

SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE 1. Partial alignment of the two catalytic domains from PpoA. A. Partial alignment of heme peroxidase domain from PpoA with *G. graminis* 7,8-LDS (Q9UUS2.1), sheep PGHS-1 (P05979.2) and sheep PGHS-2 (P79208.1). The catalytic tyrosine as well as the proximal and distal histidines, which are conserved in the four sequences and have been identified before (54), are shaded black. Mutated amino acids are labeled with an arrow. B. Partial alignment of P450 domain from PpoA with known P450 enzymes [CYP450_119 (1F4T:B), CYP450_cin (1T2B:B), CYP450_130 (2UUQ:A), CYP450_nor (1JFB:A), CYP4503A4 (1TQN:A), CYP4502C9 (1R9O:A)] and predicted CYP450 domains from different putative fungal Ppo-like enzymes [*A. niger* 1 (XP_001395220), *A. niger* 2 (XP_001401954) *A. fumigatus* 1 (EDP50447), *A. fumigatus* 2 (ABV21632.1), *A. fumigatus* 3 (EDP47075.1), *F. graminearum* 1 (FGSG_02668)^A, *F. graminearum* 2 (FGSG_10960)^A, *F. oxysporum* 1 (FOXG_12909)^A]. Amino acids matching the P450 consensus sequence according to the PROSITE database (<http://www.expasy.org/cgi-bin/nicedoc.pl?PDOC00081>) are shaded grey. Additionally, the cysteine responsible for the ligation of heme iron is shaded black. The mutated histidine and cysteine are labeled with an arrow. The alignment was carried out using CLUSTALW.

^AAnnotation code in accordance to the *Fusarium* database: (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/)

SUPPLEMENTAL FIGURE 2. Mass spectrometric analysis of products formed by PpoA from 18:2^{A9Z,12Z}. A) Electron impact mass spectrum of the Me₃Si ether methyl ester derivative of product 2 (5,8-DiHODE). B) Electron impact mass spectrum of the Me₃Si ether methyl ester derivative of product 1.

SUPPLEMENTAL FIGURE 3. Kinetic analysis of PpoA. A) Michaelis Menten plot, the initial reaction rate of O₂-consumption for the reaction of 15 µg PpoA with different linoleic acid concentrations is plotted in dependence of the substrate concentration. Reactions were performed in 50 mM phosphate buffer (pH = 7.2). B) The method of Hanes and Woolf was used for the linearization of the Michaelis Menten plot. Kinetic parameters were obtained using this method.

SUPPLEMENTAL FIGURE 4. Peroxidase activity assay. 15 µg PpoA were incubated with 100 µM 18:2^{A9Z,12Z} and 100 µM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) in 50 mM HEPES pH = 7.4 and the increase of the absorbance at 611 nm (due to oxidized TMPD) was recorded.

SUPPLEMENTAL FIGURE 5. Kinetic analysis of PpoA_H1004A. A) Michaelis Menten plot, the initial reaction rate of O₂-consumption for the reaction of 15 µg PpoA_H1004A with different linoleic acid concentrations is plotted in dependence of the substrate concentration. Reactions were performed in 50 mM phosphate buffer (pH = 7.2). B) The method of Hanes and Woolf was used for the linearization of the Michaelis Menten plot. Kinetic parameters were obtained using this method.

Supplemental Figure 1

A Alignment heme peroxidase Domain

A. nidulans_PpoA TKSFLNMLWNDLEHPPVSYLGADSMHRKADGSGNNRFWQQLGAAGSAYARSVRPKTMQSP 164
G.graminis_LDS TDGLINDLWDSDLHPPVASLGKGSFREPDSNNNIHLPSLGAANTPYARSTKPLVFQNP 163
sheep_PGHS1 MRLVLTVRNSNLIPSPPTYNIAHDYISWESFS---NVSYYTRILPSVPRDCPTPMGKGKK 169
sheep_PGHS2 MRYVLTSRSHLIESPSPPTYNHYSYKSWEAFS---NLSYYTRALPPVPPDCPTPMGVKGRK 154

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A. nidulans_PpoA SLPDPETIFDCLLRRK--EYREHPNKISSVLFYLASIIHDLFQTDPK--DNSVSKTSSY 220
G.graminis_LDS NPPDPATIFDTLMVRDPAKFRPHPNKISSMLFYLATIITHDIFQTSPR--DFNINLTSSY 221
sheep_PGHS1 QLPDAEFLSRRFLRR--KFIPDPQGTNLMFAFFAQHFTHQFFKTSKGKMGPGFTKALGHG 227
sheep_PGHS2 ELPDSKEVVKVLLRR--KFIPDPQGTNLMFAFFAQHFTHQFFKTDIERGPAFTKGKNHG 212

