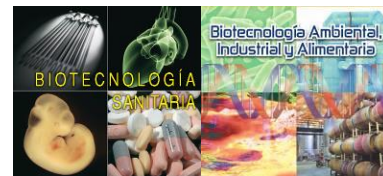


Poster

Characterization of the ECF sigma factor PP_0865 of *Pseudomonas putida* KT2442



Ernesto Valero Torres and Inés Canosa(1)

(1)Microbiology area, Department of Molecular Biology and Biochemistry /Universidad Pablo de Olavide.

Keywords: ECF sigma factor, *Pseudomonas putida*, Iron, Metal Ions.

ABSTRACT

Motivation:

Motivation: *Pseudomonas putida* is a well characterized environmental bacterium capable of removing toxic components, including heavy metals and xenobiotics from different provenance including contaminated soils or water. Also, *P. putida* has been successfully shown to efficiently work as cell factories, for the synthesis of different products of biotechnological interest (1). We are interested in the characterization of an alternative extracitoplasmic sigma factor (PP_0865) that may contribute to this detoxification ability of KT2442 (2).

Methods: We used the following methods to characterize ECF sigma factor PP_0865:

- RT-qPCR: Expression determination of PP_0865 in different mediums with different carbon availability; i.e. LB, succinate and oxaloacetate.
- β - galactosidase activity: Expression determination using a transcriptional fusion of the promoter region of PP0865 to lacZ. We assayed its activity in LB medium and a minimal medium containing succinate in the wild type background and in a *cbrB* mutant, which is a global control regulator which may regulate the ECF expression.
- Growth curve of the wild type KT2442 strains and a deletion mutant of the ECF sigma factor (MPO526) or the anti-sigma element (MPO527) in a minimal medium containing succinate as a carbon source in the presence of an excess of iron (34mM FeCl₃) and in the total absence of iron (3).
- Metal ions screening: We analysed the tolerance of strains KT2442, MPO526 and MPO527 to copper, zinc and cobalt in LB medium with a supplement of 2 mM CuCl₂, 3 mM ZnCl₂ and LB 0,6 mM CoCl₂.

Results:

- PP_0865 seems to be overexpressed in LB compared to succinate and oxaloacetate, both by RT-qPCR and β -Galactosidase analysis.
- There are no significant differences in the growth of KT2442, MPO526 or MPO527 strains in the presence or absence of iron.
- Mutant strain MPO527 seems to be more tolerant to copper than KT2442 and MPO526.

Conclusions: Preliminary data indicate that the ECF sigma factor system PP_0865-PP_0867 may be involved in the tolerance to metal ions such as copper.

REFERENCES

1. Poblete-castro, I., Becker, J. & Dohnt, K. Industrial biotechnology of *Pseudomonas putida* and related species. 2279–2290 (2012). doi:10.1007/s00253-012-3928-0
2. Llamas, A., Imperi, F., Visca, P. & Lamont, I. L. Cell-surface signaling in *Pseudomonas* : stress responses, iron transport, and pathogenicity. 38, 569–597 (2014).
3. Llamas, A. et al. The Heterologous Siderophores Ferrioxamine B and Ferrichrome