

Improving the Enzymatic Activity and Stability of a Lytic Polysaccharide Monooxygenase

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Supporting Information

Table S1. Primers used for mutagenesis

| Mutants | Sequences (5'--3') |
|---------|----------------------------------|
| Y3F-F | TTTATTAAAGAGCCGGTAAGCAGAGC |
| Y3F-R | TCCGTGACCTCCTATTGTT CG |
| I4V-F | TATG TTAAAGAGCCGGTAAGCAGAGCAT |
| I4V-R | TCCGTGACCTCCTATTGTT CG |
| K5E-F | TATATTGAAGAGCCGGTAAGCAGAGCATATAT |
| K5E-R | TCCGTGACCTCCTATTGTT CG |
| N36A-F | GCA CTGAAAGTCAGC GACGGCAG |
| N36A-R | GATGTGGGTT TCGGGTTAA CTTCT |
| N36E-F | GAA CCGCA GAGCGTTGAAGGTC |
| N36E-R | ATCAATTACT GAACCGTATT TTTGGGCT |
| V40E-F | GAA GACGGCAGCAGCGAAATCT |
| V40E-R | ACTTCAGG TTGATGTGGG TTTC |
| V40A-F | GCT GAAGGTCCGAAAGGTTCCC |
| V40A-R | GCTCTGCAGGG TTATCAATTA CTG |
| V40I-F | ATC GAAGGTCCGAAAGGTTCCC |
| V40I-R | GCTCTGCAGGG TTATCAATTA CTG |
| V40L-F | CTG GAAGGTCCGAAAGGTTCCC |
| V40L-R | GCTCTGCAGGG TTATCAATTA CTG |
| V40M-F | ATG GAAGGTCCGAAAGGTTCCC |
| V40M-R | GCTCTGCAGGG TTATCAATTA CTG |
| R55Q-F | CAG ATAGCA AGCGCAAATGGTG GTAG |
| R55Q-R | ACCATCCAGGG GGACCTG |
| N60G-F | GGT GGTGGTAGCGGTACAGATTGA |
| N60G-R | TGCGCTTGCTATACGACCAT C |
| H77R-F | CGT TGGGTTAACACAGAATATCAGAGG |
| H77R-R | GTCTGCTGTCTGTTATCTA AG |
| E124D-F | GAT CTGATTGGCACCGTTAACATG |
| E124D-R | AAATTCTCA CGGCTCAGCG |
| G158A-F | GCA GTTTGGGATGTTGCAGATACC |
| G158A-R | CAGAATGATG TGATAGCCGCT |

Table S2. Apparent kinetic parameters of the purified enzymes

| Purified enzymes | k_{cat} (min ⁻¹) | K _m (mM) |
|------------------|--------------------------------|---------------------|
| V40L-E124D | 5.15 ± 0.07 | 33.53 ± 1.6 |
| I4V-E124D | 4.74 ± 0.25 | 95.46 ± 9.5 |
| V40L-Y3F | 3.98 ± 0.05 | 30.85 ± 1.29 |
| V40L-I4V | 4.09 ± 0.27 | 53.45 ± 9.23 |
| E124D-Y3F | 5.02 ± 0.31 | 93.29 ± 9.16 |
| V40L | 5.18 ± 0.12 | 32.55 ± 3.6 |
| I4V | 3.92 ± 0.28 | 71.31 ± 7.8 |
| E124D | 3.76 ± 0.03 | 13.73 ± 0.82 |
| Y3F | 3.24 ± 0.08 | 38.33 ± 3.12 |
| V40M | 3.75 ± 0.05 | 27.69 ± 1.49 |
| K5E | 3.19 ± 0.08 | 15.59 ± 1.78 |
| V40I | 3.18 ± 0.14 | 25.36 ± 3.84 |
| V40A | 2.40 ± 0.09 | 51.46 ± 5.34 |
| R55Q | 2.48 ± 0.01 | 13.09 ± 0.41 |
| N36E | 2.55 ± 0.06 | 29.02 ± 2.43 |
| WT | 2.50 ± 0.27 | 41.33 ± 7.33 |

