The CYPome of the model xenobiotic-biotransforming fungus *Cunninghamella elegans*

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Supplementary Information

Table S1. Primers used to amplify CYP genes from *C. elegans* gDNA and cDNA.

Primers	Nucleotide sequence 5' to 3'	Tm (°C)	CYP
g6049 F	TGGCACGAACAACTTGGTAA	53.2	5206U1
g6049 R	CCAGCAGCAACTAAATCAGC	55.3	
g5706 F	GCTGATTTAGTTGCTGCTGGT	57.9	5208A2
g5706 R	CTAAACCCCGACCAATGGTA	55.9	
g5705 F	GCTGATTTAGTTGCTGCTGGT	55.3	5206D1
g5705 R	GGGAAAAACTCTGCCATTTG	55.3	
g4598 F	TGGCAGAGTTTTTCCCTTTT	55.3	5205A8
g4598 R	TGAATCCCAACACAAACTCG	57.3	
g6826 F	GACTTGCTGTGCAACTTCAAA	57.3	509Q1
g6826 R	TGGTATCTACACCAGCAGCAA	57.3	
g4063 F	GCTGACTTAATTGCTGCTGGT	53.5	5210C1
g4063 R	TGGTAATATAAGCTGCCATTC	55.3	
g3940 F	TGCACCACGTGCTATTAAGG	57.3	5313E1
g3940 R	ACGGGGATGAATGGTCATAA	57.3	
g2620 F	ATGAATGACTTTAATATTTACAATAAATTGGAAC	63.9	5313D1
g2620 R	TTAAAATTTTCTGGGCTTTAACTTCATA	63.8	

Table S2. Primers used in RT-qPCR.

Primers	Nucleotide sequence 5' to 3'	Tm (°C)	GC-content (%)	Ta (°C)
CYP F*	GGTATGAACTTTAGTTTAGAACAA	55.9	25.0	58
CYP R*	CGGATTTTCAAATCAATTGGTTTAGGT	58.9	33.3	58
CPR F*	ATTACTGACGAATCCCCTGT	55.3	45	56
CPR R*	CGTTCACCAATACGTTTAGC	55.3	45	56
SarA F	AACCTACTCTTCATCCTACCTCTGAA	61.6	42.3	60
SarA R	GCATCAACTAAGAAAACATACCAGA	58.5	34.6	60
UbcB F	ATGAGAGATTTCAAGAGACTCCAAA	58.1	36	60
UbcB R	ACAAATTTAACTGATGGTGGTTTGT	56.4	32	60
q-3940 F	ATTTGTTCCTAAAGGCACTAAGGTT	57.3	50	60
q-3940 R	TGAAATTCATTAAATCATTGGGACT	57.3	50	60
q-2620 F	AATTCTACTGGATTTGCTATGATTG	53.5	30.4	60
q-2620 R	TTCATTGATGACAGCATTAAGATAA	55.3	45	60

CYP and CPR primers were specific for CYP509A1 and a cytochrome P450 reductase, respectively.

Table S3. Conditions for RT-qPCR reactions

Step	**Temperature (^oC)**	**Time**
Pre-incubation	95 °C	5 min
45 cycles	95 °C	15 s
	~ °C	20 s
	72 °C	10 s
Melting curve	50 to 95 °C	(temperature transition 0.2 °C /s)
Cooling	40 °C	10 s

Table S4. PCR efficiency of amplified targets*

Target	**Slope**	**Y-intercept**	**E**	**Error**	**R2**
cyp5313E1	-3.5196	28.12	1.87	0.72	0.94
cyp5313D1	-3.5354	26.31	1.92	0.27	0.99
cyp509A1	-3.2309	29.58	2.04	0.08	1
ubcB	-3.5794	24.5	1.9	0.2	1
sarA	-3.7986	24.62	1.83	0.34	0.99
cpr	-4.2994	25.45	1.71	0.32	0.99

[*] PCR efficiencies were measured from calibration curves made of serial dilutions of cDNA 1, 1/4, 1/16 and 1/64 measured in duplicate.

Figure S1. Phylogenetic tree of redox partner proteins from *C. elegans* and known CYP partner proteins used for homology searches. Branch bootstrap values are presented as a percentage.

