

Online supplemental material

Table S1. Genomic organization of the siderophore biosynthetic genes *sidH*, *sidF*, *sidI*, *sidD*, *sidC* and *sidA* in various fungal species. Genes that are colocalized in the genome, i.e. organized in gene clusters, are marked in grey or black, respectively. Grey and black represent two different gene clusters. The gene accession numbers encoding orthologs to SidI, SidH and SidF correspond to the proteins listed in Table 1 and Table S2. Abbreviations: E, *Eurotiomycetes*; L, *Leotiomycetes*; S, *Sordariomycetes*; D, *Dothideomycetes*; B, *Basidiomycota*.

<i>sidH</i>	<i>sidF</i>	<i>sidD</i>	<i>sidI</i>	<i>sidC</i>	<i>sidA</i>		
AFUA_3G03410	AFUA_3G03400	AFUA_3G03420	AFUA_1G17190	AFUA_1G17200	AFUA_2G07680	<i>Aspergillus fumigatus</i>	E
NFIA_005580	NFIA_005570	NFIA_005590	NFIA_008180	NFIA_008170	NFIA_083410	<i>Neosartorya fischeri</i>	
AO090103000168	AO090103000169	AO090103000167	AO090023000529	AO090023000528	AO090011000926	<i>Aspergillus oryzae</i>	
AN6235	AN6234	AN6236	AN0609	AN0607	AN5823	<i>Aspergillus nidulans</i>	
An03g03550	An03g03540	An03g03520	An06g01320	An06g01300	An05g00220	<i>Aspergillus niger</i>	
ACLA_061010	ACLA_061020	ACLA_061000	ACLA_018340	ACLA_079690	ACLA_079680	<i>Aspergillus clavatus</i>	
TSTA_100270	TSTA_100280	TSTA_100300	TSTA_041590	TSTA_090280	TSTA_090270	<i>Talaromyces stipitatus</i>	
Pc22g20370	Pc22g20380	Pc22g20400	Pc21g23730	Pc13g05250	Pc13g05260	<i>Penicillium chrysogenum</i>	
HCBG_04177	HCBG_08322	HCBG_09033	HCBG_08321	HCBG_04660	HCBG_08320	<i>Ajellomyces capsulatus</i>	
BC1G_10565	BC1G_10564	BC1G_10567	BC1G_14541*	BC1G_03511	BC1G_03506	<i>Botryotinia fuckeliana</i>	L
SS1G_04249	SS1G_04248	SS1G_04250	SS1G_13961	SS1G_06185	SS1G_03696	<i>Sclerotinia sclerotiorum</i>	
FG03746	FG03745	FG03747	FG04333	FG11026	FG05872	<i>Gibberella zeae</i>	S
CHGG_09472	CHGG_09473	CHGG_09475	CHGG_10903	CHGG_02251	CHGG_09542	<i>Chaetomium globosum</i>	
PODANS72p146	PODANSg2355	PODANSg524	PODANSg2356	PODANSg3444	PODANSg3445	<i>Podospora anserina</i>	
NCU06960	NCU06062	NCU08441	NCU06063	NCU07119	NCU07117	<i>Neurospora crassa</i>	
MGG_14970	MGG_08289	MGG02351	MGG_08288	MGG_12175	MGG_04212	<i>Magnaporthe oryzae</i>	
SNOG_00297	SNOG_14362	SNOG_14368	SNOG_14361	SNOG_14834	SNOG_14364	<i>Phaeosphaeria nodorum</i>	D
UM01433	UM01432	UM01434	UM04803	UM05165	UM10188	<i>Ustilago maydis</i>	B

Table S2. Comparison of siderophore biosynthetic genes of *A. fumigatus* and *A. nidulans*.

gene	gene locus ^a	<i>A. nidulans</i> ortholog ^a (gene locus)	% identity (similarity) ^b	length ^a	proposed function
<i>sidA</i>	AFUA_2G07680	AN5823.4	77 (86)	498	<i>N</i> ⁵ -ornithine-monooxygenase (Eisendle et al., 2003)
<i>sidI</i>	AFUA_1G17190	AN0609.4	70 (77)	590	T AFC biosynthetic acyl-CoA ligase, PTS2 (Yasmin et al., 2011)
<i>sidH</i>	AFUA_3G03410	AN6235.4	84 (90)	270	T AFC biosynthetic enoyl-CoA hydratase, PTS1 (Yasmin et al., 2011)
<i>sidF</i>	AFUA_3G03400	AN6234.4	79 (89)	462	T AFC biosynthetic transacylase PTS1 (Schrettl et al., 2007)
<i>sidD</i>	AFUA_3G03420	AN6236.4	64 (78)	2086	fusarinine C NRPS (Schrettl et al., 2007)
<i>sidG</i>	AFUA_3G03650	AN8539.4	57 (72)	202	T AFC biosynthetic transacetylase (Schrettl et al., 2007)
<i>sidL</i>	AFUA_1G04450	AN10080.4	62 (75)	503	FC biosynthetic transacetylase (Blatzer et al., 2011)
<i>sidC</i>	AFUA1G17200	AN0607.4	55 (71)	4763	Ferricrocin NRPS (Schrettl et al., 2007)

^a predicted gene and length of protein based on gene annotations (http://www.broad.mit.edu/annotation/genome/aspergillus_group/MultiHome.html)

^b BlastP (Altschul et al., 1990) of *A. fumigatus* protein against predicted *A. nidulans* sequence

Table S3. Comparison of peroxins of *A. fumigatus* and *A. nidulans*.

