FITNESS COST ASSOCIATED TO PLASMID ACQUISITION IN
*Sinorhizobium meliloti***

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The set of plasmids and other mobile genetic elements in a given environment (i.e. the so called mobilome) represent the main genetic resource where bacteria encode and concentrate information to produce diverse adaptive responses to biotic and abiotic stimuli coming from the environment. The horizontal transfer of genetic information associated to the mobilome combined with the prokaryotic numerosity both help bacteria to preserve a splitted and transferable gene pool with redundancy within the community. In our laboratory we have studied for over 30 years the nitrogen-fixing association between the plasmid-reach rhizobia *Sinorhizobium meliloti* and *Medicago sativa* (alfalfa) which constitute a recognized model symbiotic system. In spite of the fact that *S. meliloti* and several other rhizobia carry significant amounts of extrachromosomal plasmid DNA, no data were available regarding potential fitness cost associated with the acquisition of novel plasmids by these bacteria. In a previous study we had observed that plasmid acquisition in *S. meliloti* frequently leads to minor though detectable decreases in the growth rate of the rhizobia under laboratory conditions. Now, in order to better emulate the alternating growth periods that occur under natural conditions, we investigated how repeated cycles of growth-latency-and-lag phase impact over time on the proportion of plasmid-free to plasmid-containing rhizobia during a co-cultivation in reach medium. GFP and mCherry fluorescent markers were used to monitor the relative amount of each rhizobia in the cocultures along the experiments which lasted for near a hundred generations. The results frequently showed that along the generations, the co-cultivation cultures were (nearly) linearly enriched in the plasmid-free rhizobia regarding the isogenic plasmid-containing ones. As it was reported in other bacteria, a more intense fitness cost was evident in some cases when a second additional plasmid was also present. Thus, experiments of co-cultivation with repeated cycles of bacterial growth and latency resulted in a more sensitive approach than the simple analysis of growth rates to evaluate plasmid-associated fitness costs in rhizobia. We will now evaluate whether similar effects are observed or not when plasmid-free and plasmid-containing genotypes are grown in the rhizosphere over sequential cycles of plant inoculations. Furthermore we will assess which are the biochemical changes that take place in

the host rhizobia when the novel plasmids are acquired.

Palabras clave: Plasmid - Mobilome - Horizontal gene transfer - Plant microbiome - Fitness cost