

David Leys – Understanding Bacterial Halorespiration

Bacterial halorespiration is a microbial respiratory process that uses halogenated hydrocarbons as terminal electron acceptors. Improving our understanding of this process could enable the detection and removal of harmful compounds in soil, sediment and water. This is the ultimate aim of the 5 year ERC DEHALORES project that started in 2008 in the Leys group.



E.coli bacteria that express GFP in response to the present of a chlorinated phenol.

Billions of substances fall within the wider family of chlorinated compounds. Many of these are the result of natural processes, grasshoppers for example can generate a chlorinated phenol compound to act as ant repellent. In addition to biological production, abiotic processes such as forest fires and volcanic eruptions in particular can generate a range of chlorinated products, which are broken down by several organisms in a continuous "chloride-cycle". While many of these naturally generated compounds will have little or no harmful effect on the wider environment, the

accumulation of new, man-made substances known as xenobiotics in soil, sediment and water presents significant risks. Many of these compounds have persist for long periods of time, and most of them have been found to be toxic.

Detecting the presence of these compounds themselves is an important first step to addressing this issue, with removal (ideally by the halorespiratory bacteria) being the ultimate aim. The recent sequencing of four separate genome sequences closely related to two particular bacteria - *Desulfitobacterium dehalogenans* and *D. hafniense* - has proved enormously relevant to our work. We have already made significant progress in understanding the molecular basis of transcriptional regulation of halorespiration by CprK [refs], one of the regulators found associated with the key enzyme in this process. This enzyme, CprA, is a membrane-associated, oxygen-sensitive, B12 and iron-sulfur cluster binding protein that reduces halogenated hydrocarbons. We have only recently been able to make significant progress towards understanding this aspect, thanks to the hard work of Mark Dunstan and Karl Fisher.

Our present research focuses on using CprK as a template for designing novel sensors using laboratory evolution techniques, an area in which Laura Kemp is doing her PhD. In addition, we are studying the properties of a wide range of unrelated halorespiratory regulators that could potentially serve as alternative templates. Following several months of trials, we are now able to produce the key enzyme CprA, which will allow us to elucidate its mechanism. Ultimately, we would hope to expand the substrate specificity of CprA to include xenobiotics such as dioxins, PCBs through protein engineering.

References:

- Levy, C, Pike, K, Heyes, D, Joyce, MG, Gabor, K, Smidt, H, van der Oost, J & Leys, D. (2008) Molecular basis of halorespiration control by CprK, a CRP-FNR type transcriptional regulator. *Mol. Microbiol.* **70**, 151-67.
- Mazon H, Gabor K, Leys D, Heck AJR, van der Oost J & van den Heuvel, RHH. (2007) Transcriptional activation by CprK1 is regulated by protein structural changes induced by effector binding and redox state, *J. Biol. Chem.* **282**, 11281-11290
- Joyce, MG, Levy, C, Gabor, K, Pop, SM, Biehl, DB, Doukov, RI, Ryter, JM, Mazon, H, Smidt, H, van den Heuvel, RHH, Ragsdale, SW, van der Oost, J & Leys, D. (2006) CrpK crystal structures reveal mechanism for transcriptional control of halorespiration. *J. Biol. Chem.* **281**, 28318-28325



Crystal structure of CprK in complex with DNA and a chlorinated ligand.