S1 File: A new family AA5_2 member displays dual activity preference

Supplemental Figures

A family AA5_2 carbohydrate oxidase from *Penicillium rubens* displays functional overlap across the AA5 family

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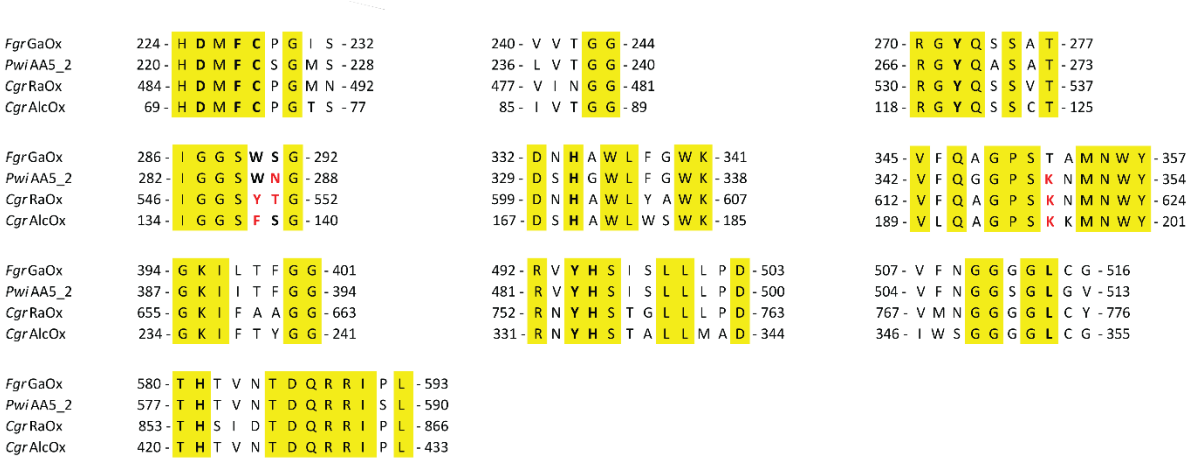
A



