Reengineering Tyrosinase by Random Mutagenesis for Activity Towards Non-native Phenol Substrates

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Tyrosinase holds great potential as a biocatalyst for the bioremediation of toxic phenols in wastewater, since it reacts with such compound to form minimally soluble pigments that can be removed readily. Furthermore, it is unique in being capable of catalyzing the hydroxylation and subsequent oxidation of phenols using only O_2 and substrate without the need for additional reducing equivalents or co-substrates. However, the development of tyrosinase as a biocatalyst has been hampered by the lack of a high-yield expression system and the lack of a suitable strategy for its reengineering. We have solved both of these problems. We have developed an *E. coli* expression system for *Streptomyces gluacescens* tyrosinase that produces enzyme that is active in vivo in high yield. With this system, we have designed a strategy for the high-throughput screening of mutant enzymes produced by random mutagenesis techniques that have altered activity towards different non-native phenol substrates.

It has long been known that tyrosinase is broadly tolerant of substituents at the *para*-position of the phenol ring, but that *meta*-substituted phenols are poor substrates, presumably for steric reasons. We have chosen this simple, steric limitation of substrate specificity as our first target for enzyme reengineering. This is an important first step, since relaxed steric constraints on substrate specificity are important for any future application of tyrosinase as a biocatalyst.