Simultaneous Improvement of Catalytic Activity and Thermal Stability by Directed Evolution

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The tyrosine phenol-lyase from *Symbiobacterium toebii* was engineered to improve both its stability and catalytic activity by applying random mutagenesis and subsequent reassembly of the acquired mutations. Activity screening of the random library produced four mutants with two-fold improved activity, while parallel screening after heat treatment at 65°C identified three mutants with half-inactivation temperatures improved by up to 5.6°C. The selected mutants were then reassembled using the staggered extension PCR method and subsequent screening of the library produced seven mutants with up to three-fold improved activity and half-inactivation temperatures improved by up to 11.2°C. Sequence analyses revealed that the stability-improved hits included A13V, E83K, and T407A mutations, while the activity-improved hits included the additional T129I or T451A mutation. Homology modeling of the enzyme structure revealed that most of the stability mutations were located around the dimer-dimer interface including the N-terminus, whereas the activity-improving mutations were located further away, thereby minimizing any interference that would be detrimental to the co-improvement of the stability and catalytic activity of the enzyme.