Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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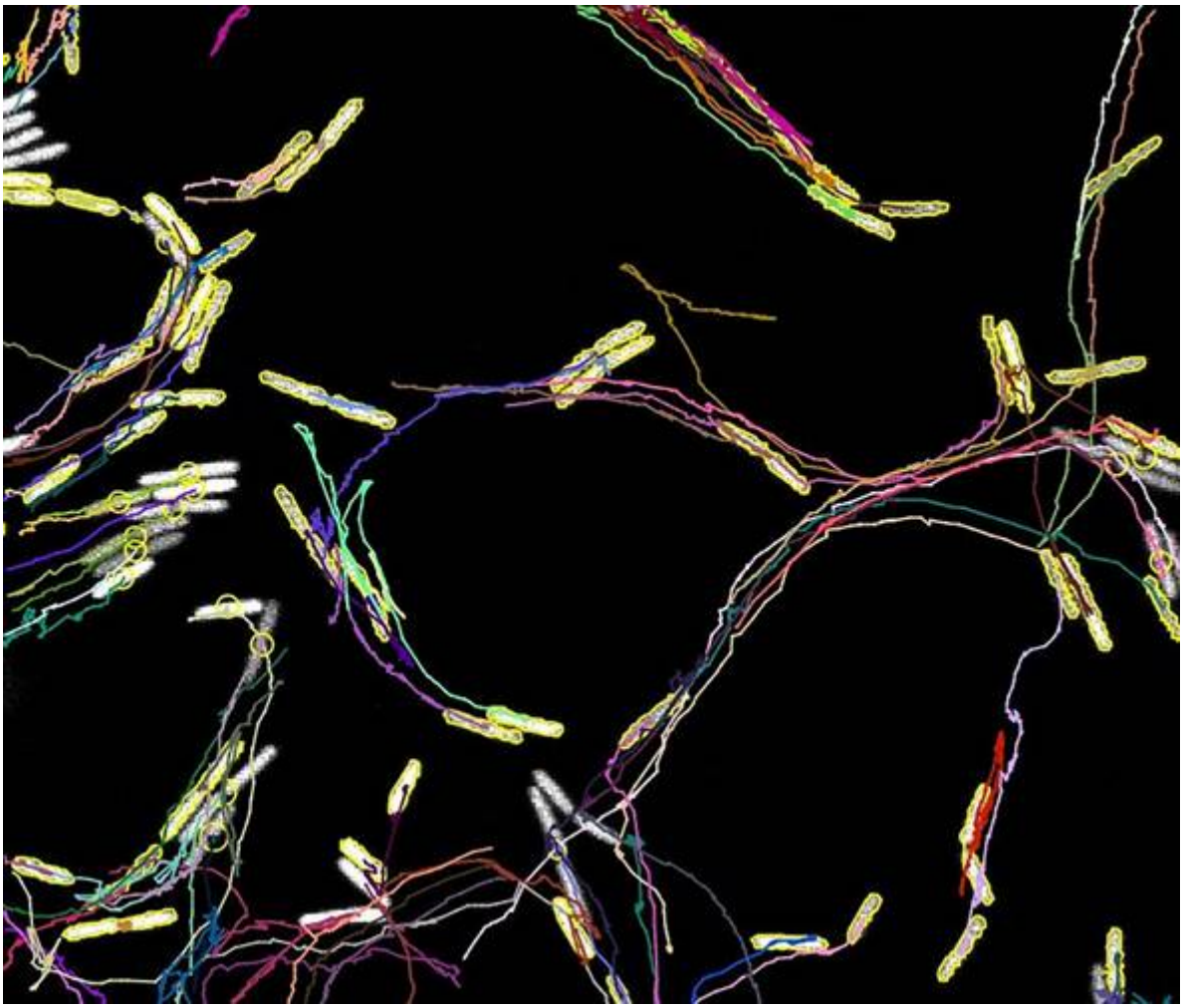

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

GENOME WIDE SCREENING OF *Pantoea agglomerans* GENES ASSOCIATED TO THE COLONIZATION OF THE RHIZOSPHERE AND ENDOSPHERE OF ALFALFA PLANTS BY USING A TNSEQ PHENOMIC APPROACH

Erdozain Bagolin S.[1] - Pagnutti A.[1] - Lozano M.[1] - Becker A.[2] - Lagares A.[1]

1) Instituto de Biotecnología y Biología Molecular (IBBM), Universidad Nacional de La Plata - La Plata - Argentina.
2) Zentrum für Synthetische Mikrobiologie (SYNMIKRO), Philipps Universität - Marburg - Germany
Contacto: sofierdozain@gmail.com

Plants are organisms extensively colonized by diverse microorganisms which constitute their so-called microbiome. Under such associative strategy, it is the whole system—known as the "holobiont"— that adapts to the environment and evolves. For this reason, rhizospheric-phylospheric and endophytic microbiomes are currently the target of intense investigations aiming to elucidate how plants and their associated microbes communicate to promote the optimal fitness of the holobiont. Understanding the basis of sustainable associations between plants and their complex microbiomes constitutes the basis for delineating novel practical interventions to enhance growth and health in natural plants and agricultural systems.

Pantoea strains are known to be natural endophytes of *Medicago sativa* plants and seeds as well as many other plant species. The characterization of the colonization of alfalfa by *Pantoea agglomerans* LPU12 showed that is present in high numbers both in the rhizosphere (ca. 105 CFU/plant) and also in the inner plant tissues (ca. 107 CFU/g of wet root), thus constituting an ideal system to investigate plant root colonization by an ubiquitous bacteria. To gain insight into the rhizospheric and endophytic lifestyle of a model plant-associated soil bacterium we implemented TnSeq experiments using *P. agglomerans* LPU12 and alfalfa as the host plant.

By using a Tn Mariner mutant library of *P. agglomerans* LPU12 for the TnSeq experiments we identified 51 genes implicated in rhizosphere colonization, with most of them causing negative effects upon mutation, suggesting that evolution tends to minimize/exclude genomic information that affected access to the rhizosphere. Regarding colonization of the endosphere, a significant bottleneck was observed where only a limited number of bacterial cells infect the inner tissues of the plant to find the final population density above mentioned. We found that these “founders” cells enter the plant root resulting in an estimated amount of only 103-104 endophytic founders/plant. TnSeq data also showed that the initial degree of endophytic diversity expressed by the founders didn’t increase in one-month-old plant roots, even when they stayed in contact with the bacterial diversity present in the initially inoculated support. Although the

observed bottleneck imposed a severe restriction on the identification of genes associated with endophytic colonization, at least 60 genes could be identified. In contrast to the rhizospheric markers, several mutants showed an increased colonization phenotype (ca. 50%), stating that purifying selection of those genes could not take place likely due to their need during the bacterial life in plant-free environments.

The results of this work show the presence of a limitation in the infection of the plant beyond its capacity to host a given population density and open the door to investigate the factors that lead the plant to limit the bacterial diversity that inhabits it.

Palabras clave: Plant colonization - Endophytes - TnSeq - Microbial ecology