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B

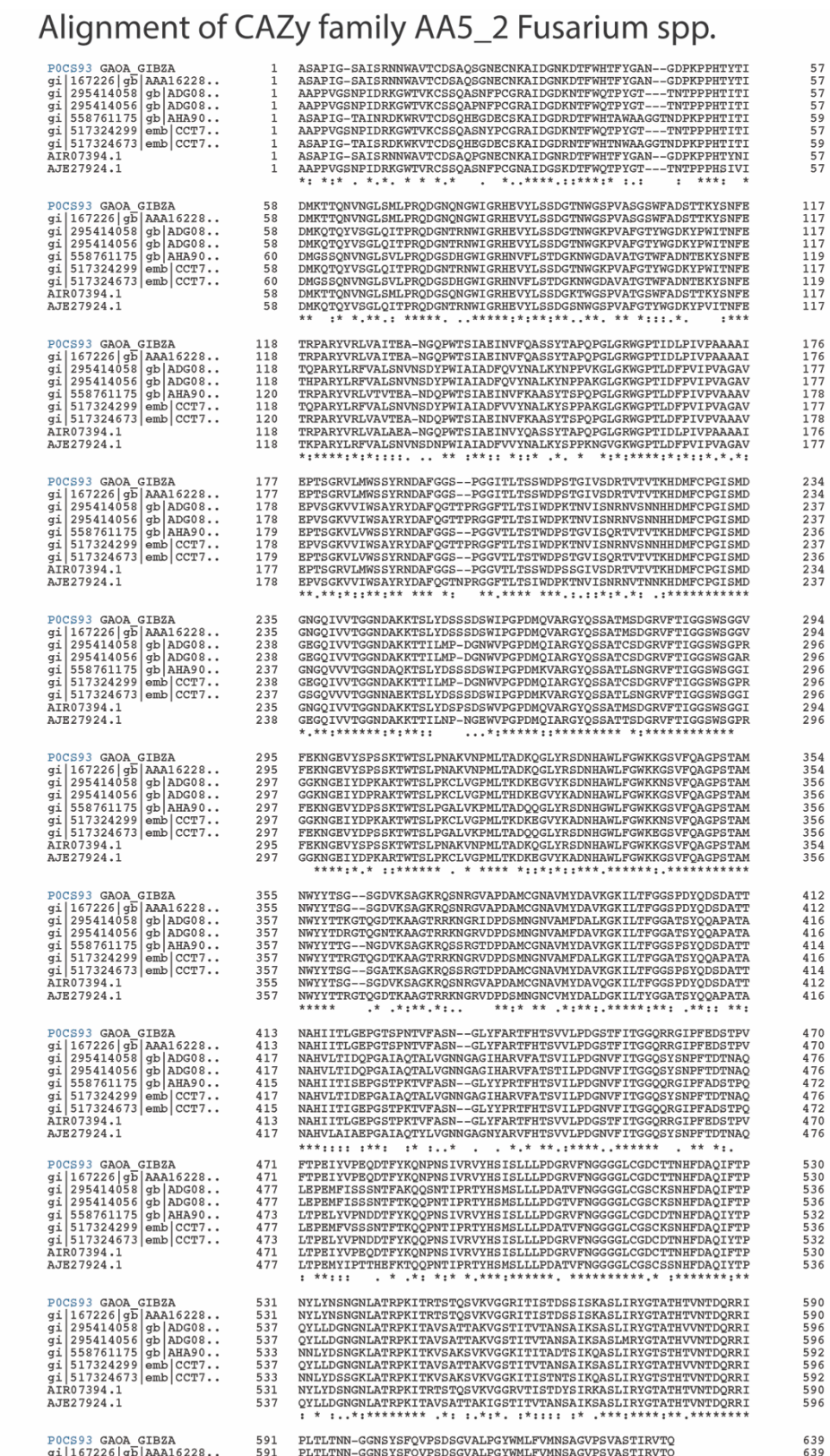

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Fig A. Analysis of AA5_2 Sequences. (A) Sequence conservation between *Fgr*GaOx, *Pru*AA5_2A, *Cgr*RaOx and *Cgr*AlcOx in within 10 sequence stretches recognized for having identical amino acids in the 9 analyzed *Fusarium spp*. Yellow highlights identical amino acids throughout the four sequences of *Fgr*GaOx, *Pru*AA5_2A, *Cgr*RaOx and *Cgr*AlcOx. Amino acids that also occur in Table 1 are in bold, red positions denotes variances. The placements of the sequence segments in the structure of *Fgr*GaOx are highlighted in Figure 2C. (B) Sequence alignment of the 9 Fusarium spp. in subfamily AA5_2 used to identify the 10 conserved sequence segments and information presented in Table 1. The conserved sequence segments were defined as strings of 4 or more consecutive and conserved amino acids in alignment of the *Fusarium spp.* sequences.

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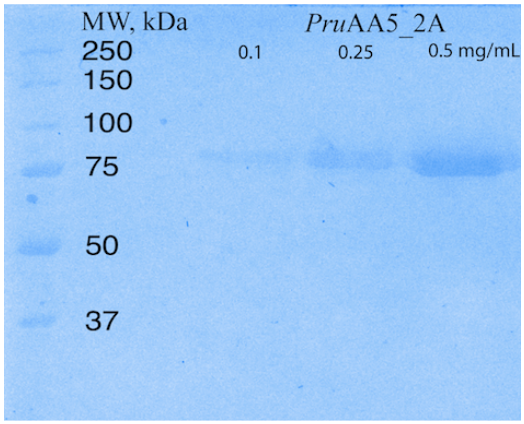

Fig B. SDS-PAGE of pure *Pru*AA5_2A after production and purification. The molecular weight of *Pru*AA5_2A was estimated to be 75 kDa, indicating that the purified enzyme is glycosylated.

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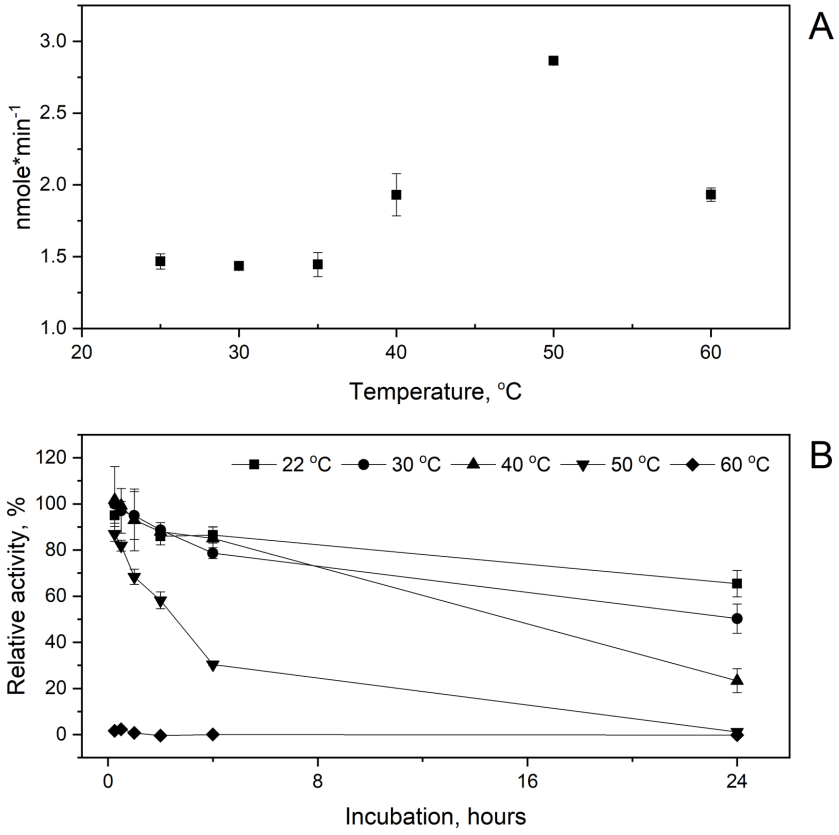

