A new class IV cytochrome P450 enzyme: an evolutionary tale and an *in silico* analysis

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A new member of class IV cytochrome P450 (P450) (Roberts *et al*., 2002) was identified in *Acinetobacter radioresistens* S13. As such this is expected to be a self-sufficient enzyme consisting of a N-terminal catalytic P450 domain fused to a C-terminal reductase domain. Interestingly phylogenetic analysis places the sequence of the P450 domain close to that of class I P450 from *Rhodococcus jostii*. The reductase domain is close to a *R. jostii* oxidoreductase containing FMN, NADH and 2Fe2S centre. *R. jostii* genes coding for class I P450 (*cyp*x116) and the oxidoreductase (*oxred*116) are located in a plasmid in adjacent position. *R. jostii* does not possess genes coding for class IV P450 proteins and *A. radioresistens* does not have class I P450 proteins. Therefore, it can be speculated that *A. radioresistens cyp*x gene is the result of: (1) a horizontal gene transfer of the *R. jostii* DNA fragment harbouring *cyp*x116 and *oxred*116, (2) gene fusion, and (3) integration of the fused new *cyp*x gene in the *A. radioresistens* chromosome.

In the absence of any published crystal structure for this class of P450 enzymes, an *in silico* 3D model was generated based on homology modeling with class I P450 from *Bacillus subtilis* (3EJB). The P450 enzyme from the latter bacterium is known to catalyze the oxidation of fatty acids (Cryle *et al.* 2003). *In silico* docking experiments showed, on the basis of the binding energies, that stearic and palmitic acid could be putative substrates of this enzyme. Moreover, the expression of S13 *cyp*x was induced by the presence of these molecules in the bacterial growth medium. Therefore, both *in silico* and *in vitro* experiments support turnover of these fatty acids by this novel class IV P450 enzyme.