

Supplementary materials to

An Engineered Thermostable Laccase with Great Ability to Decolorize and Detoxify Malachite Green

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Table S1. Primers for site-directed mutagenesis of Mut2.

Primer	Sequence
H78A-F	ACAAGTT <u>GC</u> AGGCAACAACATTCTGG
H78A-R	TTGTTGCCTGCAACTTGTCGAACGGT
C119A-F	GCGGAT <u>GC</u> AATACCCGGTTCTGTTGG
C119A-R	CGGGTATGCATCCGCCACGAGGAC
H136A-F	CGGTGG <u>CA</u> GTTGGCTGGCGTGGTG
H136A-R	AGCCAAGTGCACCGCAGCAACGAC

The underlined bases indicate the mutated sites.

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WT      1  MKMARNGKISYLEPEWITGSLRAGFTTRNGGISRAPYNSLNLGLNTEDPRPNVEGNRSTLVRAFGLLEVHQ
Mut1    1  MKMARNGKIYYLEPEWITGSLRAGFTTRNGGISRAPYNSLNLGLNTGDPNPVEGNRERLVRAFGLEPHQ
Mut2    1  MKMARNGKIYYLEPEWIHGSVRAGFTTRNGGISRPPYNSLNLGLNTGDPNPVEGNRERLVRAFGLEPHQ
Mut3    1  MKMARQGKIYYLEPEWIHGSVRAGFTTRNGGISRPYESLNGLNTGDPRENVEGNRERLVRAFGLEPHQ

WT      71  LLTVRQVHGNNILVVDEPNPDLSHFQQVECDAIISNQPGMMIGVLVADCYPVLLAAPAQGVVAHVHVGWR
Mut1    71  LLTVRQVHGNNILVVDEPNPDLSHFQQVECDAIISNQPGMMIGVLVADCYPVLLAAPANGVVAHVHVGWR
Mut2    71  LLTVRQVHGNNILVVDEPNPDLSHFQQVECDAIISNQPGMMIGVLVADCYPVLLAAPANGVVAHVHVGWR
Mut3    71  LLTVRQVHGNNILVVDEPNPDLSHFQQVECDAIISNQPGMMIGVLVADCYPVLLAAPANGVVAHVHVGWR

WT      141  GAVAQIIQRTVDALQEQQFGVCPPELLAAVGP GIGAHAYEVD RPVRDQFRKAGLPWAGVAEESRLGHWKLD
Mut1    141  GAVAQIIQRTVDALQEQQFGVRPEDLLAAVGP GIGACCYEVD RPVRDQFRKAGLPWEVAEESRLGHWKLD
Mut2    141  GAVAQIIQRTVDALQEQQFGVRPEDLLAAVGP GIGACCYEVD RPVRDQFRKAGEPWEVAEESRDGHWKLD
Mut3    141  GAVAQIIQRTVDAMQEQQFGVRPEDLLAAVGP GIGACCYEVD RPVRDQFRKAGGPWEVAEFSRDGHWKLD

WT      211  LRETCRRQLLTAGVVRTHIDLAEECTCCHRELLFSHRRDNGRTGRQLGFILLT
Mut1    211  LRETCRRQLLDAGVPREHIDLAEWCTCCHRELFFSHRRDNGRTGRQLGFILLT
Mut2    211  LREACRRQLLDAGVKAEHIDLAEWCTCCHRELFFSHRRDNGRTGRMLGFILLT
Mut3    211  LREACRRQLEDAGVKREHIYLAEWCTCCHRELFFSHRRDNGRTGRMLGFILLT

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Figure S1. Sequence alignment of GhIac WT, Mut1, Mut2, and Mut3. The mutated residues are colored red.

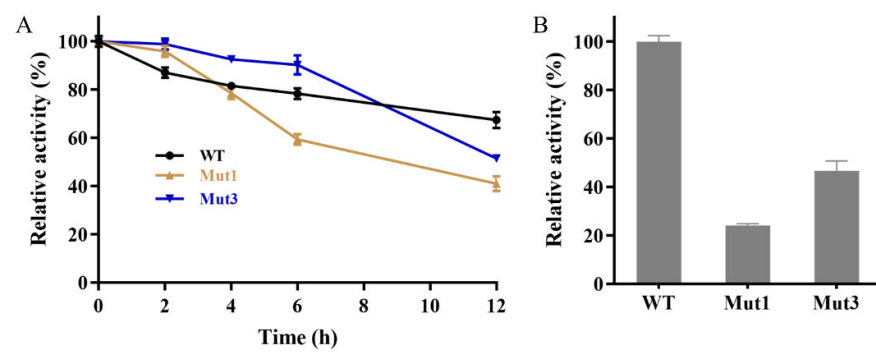


Figure S2. Thermostability at 50 °C (A) and activity against ABTS (B) of Mut1 and Mut3.

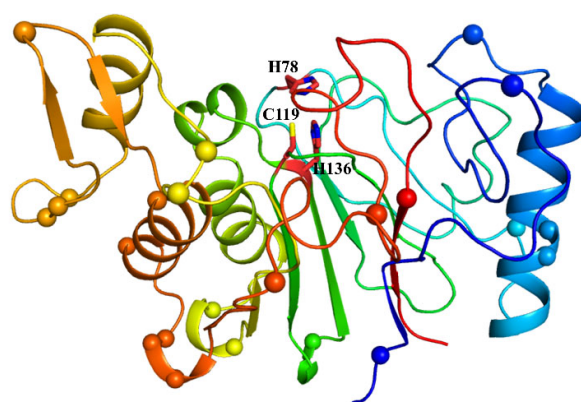


Figure S3. Structural mapping of the mutated residues of GhLac Mut2. The mutated residues are shown as spheres. H78, C119, and H136 are labeled.

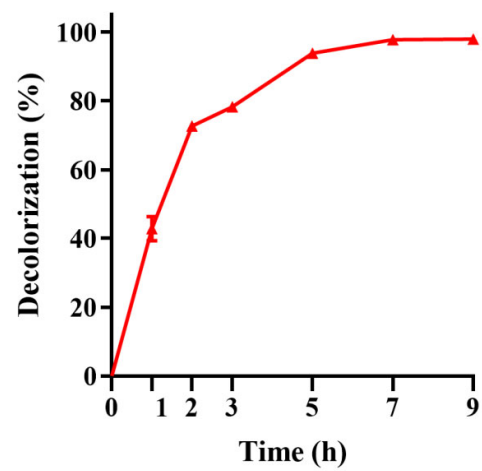


Figure S4. MG decolorization catalyzed by Ghlac WT. MG at the concentration of 100 mg/L was decolorized by 40 U/L of Ghlac WT with 0.1 mM ABTS at 70 °C.