Fig C. Effect of temperature on *Pru*AA5_2A activity. (A) Activity on 300 mM raffinose in 20 mM MOPS (pH 7.5). (B) Residual activity at 30 °C after 15, 30 min or 1, 2, 4 and 24 hours at 22, 30, 40, 50 and 60 °C in 20 mM MOPS (pH 7.5). n=4; error bars indicate standard deviation.

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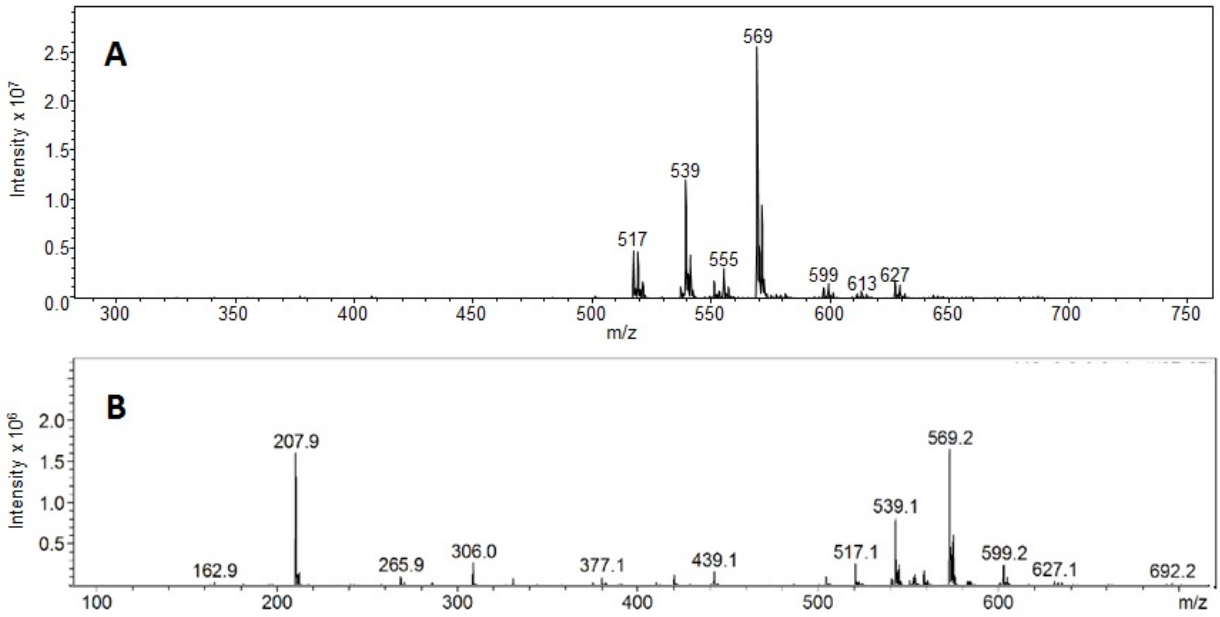

Fig D. Analysis of oxidized products by mass spectrometry. (A) Negative mode ESI-MS spectra of oxidized raffinose produced by *Cgr*RaOx -catalyzed reaction (3), and (B) by *Pru*AA5_2A -catalyzed reaction. m/z 517, uronic acid; m/z 539, unoxidized raffinose (Cl^- adduct); m/z 569, aldehyde product reacted with methanol (Cl^-adduct); m/z 207.9, MOPS buffer.

