

Supplemental Figures

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

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A

<i>Fgr</i> GaOx	224 - H D M F C P G I S - 232	240 - V V T G G - 244	270 - R G Y Q S S A T - 277
<i>Pwi</i> AA5_2	220 - H D M F C S G M S - 228	236 - L V T G G - 240	266 - R G Y Q A S A T - 273
<i>Cgr</i> RaOx	484 - H D M F C P G M N - 492	477 - V I N G G - 481	530 - R G Y Q S S V T - 537
<i>Cgr</i> AlcOx	69 - H D M F C P G T S - 77	85 - I V T G G - 89	118 - R G Y Q S S C T - 125
<i>Fgr</i> GaOx	286 - I G G S W S G - 292	332 - D N H A W L F G W K - 341	345 - V F Q A G P S T A M N W Y - 357
<i>Pwi</i> AA5_2	282 - I G G S W N G - 288	329 - D S H G W L F G W K - 338	342 - V F Q G G P S K N M N W Y - 354
<i>Cgr</i> RaOx	546 - I G G S Y T G - 552	599 - D N H A W L Y A W K - 607	612 - V F Q A G P S K N M N W Y - 624
<i>Cgr</i> AlcOx	134 - I G G S F S G - 140	167 - D S H A W L W S W K - 185	189 - V L Q A G P S K M N W Y - 201
<i>Fgr</i> GaOx	394 - G K I L T F G G - 401	492 - R V Y H S I S L L L P D - 503	507 - V F N G G G G L C G - 516
<i>Pwi</i> AA5_2	387 - G K I I T F G G - 394	481 - R V Y H S I S L L L P D - 500	504 - V F N G G S G L G V - 513
<i>Cgr</i> RaOx	655 - G K I F A A G G - 663	752 - R N Y H S T G L L L P D - 763	767 - V M N G G G G L C Y - 776
<i>Cgr</i> AlcOx	234 - G K I F T Y G G - 241	331 - R N Y H S T A L L M A D - 344	346 - I W S G G G G L C G - 355
<i>Fgr</i> GaOx	580 - T H T V N T D Q R R I P L - 593		
<i>Pwi</i> AA5_2	577 - T H T V N T D Q R R I S L - 590		
<i>Cgr</i> RaOx	853 - T H S I D T D Q R R I P L - 866		
<i>Cgr</i> AlcOx	420 - T H T V N T D Q R R I P L - 433		

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

Fig A. Analysis of AA5_2 Sequences. (A) Sequence conservation between *FgrGaOx*, *PruAA5_2A*, *CgrRaOx* and *CgrAlcOx* 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 *FgrGaOx*, *PruAA5_2A*, *CgrRaOx* and *CgrAlcOx*. 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 *FgrGaOx* 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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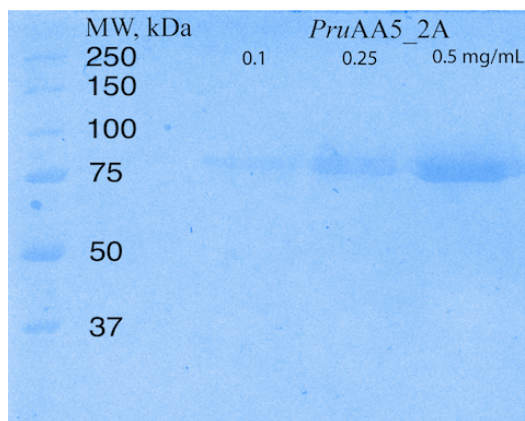


Fig B. SDS-PAGE of pure *PruAA5_2A* after production and purification. The molecular weight of *PruAA5_2A* was estimated to be 75 kDa, indicating that the purified enzyme is glycosylated.

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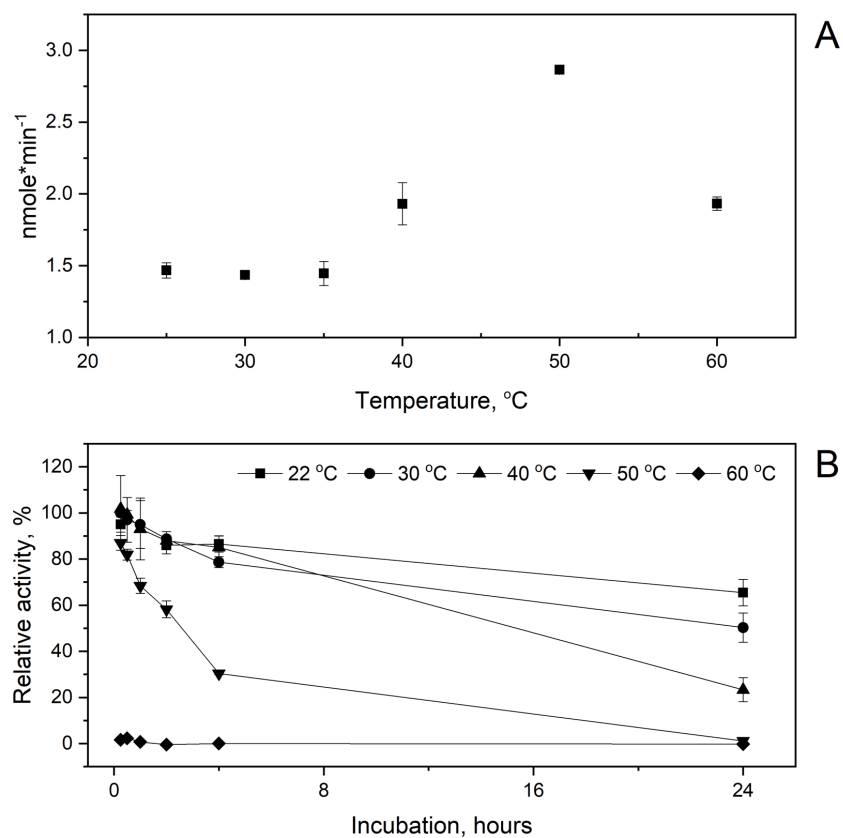