| | | | | | |
|----------------|--|-----|----------------|--|-----|
| AA-CBM33 .SEQ | | 0 | AA-CBM33 .SEQ | WVQNTRQCFNEDHDTIATISWYWIDRKNPENKE | 117 |
| AOAOWTY051.SEQ | MNTFEAKKLSPKGISA.....AIIAAGINIGISMAIN.RYN | 34 | AOAOWTY051.SEQ | WFKNITITGSNITFVKDIAAHLSKSMWYVYDNEKGWNENKE | 143 |
| AOALH8E454.SEQ |MAQRSFNW.CKWMVSCGMMLIVGACTLIVFESASA | 33 | AOALH8E454.SEQ | WHRVNIRGNNITCQYDIAHATKENVYVYDNEKGWNENKE | 137 |
| AOA521ABL2.SEQ |MTIGIRGKSTLLITCGMMLMNGFILEDERSA | 34 | AOA521ABL2.SEQ | WFRNVLNGKNNITCQHDTIAHATKENVYVYDNEKGWNENKE | 138 |
| AOA559IZW6.SEQ | .MIQTITLLEFKSVFELAFAMTLLLAISVVFQCSA | 39 | AOA559IZW6.SEQ | WDTTILSGQNTISCHDTIAHATKENVYVYDNEKGWNENKE | 145 |
| A9WQI8.SEQ |MKKNNFKVFAGA.....ITVVAIMLGVAAM.....A | 27 | A9WQI8.SEQ | WYKNLILATGACRFDTIATIAHATKENVYVYDNEKGWNENKE | 133 |
| Q9KDB1.SEQ |HVRQNLTKCCR.....LIVVITAVLGMLLAGIQVWSA | 33 | Q9KDB1.SEQ | WSKVELLSGQTCFDTIATIAHATKENVYVYDNEKGWNENKE | 138 |
| Q62YNT.SEQ |MYRLPTTTEST.....VFLAAGLWNMSIVOCFRA | 31 | Q62YNT.SEQ | WFKHINTGEGHTFDTIATIAHATKENVYVYDNEKGWNENKE | 140 |
| Q81CE4.SEQ |MKKNSLQKMVKVILSGGVLTGLLTGFSEKSP | 34 | Q81CE4.SEQ | WKRVILIDGGINIPTKPKDIAHSKENVYVYDNEKGWNENKE | 138 |
| Q81CG6.SEQ | MNNREFLKQLQNMMRNNKSLGAVALTAGIISTTLIQNAYA | 40 | Q81CG6.SEQ | WFRNITIGENATCQHDTIAHATKENVYVYDNEKGWNENKE | 149 |
| W4QLS0.SEQ |MFSLRKMKKIR.MVGVTIVAGLTSFVGGQAVSA | 33 | W4QLS0.SEQ | WAVVLLSGANSFTHIATIAHATKENVYVYDNEKGWNENKE | 138 |
| W7CCV2.SEQ |MANITSA | 7 | W7CCV2.SEQ | WIKQDQKICGIPHTIATIESTWYWYMTHQEWVNAE | 127 |
| Consensus | | | Consensus | w g f w t h w y y t w | |
| AA-CBM33 .SEQ | HGTKEPGVSPGVGALEKQINGWTAQAKYGSVIDNQSV | 40 | AA-CBM33 .SEQ | ISIDEFEI.....IGTVNHGSKADTNLTKIKHETPSGYH | 155 |
| AOAOWTY051.SEQ | HGTVERPSPRPAACGQNYGAINLME.....GNIMTEQSDL | 69 | AOAOWTY051.SEQ | FNADLIEL.....IATVHEHGSAASNNIATITWPSGYH | 181 |
| AOALH8E454.SEQ | HGTIESASRSHYLCRQG...LNNDG.....GCIERWEQSV | 65 | AOALH8E454.SEQ | LAAQAEEL.....FCHYIDGCKRFNTWTCNCVTFPSGYH | 175 |
| AOA521ABL2.SEQ | HGTIESPKSYRYLCRQG...ENINC.....GIVQVEQSV | 66 | AOA521ABL2.SEQ | LSKDALEEEPFCCYINCDAKKEPSITVTCNCVTFPSGYH | 178 |
| AOA559IZW6.SEQ | HGTIESPKSYRYLCRQG...ENINC.....GIVQVEQSV | 71 | AOA559IZW6.SEQ | IIGSDLEEE.....FTCYNGGAKPMKVNTTCNCVTFPSGYH | 183 |
| A9WQI8.SEQ | HGTIESPKSYRYLCRQG...LNNDG.....GIVQVEQSV | 59 | A9WQI8.SEQ | IISDSELEEE.....IATVHKHGSFASNNUITVWIAHRSGYH | 171 |
| Q9KDB1.SEQ | HGTIESPKSYRYLCRQG...LNNDG.....GIVQVEQSV | 65 | Q9KDB1.SEQ | IIDDELIPIPYEQVYGCARPEKVTIEVWII.PPTGYH | 177 |
