THE PHOSPHOTRANSFERASE SYSTEM PTS^{Ntr} AS THE CENTRAL REGULATOR OF ATP-DEPENDENT TRANSPORTERS

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ABC transporters are essential for an effective symbiosis in *Rhizobium leguminosarum*

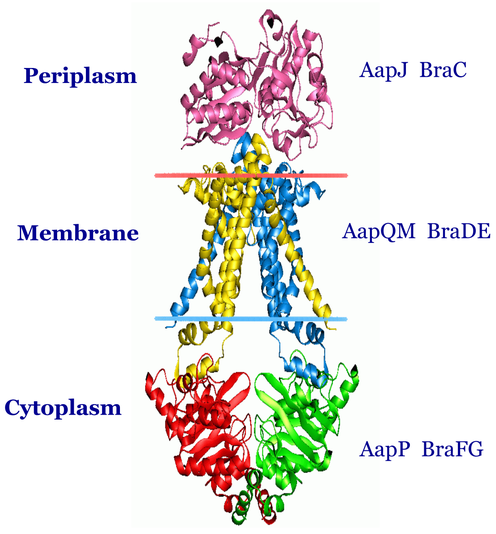
The specific *Rhizobium*–legume interaction requires a complex coupling of biochemical and morphological factors between both partners. In the case of rhizobia, one of these essential factors is nutrient acquisition, which is mediated by ABC (ATP-binding cassette transporters). This family of transporters is the largest group of membrane transport systems in living organisms and are highly represented in rhizobial genomes.

Presence of ABC transporters in different bacteria

Nº of ABC transporters	Bacterial genome
269	***Rhizobium leguminosarum***
200	*Sinorhizobium meliloti*
216	*Mesorhizobium loti*
240	*Bradyrhizobium japonicum*
67	*Escherichia coli*
124	*Pseudomonas aeruginosa*

General L-amino acid permease (**Aap**) – *aapJQMP*

Broad range amino acid transport (**Bra**) - *braCDEFG*



Belonging to this class of transporters, two broad range amino acid ABC transporters (Aap and Bra) of *R. leguminosarum* have been shown to be crucial in the bacteroid for an effective symbiosis, as pea plants inoculated with a double mutant defective in these two transporters are severely nitrogen starved.

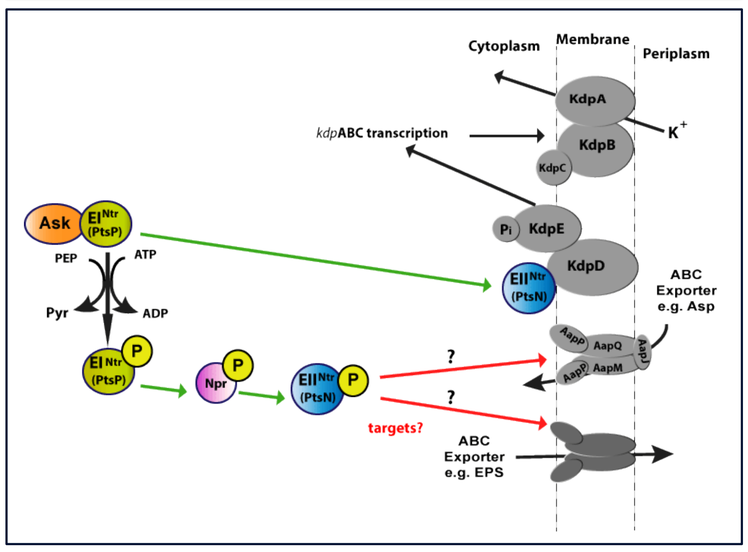
Symbiotic phenotype of *aap*$^-$/*bra*$^-$ mutant compared to wild type

Uninoculated / RU1357 / *A34* (*aap*$^-$/*bra*$^-$)/ (wild type)

Peas grown for 40d on N-free solution

ATP-dependent transporters are controlled by the PTS^{Ntr} system in *Rhizobium leguminosarum*

