Regulation of bacterial metabolism by the phosphotransferase system PTS^{Ntr}

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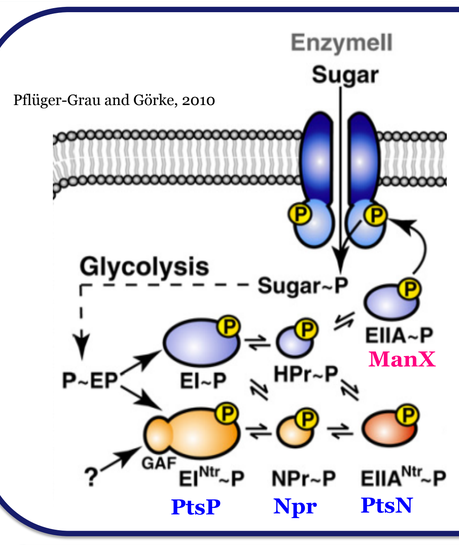


Carbohydrate-PTS
Gram(+) and Gram(-) bacteria

Nitrogen-PTS
Only in Gram(-) bacteria

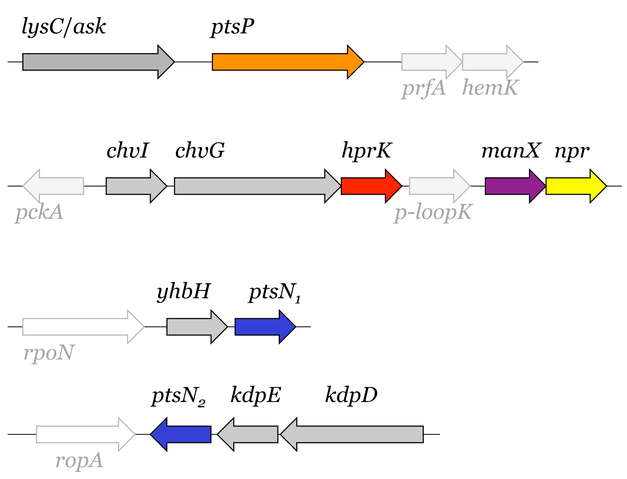
PTS: Phosphoenolpyruvate:carbohydrate-phosphotransferase system

The PTS system is the key signal transduction pathway involved in the regulation of carbon metabolism in bacteria. It acquires phosphate from phosphoenolpyruvate (PEP) and passes it through the different components of the system, with the ultimate acceptor being a sugar available in the environment, which is phosphorylated upon transport by membrane components. Alpha-proteobacteria, such as rhizobia, retain a sugar PTS (EIIA-ManX) but do not have the transport components (EII).

In the case of Gram(-) bacteria, PTS^{Ntr} represents an alternative system encoded by the genes *ptsP, npr* and *ptsN*. PTS^{Ntr} is found in the same genomic region as the sigma factor *rpoN*, which enables the transcription of the nitrogen fixation genes. This system preserves the phosphotransfer components, but lacks the permeases, suggesting an exclusively regulatory role.

The establishment and maintenance of an effective N-fixing symbiosis is intimately interconnected with the metabolism of the plant and requires a complex coupling of biochemical and morphological factors between rhizobia and their host. The success of this interaction relies on a fine-tuned coordination of intracellular and extracellular signals.

Is PTS^{Ntr} the system allowing N-fixing bacteria to adapt their metabolism and thrive in different nutritional niches?

