Adaptive evolution of drug targets in producer and non-producer organisms

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Supporting Information

Table S1. Sequences generated in this study. a) MPA production on CYA media. – means no production, + medium and ++ high production (Frisvad and Samson, 2004)). b) The extracted *imdA* and *imdB* sequence parts for these organisms do not overlap and are therefore not included in Figure 5 B.

Taxon name	IBT number	Other collection numbers	MPA prod [a]	DNA sequences (GenBank accession #)		
				IMPDH-A	IMPDH-B	*β-tubulin*
P. allii[a]	20212	CBS 875.95	–	JN112053	JN112066	JN112035
P. albocoremium[b]	21596	-	–	JN112054	JN112065	JN112036
P. aurantiogriseum[b]	11325	CBS 792.95	–	JN112050	JN112062	JN112033
P. brevicompactum[b]	18329	CBS 110067	++	JN112046	JN112068	JN112040
P. caseifulvum[a]	19782	CBS 108956	–	JN112051	JN112067	JN112037
P. crustosum	21518	CBS 101025	–	JN112048	JN112061	JN112030
P. cyclopium[b]	16769	CBS 110337	–	JN112049	JN112063	JN112034
P. echinulatum[b]	21839	CBS 112286	–	JN112052	JN112064	JN112032
P. roqueforti	14756	-	+	JN112045	JN112058	JN112039
P. roqueforti[b]	24748	-	–	JN112044	JN112059	JN112038
P. viridicatum	21551	CBS 101034	–	JN112047	JN112060	JN112031

Table S2. PCR primers

Primer	Sequence (5' to 3')[a]
BGHA358	AGGCCAGCUCGTGAAAGCCTTCCGTGGTATG
BGHA359	AGCTGGCCUTCGTTGCTGACAAAGTACTC
BGHA360	AGGTCAGCUGGTCAAGGCCTACCGCGGTATG
BGHA361	AGCTGACCUTCATTGCTAACGTAGTACTC
BGHA430	AAGCCGATGUAACCGGGCAGGATCAGGA
BGHA431	ACATCGGCTUCCCCGCTTCCGATGTCTCATTG
BGHA432	AGGGTTCCGTTCUCAGTGACAGGGAAGCCACCGAAG
BGHA433	AGAACGGAACCCUTAAGTCCAAGCTCGTTGGA
BGHA434	AAGGTGAUAGAACCGGGCAAAATCAGGA
BGHA435	ATCACCTUCCCGGCATCGGATGTTTCGT
BGHA436	AGGGTTCCCTTTUCGGTCACCGGGAACCCGCCGA
BGHA437	AAAAGGGAACCCUCCTCTCGAAGCTTCTCGGA
BGHA454	ACGGAAGGCCUTGACGAGCTGGCCCTCGTTG
BGHA455	ATTGGGGTGGCCTCUCTCATGAGCACTGAAGTTCTTTC
BGHA505	AGCCGATGUAGCCGGGCAAGATCAGGA
BGHA506	ACATCGGCUTCCCTGCCTCCGATGTGT
BGHA507	AGTTCCGTUCTCAGTGACAGGGAAACCTCC
BGHA508	AACGGAACUCTTCGCTCCAAGCTCGTT
BGHA509	AACCGATAUATCCCGGAAGAATGAGGAA
BGHA510	ATATCGGTUTTCCTGCTTCCGATGTTAC
BGHA511	ATTTTCAGUAACTGGGAAGCCGCCGAA
BGHA512	ACTGAAAAUGGAACTCTCCGCTCAAAG
BGHA527	ATATGGCUGCCGCGCGGCACC
BGHA528	AGATCCGGCUGCTAACAA
BGHA529	AGCCATAUGGTCGAAATCCTGGACTA
BGHA530	AGCCGGATCUAAGAAGAGTACAACTTCTTCTCG
BGHA539	AGCCATAUGCCTATCACCGCCAGCGAC
BGHA540	AGCCGGATCUACGCGTAAAGCTTCTTGTCG
BGHA541	AGCCATAUGGTCGAAGTTCTGGATTATAC
BGHA542	AGCCGGATCUAAGAGTACAACTTCTTCTC
BGHA543	AGCCATAUGCCTATCACCGCCGGCGAC
BGHA544	AGCCGGATCUACGAGTAAAGCTTCTTGTC
BGHA545	AGCCATAUGCCAATTGCCAACGGTGAC
BGHA546	AGCCGGATCUAAGAGTAGAGCTTCTTGTC
BGHA667	ACGGTTCCGUTGTTCACACCGGTGTGCA
BGHA668	ACGGAACCGUTCGCTTTGAAATGCGCAG
BGHA669	ACCTGGCCGUTGTCGACACCAGCGTGCA
BGHA670	ACGGCCAGGUTCGCTTTGAGATGAGAAG
BGHA236HC	ATGCCIATYNCCRMCGGIGAYKC
BGHA246HC	CRGCCTTCTTRTCCTCCATGG
BGHA531	AGCTTCTTGTCGTAGCTGTG
BGHA532	GTCAAGGGTCTSGCYATGG
BGHA240 HC	ATGGTCGADRTYCWGGAYTAYACC
BGHA241 HC	GARGCRCCRGCGTTMTTG
BGHA343	GAGCGYATGARYGTYTAYTTCA
BGHA344	GTGAACTCCATCTCRTCCATACC