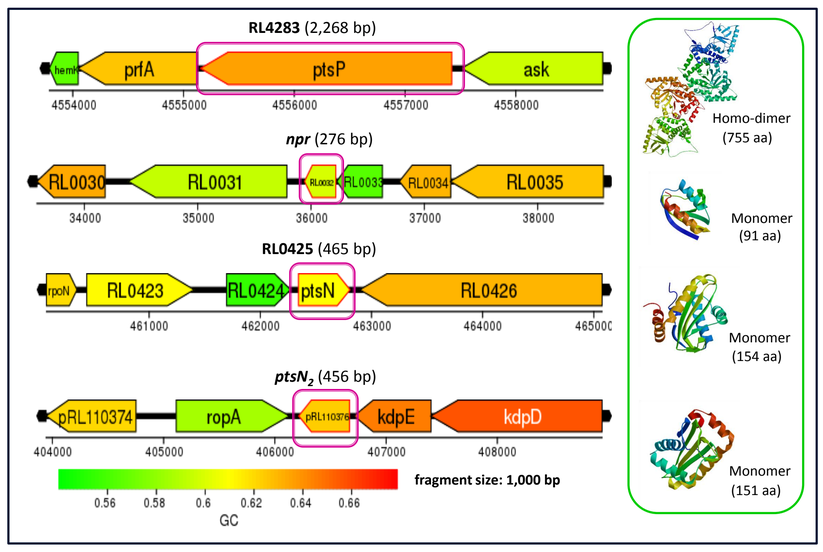
Model for PTS^{Ntr} regulation in *Rlv* 3841



In *R. leguminosarum* ABC transporters are regulated by PTS^{Ntr}, a phosphotransferase system that connects the metabolic status of the cell with a diverse number of physiological processes. Derived from the sugar PTS and only present in Gran-negative bacteria, PTS^{Ntr} acquires phosphate from phosphoenol pyruvate (PEP) and passes it from PtsP to Npr and PtsN. The absence of the permease components EIIB and EIIC involved in solute traslocation from the sugar PTS suggests an exclusively regulatory role for PTS^{Ntr}.

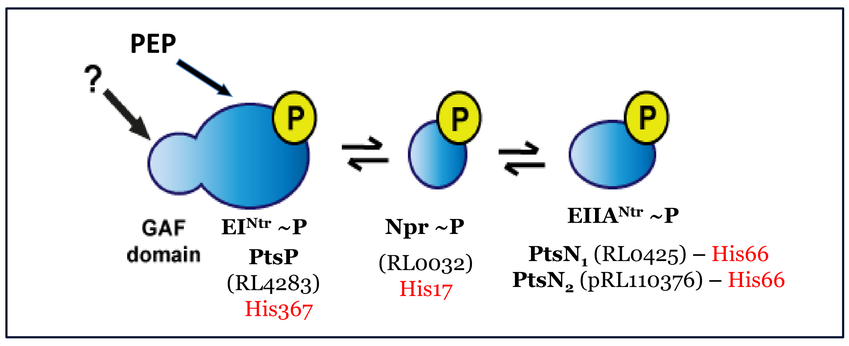
All the PTS^{Ntr} components have been recently identified in *R. leguminosarum*, but unlike *E. coli*, the genes are located in different genomic regions and there are two copies of *ptsN* (N_1 and N_2). The only target that has been identified so far for $PtsN_1$ in *R. leguminosarum* is the sensor kinase KdpD, modulating the high-affinity K+ transporter KdpABC and controlling K^+ homeostasis. Whereas this interaction relies on the non-phosphorylated form of $PtsN_1$, the targets for the phosphorylated version of this protein remain unknown.

Characterization of PTS^{Ntr} system in *Rhizobium leguminosarum 3841*



Generation of PTS^{Ntr} tagged proteins to determine the phosphotransfer pathway between PTS^{Ntr} components and to identify $PtsN_1$ targets

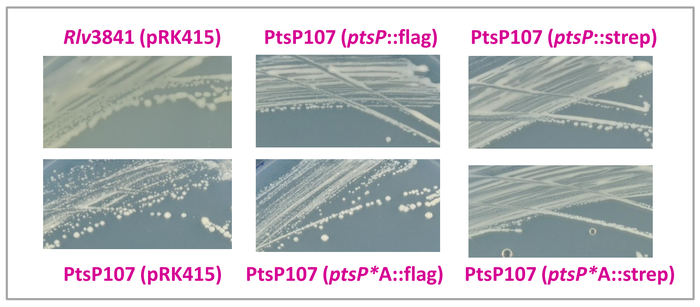
PTS^{Ntr} phosphotransfer pathway and phosphorylation sites in *Rlv*3841



