

Supplementary Materials

Characterization of a New Glyoxal Oxidase from the Thermophilic Fungus *Myceliophthora thermophila* M77: Hydrogen Peroxide Production Retained in 5-Hydroxymethylfurfural Oxidation

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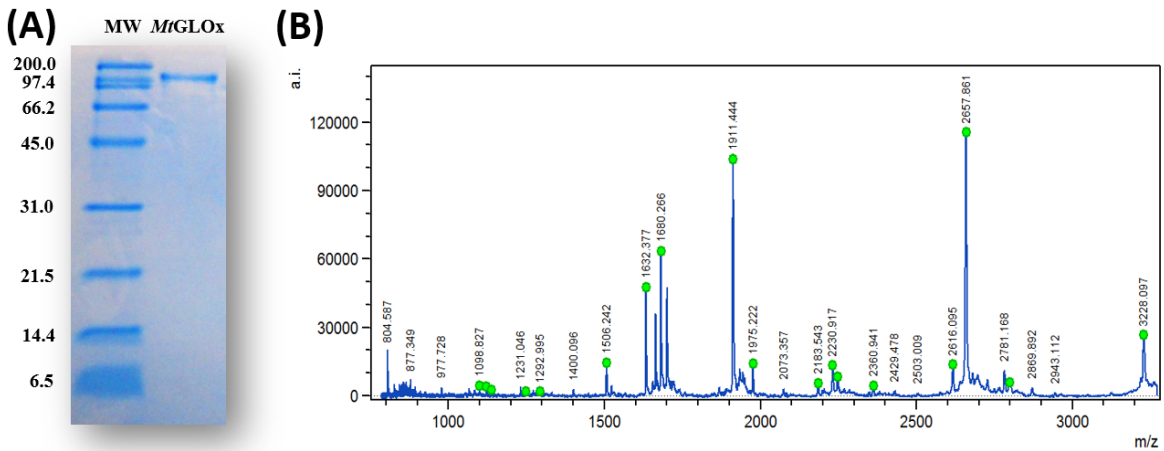
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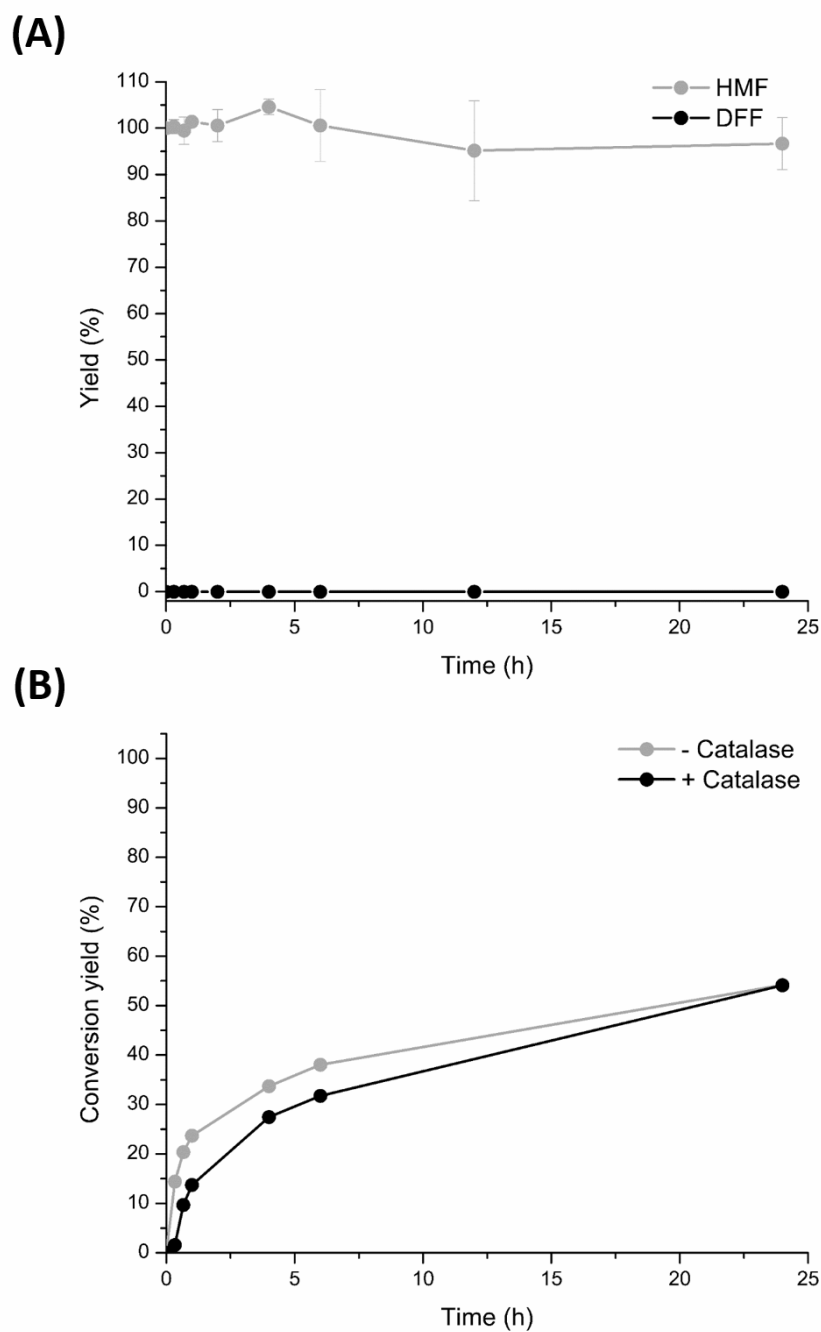