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A. nidulans_PpoA LDLSPLYGNQNDEQNLVRTFKDGKLKPDCFA-----TKRVLGFPPGVGVLLIMFN 270
G.graminis_LDS LDLSPLYGRNHDEQMAVRTGKDGLLKPDTSF-----SKRVIGFPPGVGAFLIMFN 271
sheep_PGHS1 VDLGHIYGDNLERQYQLRLFKDGKLKYQMLNGEVYPPSVEEAPVLMHYPRGIPPQSQMAV 287
sheep_PGHS2 VDLSHVYGESLERQHNRRFLFDGKMKYQMINGEMYPPTVKDQVEMIYPPHPEHLKFAV 272

:***. :**. : . : * *** : * : . : : * : :

A. nidulans_PpoA RFHNYVVDQLAIAINECGRFTPDE-SNVDEYAK---YDNNLQTGRLVTCGLYANIILKD 326
G.graminis_LDS RFHNYVVTQLAKINEGGRFKRPTTPDDTAGWET---YDNSLFQTGRLITCGLYINVILGD 328
sheep_PGHS1 GQEVFGLLPGMLMYATIWLREHNRVC DLLKAEPHTWGDEQLFQTARLLIGETIKIVIE 347
sheep_PGHS2 GQEVFGLVPGLMMYATIWLREHNRVC DLLKQEHPEWGDEQLFQTSLILIGETIKIVIED 332

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A. nidulans_PpoA YVRTILNINRTDSTWSLDPRMEMKDGLGEAAAMATGNQVSAEFNVMVRWHACISKRDEK 386
G.graminis_LDS YVRTILNLNRANTTNLDPRTKEGKSLLSKPTPEAVGNQVSVEFNLIYRWHTCTISERDDK 388
sheep_PGHS1 YVQQLS---GYFLQLKFDPELLFG-----AQFQYRNRIAMEFNFQIYHWHPPLMPDSFR- 396
sheep_PGHS2 YVQHLS---GYHFKLKFDPELLFN-----QQFQYRNRIAEFTNTLYHWHPPLPDVFQ- 381

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A. nidulans_PpoA WTEDFHREIMPGVPDSTLSMQDFVAGLGRWQAGLPQEPLERPFSGLQRKPDGAFNDDLV 446
G.graminis_LDS WTTNAMREALGGQDPATAKMEDVMRALGMFEKNIPEPEKRTLLAGLITQSDGAFDDTELV 448
sheep_PGHS1 -----VGPQDYSYEQFLFNT---SMLVDYVGEALVDAFSRQPAGRIG---- 435
sheep_PGHS2 -----IDGQEYNYQQFIYNN---SVLLEHGVTQFVESFTRQIAGRVA---- 420

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B Alignment P450 Domain

A. nidulans_PpoA -----IFISSHAACMSILENQETFKTWGRKIEFLMQRDKH 689
A.niger_1 EQIAKEIGTIDRYSQEDPKPPPRTVMLANHATIIEVLKDQDTFRVPWARYLNDMIPGKRF
A.niger_2 -----FISSNAACISVLNQDQEAFKVTWGSKIEFLMRHNNH
A.fumigatus_1 -----FINSHSAMCISLSDQETFKVTWGSKIEFLMRHNNQ
A.fumigatus_2 -----FSWDRPSYIPQRI SVF DYANVRHILQDASNRFRVMWGEATAYVFGSK--
A.fumigatus_3 -----FSWDRPSYIPQRI SVF DYANVRHILQDASNRFRVMWGEATAYVFGSK--
F.graminearum_1 -----
F.graminearum_2 -----DRFDFSRPAPTATRINIVGYKA AKYILEDQAKYRVCWE EGLKHL MGEG--
F.oxy sporum_1 -----ADRFDFSRP EPTATRINIVGYNAAKYILEDQAKYRVCWE EGLKHL MGEA--
CYP450_119 -----MYDWFSEMRKKD PVYD GNQVFSYR YTKEV LNNFS--
CYP450_cin -----TSLFTTADHYHTPLGPDGTPHAF FEALRDEAETTPIGWSE
CYP450_130 -----MHHHHHHHMITSVMSHEFQLATAETWPNPWPMYRALRDHDPVHHVPPQR
CYP450_nor -----TMASGAPSFPFSRASGPEPPA EFAKL R ATNPV SQV KLF DG
CYP450_3A4 -----MALYGTHSHGLFKKL GIPGPTPLPFLGNILSYHKGFCMF DMECHK
CYP450_2C9 -----MAKKTSSGRGKLP GP TPLPVIGNILQIGIKDISKSLTNLSK