Gene	gene locus ^a	deletion strain	<i>A. fumigatus</i> ortholog ^a	% identity (similarity) ^b	length ^a	proposed function
<i>pexC</i>	AN2281.4	<i>pexC::bar</i>	AFUA_5G06300	70 (82)	530	peroxisome membrane biogenesis
<i>pexE</i>	AN10215.4	Δ <i>pexE</i>	AFUA_8G05240	71 (76)	655	PTS1 protein import receptor
<i>pexF</i>	AN2925.4	<i>pexF23</i>	AFUA_3G08000	78 (87)	1476	AAA ATPase, Pex5 recycling
<i>pexG</i>	AN0880.4	<i>pexG14</i>	AFUA_1G15400	85 (92)	355	PTS2 protein import receptor
<i>pexK</i>	AN1921.4	Δ <i>pexK</i>	AFUA_6G07740	87 (96)	235	peroxisome proliferation
<i>antA</i>	AN0257.4	<i>antA15</i>	AFUA_1G03440	83 (91)	336	peroxisomal ATP carrier

^a predicted gene and length of protein based on gene annotations (http://www.broad.mit.edu/annotation/genome/aspergillus_group/MultiHome.html)

^b BlastP (Altschul et al., 1990) of *A. fumigatus* protein against predicted *A. nidulans* sequence

Table S4. Strains used in this study

	genotype	reference
<i>Aspergillus fumigatus</i>		
ATCC46645	wild type (wt)	American type culture
Δ sidH	wt, sidH::hph/tk	(Yasmin et al., 2011)
Δ sidF	wt; sidF::hph	(Schrettl et al., 2007)
Δ sidI	wt; sidI::hph/tk	(Yasmin et al., 2011)
sidI-gfp	wt; sidI::sidI-gfp, ptrA	this study
gfp-sidH	Δ sidH, gfp-sidH(p), ptrA(p)	this study
gfp-sidF	Δ sidF, gfp-sidF(p), ptrA(p)	this study
gfp-sidH Δ PTS1	Δ sidH, gfp-sidH Δ PTS1(p), ptrA(p)	this study
gfp-sidH/rfp-pts1	gfp-sidH, rfp-pts1(p), phleo(p)	this study
<i>Aspergillus nidulans</i>		
WGTRAN, wt	argB2::pTran, argB; bgA0; biA1	(Brakhage & Turner, 1992)
pexC::bar	biA1 niiA4 pyroA4 pexC::bar	(Hynes et al., 2008),
Δ pexE	yA1, su-ad ad E20, pyroA4, areA102, riboB2,	(Hynes et al., 2008),
pexF23	yA1, pabaAI, PexF(6)23	(Hynes et al., 2008), BU223
pexG14	yA1, pabaAI, PexG(7)14	(Hynes et al., 2008), BU414
Δ pexK	yA1, su-ad ad E20, pyroA4, riboB2, areA102	(Hynes et al., 2008),
pexK-gfp	nkuAD pyroA4, pexK::gfp(PyroA+)	(Hynes et al., 2008),
pexCc	pexC::bar, pl4(pyroA+) pexC	Hynes unpublished,
pexEc	Δ pexE, pl4(pyroA+) pexE	Hynes unpublished,
Δ pexE/pexF23	yA1, pabaAI, pyroA4, PexG14, pexE(5) Δ (ribo+)	Hynes unpublished,
wt gfp-sidH	wt, gfp-sidH(Af)(p); ptrA(p)	this study
Δ pexE gfp-sidH	Δ pexE gfp-sidH(Af)(p), ptrA(p)	this study
pexG14 gfp-sidH	pexG14 gfp-sidH(Af)(p), ptrA(p)	this study
wt gfp-sidI(Nc)	wt, gfp-sidI(Nc)(p), ptrA(p)	this study
Δ pexE gfp-sidI(Nc)	Δ pexE gfp-sidI(Nc)(p), ptrA(p)	this study
Δ pexE/pexF23	yA1, pabaAI, pyroA4, PexG14, pexE(5) Δ (ribo+)	Hynes unpublished,
antA15	yA1, pabaAI, antA15	(Hynes et al., 2008),
wt sidI-gfp	wt, sidI-gfp(Af)(p); phleo(p)	this study
pexG14 sidI-gfp	pexG14 sidI-gfp(Af)(p), phleo(p)	this study
<i>Neurospora crassa</i>		
74-OR23-1A(FGSC)	wild type (A)	American type culture

