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INDOLE-3-ACETIC ACID AFFECTS *RHIZOBIUM*-LEGUME NITROGEN FIXING SYMBIOSIS

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The nitrogen fixing soil bacteria Rhizobia naturally synthesise indole-3-acetic acid (IAA), the most abundant naturally occurring auxin, and bacterial IAA production has been proposed to play a role in the *Rhizobium*-legume symbiosis. To demonstrate that auxin synthesis affects root nodule development and function, we have introduced in different strains of Rhizobia a second pathway for the IAA biosynthesis under the control of a stationary phase induced promoter, the *rolA* promintron (Pandolfini, 2000), active both in free-living bacteria and in bacteroids. The newly introduced genes are the *iaaM* gene coding for a tryptophane monoxidase, which converts tryptophan to indoleacetamide and the *tms2* gene coding for a hydrolase which turns indoleacetamide into IAA. When we express IAA by the *promintron-iaaMtms2* construct, it is locally delivered to the plant root and root nodules are bigger in size, reduced in number per plant, heavier as dry weight and show an enlarged and more active meristem. Plants nodulated by IAA-overproducing rhizobia increase nitrogen fixation, dry weight and seed dry weight production.

We modulated the expression of the *iaaMtms2* construct by introducing in the promoter sequence a number of site directed mutations, altering the transcriptional activity. This promoter is the only stationary phase promoter so far isolated and active in *Rhizobiaceae*. We introduced the reporter gene GUS downstream the promintron sequence to investigate its activity in the free-living condition by means enzymatic assays, and during the bacteroid stage by means at hystological GUS assays. We found that the mutations in the promintron increasing the homology of the –10 and–35 regions to the consensus sequences recognised by *E.coli* vegetative sigma factor (sigma70), alter positively the promoter activity in both exponential and stationary growth phase.

We performed a metabolic analysis of the first sequenced *Rhizobium* strain**,** *Sinorhizobium meliloti* 1021 treated with IAA or harbouring the promintron-*iaaMtms2*, as a first step towards a better understanding of the symbiosis with its host plant *Medicago*. We observed a significant increase in nitrogen fixation measured as acetylene reduction activity in *Medicago* nodules elicited by the IAA-overproducing *S. meliloti* and a higher meristematic activity of these nodules. Enzymatic analyses revealed that cells of the wild-type strain treated with IAA and those over-producing IAA, posses a more active tricarboxylic acid (TCA) cycle, the main energy-producing pathway.

Based on the complete *S. meliloti* genome sequence we established DNA microarrays as comprehensive tools for systematic gene expression analysis in *S. meliloti* 1021. In order to have a more global view of the response of *S. meliloti* to IAA action we carried out parallel hybridizations based on different RNA samples. These RNA derived from the following cells: over-producing IAA, IAA-treated, ICA-treated (ICA, indole-3-carboxylic acid, is structurally similar to IAA) and 2,4-D-treated (2,4-D, 2,4-dichlorophenoxyacetic acid, is functionally similar to IAA).