A. nidulans_PpoA QYGKDFMLSGDRPPNAASR--KMMGSALYR---DEWEAEVKN-----FYEQTTLKL 735
A.niger_1 N---DYM LGGDGPVNAAQK--KLVKSILFSP--DQFNQLLSQ-----TTVRLGKEL
A.niger_2 PYGRDFMLSGDKPPNAASR--RMMGSALYR--DKWESEVKR-----FYEDITIKL
A.fumigatus_1 PYGRDFMLSGDRTPNAMS R--QMMGKALYR--DKWETEVKR-----FYENITLKL
A.fumigatus_2 --GWD FMLS G DAP THAN QR--NIMS RALYR--GQWHD AVKQ-----FYLDITQQL
A.fumigatus_3 --GWD FMLS G DAP THAN QR--NIMS RALYR--GQWHD AVKQ-----FYLDITQQL
F.graminearum_1 -----
F.graminearum_2 -----GGRFMLS G D T AL HA QQR--KCMGRLLY N--DTW RNAV KS-----FYSTTAEML
F.oxy sporum_1 -----GGRFMLS G D T AL HA QQR--KCMGKLLY N--DTW RNAV KS-----FYATTAEKL
CYP450_119 -----KFSSDLTG YHERL--EDLRNGKIR-----FDI PTR-----YTMLTSDPP
CYP450_cin AYGGHWWVAGYKEIQAVI QN TKA FSNKG VTF--PRYETGEFE-----LMMAGQDDP
CYP450_130 PEYDYYVLSR HADVWSAARDHQTFSSA QGLT--VNYGELEMIGLH---DTPPMVMQDPP
CYP450_nor --SLAWLVTKHKDVCVATS-EKLSKVRTRQGFPELSASGKQAAK---AKPTFVMDPP
CYP450_3A4 KYGKVWGFYDGQQPVLA ITDPDMIKTVL VKECYSVFTNRRPFGPVG--FMKSAISIAEDE

CYP450_2C9

VYGPVFTLYFGLKPIVVLHGYEAVKEALIDLG-EEFSGRGIFPLAERANRGFGIVFSNGK

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A. niger 1
A. niger 2
A. fumigatus 1
A. fumigatus 2
A. fumigatus 3
F. graminearum 1
F. graminearum 2
F. oxysporum 1
CYP450_119
CYP450_cin
CYP450_130
CYP450_nor
CYP450_3A4
CYP450_2C9

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LRQHSYQILGG-VNQVD--IVRDVANYAQVHFCANVFS-LPLKTESNPRGIFAESELYEIL
LHRYSYKLAG-VNQVD--VVRDIANLAQVHFCASVFS-LPLKTESNPRGIFTSELYQIM
LTEKSCRIGN-VNQVD--ISRDVGNLAHVFASNVFS-LPLKSREHPHGIITAHEMFEAM
LTEKSCRIGN-VNQVD--ISRDVGNLAHVFASNVFS-LPLKSREHPHGIITAHEMFEAM
-----DQID--IIRDVAIPLNAQLLSDMFY-FDMRTDENPDGEELGAELYRSL
LNEKSYTLAG-KKQVD--VVRDVGNVAHTHFVARMFN-LPLKTQNPKGVFSEQELYMIL
LAEKSYRLAG-KMQVD--VVRDVGNVAHTHFVARMFN-LPLKTSENPKGVFSEQELYMIL
LHDELRSMSADIFSPQ--KLQTLTETIRETRSLDS-IDPREDD----IVKKLAVPL
VHKKYRQLVAKPFSPE--ATDLFTEQLRQSTNDIIDARIELGED----AATWLANEI
VHTEFRKLVSRGFTP--QVETVEPTVRKFVVERLEKLRANGGD----IVTELFKPL
EHMHQRSMVEPTFPE--AVKNLQPYIQRSTVDDLEQMVKQKGANGP--VDLVKEFALPV
EWKRLRSLLSPTFTSG--KLKEMVPIIAQYGDVLVRN--LRREAETGKPVTLKDVFGAYS
WKWEIRRFLSLMLRNFGMGKRSIEDRVQEEARCLVEELRKTASPCDPTFILGCAPCNVI