Table S3. *E. coli* expression constructs and cloning strategy. [a]Underlined sequence will be removed and lead to single stranded overhang during USER cloning.

Construct	Expressed enzyme	Primers for IMPDH amplification		
		Exon 1	Exon 2	Exon 3
P113	*P. brevicompactum* IMPDH-A	539/505	506/507	508/540
P114	*P. brevicompactum* IMPDH-B	Amplified from cDNA with 529/530		
P115	*A. nidulans* ImdA	545/509	510/511	512/546
P116	*P. chrysogenum* IMPDH-A	543/430	431/432	433/544
P117	*P. chrysogenum* IMPDH-B	541/434	435/436	437/542
P118	*P. brevicompactum* IMPDH-A with Y411F	539/455 and 540/454 and P113 as PCR template		
P119	*P. brevicompactum* IMPDH-B with F411Y	529/361 and 530/360 and P114 as PCR template		
P120	*A. nidulans* ImdA with Y411F	545/359 and 546/358 and P115 as PCR template		
P135	Chimeric IMPDH of *P. brevicompactum* IMPDH-B (N-term) and *P. brevicompactum* IMPDH-A C-term	*P. brevicompactum* IMPDH-B and ImdA fragments amplified with primer-pairs 529/667 and 668/540, respectively.		
P136	*P. brevicompactum* IMPDH-B with F411Y and IMPDH-A C-term	Same as for P135 but using P119 as PCR template for IMPDH-B fragment		
P137	Chimeric IMPDH of *P. brevicompactum* IMPDH-A (N-term) and *P. brevicompactum* IMPDH-B C-term	*P. brevicompactum* IMPDH-A and IMPDH-B fragments amplified with primer-pairs 539/669 and 670/530, respectively.		
P138	*P. brevicompactum* IMPDH-A with Y411F and with IMPDH-B C-term	Same as for P137 but using P118 as PCR template for IMPDH-A fragment		

Table S4. Concentrations of IMP, NAD^+ used for determination of IC_{50} (MPA)

Enzyme	IMP (mM)	NAD^+ (mM)
*An*ImdA	0.5	0.5
*Pb*IMPDH-A	3	1
*Pb*IMPDH-B	3	1
*Pc*IMPDH-A	1	1
*Pc*IMPDH-B	1	1
Mutant proteins	1	1

Table S5. Putative genes flanking the MPA cluster in *P. brevicompactum*.
Further comparison of the regions further away from the MPA cluster in *P. brevicompactum* reveal other genes homologous to genes from *P. chrysogenum* however, they are located in loci of many different contigs.

