Improving the indigo carmine decolorization ability of a *Bacillus amyloliquefaciens* laccase by site-directed mutagenesis

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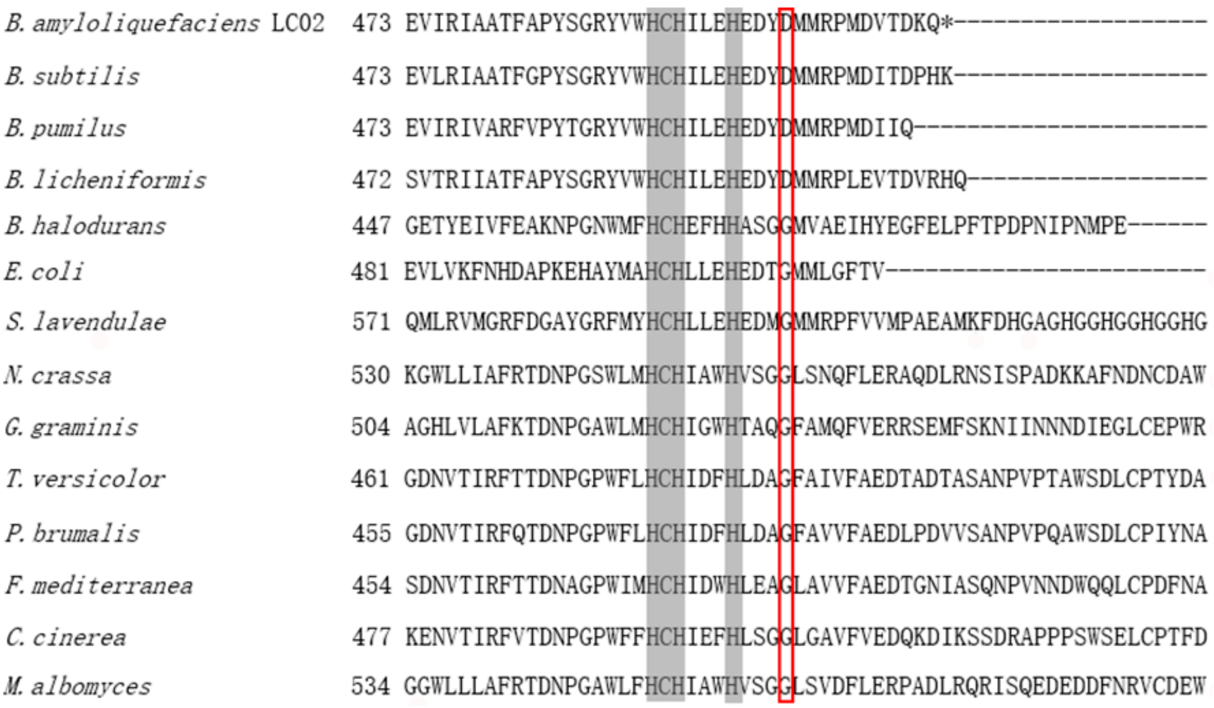
Figure S1. Homology model of the wild-type laccase from *B. amyloliquefaciens*. Aspartic acid 501 is shown as sticks and the copper atoms are highlighted in orange.