A. nidulans_PpoA

A. niger 1
A. niger 2
A. fumigatus 1
A. fumigatus 2
A. fumigatus 3
F. graminearum 1
F. graminearum 2
F. oxysporum 1
CYP450_119
CYP450_cin
CYP450_130
CYP450_nor
CYP450_3A4
CYP450_2C9

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ALVFASIFYDADVGTSFQLNQTARDVTQQLG---ELTMANVDFVN-----
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AVIFTAIFFDAEPVKSFELRHKAAREAANKLG---RLVELNVKAIK-----
AVIFTAIFFDAEPVKSFELRHKAAREAANKLG---RLVELNVKAIK-----
LDIRVWGVNNNDPGQAWNRRAQEGVKRIY---DSTKKLVSEVEVARPRG-----
AVIFVCIFFDIDPAKSFPLRQQAAREVAQQLG---KIVEMNVKLAT-----
AVIFVCIFFDIDPAKSFPLRQGAREVAQKLG---GIIEMNVKLAN-----
PIIVISKILGLPIEDKEFKKEWSDLVAFRLG---KPGEIFELGKK-----
PARLTAILLGLPPEDGDTYRRWWVWAITHVENP---EEGAEIJAELVAH-----
PSMVVAHYLGVPEEDWTQFDGWTQAIAVAANAV--DGATTGALDAVG-----
PSYIITYLILGVPFNDLEYLTQQNAIRTNGSST--AREASAANQELLD-----
MDVITSTSFGVNIDSNNPQDPFVENTKKLLR--FDFLDPFFLSITVFPFLIPILEVNL
CSIIIFHKRFDYKDQQFLNLMEKLNENIKILSSPWIQICNNFSPIIDYFPGTHN---KL

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F. oxysporum 1
CYP450_119
CYP450_cin
CYP450_130
CYP450_nor
CYP450_3A4
CYP450_2C9

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-----TRMLGKLTGYQSEVSTLRWYGNNVVQMMEM-----DMTAAGTAEV
-----KAGFIANLVSSLH-RDVLSEYGIHMIQRLLDS-----NVPPAEIVWT
-----KTGFIANLVNSLH-RDVLSEYGVHMIQRLLDS-----GMPAPEIVWT
-----SSGLIATLLGNMPANRNALFEYGVHMVERLLQS-----GLDPEQVTWS
-----SSGLIATLLGNMPANRNALFEYGVHMVERLLQS-----GLDPEQVTWS
----IGLISAVSNRIGAKSYLKKDSLRSCLKLVEELLAQ-----GSSIDHVTDN
----SVGIKGLFTSKPNKNDPLAAYGENMAKGLKKA-----GLSIDDIVWS
----SIGVKGLFTSKPDKNDDPLAAYGENMAKGLKKA-----GLSTEDIVWS
----YLELIGYVKDHLNS-----CTEVVSRVVNS-----NLSDEIKLGY
----ARTLIAERRTNPGN---DIMSRVIMSKIDGE-----SLSEDDLIGF
----SMMAYFTGLIERRRTEPADDASHLVAAGVGADGD-----TAGTLSILAF
----YLAILVEQRLVLPK---DIISKLCTEQVKPG-----NIDKSDAVQI
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LKNVAFMKSYILEKVKEHQESMDMNNPQDFIDCFLMKMEKEKHNPQS--EFTIESLENT

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A. niger 1
A. niger 2
A. fumigatus 1
A. fumigatus 2
A. fumigatus 3
F. graminearum 1
F. graminearum 2
F. oxysporum 1
CYP450_119
CYP450_cin
CYP450_130
CYP450_nor
CYP450_3A4
CYP450_2C9