Gene	Region on *P. brevicompactum contig* (*Pb* BAC 1E13+1C23)*		Size (aa)	BLASTx homolog	Predicted function	E-value	Identity (%)
orf1	0 -	4139	987	*P. chrysogenum,* Pc22g22260	C2H2 finger domain protein	0	87
orf2	4513 -	5736	407	*P. chrysogenum,* Pc22g22270	DNA mismatch repair protein	1.15E-151	76
orf3	7554 -	9212	514	*P. chrysogenum,* Pc22g22280	WD repeat protein	0	94
orf4	10249 -	10913	191	*P. chrysogenum,* Pc22g22290	IgE-binding protein	5.97E-75	82
orf5	12809 -	1399	360	*Neosartorya fischeri*, XP_001265454	nucleoside diphosphatase	1.42E-113	71
				P. chrysogenum, Pc22g22320		1.37E-93	72
MPA cluster (17830 – 43315)							
orf13	50594 -	51894	368	*P. chrysogenum,* Pc21g04710	phospho-2-dehydro-3-deoxyheptonate aldolase	0	94
orf14	52993 -	53878	256	*P. chrysogenum,* Pc21g04720	unknown	4.00E-91	72
orf15	54299 -	55162	251	*P. chrysogenum,* Pc21g04730	unknown	1.61E-63	60
orf16	55381 -	56121	246	*Aspergillus fumigatus,* XP_001481519 (no hits in *P. chrysogenum*)	glycosidase hydrolase	1.00E-61	57
orf17	57170 -	59032	597	*P. chrysogenum,* Pc21g04750	C6 transcription factor	0	81
orf18	60261 -	62432	539	*P. chrysogenum,* Pc21g04770	NADH-cytochrome B5 reductase	6.63E-115	76
orf19	63597 -	65642	625	*P. chrysogenum,* Pc21g04800	unknown	0	73
orf20	67638 -	68559	286	*P. chrysogenum,* Pc12g02580	unknown	3.54E-44	62
orf21	68851 -	72867	1255	*P. chrysogenum,* Pc12g02550	myosin protein	0	93
orf22	74083 -	79557	1181	*P. chrysogenum,* Pc22g21120	carboxypeptidase	0	79
orf23	79786 -	82071	689	*P. chrysogenum,* Pc12g02500	arginine permease	0	85
orf24	84043 -	85074	293	*Aspergillus niger,* XP_001391260 (no hits in *P. chrysogenum*)	phytanoyl-CoA dioxygenase	3.30E-130	74
orf25	85889 -	91912	1907	*Aspergillus terreus,* XP_001209574	unknown	0	37
				P. chrysogenum, Pc21g03870		9.85E-142	27
orf26	93143 -	100908	2119	*Penicillium marneffei*, XP_002147174	beta-galactosidase	0	74
				P. chrysogenum, Pc22g14540		0	38
orf27	101641	103365	574	*P. chrysogenum,* Pc21g21450	unknown	0	61

Figure S1. Detailed graphical representation showing principle of PCR based USER cloning of a gene of interest (GOI) into pET28a. A pET28a USER compatible vector fragment, pET28aU, is made by PCR amplification using uracil containing primers (527 and 528) followed by DpnI treatment to remove PCR template. Likewise, the GOI fragment to be inserted into pET28aU is amplified with primers containing uracil. The primer tails were designed to allow seamless USER fusion and the uracil in the 5'-end of the GOI fragment corresponds to the T of the start-codon (ATG) of the GOI; and the uracil in 3'-end of the GOI fragment is complementary to the second nucleotide in the stop codon (TAG) of pET28a. USER fusion of the GOI PCR fragment and pET28aU is performed by mixing the two fragments followed by USER enzyme treatment to remove uracil residues. This step generates ends with single stranded DNA overhangs that allow for orderly fusion of the fragments. Following USER treatment the cloning mix is transformed into *E. coli*.

