Poster

Characterization of the histidine quinase protein CbrA and the role of the CbrX in *P. putida* KT2442.

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ABSTRACT

Motivation: The γ-proteobacteria *Pseudomonas*, is present in a wide ecological niches due to metabolic, physiological and genetic versatility. It bears a very high number of regulatory systems that may allow them to adapt to many environmental conditions. Within this genus, *P. putida* KT2440 serves as a model microorganism of biotechnological interest. One of these regulatory two-components systems, unique in the pseudomonads, is CbrAB where CbrA is a histidine kinase sensor protein and CbrB a transcriptional activator of σ^N-dependent promoter, many involved in the assimilation of different C sources [1,3]. In this project, we will characterise the role of CbrA in the reception of the environmental signal to activate Cbr system when there is limited in C availability. In addition, we will study the role of an open reading frame upstream and overlapping with *cbrA*, called *cbrX*, and its involvement in transcriptional/translational regulation of CbrA.

Methods: A deletion mutant of *cbrA* and *cbrX* (Δ*cbrXA*) has been constructed (MPO494) in *P. putida* KT2442, and the phenotypic characterization of its ability to grow in a minimal medium using different C sources (succinate, citrate, histidine, glucose) has been evaluated. Also the transcriptional activation of three different targets of the Cbr regulatory system has been studied by analysis of the β-galactosidase activity of a transcriptional fusion to the promoter regions of *crcZ*, *crcY*, *PP2810* [2]. Complementation of the mutant at the Tn7 integration site with the complete sequence *cbrXA*, and different constructs bearing *cbrA* or *cbrX* have also been constructed and their phenotypes analysed. Finally, a truncated form of CbrA expressed from heterologous P_{tac} promoter, which lacks 13 transmembrane domains, that is presumably not anchored to the inner membrane has also been constructed.

Results: The Δ*cbrXA* deletion mutant MPO494 shows a longer lag phase when growing in succinate and glucose as C source, and even longer when growing on citrate medium, when compared to the wild-type strain KT2442. MPO494 is not able to use histidine as C source. Complementation of the MPO494 with *cbrXA* sequence fully recovers the wild-type phenotype. The activation of *crcZ*, *crcY* and *PP2810* genes is 26 to 20 fold lower in a medium containing succinate or oxalacetate as C source in a mutant background compared to the KT2442, but it is fully complemented when the *cbrXA* sequence is supported in *trans*. The effects of *cbrX* on the CbrA expression/activity is currently being analysed.

REFERENCES

[1] Amador, C. I., I. Canosa, F. Govantes & E. Santero, (2010) Lack of CbrB in *Pseudomonas putida* affects not only amino acids metabolism but also different stress responses and biofilm development. *Environ microbiol*, 12, 1748-1761.

[2] Miller, J. H., (1992) A short course in bacterial genetics: a laboratory manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y.

[3] Nishijyo, T., D. Haas & Itoh, (2001) The CbrA-CbrB two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. *Mol microbiol*, 40, 917-931.