

P014 A novel method of variant cytochrome cd_1 expression
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Paracoccus pantotrophus cytochrome cd_1 nitrite reductase is a soluble tetraheme homodimer located in the periplasm that catalyses the one electron reduction of nitrite to nitric oxide. The formation of nitric oxide is the first committed step of the denitrification chain. Each monomer of cytochrome cd_1 nitrite reductase carries a covalently bound c-heme and a non-covalently bound d_1 -heme, a cofactor unique to this class of enzyme. The genes for d_1 -heme biosynthesis are located downstream of the cytochrome cd_1 structural gene (*nirS*) within the *nir* operon. Expression of the *nir* operon in *P. pantotrophus* and many other bacteria is dependant on the production of nitric oxide. Nitric oxide is thought to activate the transcription factor NNR which binds upstream of the *nir* operon. Mutagenic studies of *P. pantotrophus* cytochrome cd_1 reveal two categories of variant enzyme: functional and non-functional. A functional enzyme will catalyse the production of nitric oxide which will switch on the biosynthesis of d_1 heme whereas a non-functional enzyme will not. Expression of a non-functional enzyme results in the production of a semi-apo protein (carrying a c-heme but no d_1 -heme). To produce a non-functional holoenzyme nitric oxide needs to be present in order to induce d_1 -heme biosynthesis. A novel strategy has been developed to introduce nitric oxide into the expression system and hence express non-functional forms of holo-cytochrome cd_1 .