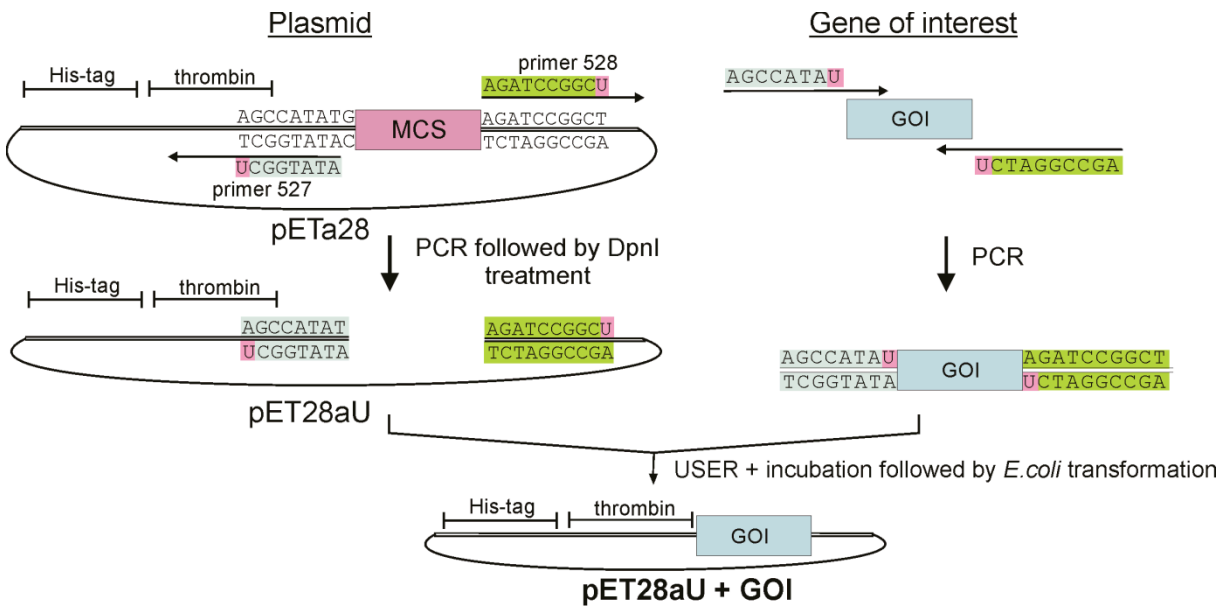

Figure S2. SDS-PAGE of purified recombinant fungal proteins. Lanes are as marked. All enzymes were purified to >90% purity with the exception of *Pc*IMPDH-B, which was partially purified to ~45% purity due to poor stability. Note that the lower molecular weight bands in the *P*cIMPDH-B sample are also IMPDH as demonstrated by Western blot analysis. The concentrations of all enzymes were verified by titration with MPA as described in the manuscript. This proteolysis could not be prevented with the addition of protease inhibitor cocktails to the lysate. P135 = *Pb*IMPDH-B with *Pb*IMPDH-A C-terminus, P136 = *Pb*IMPDH-B with *Pb*IMPDH-A C-terminus + F411Y mutation, P137 = *Pb*IMPDH-A with *Pb*IMPDH-B C-terminus, P138 = *Pb*IMPDH-A with *Pb*IMPDH-B C-terminus + Y411F mutation.

