Identification of genes regulated by the transcriptional activator SyrM2 in *Rhizobium* sp. NGR234 by RNA sequencing.

Evaristo, J.A.M.\(^1\); Barbosa, P.K.A.\(^1\); Tadra-Sfeir, M.Z.\(^2\); Faoro, H.\(^2\); Pedrosa, F.O.\(^2\); Souza, E.M.\(^2\); Bonatto, A.C.\(^1\); Wassem, R.\(^1\)

\(^1\)Departamento de Genética, Universidade Federal do Paraná, Curitiba-PR, Brazil.  
\(^2\)Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba-PR, Brazil.

**Introduction:** *Rhizobium* sp. strain NGR234 is a unique bacterium that forms nitrogen-fixing nodules with more legumes than any other microsymbiont. Infection of legumes by NGR234 and subsequent development of nitrogen-fixing nodules are dependent on the coordinated actions of Nod factors, proteins secreted by a type III secretion system (T3SS) and modifications of surface polysaccharides. The production of these signal molecules is dependent on plant flavonoids which trigger a regulatory cascade controlled by the transcriptional activators NodD1, NodD2, SyrM2 and TtsI.

**Material and Methods:** Aiming to detect genes regulated by the transcriptional activator SyrM2, the wild type NGR234 and the mutant *syrM2*- strains were cultivated for 24h in minimal medium enriched with flavonoids. The cells were collected and total RNA was purified, depleted of ribosomal RNAs and used to construct the RNAseq libraries. The cDNAs were sequenced using a SOLiD platform and the 50-mer reads generated were mapped against the symbiotic plasmid of NGR234 using the software Shrimp. Afterwards, the mapped reads were imported into the CLC Genomics Workbench software and mapped against the CDS files of the symbiotic plasmid of NGR234. Statistical analysis revealed a set of differentially expressed genes. **Results:** A list of differentially expressed genes was obtained and contained genes with length coverage of at least 3 times, fold change ≥ |2| and p-value lower than 0.05. Genes coding for type III secretion system proteins RhcT, NopB, RhlC, NopJ and NopA, the LysR family transcriptional regulator NodD2, nodulation protein NodI, NodS and NodJ were more expressed in the wild type strain. However, the transcriptional regulator SyrM2, nodulation protein NopP and the T3SS-RhcU were more expressed on the mutant *syrM2*. **Conclusions:** Our approach has allowed the underpinning of SyrM2 target genes and also confirmed the effect of SyrM2 on the expression of previously described target genes.

Keywords: transcriptomics, *Rhizobium* sp. NGR234, Next Generation Sequencing. Supported by: CAPES and INCT.