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CWLTAvgvg-APVGLIADVLQYYLRPENIDHWKR1QNLVSQPDNS---GSIDKLLRQ
HLLPTAGGMVANQAQLFSQCLDDYYLSEEGSGHLPEINRLAKEDT-----TEADDELLR
HVLPTAGGMVANQAQLFSQCLDDYYLSEEGSGHLPEINRLAKEDT-----TEADDELLR
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LWLTAFFGIG-VPVTAFFEVLAFFLRENASIWAEVQAIQKGD-----DATLHA
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TFTMTGGNDTVTGMLGGSMPLLHRPDPQRRLNLLDDP-----EGIPD
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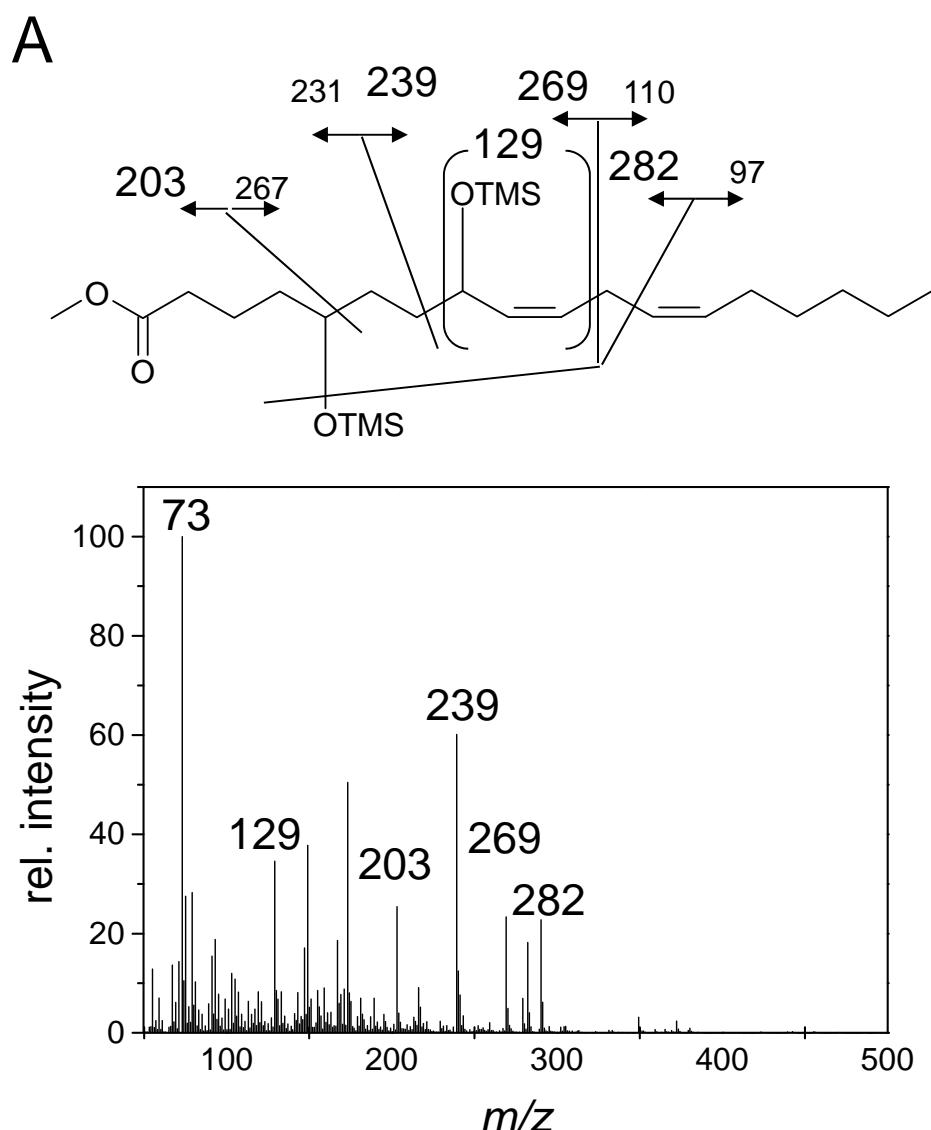
A. nidulans_PpoA