| Q62YNT.SEQ | HGTIESPKSYRYLCRQG...LNNDG.....GIVQVEQSV | 66 | Q62YNT.SEQ | IIGADEFEI.....IGAVPHIGSFASNNUISHIVIPIGLGYH | 178 |
| Q81CE4.SEQ | HGTIESPKSYRYLCRQG...VNVNQ.....GIVQVEQSV | 66 | Q81CE4.SEQ | IISRSDELDIVFFYVWII.GCARFGTIVTCANETFPSGYH | 178 |
| Q81CG6.SEQ | HGTIESPKSYRYLCRQG...VNVNQ.....GIVQVEQSV | 75 | Q81CG6.SEQ | IIGADEFEI.....IGTVQHIGSAASNNIUTKINVTFPSGYH | 187 |
| W4QLS0.SEQ | HGTIESPKSYRYLCRQG...VNVNQ.....GIVQVEQSV | 65 | W4QLS0.SEQ | IISQDFDIEVFFYEQVYGCARPEEKVTIEVWII.PPTGYH | 177 |
| W7CCV2.SEQ | HGTIESPKSYRYCAQQLDACSIISWDAGIAAHRGVITQQSV | 47 | W7CCV2.SEQ | IISDSELEI.....IGTIGHIGSAASNNIUTKINVTFPSGYH | 165 |
| Consensus | hg p r | g p | Consensus | x g h p i gy | |
| AA-CBM33 .SEQ | FGPKGHPAGEFPGFRIASANGGGQIDFG.....IUDCAIR | 77 | AA-CBM33 .SEQ | ILGVWQVDTDSNAFNTVIDVWIT..... | 178 |
| AOAOWTY051.SEQ | BPKGHPCSGEADCKLIAASAGCLEDG.....IUDCAIR | 103 | AOAOWTY051.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGNV.....DIEA | 213 |
| AOALH8E454.SEQ | BPKGHPCSGEFPGFPGCAGLSTS.....R.FF.....IUDCAIR | 97 | AOALH8E454.SEQ | ILGVWQVDTDSNAFNTVIDVNLVNGV.....SN | 203 |
| AOA521ABL2.SEQ | BPGTTPFKGHPFGCAGLSTS.....VFF.....IUDCAIR | 98 | AOA521ABL2.SEQ | ILGVWQVDTDSNAFNTVIDVNLVNGD.....SG | 206 |
| AOA559IZW6.SEQ | BGLKGPRAFGAAGCGITISAGRQYTS.....IUDCAIR | 105 | AOA559IZW6.SEQ | ILGVWQVDTDSNAFNTVIDVNLVNGV.....SA | 211 |
| A9WQI8.SEQ | BPKGHPAKSGSACKLIAASAGCQFGG.....IUDCASTR | 93 | A9WQI8.SEQ | VYAVWQVDTDSNAFNTVIDVNLVNIKAS.....SA | 198 |
| Q9KDB1.SEQ | BPKGHPAKSGSACKLIAASAGCQFGG.....IUDCASTR | 98 | Q9KDB1.SEQ | IIGAVWQVDTDSNAFNTVIDVNLVNGD.....PSFQ | 213 |
| Q62YNT.SEQ | BPKGHPHSGEAUGQIISASAGGLEGG.....IUDCASTR | 100 | Q62YNT.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGV..... | 203 |
| Q81CE4.SEQ | BPGIGHPGLQGSGCAGAGAG.....H.FF.....IUDCAIR | 98 | Q81CE4.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGL.....SS | 207 |
| Q81CG6.SEQ | BPKGHPGGFVGQIISASAGGKGG.....IUDCASTR | 109 | Q81CG6.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGV.....DIEA | 219 |
| W4QLS0.SEQ | BPKGHPAEAVGAVACCGIISASAGG.VFF.....IUDCASTR | 98 | W4QLS0.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGV.....EGFD | 212 |
| W7CCV2.SEQ | BPKGHPILGFIQDSTIASANGVLDGGNIGSN.....IUDCAIR | 87 | W7CCV2.SEQ | ILAWWDVADDSNAFNTVIDVNLVNGEGTTPTEPSEPSSESVT | 205 |
| Consensus | e p dg | l q | Consensus | w dt nafy dv | |

