Molecular and biochemical characterization of a new thermostable bacterial laccase from Meiothermus ruber DSM 1279 - DTU Orbit (08/11/2017)

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A new laccase gene (*mrlac*) from *Meiothermus ruber* DSM 1279 was successfully overexpressed to produce a laccase (Mrlac) in soluble form in *Escherichia coli* during simultaneous overexpression of a chaperone protein (GroEL/ES). Without the GroEL/ES protein, the Mrlac overexpressed in *E. coli* constituted a huge amount of the total cellular protein, but the enzyme was localized in the insoluble fraction with no activity in the soluble fraction. Co-expression of the Mrlac with the *E. coli* GroEL/ES drastically improved proper folding and expression of active Mrlac in the soluble fraction. Spectroscopic analysis of the purified enzyme by UV/visible and electron paramagnetic resonance spectroscopy confirmed that the Mrlac was a multicopper oxidase. The Mrlac had a molecular weight of ~ 50 kDa and exhibited activity towards the canonical laccase substrates 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), syringaldazine (SGZ), and 2,6-dimethoxyphenol (2,6-DMP). Kinetic constants K_m and k_{cat} were 27.3 μM and 325 min_{-1} on ABTS, 4.2 μM and 106 min^{-1} on SGZ, and 3.01 μM and 115 min^{-1} on 2,6-DMP, respectively. Maximal enzyme activity was achieved at 70° C with ABTS as substrate. In addition, Mrlac exhibited a half-life for deactivation at 70° C and 75° C of about 120 min and 67 min, respectively, indicating that the Mrlac is intrinsically thermostable. Finally, Mrlac was efficient in catalyzing the removal of 2,4-dichlorophene (DCP) in aqueous solution, a trait which makes the enzyme potentially useful for environmentally friendly applications.

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