A. niger 1
A. niger 2
A. fumigatus 1
A. fumigatus 2
A. fumigatus 3
F. graminearum 1

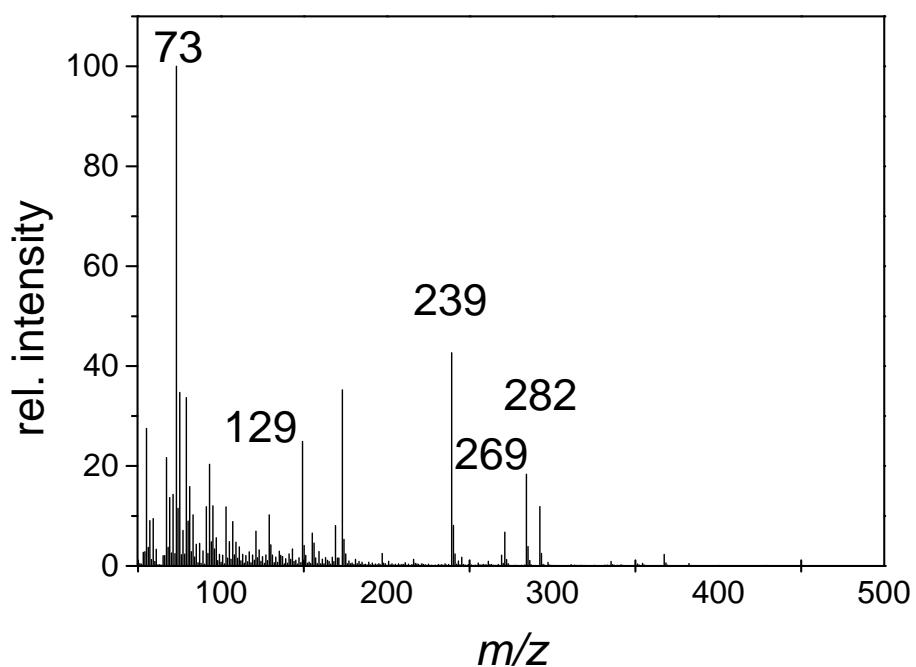
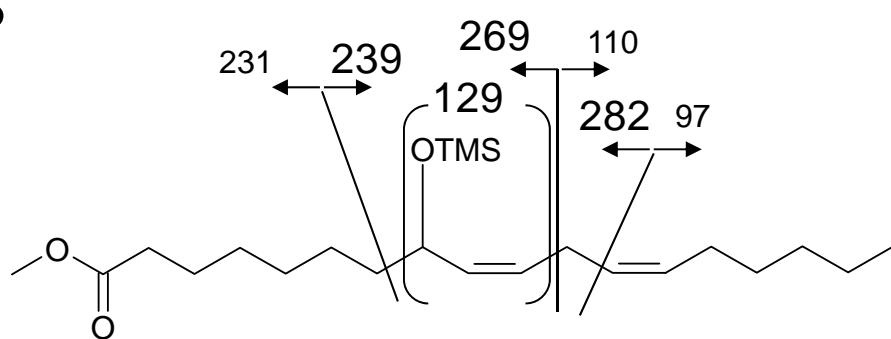
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F. graminearum 2 YAMEGIRMA--TFGLYREATTADVIQEDDGREVVKAGDRVVFVFTAAKDPNIF--PD
F. oxysporum 1 YAMEGIRMA--TFGLYREATGPDTIHEDDGRSIPVNAGDRVVFVQAQDPKIF--PN
 CYP450_119 AIEEALRYSPP-VMRTVRKTKEVRKLG----DQTIEEGEYVRWVIASANRDEEVF--HD
 CYP450_cin AVDELLRLFYG--PAMVGRLLTQEVTVG----DITMKPGQTAMLWFPIASRDRSAF--DS
 CYP450_130 AVEELLRLTSP-VQGLARTTTRDVTIG----DTTIPAGRRLVLLYGSANRDERQYG-PD
 CYP450_nor FVEELCRYHTASALAIKRTAKEDVMIG----DKLVRANEGLIASNQSANRDEEVFENPD
 CYP450_3A4 VVNFTLRLFP-IAMRLERVCKKDVEIN----GMFIPKGVVVMIIPSYALHRDPKYW--TE
 CYP450_2C9 VVHEVQRYIDLPLSLPHAVTCDFR----NYLIPKGTTILISLTSVLHDNKEF--PN
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A. nidulans_PpoA PEKVKLDRDMNL-----YAHFGFGPHKCLGLDLCKTGLSTMLKVLGR-LDNLRRAP 1033
A. niger 1 AETFKLDRPSNA-----YIHFGGYGAHECLGKEIGLTFAVSMLRVLAG-LKYLRRAP
A. niger 2 PEKVKLDRDMSL-----YAHFGFGPHQCLGMGICKLALTMLRVVGR-LDNLRRAP
A. fumigatus 1 PDKVKLDRDMNL-----YAHFGFGPHQCLGLGLCKTALTMLKVIGR-LDNLRRAP
A. fumigatus 2 PEEVDLNRPMES-----YIHGVGPHTCLGSEASKVALTTMLRVVGR-LDNLRRAP
A. fumigatus 3 PEEVDLNRPMES-----YIHGVGPHTCLGSEASKVALTTMLRVVGR-LDNLRRAP
F. graminearum 1 ADKFNAQRKQE-----VSASF SYGQHECVAKDVALAFVTGLIKLVAD-LKELRRAP
F. graminearum 2 PEVVDPRRPLDS-----YIHGVGPHACLGRDISQVALTELFRALFR-KKGLRRVA
F. oxysporum 1 PGVVDPKRPLDK-----YIHGVGPHACLGRDISQVALTELFRAVFR-KKGVRRVP
 CYP450_119 GEKFIPDRNPNP-----HLSFGSGIHLCLGAPLARLEARIAIEFSKRFRHIEILD
