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Title: Characterization of a mildly alkalophilic and thermostable recombinant Thermus thermophilus

laccase with applications in decolourization of dyes

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Thermus thermophilus laccase

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Abstract:

Objective: To examine the potential for applications of TthLAC, a monomeric (~ 53 kDa) laccase encoded by the genome of Thermus thermophilus (strain HB 27) which can be produced at low cost in Escherichia coli. Result: Functional, thermostable and mildly alkalophilic TthLAC of high purity (> 90%) was produced through simple heating of suspended (TthLAC overexpressing) E.coli cells at 65 °C. For reactions of short duration (< 1 h) the temperature for optimal activity is ~ 90 °C. However, TthLAC undergoes slow partial unfolding and thermal inactivation above 65 °C, making it unsuitable for long incubations above this temperature. With different substrates, optimal function was observed from pH 6 to 8. With the substrate, ABTS, catalytic efficiency (Km) and maximum velocity (Vmax) at 60 °C and pH 6.0 were determined to be 2.4 × 103 μ M and 0.04 × 103 μ M/min respectively. Ultra-pure, affinity-purified TthLAC was used to confirm and characterize the enzyme's ability to oxidize known (laccase) substrates such as ABTS, syringaldazine and 4-fluoro-2-methylphenol. TthLAC decoloured up to six different industrial dyes, with or without the use of redox mediators such as ABTS. Conclusions: Unlike versatile laccases from most other sources, which tend to be thermolabile as well as acidophilic, TthLAC is a versatile, thermostable, mildly alkalophilic laccase which can be produced at low cost in E.coli for various redox applications.

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