Figure S1. Sequences alignment of *BaLPMO10A* (AA-CBM33) and its homologs. The multiple amino acid sequence alignment was generated using the DNAMAN software.

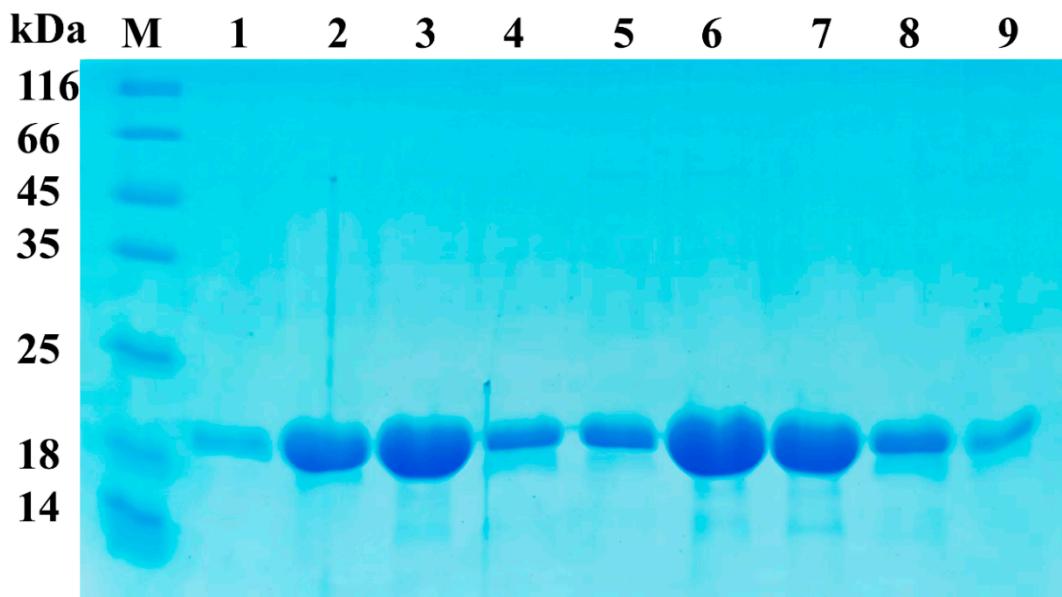


Figure S2. SDS-PAGE of the purified proteins; (M: standard protein marker), the lanes 1, 2, 3, 4, 5, 6, 7, 8, and 9 represents H77R, WT, V40L, N36E, V40I, I4V, E124D, Y3F, and V40M respectively. The remaining mutants have a similar purity. But they were not run on the same SDS-PAGE and thus are not shown.