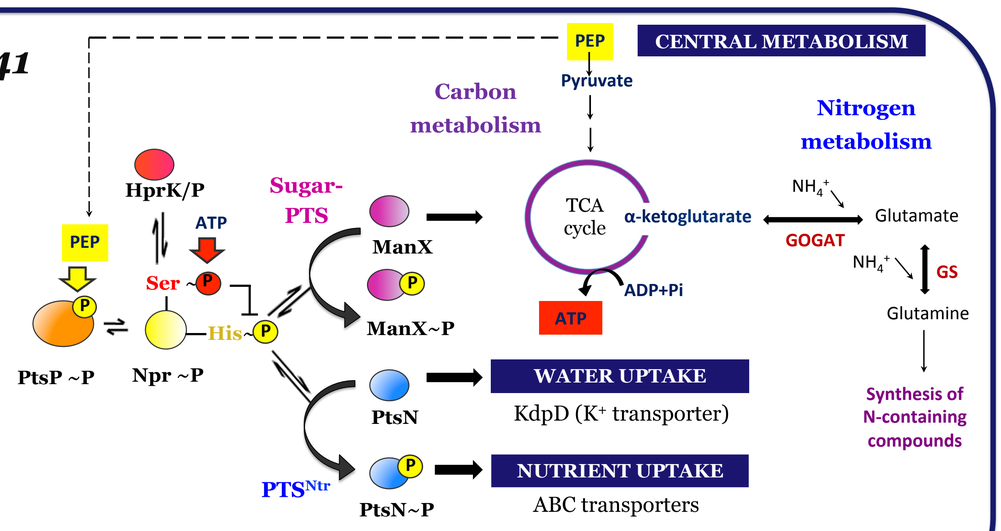


PTS^{Ntr} genomic organization

PTS^{Ntr} in *Rhizobium leguminosarum 3841*

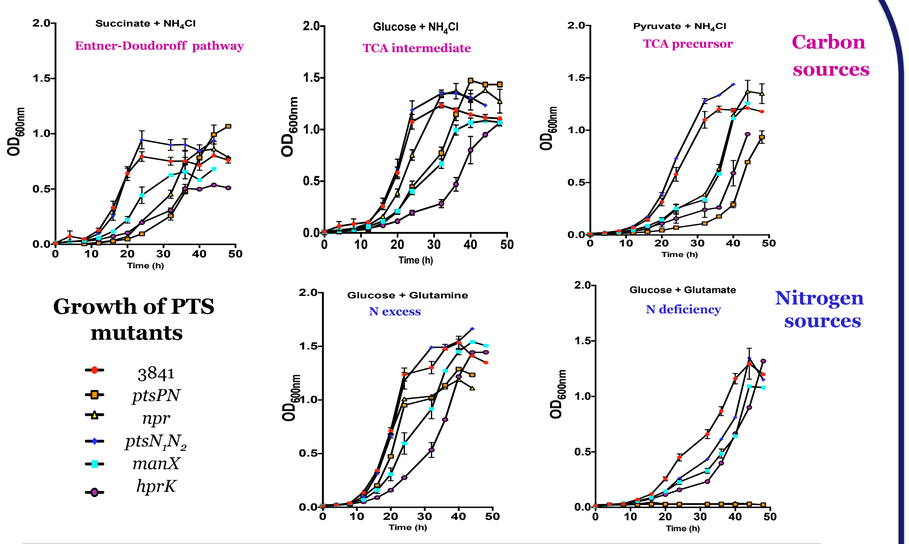
PTS^{Ntr} components are organized in four different operons, including two copies of *ptsN*. *npr* is located downstream of *manX*, encoding an EIIA^{Man} homologue, thus linking PTS^{Ntr} and the carbohydrate-PTS.

ManX and PtsN exert a differential regulation depending on their phosphorylation status, which is modulated by Npr. Npr transfers phosphate when it comes from PEP through PtsP, but it does not when it is phosphorylated by the kinase HprK.



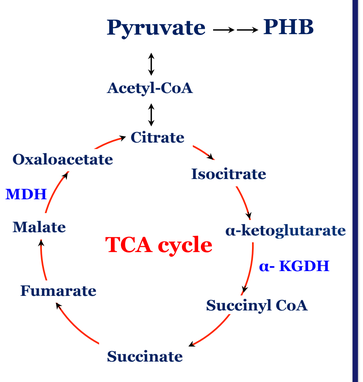
Working model in *R. leguminosarum* 3841

Central metabolism



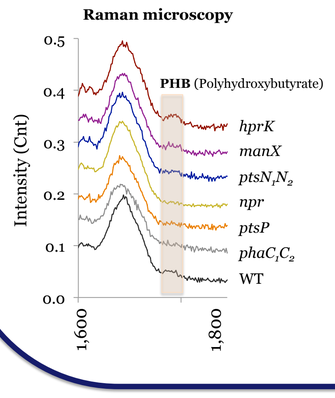
Description	Oxygen consumption ((umol/ml/min) OD_{600nm})		MDH activity	αKGDH activity
	Glucose	Succinate	(nmol/min/mg protein)	(nmol/min/mg protein)
Wild type	4.92 ± 0.85	6.06 ± 0.44	949.8 ± 60.8	16.5 ± 1.7
npr	4.18 ± 0.51	5.76 ± 1.31	910.4 ± 50.4	20.1 ± 1.0
manX	3.20 ± 0.72	**1.94 ± 0.63**	**375.5 ± 30.0**	**4.3 ± 1.0**

manX and *hprK* mutants show a reduced growth on several carbon sources and a compromised oxygen consumption when grown on succinate, indicating *manX* interacts with the TCA cycle. Accordingly, MDH and αKGDH activities are drastically reduced in a *manX* mutant.



