N=N

6th European
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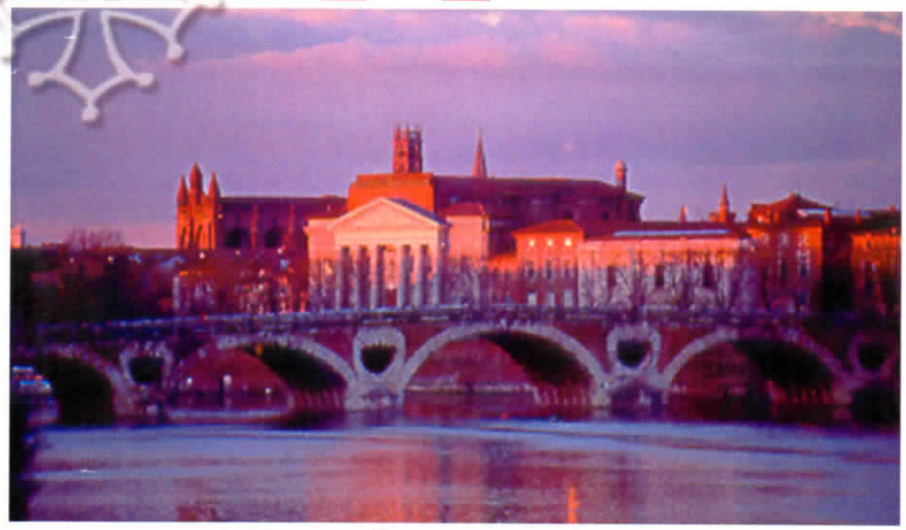

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P5.13 Genomic analysis of ECF-sigma factor in Rhizobium etli

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Rhizobia in free-living soil or as a nitrogen-fixing bacteroid, are continuously challenged by changes on nutrient availability and by exposure to different conditions. Alterations in the rhizosphere may provoke osmotic, oxidative or temperature shocks; even during the infection process, the plant produces superoxide ions and hydrogen peroxide against the bacteria. Moreover, microaerobiosis and carbon limitation are experienced by the bacteroid in nodule.

Different strategies have been development by the bacteria to respond to these changing environments. One of them is the transcriptional regulation of genes involved in one process by an alternative sigma (σ) factor, which determine the promotor specificity of the RNA polymerase. The extracytoplasmic function (ECF) σ-subgroup of the σ^{70} family is a class of small proteins (24 kDa), which are activated by environmental conditions. Generally, the ECF-σ factors are cotranscribed with one or more negative regulators, one of these include a transmembranal anti-σ factor that binds and inhibits the σ-factor.

In Rhizobia, several ECF σ-factor are present: *Sinorhizobium meliloti* has 11, *Mesorhizobium loti* has 19, *Agrobacterium tumefaciens* has 12, *Bradyrhizobium japonicum* has 18 and preliminary results indicate that *Rhizobium etli* has about 12. One unresolved question is, why members of the Rhizobia family have several ECF σ-factors, and what is the role of them?

In order to gain an insight, two approaches are following: a genetic analysis in *R. etli*, which include the gene inactivation of each ECF σ-factor as well as its gene expression regulation; and a comparative genomic analysis in Rhizobia. The advances in both approaches will be presented.

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P5.14 Identification and analysis of additional components of the nitrogen regulatory network in enteric bacteria

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In enteric bacteria, the NtrBC two-component system controls transcription of genes involved in nitrogen assimilation. Signals that indicate carbon and nitrogen status determine the ability of PII proteins to regulate the kinase and phosphatase activities of NtrB and, as a result, the activity of response regulator NtrC. The nitrogen status of the cell is sensed by UTase/UR enzyme, encoded by *glnD*, which uridylylates/de-uridylylates PII proteins (encoded by paralogous genes *glnB* and *glnK*) according to the glutamine levels. In addition to transcriptional regulation, UTase/UR and PII proteins also play a role on metabolic regulation (by affecting glutamine synthetase activity) and ammonium transport (by interaction with AmtB).

To search for additional regulatory connections we have constructed and screened *Escherichia coli* and *Klebsiella pneumoniae* libraries for proteins interacting with NtrB, GlnB, and UTase/UR proteins. The relevance of some of the newly identified interactions has been confirmed by additional approaches. Results obtained so far indicate that the paradigmatic Ntr system is part of a wider regulatory network, with the UTase/UR emerging as a rather promiscuous signal transduction protein.