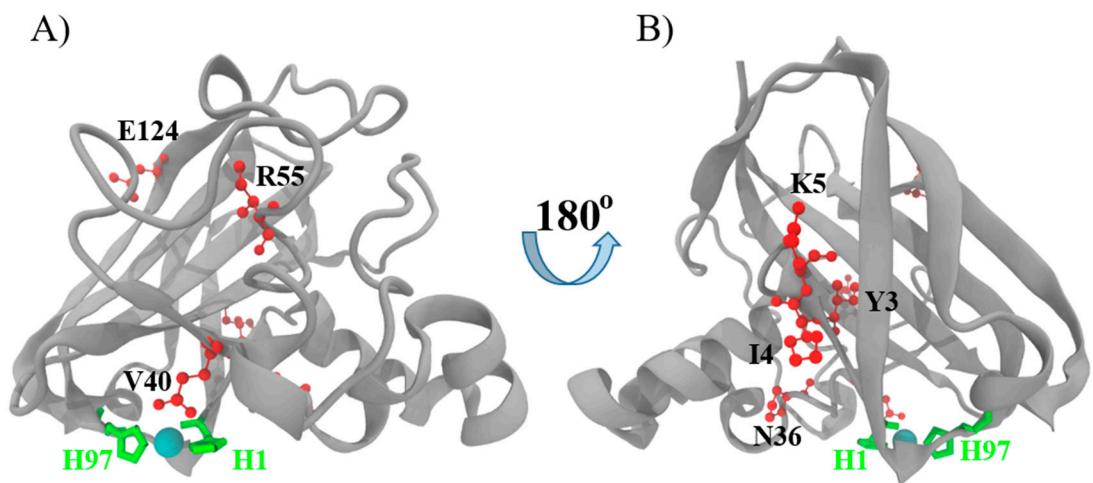


Figure S3. Structural representation of *BaLPMO10A* (PDB 2YOX). The active site is close to H1, showing copper active site (side chains colored green) and higher activity mutation site (side chains colored red). The distances from the higher activity mutation site's C α V40, I4, Y3, E124, K5, R55 and N36 to copper ions is 8.0 Å, 15.9 Å, 13.8 Å, 24.2 Å, 19.1 Å, 17.8 Å and 11.5 Å respectively.

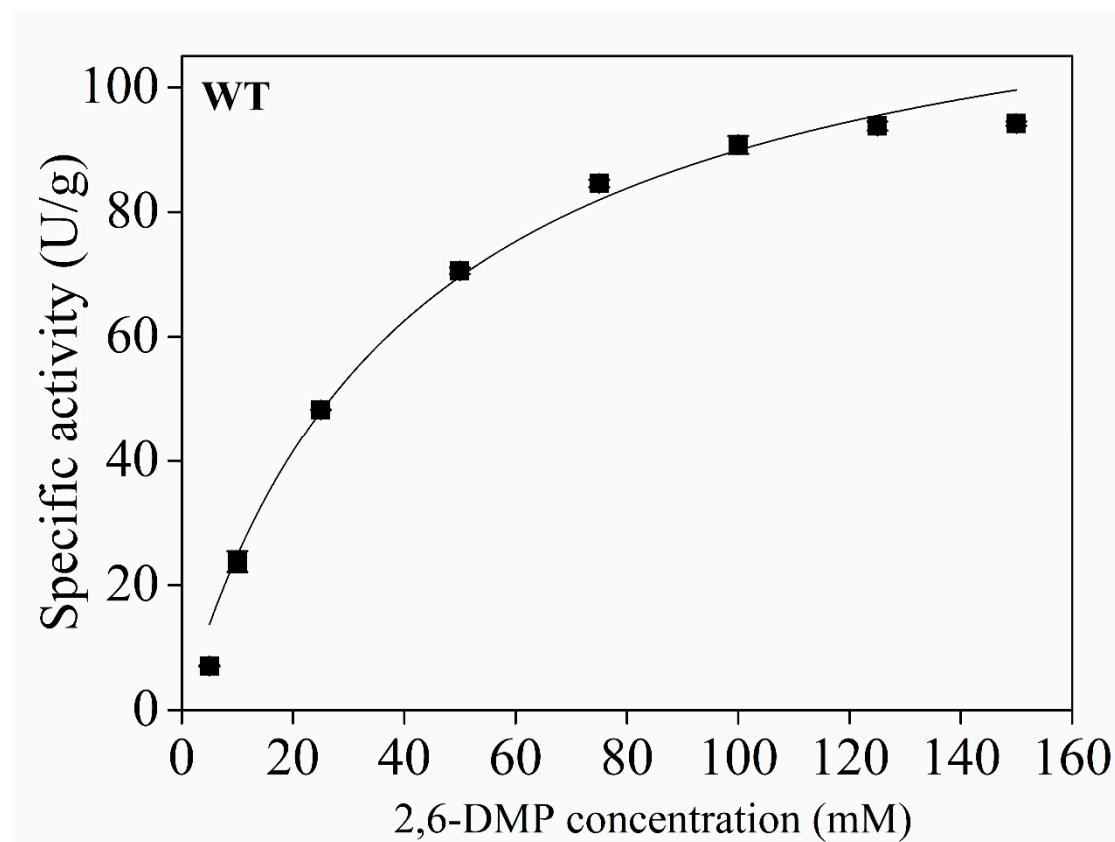


Figure S4: Apparent kinetic parameters of the purified enzyme wild type (WT). It is generated and analyzed by fitting using the OriginPro software.