 CYP450_cin PDNIVIERTPNR-----HLSLGHGIHRCGLGAHLIRVEARVAITEFLKRIPEFSLDP
 CYP450_130 AAELDVTRCPNR-----ILTFSHGAAHCLGAAAARMQCRVALTELLARCPDFEVAE
 CYP450_nor EFNMNRKWPQD-----PLGFVGDHRCIAEHLAKAELTTVFSTLYQKFPDLKVAV
 CYP450_3A4 PEKFLPERFSKKNKDNIDPYIYTPFGSGPRNCIGMRFALMNKALIRVLQNFSFKPCKE
 CYP450_2C9 PEMFDPHIFLDEGGNFKKSKYFMPFSAGKRICVGEALAGMELFLFLTSILQNFNLKSLVD
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A. niger 1 GDMGMLKSI--VDGRRVYLNDSSWSWMTRDPTNGGIMSTQYSFGQHHHHQYHEITRSSS
A. niger 2 GSQGQLKKIAGPGGIMMTADQSSYWPFNSTMKIQWD-----GDLPSL
A. fumigatus 1 GGQGKLKKLSPGGIAMYMTPDQTAFFPFPTTMKIQWD-----GDLPEV
A. fumigatus 2 GAQGELKKIIPREHGFYTMYREDQSSFYPFPMWSWLHYD-----GEIPGK
A. fumigatus 3 GAQGELKKIIPREHGFYTMYREDQSSFYPFPMWSWLHYD-----GEIPGK
F. graminearum 1 GQMGTVKTIR-VGTEKAYLNDSSWSYLGFDASTWKVHFN-----GHGKGNFEGDRVPTS
F. graminearum 2 GAQGELKKVPRPGGFFVYMTEDWGSIWPFPTSMKVTD-----E-----
F. oxysporum 1 GAQGELKKVPRPGGFFVYMTEDWGSIWPFPTSMKVTD-----E-----
 CYP450_119 TEKPVNEVLNGYKRLVVRLLKSNE-----
 CYP450_cin NKECEWLMGQVAGMLHVPIIFPKGKRLSE-----
 CYP450_130 SRIVWSGGSYVRRPLSVPFRVTSSR-----
 CYP450_nor PLGKINYTPLNRDVGIVDLPVIF-----
 CYP450_3A4 TQIPLKLSLGGLLQPEKPVVLKVESRDGTVSGAHHHH-----
 CYP450_2C9 PKNLDTTPVNGFASVPPFYQLCFPIIHHHH-----
A. nidulans_PpoA KEDF----- 1081
A. niger 1 SLPSMLGDDESYGRFSGTLN-
A. niger 2 ATN-----
A. fumigatus 1 KE-----
A. fumigatus 2 EQPVRGDFVCNVPGHWQN---
A. fumigatus 3 EQPVRGDFVCNVPGHWQN---
F. graminearum 1 NTPIQFYSLQKRKDELLRR-----
F. graminearum 2 -----
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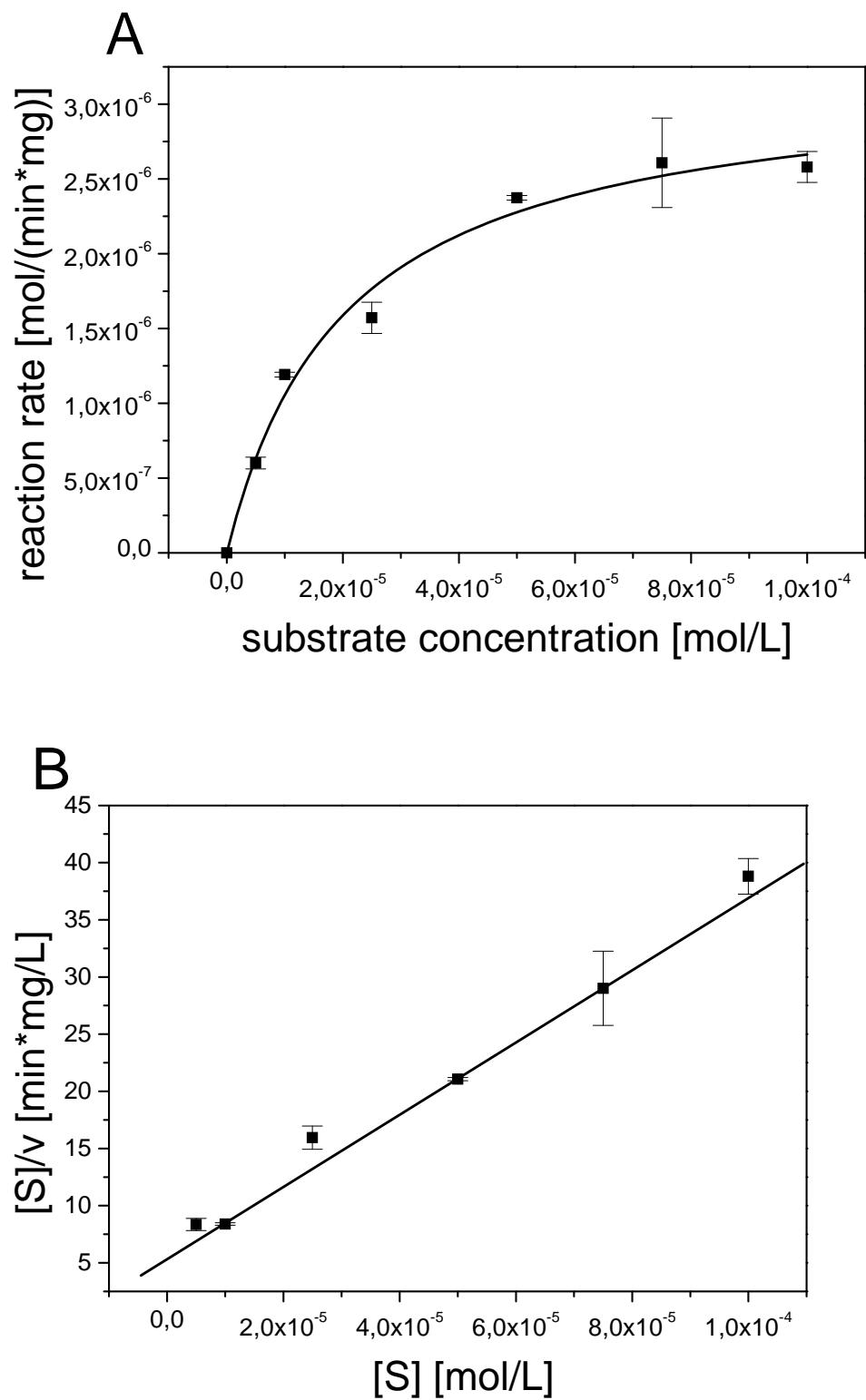
Supplemental Figure 2