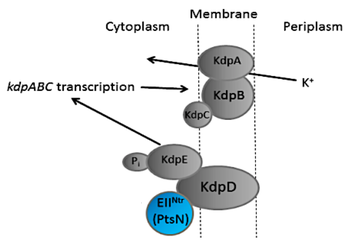
Malate dehydrogenase (MDH)

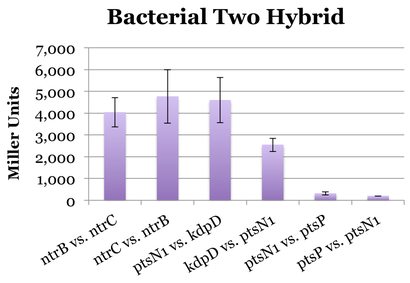
Alpha-ketoglutarate dehydrogenase (α-KGDH)



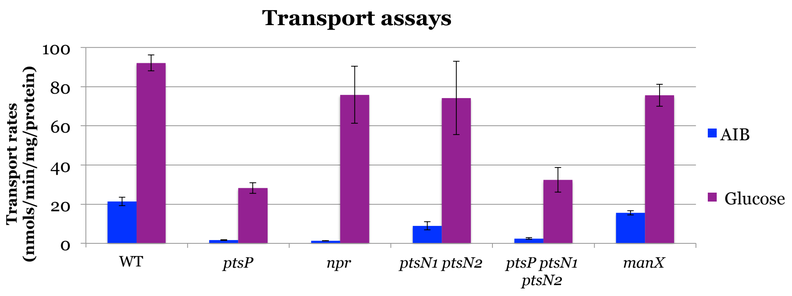
PTS^{Ntr} system controls PHB production, as mutants on this system have a significantly decreased production of this carbon storage compound.

Water and nutrient uptake

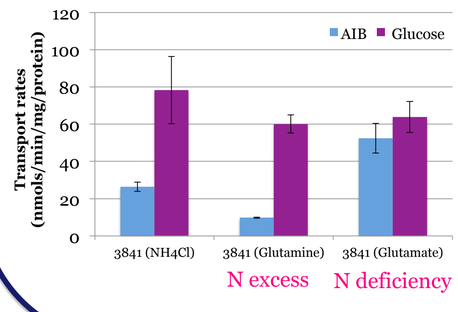




PtsN$_1$ and KdpD physically interact, indicating that non-phosphorylated PtsN$_1$ is required for full activation of the high affinity K$^+$ transporter KdpABC.

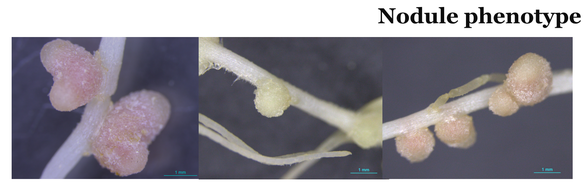


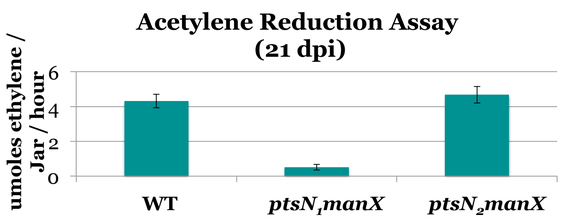
The PTS^{Ntr} system also controls ABC transporters, as PTS mutants have a reduced transport rate on different nutrients that need an ATP-dependent transporter.



Nitrogen availability drives PTS^{Ntr} regulation, as glutamine decreases the rate of transport.

Symbiosis





An active sugar-PTS and PTS^{Ntr} systems are needed together for an effective symbiosis, as a mutant in both *ptsN$_1$* and *manX* renders white nodules unable to fix nitrogen. Whereas *ptsN$_1$* exerts an important role in symbiosis, *ptsN$_2$* seems to be dispensable.

CONCLUSIONS

- *manX* mutant is growth defective and has a reduced MDH and αKGDH activitiy , indicating an interaction with the TCA cycle.
- PTS^{Ntr} system is involved in PHB production.
- PTS^{Ntr} controls K$^+$ homeostasis and ABC transporters. The control of these transporters seems to be exerted by nitrogen availability.
- PtsN$_1$ and ManX are essential for symbiosis.

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