The regulatory role of ManX in the central metabolism of *Rhizobium leguminosarum* bv. 3841

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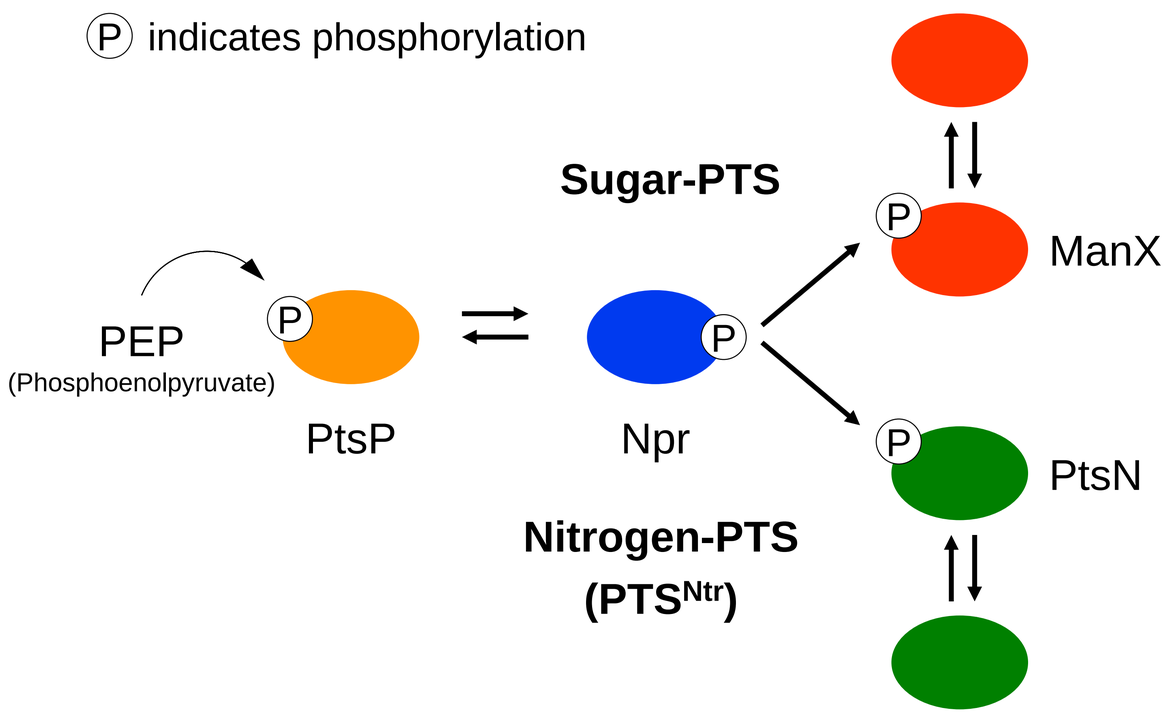
Rhizobia are α-proteobacterial soil microbes capable of free-living and symbiotic lifestyles. During symbiosis, they enter the roots of legume plants and become specialized nitrogen-fixing quasi-organelles. The switch between these two lifestyles requires large changes in metabolism, partially mediated by the PTS system.[1] We investigated the regulation and function of the ManX protein, required for symbiosis and part of the PTS system.

The PTS system in *R. leguminosarum*

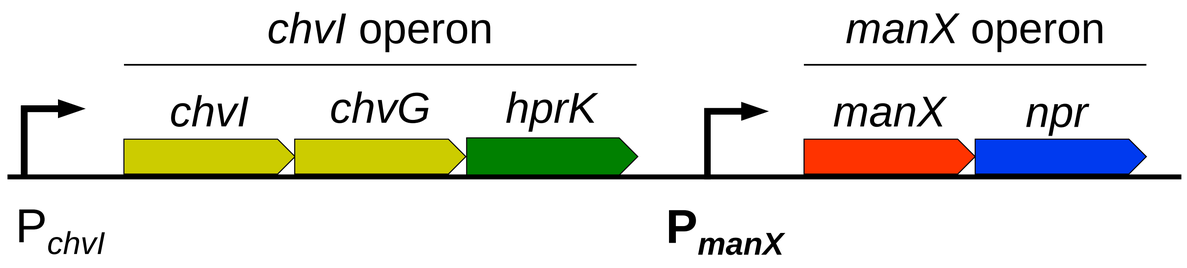
- The phosphoenolpyruvate phosphotransferase (PTS) system is key in regulating lifestyle switches [2]



- The system has two main effector proteins. PtsN (green) regulates nitrogen metabolism, while ManX is believed to regulate carbon metabolism
- The Npr protein controls the branching between these two terminal phosphorylation acceptors [3]

