

Talk

## Molecular analysis of the symbiosis between *Nostoc punctiforme* and *Oryza sativa*



Sánchez del Solar, Carmen\* (1), Mariscal, Vicente (1), Álvarez, Consolación (1)

(1) Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, cicCartuja, Avda. Américo Vespucio 49, 41092 Seville, Spain

Tutor académico: Ramos Barrales, Ramón

**Keywords:** symbiosis; nitrogen fixation; cyanobacteria

### ABSTRACT

*Nostoc punctiforme* is a filamentous, diazotrophic cyanobacteria capable of forming symbiotic relationships with diverse plant species (Álvarez et al., 2020; Álvarez et al., 2023). Given the widespread use of synthetic nitrogen fertilizers in rice cultivation and their associated environmental issues, biological nitrogen fixation (BNF) by cyanobacteria presents a promising alternative (Álvarez et al., 2023). However, the molecular mechanisms underpinning the symbiotic association between *N. punctiforme* and rice remain poorly understood. In well-characterized symbiotic interactions, such as those occurring between legumes and nitrogen-fixing rhizobial bacteria, the common symbiotic signalling pathway (CSSP) is activated. This process begins with *Rhizobium* secreting Nod factors in response to flavonoids released by compatible plants. Álvarez et al. (2022) demonstrated for the first time the expression of Nod-like proteins in *N. punctiforme* and the participation of the CSSP pathway in rice during symbiosis.

Based on these previous results, this study aims to elucidate the genetic basis of this interaction by characterizing orthologous genes in *Nostoc* related to the *nodD* regulatory gene in *Rhizobium leguminosarum*, which play a pivotal role in symbiosis by regulating the expression of the genes involved in the generation of nod factors. Bioinformatic analyses were conducted to identify homologous sequences of *nodD* from *R. leguminosarum* in *N. punctiforme*, and structural modelling compared their potential functional roles. Three key *nodD* homologs with conserved DNA-binding and inducer recognition domains were found in *N. punctiforme*, called *nodD1*, *nodD2* and *nodD3*. Using homologous recombination, loss-of-function *nodD* gene mutants were generated in *N. punctiforme* and characterized. Genetic characterization revealed the maintenance of wild type copies of genes *nodD1* and *nodD3* in their respective mutants, suggesting that those genes may be essential.

Preliminary symbiosis analysis results with different symbiotic plant species seem to indicate reduced root adherence, highlighting their possible involvement in establishing symbiosis. Further symbiosis assays with rice are needed to evaluate endophytic colonization efficiency. These insights contribute to understanding microbial symbiosis and support the development of cyanobacteria-based biofertilizers for sustainable agriculture.

### REFERENCES

- Álvarez C, Navarro JA, Molina-Heredia FP, Mariscal V. (2020) Endophytic colonization of rice (*Oryza sativa* L.) by the symbiotic strain *Nostoc punctiforme* PCC 73102. *Mol. Plant Microbe Interact.* 33 (8): 1040-1045. <https://doi.org/10.1094/MPMI-01-20-0015-SC>
- Álvarez, C., Brenes-Álvarez, M., Molina-Heredia, F. P., & Mariscal, V. (2022). Quantitative proteomics at early stages of the symbiotic interaction between *Oryza sativa* and *Nostoc punctiforme* reveals novel proteins involved in the symbiotic crosstalk. *Plant & Cell Physiology*, 63(10), 1433–1445. <https://doi.org/10.1093/pcp/pcac043>
- Álvarez, C., Jiménez-Ríos, L., Iniesta-Pallarés, M., Jurado-Flores, A., Molina-Heredia, F. P., Ng, C. K. Y., & Mariscal, V. (2023). Symbiosis between cyanobacteria and plants: from molecular studies to agronomic applications. *Journal of Experimental Botany*, 74(19), 6145–6157. <https://doi.org/10.1093/jx>