(C)
QLSIPTDLPDSWEYQGCYTDVPGRTINSASYADGTNMTNAACLSYCASKGFPYAGTEYSVEFCGTTLA
SSSAKVADSECNMPCSGAPSEPCGAGSRLSLFHSSAVTGPAANPGVNDFTHLGCYAEKGTGRALTYNP
GLPGADMTVAKCTAACRAANYILAGVEYGGECYCGNTIANGGAPADSGCSMVCNGNSTEFCGGPDRLN
VYSYKNQYEPTATSTTGAGSTSSSSVPSATGLPEGWSYQGCWIDGK**QGRILPYQLPDSQTNSRAACAN**
ACAEAGYTVSGTEYAVQFCGDAIHNGGVETDEADCSTPCPGAPGEK**CGAGDRLSIVSRGPPKIYAPPA**
PIEKIGDWEYQGCAEDNINDKRTFFWQIFFNDIMTPEMCLDRCAEFGYHAAGLEYGQECYCGDPANMA
THGATFRPESECNVVCAGNSTAICGGLARLTTYFWIGTPFYSWDFPQDWR**AGKYEFLVDGVNIPLIHTET**
ITGKVSFISKGATGPGNETGAYEFDPATLEFRELHIKTDVFCAASVTLDPKAGRQLNVGGWAGEATYGTR
LYWPDGAPGVPGTHDWQENVNLHLQAGRWYPSVLVLTNGSVMVVGGLIGSNDAATPSIILPYTGTP
PLYMDWLDRTHPNLYPFLCILPGGGIFVQYWNEAR**ILDVTFDTVKTLDPAGAPNDPK**GGRTYPLEG
TAVLLPQKYPTDPLGLVLCGGSTEGPGNALDNCVSIYPEADEPEWQIERMPSFRVMTCMAPLPDGTYLI
ANGALHGVA GFGLGVGNL NALLYDPSKPLGSRITVAANTTIAR**MYHSEAITLLDGRVLISGSNPEDGVN**
PEEYRVEVFLPPYLLAGKPRPTFTLENRDWAHGQTGIPFTLGSPARNGDITATLLGSVASTHGNSMGAR
TLMPRVSCRGTSTVDAPPTANICPPGWYQFFVLDGGIPAVGVYVRI**IGGDAGQIGNWPQAPDFSVPGV**

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Figure S1. Identification of the purified *MtGLOx* heterologously expressed and secreted by *Aspergillus nidulans*. (A) SDS-PAGE showing the purified *MtGLOx*. MW: molecular weight. (B) MALDI-TOF/MS peptide mass fingerprint analysis of *MtGLOx*. Mass spectrum profile in the *m/z* range 800-3300 Da. The peptide mass fingerprint was made from fragments of *MtGLOx* after tryptic digestion. Green dots mark the expected tryptic masses that matches the theoretical *m/z* with a maximum 2 Da tolerance. (C) Protein identification and sequence coverage after *m/z* list analysis. The sequence coverage of the tryptic fragments is shown in bold red (31% coverage).

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Figure S2. Reaction controls of the time course oxidation of HMF by *MtGLOx*. (A) Effect of HMF incubation in 100 mM Bis-Tris pH 6.0. (B) Effect of catalase on time course conversion of HMF to DFF. The time course oxidation of HMF (1 mM) by *MtGLOx* (0.5 μ M) in 100 mM Bis-

73 Tris pH 6.0. The reaction was monitored by DFF quantification by High Performance Liquid
74 Chromatography (HPLC), using a Bio-Rad Aminex HPX-87H column with and without 670
75 U/mL of catalase from *Aspergillus niger* (Sigma-Aldrich).