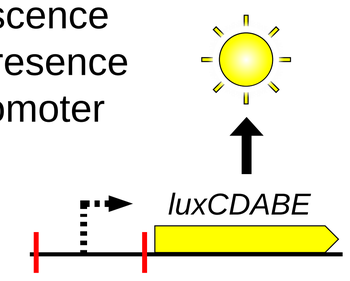
manX and *npr* form a discrete operon

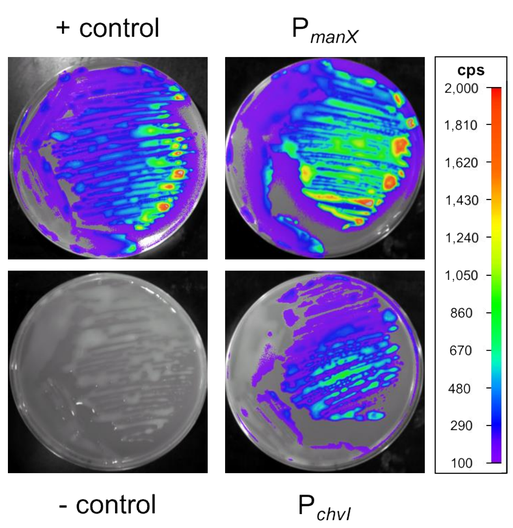
- The *manX* and *npr* genes were believed to be part of the *chvI* operon [4]



- Regions in the *chvI* operon were fused to a luminescence cassette to check for promoter activity

Colony luminescence indicates the presence of an active promoter

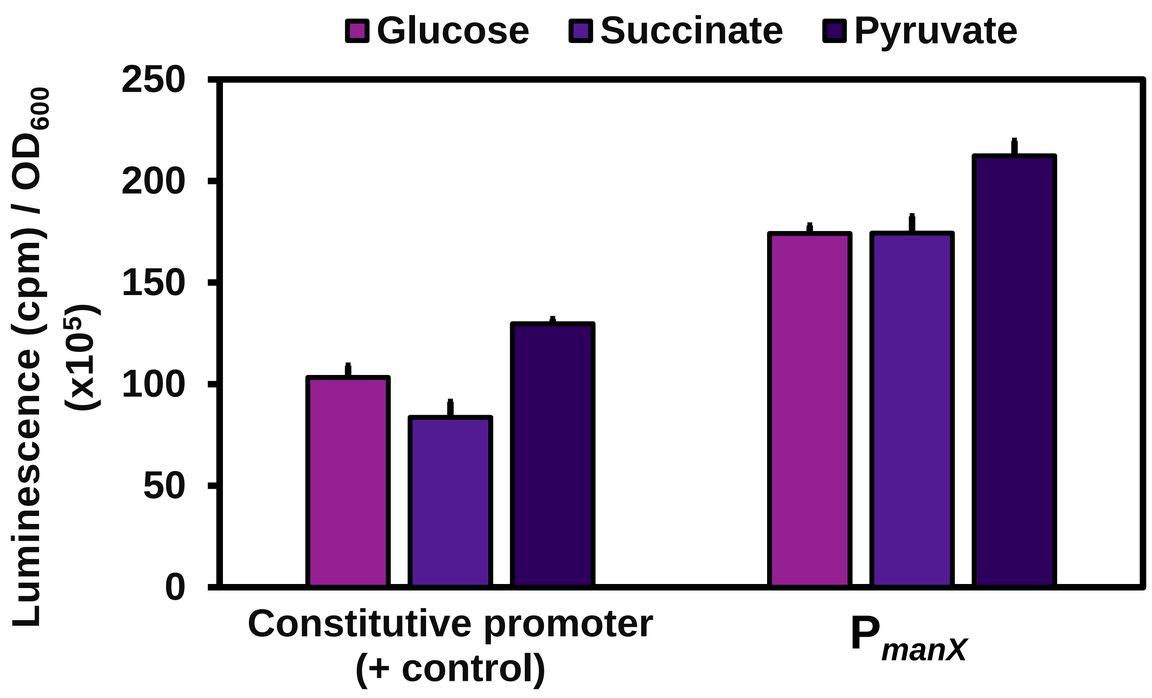




- A promoter was found upstream of *manX* (P_{manX}), separating it and *npr* from the *chvI* operon

manX is expressed regardless of carbon source

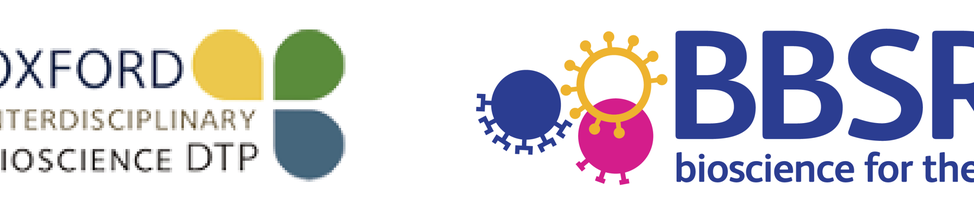
- In other organisms, *manX* expression is regulated based on available carbon sources



- In *R. leguminosarum*, no significant changes in *manX* expression were observed across the three carbon sources tested

ManX interacts with malate dehydrogenase

- We found a transient interaction between ManX and malate dehydrogenase (MDH) through bacterial two-hybrid assays (BACTH)



- This suggests ManX may regulate central metabolism by controlling MDH activity

References

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2. Walshaw, D. et al. (1997) Microbiology 143:2209–2221.
3. Dozot, M. et al. (2010) PLoS ONE 5:1:16
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