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Characterization of recombinant *Phanerochaete chrysosporium* cellobiose dehydrogenase mutants with increased oxidative stability from *Pichia pastoris* KM71H strain

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Phanerochaete chrysosporium is a white rot fungi and it has been known to secrete flavocytochrome enzyme cellobiose dehydrogenase (CDH, EC 1.1.99.18) which contains two domains, a flavine domain and cytochrome domain. Flavine domain contains FAD as prostetic group and its catalytically active domain, whereas cytochrome domain serves as electrone acceptor. Cellobiose and lactose, as well as other β – 1,4 – linked disaccharides and oligosaccharides, have been oxidized by the cellobiose dehydrogenase to their corresponding lactones [1-3]. CDH can be used for constructing biosensors and therefore directed evolution has been used to produce more active and stable variants of the enzyme. Wild type CDH enzyme was expressed in *S.cerevisiae* INVSc1 cells and used for creation of saturation mutagenesis libraries at M65, M685 and M738 and screening for increased oxidative stability. More stable mutants that were found were recloned into *Pichia pastoris* KM71H strain for higher expression yield. They were afterwards, expressed in *Pichia*, purified and kineticaly characterized.

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