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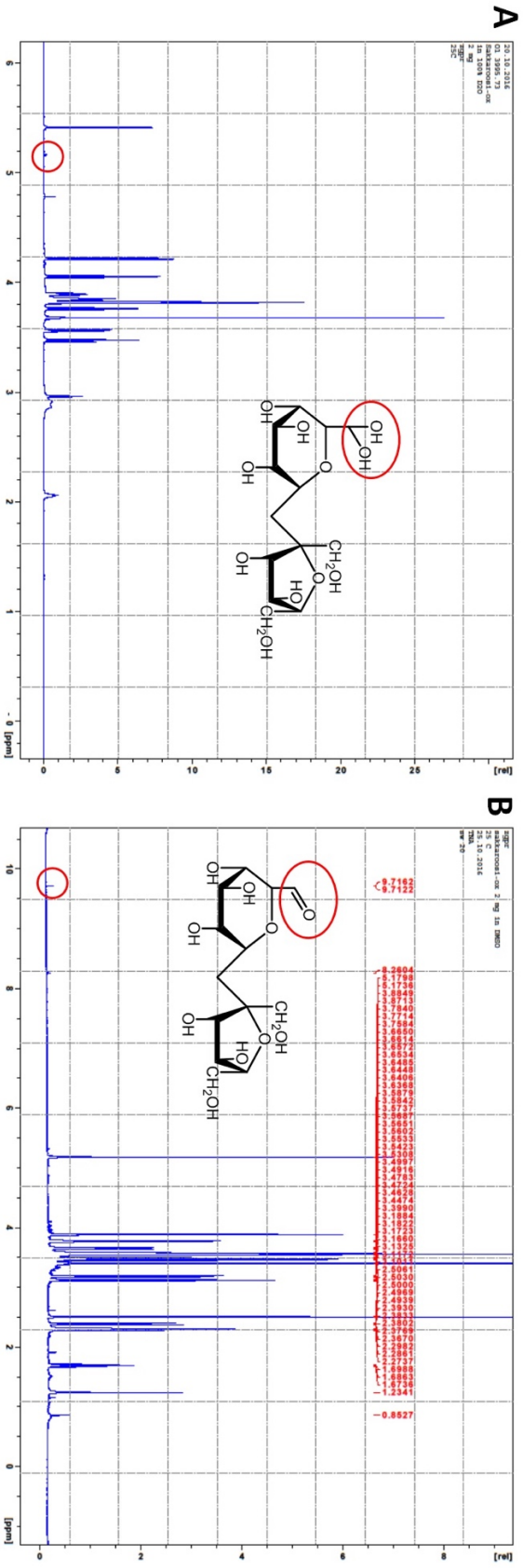

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Fig E. **^{1}H NMR spectra of sucrose oxidation products.** (A) Analyzed in D_2O, and (B) DMSO-d_6. Chemical shifts indicating the oxidized product formation and putative oxidation sites as hydrates (A) and aldehydes (B) marked with a ring. Due to low degree of oxidation the final structure of the oxidation product could not be determined.

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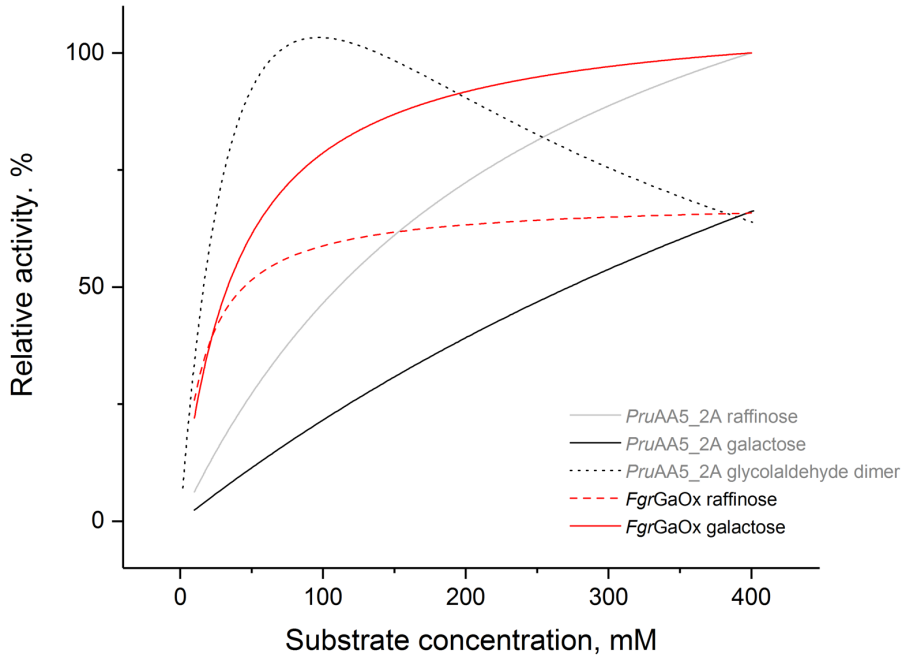

Fig F. Comparative plot of *Pru*AA5_2A and *Fgr*GaOx substrate kinetics using the calculated Michealis-Menten plot from kinetical analysis. The activity axis (y-axis) were converted to relative activity for easy comparison. Actual kinetic parameters are present in Fig 6 for *Pru*AA5_2A and the discussion for *Fgr*GaOx. No activity on glycolaldehyde dimer was detected for *Fgr*GaOx.