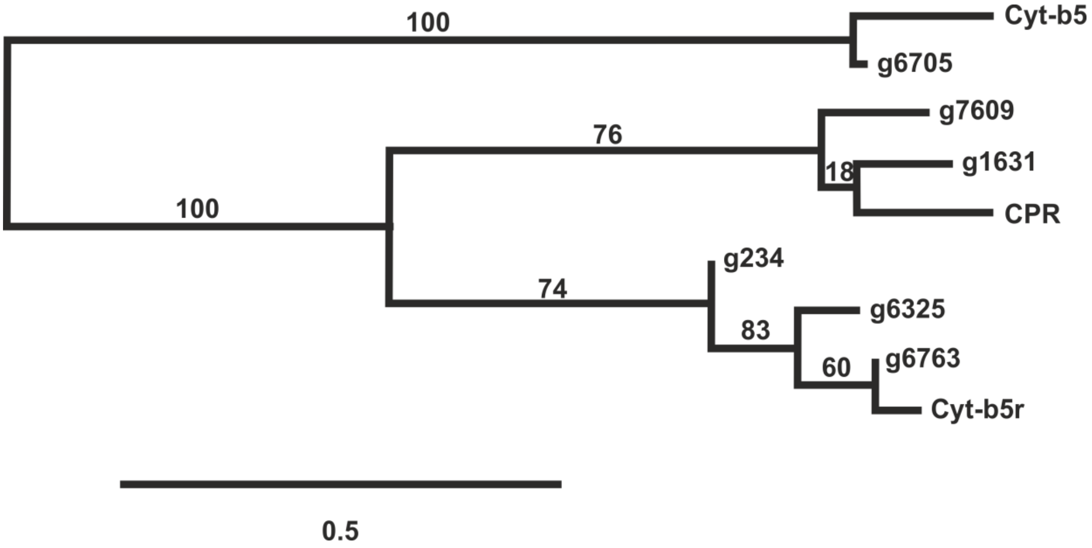

Figure S2. Alignment of the amino acid sequences of CPRs from *C. elegans* (G1631, G7609 and G4301) with those of *Mucor ambiguous* (GAN1069.1) and *Rhizopus stolonifer* (RCH89036.1). FMN domain is in red, NADPH-binding domain is in green and the FAD domain is bold.

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G1631            --MLKSNNQVFEHQLMLVLGTISVGGLLWVAKGLLFNDSNKSSDQKP--LI--------- 47
G7609            MTTRRSNNRVLGTYHLIVLGAIGLGTIAWFARNKFF----GSSDTDDNKIISNSETSTTS 56
G4301            --MAQQSPAVIDTLDLILLGSIGLGTIAWFTRRQISERLFGTGQSNA------------- 45
GAN10969.1       --MTRSSQHLLDSLDIIFLGTIGLGTIAWFARHQIADKLFKSSKPEI------------- 45
RCH89036.1       --MAKTSHHLLDTLDIVLLGTIGLGTIAWFARHQIANRLFQSNKSSE------------- 45
                     : .  ::    ::.**:*.:* : *.::  :      :.. .

G1631            PDVKTMEKSEEEQEVGNFVKLMKEQNRKVIFFYGSQTGNAENYCYQLSKECKKRYGIQPM 107
G7609            SDTTAPKPNVPAKPERNFVKVMQQQGRRVIFFYGSQTGTAEDYASRLAKECSQKYGISAM 116
G4301            T--SKPTTPQAPKRERNFVKVMEQQGRKVIFFYGSQTGTAEDFASRLAKQCSQKYGVSCM 103
GAN10969.1       KP-ASDAKTGPPKKERNFVKVMQQQGRRVIFFYGSQTGTAEDYASRLAKECSQKYGVSSM 104
RCH89036.1       PNTSTSDEAKSPKKERNFVKVMQEQGRRVIFFYGSQTGTAEDYASRLAKECSQKYGVSAM 105
                             :   ****:*::*.*:**********.**::. :*:*:*.::**:. *

G1631            VADIETYDLKYLDLLTEDNLAVFIVSTYGEGDPTDSAINFWEL-IHNENPNFSKCD-GNP 165
G7609            TADIELYDLNYLDTLPEDNLVFFVMATYGEGEPTDNAVDFWEL-INDETPQFSQLANVED 175
G4301            TADIEMYDLSYLDTLSEDSLVCFVMATYGEGEPTDNAVDFWEQFITDESPVFSQGG---- 159
GAN10969.1       AADIEQYDMSYLDTVPEDFLVFFVMATYGEGEPTDNAVDFWDL-VSEEQPQFSEAE-DEE 162
RCH89036.1       TADIEQYDLSYLDTVPEDCLVFFVVATYGEGEPTDNAVEFWDL-ITDEEPQFSTSD---- 160
                 .**** **:.*** : ** *. *:::*****:***.*::**:  : :* * **