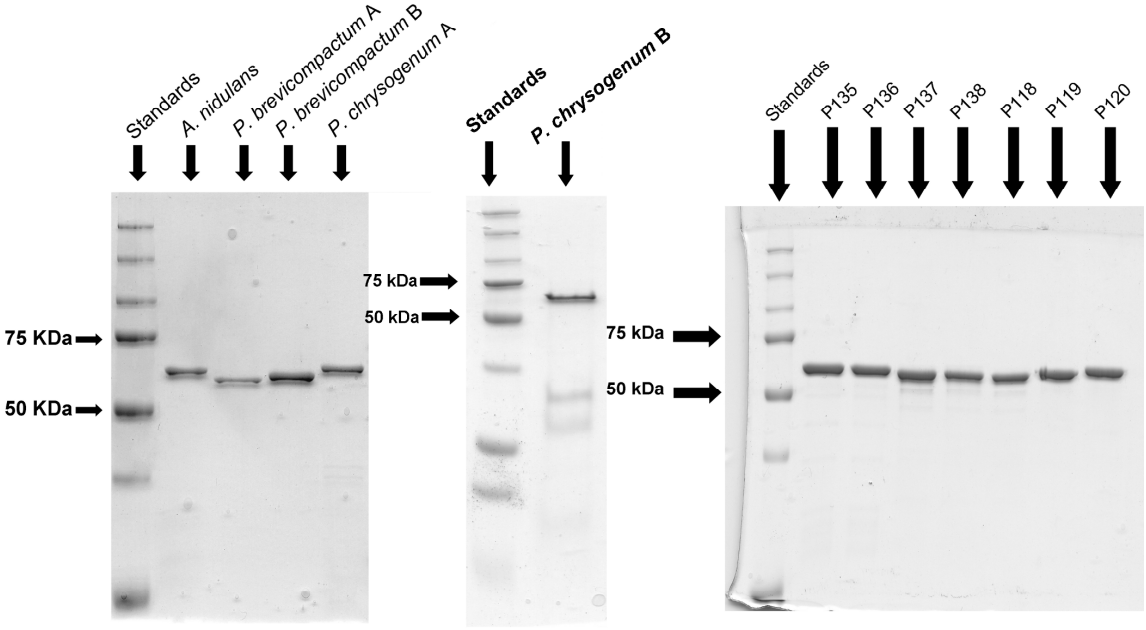

Figure S3. Determination of IMPDH oligomeric state using gel filtration chromatography on a S200 Sephacryl column. *An*ImdA (dotted line) elutes at 39.3 ml, *Pb*IMPDH-A (solid line) elutes at 39.2 ml, and *Pb*IMPDH-B (dashed line) elutes at 39.0 ml. All three proteins elute at ~ 220 kDa, consistent with being tetramers. The elution volume of the standards are as follows: blue dextran: 36 ml, thyroglobulin: 670 kDa, 36.2 ml, bovine gamma-globulin: 158 kDa, 44 ml, chicken ovalbumin: 44 kDa, 56.9 ml, equine myoglobin: 17 kDa, 71.8 ml, and vitamin B12: 1.35 kDa, 117 ml.

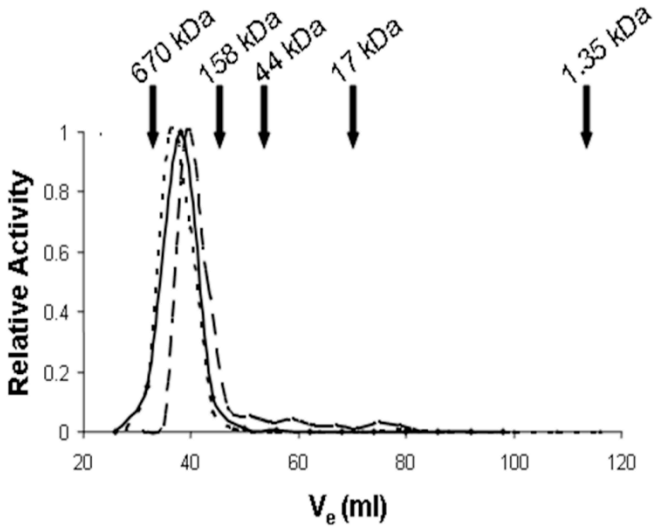

Figure S4. Rooted cladogram based on β-tubulin cDNA sequences (950 bp) from 14 species from *Penicillium* subgenus *Penicillium* and two filamentous fungi outside. *P*.: *Penicillium* and *A*.: *Aspergillus*. MPA production is indicated by "+" or "-". The gray line indicates organisms where both IMPDH-A and IMPDH-B genes have been detected. Bootstrap values (expressed as percentage of 1000 replications) are shown at the branch points, and the scale represents changes per unit. *Coccidioides immitis* has been used as outgroup.