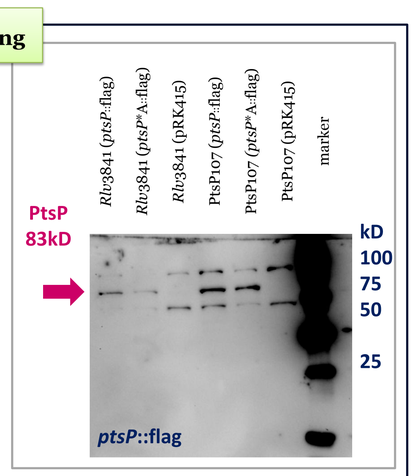
The objective of the project is to determine the phosphotransfer pathway between PTS^{Ntr} components. Biochemical interactions between PTS^{Ntr} components will be checked *in vivo* by means of strep and flag tagged proteins cloned into pRK415, a broad host range vector. Furthermore, because PtsN is able to interact with different proteins depending on its phosphorylation status, these complementation analyses have also been carried out with the non-phosphorylated versions of the PTS^{Ntr} proteins. They have been obtained through site-directed mutagenesis, replacing the histidine found at the phosphorylation site by an alanine.

It was previously shown that *ptsP and ptsN1/N2* mutant formed dry colonies and grew poorly on organic nitrogen or dicarboxylates. PTS^{Ntr} tagged proteins have been confirmed to be fully functional checking their surface phenotype on agar plates compared to the wild type. In all cases, flag and strep-tagged proteins have complemented *ptsP* and *ptsN*$_1$ mutants. As it is shown in the figure, in the case of flag-tagged proteins, PstP and $PstN_1$ have been identified in whole cell extracts using Western Blotting. Interacting partners will be identified by pull down assays followed by MALDI-TOFF Mass Spectrometry (MS) analysis with these tagged versions of the PTS^{Ntr} proteins.

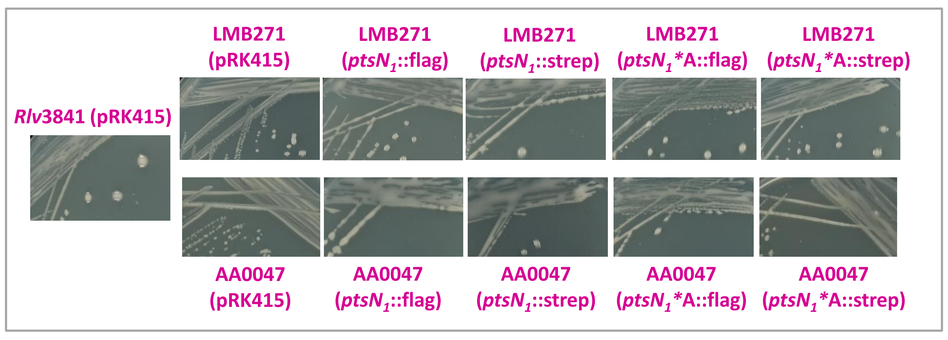
PtsP mutants: surface phenotype and protein identification after Western Blotting



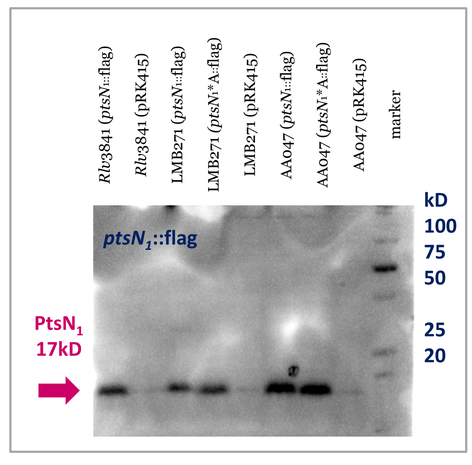
Wild type strain: *Rlv*3841
PtsP mutant: PtsP107 (Tn5::*ptsP*)



$PtsN_1$ mutants: surface phenotype and protein identification after Western Blotting



Wild type strain: *Rlv*3841
$PtsN_1$ mutant: LMB271 (*ptsN*$_1$::ΩSpec)
$PtsN_1$ /$PtsN_2$ double mutant: AA0047 (*ptsN*$_2$ markerless in *ptsN*$_1$:: ΩSpec background)



CONCLUSIONS

♣ Strep and flag tagged proteins have been cloned into pRK415 vector and the complemented mutants have been confirmed to be fully functional.

♣ Flag –tagged PstP and PtsN1 versions have been effectively detected in Western Blotting.

As PTS^{Ntr} is not only widely distributed in bacteria, but also constitutes an essential regulatory system in *Rhizobium*, it is important to determine if PTS^{Ntr} acts as a global regulatory system and to determine which genes are controlled by this system.

REFERENCES

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