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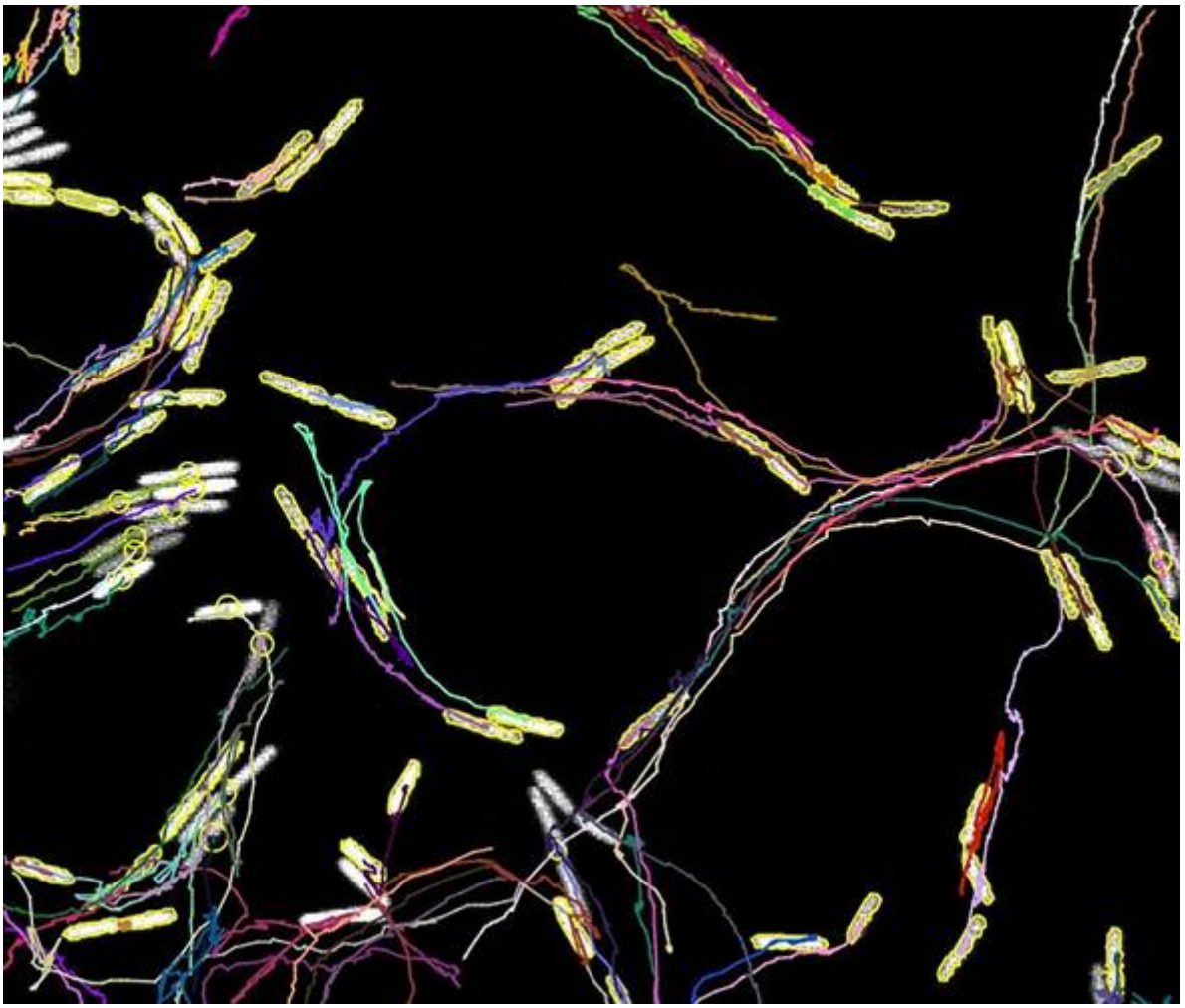

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

EXPLORING BACTEROID DIFFERENTIATION IN *Medicago truncatula* : A PROTEOMIC APPROACH TO RHIZOBIUM SYMBIOSIS

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Rhizobium-legume symbiosis is an intricate relationship between plants and bacteria. The nodulation process in plants begins when it releases chemical signals that are detected by rhizobia. This activates the nodulation genes in the bacteria. Inside the nodules, the rhizobia differentiate into bacteroids, becoming capable of converting molecular nitrogen into ammonium, a form of nitrogen that the plant can assimilate. The differentiation into bacteroids is a key process for efficient biological nitrogen fixation. Numerous genes involved in this process have been described whose expression is either decreased or increased in nodules, however, it is still unknown which ones are responsible for these processes to happen. *Medicago truncatula* is commonly used as a model plant for studying rhizobium-legume symbiosis. The rhizobium *Ensifer meliloti* produces nodules on *M. truncatula* with differentiated bacteroids for efficient biological nitrogen fixation (BNF). Nevertheless, there are other rhizobia, such as *Rhizobium favelukesii*, that can also nodulate *M. truncatula*. While *R. favelukesii* is highly competitive in occupying nodules under acidic conditions, it is very inefficient in BNF. By comparing the development of efficient and inefficient nodules in the BNF, we aimed to identify determinants involved in the differentiation of bacteria into bacteroids, which is why we proposed to perform a proteomic analysis. These studies will allow us to determine which proteins are expressed in each case and which are delivered by the plant into bacteria and bacteroids within the nodule. To address this proteomic analysis, we performed experiments with *M. truncatula* plants infected with both rhizobia. 31 days post-inoculation (dpi), nodules were collected. Bacteria were separated from the bacteroids by density gradients. Proteins were extracted and digested with trypsin. The analysis of the data obtained by Data-Independent Acquisition (DIA) shotgun proteomics allowed us to identify ca. 2750 bacterial proteins from *R. favelukesii* and 2300 from *E. meliloti*. In the bacteroid-enriched fraction, ca. 60 proteins from the plant were exclusively identified in nodules of *E. meliloti*, and almost ca. 1000 proteins in nodules of *R. favelukesii*. When plant differential proteins were evaluated, 42 proteins were found to be overexpressed in *E. meliloti* nodules meanwhile 960 in *R. favelukesii* nodules. Remarkably, 75% of the proteins overexpressed in *E. meliloti* nodules belong to the nodule-specific cysteine-rich peptides (NCRs) group, while in the proteins overexpressed in *R. favelukesii* nodules were not detected. NCRs are of high importance since they are specific peptides secreted by the plant to bacteroids that induce bacterial

differentiation. Studying the function of the proteins that are differentially expressed will help us to identify determinants involved in the process of bacterial differentiation.

Palabras clave: Rhizobia – Nodules – Symbiosis – Proteomics