Fig C. Effect of temperature on *PruAA5_2A* activity. (A) Activity on 300 mM raffinose in 20 mM MOPS (pH 7.5). (B) Residual activity at 30 °C after 15, 30 min or 1, 2, 4 and 24 hours at 22, 30, 40, 50 and 60 °C in 20 mM MOPS (pH 7.5). n=4; error bars indicate standard deviation.

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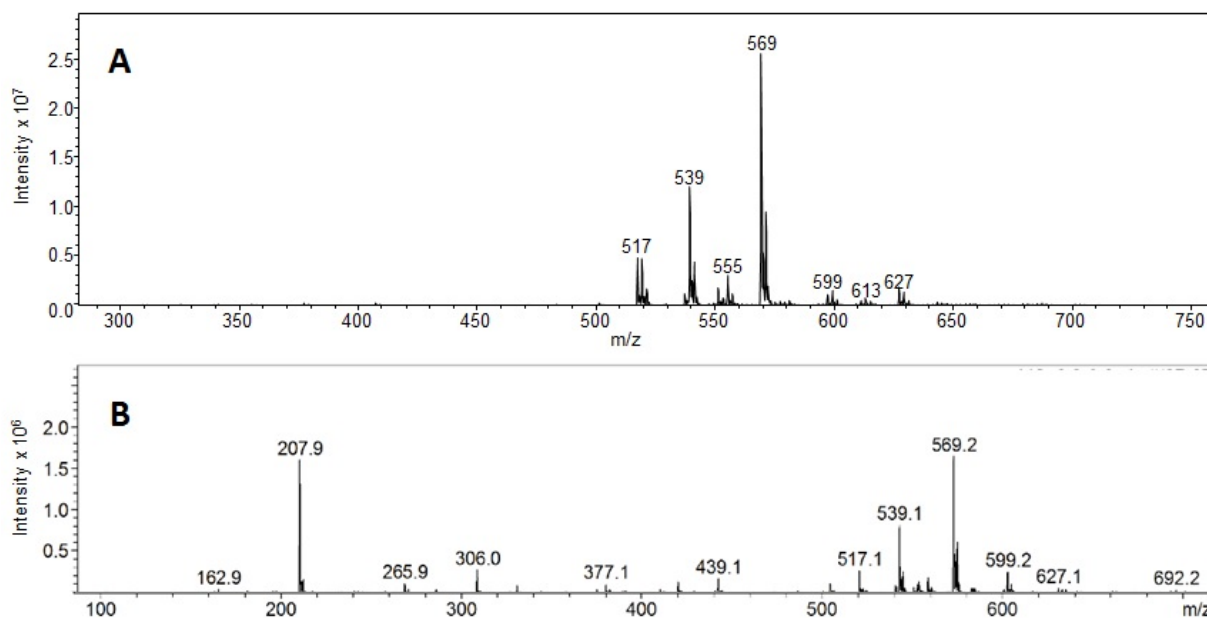
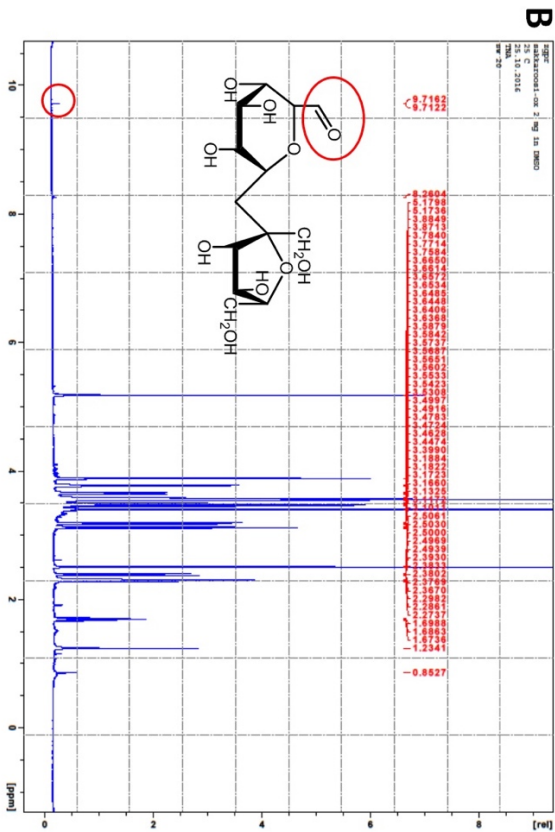
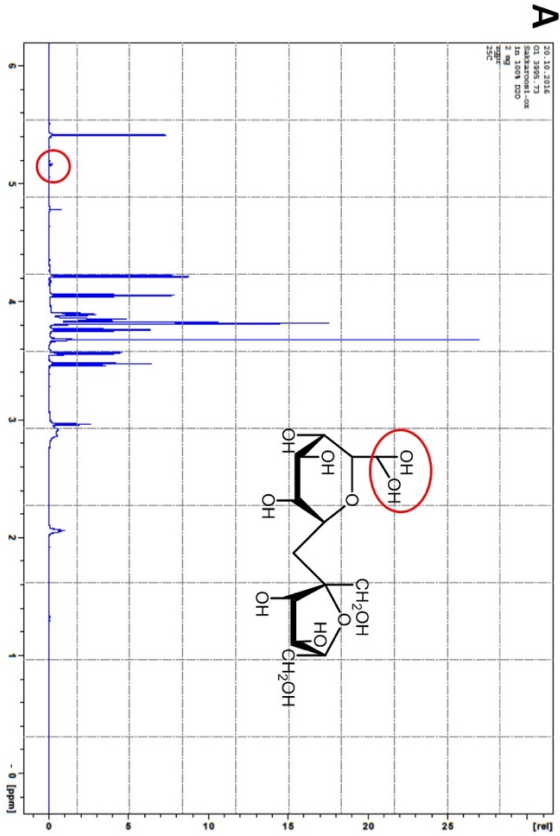


