



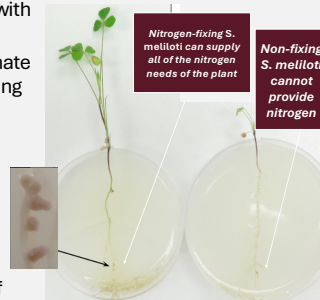
# The Interplay of Terminal Differentiation of Symbiotic *Sinorhizobium meliloti* and Nitrogen Fixation in Legume Plants

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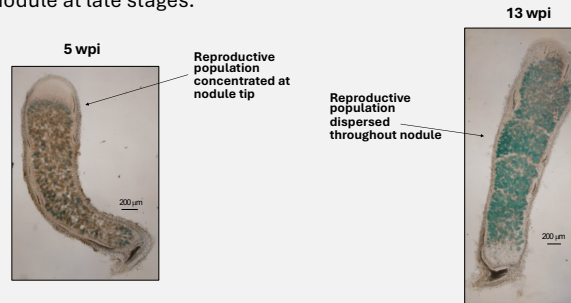
## Plant-bacterial symbiosis

- Rhizobial bacteria form a symbiosis with legume plant roots and provide the plant with fixed nitrogen.
- Medicago sativa* (alfalfa) forms indeterminate nodules when infected by the nitrogen-fixing bacterium *Sinorhizobium meliloti*.
- Indeterminate nodules are characterized by sustained growth of nodule meristem and continual invasion of infection threads.
- There is a question in the field as to whether terminal differentiation of bacteroids always precedes expression of the nitrogen-fixing enzyme nitrogenase.
- Bacteroids must differentiate in order to fix nitrogen, but differentiation renders them unable to reproduce.



## The reproductive population increases as the nodule ages

- div-J is a cell cycle reported gene used to track the dividing population of *S. meliloti* in indeterminate alfalfa nodules.
- Expression of the div-J cell cycle marker in older nodules suggests that non-terminally differentiated bacteria take over the nodule at late stages.

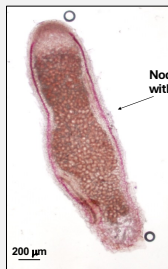
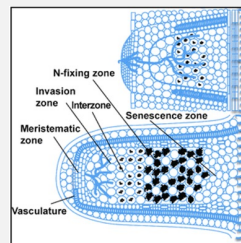


## Questions

- The scientific consensus is that terminal differentiation proceeds nitrogenase expression.
- We question if this is always the case.
- Does the plant sensing some level of nitrogen fixation proceed the induction of a signaling cascade?
- If there are a subset of cells in which nitrogenase expression proceeds terminal differentiation, we can use a marker system to recover members of the reproductive population that have expressed nitrogenase.

## Terminal differentiation & the reproductive population

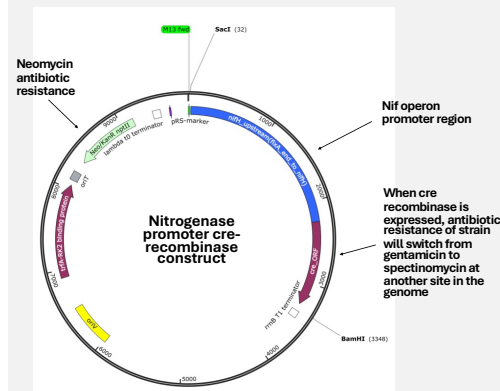
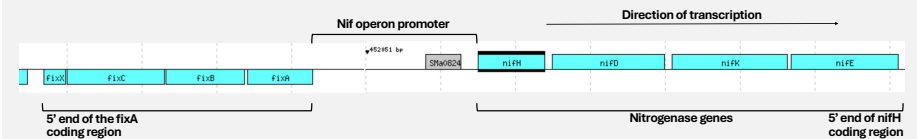
- Alfalfa form indeterminate nodules with nitrogen-fixing *S. meliloti*.
- In young nodules, the reproductive bacterial population is located at the nodule tip.
- Bacterial cells terminally differentiate and express nitrogenase to fix nitrogen for the host plant.
- The host plant regulates nitrogenase expression and terminal differentiation.
- The reproductive population benefits the bacteria, which represents the population that can eventually recolonize the soil.
- The terminally differentiated bacterial population benefits the plant through nitrogen-fixation and nitrogenase expression.



### Bacterial reproductive population

- In the indeterminate nodule, nitrogen-fixing bacteria are terminally differentiated.
- The primary goal of the research is to track the reproductive dividing population relative to the terminally differentiated population.
- The reproductive bacterial population benefits from symbiosis with the plant.

## Monitoring nitrogenase expression with a recombinase marker system



- Cre expression from the nitrogenase promoter will switch antibiotic resistance from gentamicin to spectinomycin in each cell.
- If there are no spectinomycin-resistant colonies recovered from the nodule, this suggests nitrogenase is only expressed in terminally differentiated cells.
- If spectinomycin-resistant colonies are recovered from the nodule, nitrogenase expression has occurred in non-terminally differentiated cells.
- The proportion of gentamicin to spectinomycin-resistant colonies recovered will allow us to determine a relative estimate of nitrogenase expression in non-terminally differentiated cells.