G1631            QPLSKLRFFAFGLGNSTYEHFNAAVVGVDRELTRLGATRLGEIGKGDDDACLDDDFAHWQ 225
G7609            KPLKNVRYLVFGLGNKTYEHYNSVGRTVDKKLTDFGATRIGERGEGDDDGSLEEDFLAWQ 235
G4301            ETLENLRYLMFGLGNKTYEHYNAVARILDKKLTGLGAKRIGERGEGDDDGSLEEDFLAWQ 219
GAN10969.1       APLKNLRYVAFGLGNKTYEHYNEVIRNIDKRLTCMGAKRIGERGEGDDDGSLEEDFLAWQ 222
RCH89036.1       -RLENLRYITFGLGNKTYEHYNEVVRKIDHRLLALGAKRIGERGEGDDDGSLEEDFLAWQ 219
                   *.::*:. *****.****:* .   :*:.*  :**.*:** *:****..*::**  **

G1631            ESFWPLFGEAVSQLIDGEADEGNHGDQYAYEVTDVNVDDNDGFYYQGEL---GSDRTQKL 282
G7609            ETMWPAFCQALGIDE---NNIQLGPRQAAFKVEELTTFDDSSVYY-GEIAENLKSKGKIV 291
G4301            ESMWPTFCNALGVDE---NNAQQGPRQASYSVDELEEYQNDDVYF-GELGTISKDSSRVV 275
GAN10969.1       EEMWPAFCEALGVDE---SSASSGPRQASFAVEELTEFDKAKVYV-GELSEWLKEGARVV 278
RCH89036.1       EEMWPAFCEALGVDE---ANAQLGPRQAIFSVEELTEFDKAKVYF-GELSEWLKEGARVV 275
                 * :** * :*:.       .      *  : * ::   :.  .*  **:    ..  : :

G1631            FDAKNPYPATVQIRDLTPATQDDRHCLHIDFDLADSGLTYKTGDHLGVWPINNELEVNLV 342
G7609            YDAKRPYNAPIQSRELFQD-GGDRHCLHVDIDISGTNLNYQTGDHVAIWPTNNEIEVIRL 350
G4301            YDAKRPYNAPITTRELFN--SSERHCLHVDIDISGTNLSYQTGDHVAMWPTNNEDEVLRL 333
GAN10969.1       YDAKRPYNAPITSTDLFK--GGDRHCLHMEIDISGSNLTYQTGDHVAIWPTNNEIEIERL 336
RCH89036.1       YDAKRPYNAPITSRDLFK--NSDRHCLHMEIDISDTNLSYQTGDHVAIWPTNSEVEVERL 333
                 :***.** * :   :*     .:*****:::*::.:.*.*:****:.:** *.* *:  :

G1631            SSIFGWNHDGILDKVISVKPTDPT--GKVPFPQPTTLRTALRHYLDIAAIPSRSVFELLI 400
G7609            ASILGLKD--KLDTVIMVNAVDSAASKQHPFPVPTTYRTIFRHYLDICSIASRQTLMSLV 408
G4301            ATILGLQE--KLDTVISVKAIDPAAPKQNPFPVPTTYRAIFRHYIDICAPASRQSLMSFV 391
GAN10969.1       AKVLGLSD--KLDTVIRVQALDAAASKQFPFPVPTTYRAIFRHYLDICAAVSRQTLMSLI 394
RCH89036.1       AQVLGLMD--KLDTVIRVQAIDATASKQHPFPVPTTYRAVFRHYLDICAVVPRQTLMSLI 391
                 : ::*  .   **.** *:  *  :   : *** *** *: :***:**.:   *.  :  ::

G1631            PHVP-EQIKAALQKIIDDKEVYNKLVVDEVRNFGQVLSHILTSNGYT---DIENALAKVP 456
G7609            EYAPTEESKANLLRLSKDKDEYHVHVHDSVRNLGEVLQYVCGNNDHTDPEAHSGAFANVP 468
G4301            EFAPTDAAKDLLKLLATDKDEYRLKVGEAVRNLGEVLELVSGKD------AQPGSFSSVP 445
GAN10969.1       EYAPTEKSKDILRKLATDKDEYRVRVGDVTRNLGEVLEMLAESES----LAVEGSFSSVP 450
RCH89036.1       EYAPTEKSKEVLRKLATDKEEYRLRVGDMTRNLGEVLEMLAESES----MDPKNSFANVP 447
                  ..* :  *  *  :  **: *.  * : .**:*:**. :  .:         .:::.**