Fig D. Analysis of oxidized products by mass spectrometry. (A) Negative mode ESI-MS spectra of oxidized raffinose produced by *Cgr*RaOx -catalyzed reaction (3), and (B) by *Pru*AA5_2A -catalyzed reaction. m/z 517, uronic acid; m/z 539, unoxidized raffinose (Cl⁻ adduct); m/z 569, aldehyde product reacted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

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Fig E. ¹H NMR spectra of sucrose oxidation products. (A) Analyzed in D₂O, and (B) DMSO-d₆. 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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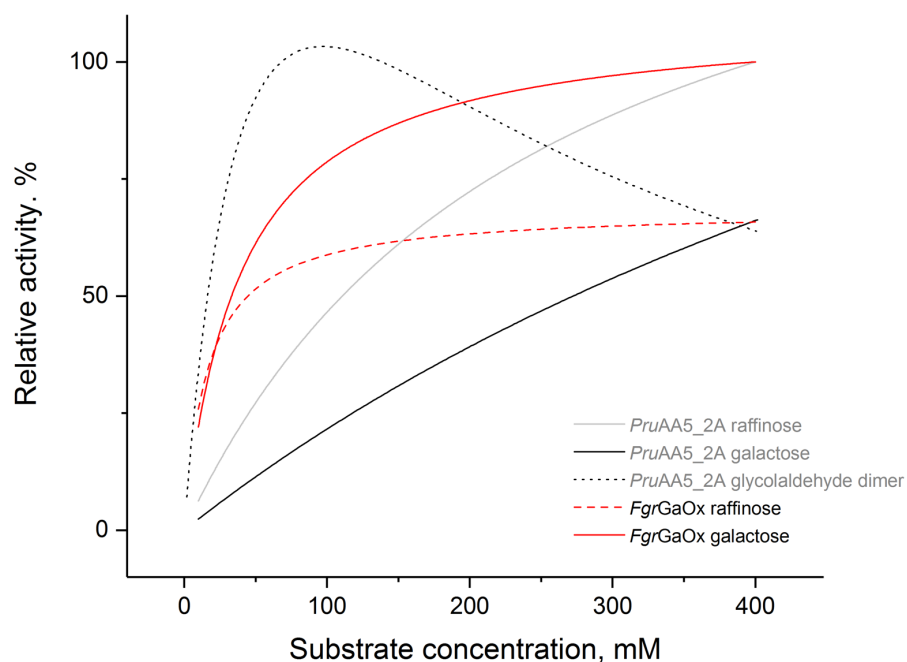


Fig F. Comparative plot of *PruAA5_2A* and *FgrGaOx* substrate kinetics using the calculated Michealis-Menten plot from kinetical analysis. The activity axis (y-axis) were converted to relative activity for easy comparison. Actual kinetic parameters are present in Fig 6 for *PruAA5_2A* and the discussion for *FgrGaOx*. No activity on glycolaldehyde dimer was detected for *FgrGaOx*.