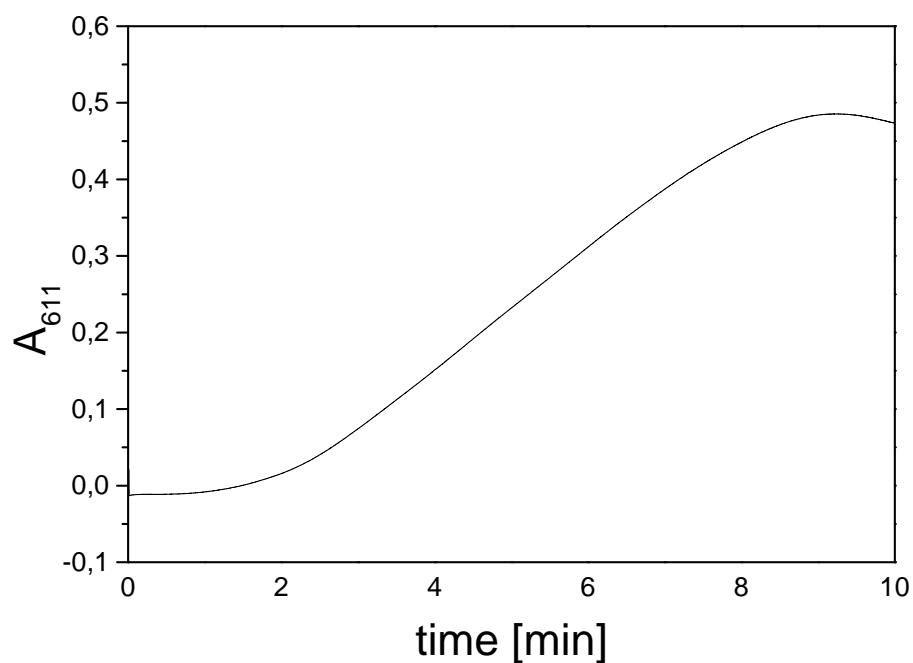
B



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