G1631            INIIIECYTRLQPRYYSISSSSSESGSIVTATAVTLKYNP--TPDRTVYGVNTNYLWSIY 514
G7609            FDLIIESISRLQPRYYSISSSSKEQPNIISATCVTLEYQPKPTPERTVYGVNTNYLWKIH 528
G4301            FDLIVETIPRLQPRYYSISSSSKENPSIISATCVTLAYQPDPTPDRTVYGVNTNFLYRIH 505
GAN10969.1       FDLIVESVSRLQPRYYSISSSSKESPKKITVTVVTLQYTPETSSPRTVYGVNTNYLWRLH 510
RCH89036.1       FDLIVEAVSRLQPRYYSISSSSKEDPKKITVTAVTLQYTPDLSSPRTVYGVNTTYLWRLH 507
                 :::*:*   *************.*. . ::.* *** * *  :  *******.:*: ::
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G1631           QKLNPSVANPNHPPYAIEGPHQAYFQKNGDTIITKLPVHIRQSTFRLPTDASTPVVMVGP 574
G7609           SIVHQVDDGRSYPHYDIAGPRQQLFESSL--SHAKIPIHVRRSQFKLPRNSKVPVIMVGP 586
G4301           MQNNDDSVIQGLPKYDLAGPRKAFLNGQG--QSHKLPIHIRRSQFKLPRNTSCPVIMIGP 563
GAN10969.1      ESINGIEPDASFPHYSITGPRNCLYDTEA--KAARVPVHVRRSQFKLPRNPTVPIIMVGP 568
RCH89036.1      EAINGLEPDTASPRYYLGGPRDGLFDVQA--KQARVPVHVRRSQFKLPRNPTVPIIMVGP 565
                   :        * * : **:.   : .      ::*:*:*:* *:** : . *::*:**

G1631           GTGVAPFRGFVRERVYQKQVEKQQVGTTLLFFGCRRSNEDYLYADEWPELFNQLDNESRI 634
G7609           GTGVAPFRGFVRERALLKQK-GQEVGPTVLFFGCRHSEKDFIYKDEWPELFNTLGEESQL 645
G4301           GTGVAPFRGFVRERALQKKE-GKSVGPTILFFGNRHSQHDFLYSDEWPELFNTLGEDSKL 622
GAN10969.1      GTGVAPFRGFVRERALQKKE-GKPIGPTILYFGCRNSAEDFLYEEEWPELFETLGESSRI 627
RCH89036.1      GTGVAPFRGFVRERALQKKE-GKPVGTTVLFFGCRHPEQDFLYADEWPELFETLGGDSRL 624
                **************.  *:   : :* *:*:** *.  .*::* :******: *. .*::

G1631           INAFSRETDKKVYVQHRVRENGEEVWDLLANQGGYLYVCGDAKRMAKDITQTILDLAKHF 694
G7609           ITAFSRETDQKVYVQHRLKEYGQQMWDYI-QQGAYIYVCGDAKNMAHDVNQAFIQFAQEF 704
G4301           ITAFSRESEHKVYVQHRLEENGKEIWQLL-EKGAYIYVCGDARNMARDVNQTFVNLAMEY 681
GAN10969.1      ITAFSRETAQKVYVQHRLMENGPEMWDLL-EKGAYVYVCGDAKVMARDVNQTFVRFAQQF 686
RCH89036.1      ITAFSRETDKKVYVQHRLLEHGQAMWNLL-EKGAYVYVCGDAKVMARDVNQTFVQFAKDL 683
                *.*****: :*******: * *  :*: : ::*.*:******: **:*:.*::: :*  .

G1631           GKLDDEAALAFVQGLRKAGRYQEDVWA        721
G7609           GGRDEVKANAFFKQLRNTGRYQEDVWS        731
G4301           GEKTEQKALDYVKSLRNTGRYQEDVWS        708
GAN10969.1      GGHDEEKAQDYVKNLRNTGRYQEDVWS        713
RCH89036.1      GGLDEAEAQDYVKNLRNTGRYQEDVWA        710
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Figure S3. Amplification of *cyp5313D1* from cDNA of *C. elegans* DSM1908. The expected amplicon size was 1,539 bp.

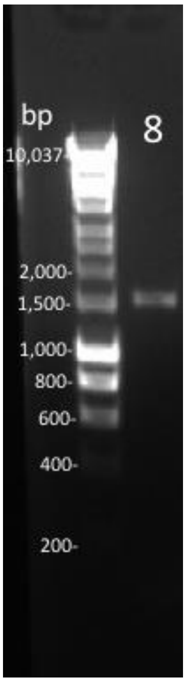

Figure S4. Full Western blot corresponding to Fig 3. The upper blot was developed with anti-his antibodies and the lower blot probed with anti-myc antibodies.