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B. amyloliquefaciens LC02  473 EVIRIAATFAPYSGRYVWHCHILEHEDYDMMRPMDVTDKQ*-------------------
B. subtilis                473 EVLRIAATFGPYSGRYVWHCHILEHEDYDMMRPMDITDPHK-------------------
B. pumilus                 473 EVIRIVARFVPYTGRYVWHCHILEHEDYDMMRPMDIIQ----------------------
B. licheniformis           472 SVTRIIATFAPYSGRYVWHCHILEHEDYDMMRPLEVTDVRHQ------------------
B. halodurans              447 GETYEIVFEAKNPGNWMFHCHEFHHASGGMVAEIHYEGFELPFTPDPNIPNMPE------
E. coli                    481 EVLVKFNHDAPKEHAYMAHCHLLEHEDTGMMLGFTV------------------------
S. lavendulae              571 QMLRVMGRFDGAYGRFMYHCHLLEHEDMGMMRPFVVMPAEAMKFDHGAGHGGHGGHGGHG
N. crassa                  530 KGWLLIAFRTDNPGSWLMHCHIAWHVSGGLSNQFLERAQDLRNSISPADKKAFNDNCDAW
G. graminis                504 AGHLVLAFKTDNPGAWLMHCHIGWHTAQGFAMQFVERRSEMFSKNIINNNDIEGLCEPWR
T. versicolor              461 GDNVTIRFTTDNPGPWFLHCHIDFHLDAGFAIVFAEDTADTASANPVPTAWSDLCPTYDA
P. brumalis                455 GDNVTIRFQTDNPGPWFLHCHIDFHLDAGFAVVFAEDLPDVVSANPVPQAWSDLCPIYNA
F. mediterranea            454 SDNVTIRFTTDNAGPWIMHCHIDWHLEAGLAVVFAEDTGNIASQNPVNNDWQQLCPDFNA
C. cinerea                 477 KENVTIRFVTDNPGPWFFHCHIEFHLSGGLGAVFVEDQKDIKSSDRAPPPSWSELCPTFD
M. albomyces               534 GGWLLLAFRTDNPGAWLFHCHIAWHVSGGLSVDFLERPADLRQRISQEDEDDFNRVCDEW
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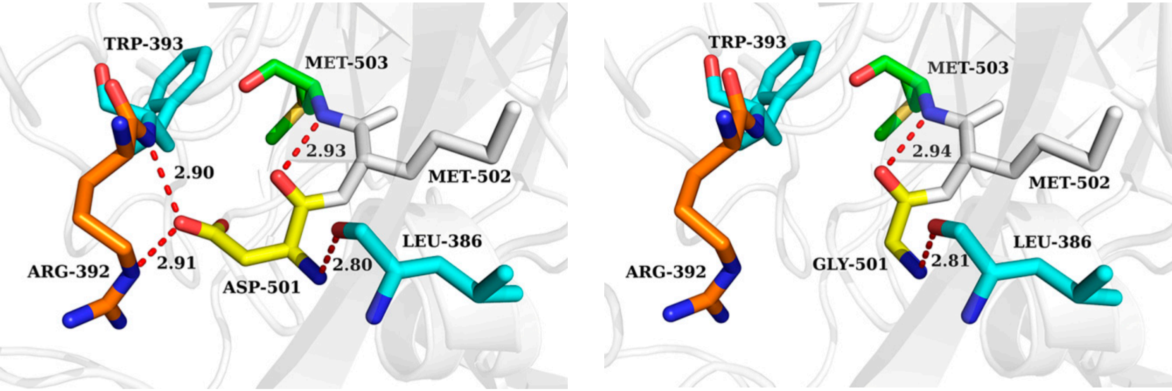
Figure S2. Protein sequence alignment of fungal and bacterial laccases. The highly conserved regions of copper binding sites are represented with gray background. The conserved glycine residue is framed. Laccase protein sequences: *Bacillus amyloliquefaciens* LC02 (ADZ57285); *B. subtilis* (AID81987); *B. pumilus* (AFV60743); *B. licheniformis* (BAU80729); *B. halodurans* (AAP57087); *Escherichia coli* (BAB96698); *Streptomyces lavendulae* (BAC16804); *Neurospora crassa* (AAA33591); *Gaeumannomyces graminis* (CAD24842); *Trametes versicolor* (BAA23284); *Polyporus brumalis* (ABN13591); *Fomitiporia mediterranea* (EJC99977); *Coprinopsis cinereal* (ABP81837); *Melanocarpus albomyces* (CAE00180).



(**a**) (**b**)

Figure S3. Partial structure of the wild-type laccase (a) and D501G variant (b). Hydrogen bonds are shown as red dashes.