Table S5. Plasmids used in this study

Name	purpose	reference
psidD-COS	cosmid carrying <i>sidD</i> and adjacent <i>sidH</i> and <i>sidF</i>	(Schrettl et al., 2007)
pPhleo	the phleomycin resistance marker gene <i>ble</i> in bluescript	(Punt et al., 1987)
pUCGH	carrying EGFP-encoding gene	(Langfelder et al., 2001)
pCAME703-AoHapX-full	expression vector for N-terminal GFP tagging	(Goda et al., 2005)
pSK275	pyrithiamine resistance gene <i>ptrA</i>	(Kubodera et al., 2000)
pGfp	EGFP-encoding gene in pGEM-5zf(+)	this study
pSidI-Gfp	C-terminal EGFP tagged <i>A. fumigatus</i> SidI truncation in pGEM-5zf(+)	this study
pSPGfp	C-terminal EGFP tagged <i>A. fumigatus</i> SidI truncation and resistance gene <i>ptrA</i>	this study
psidH ^c	functional <i>sidH</i> copy for complementation	this study
pGFP-SidH	expression plasmid encoding N-terminal GFP-tagged <i>A. fumigatus</i> SidH	this study
pGFP-SidH Δ PTS1	expression plasmid encoding N-terminal GFP-tagged <i>A. fumigatus</i> SidH lacking PTS1	this study
pGFP-SidF	expression plasmid encoding N-terminal GFP-tagged <i>A. fumigatus</i> SidF	this study
pGFP-NcSidI	expression plasmid encoding N-terminal GFP-tagged <i>N. crassa</i> SidI	this study
pSidI-Gfp2	Expression plasmid encoding C-terminal GFP-tagged <i>A. fumigatus</i> SidI (full length)	this study
pSK379-RFP-PTS1	Expression plasmid encoding RFP-PTS1	(Beck & Ebel, 2013)

Table S6. Oligonucleotides used in this study.

primer	sequence 5' to 3'
oAf538RAC1-f	CAC TGT GGC TTT CGC TTC
oGFP1	GAC CAC ATG AAG CAG CAC
ogfp4	AAC CAC TAC CTG AGC AAC
osidHgfp1	ACA <u>AGA TCT</u> TCA ACA GAA GCA CAT CCA AC
osidHgfp2	CAC <u>GCG GCC GCT</u> CAC CTC CAC TGT GGC TTT CG
osidHgfp3	CTG <u>GCG GCC GCT</u> TCA CAA CTT AGA CGG CC
ogfpsidF2	ATA <u>GCG GCC GCT</u> TAC AGC TTT GCA TCC CAC
ogfpsidF3	ATA <u>GGA TCC GCG</u> ACT CAA AGC AGC ACC
oAf ₅₃₈ AT1-r	AGT TTT GAG CGA GAG GGG
oAfsidI-1	CAG <u>TGG ATC CTT</u> TTT CCT TTA TTG CTG CC
oAfsidI-2	CTA CGG ATG TCT GCA ACC
oNcSidI-1	AAT AAT <u>AGA TCT</u> TCA CCG GCG TTG GTA
oNcSidI-2	TTA <u>GCG GCC GCT</u> CCA TCT TCT TCC CTC AG
oAfsidIgfp1	ATA <u>GAT ATC</u> AGC CGG ACA AGA AAC AAG
oAfsidIgfp2	TAT <u>ATC GAT</u> GTT TGT GGT TTC CGT TGG

Introduced restriction sites are underlined.

Table S7. Homologs of *Basidiomycetes* to *A. fumigatus* SidF identified by a BlastP search (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi?organism=fungi).

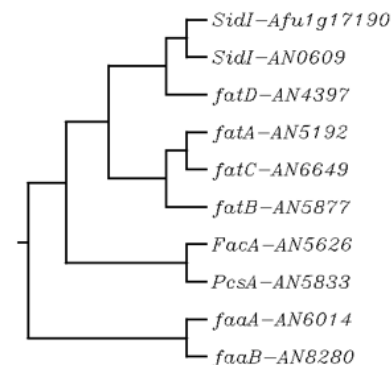
Protein		Score (Bits)	E Value
XP_001731607.1	[<i>Malassezia globosa</i>]	205	3e-59
XP_757579.1	[<i>Ustilago maydis</i>]	182	1e-50
EGO03326.1	[<i>Serpula lacrymans</i> var. <i>lacrymans</i>]	173	9e-48
XP_003035019.1	[<i>Schizophyllum commune</i>]	171	4e-47
XP_003322498.1	[<i>Puccinia graminis</i> f. sp. <i>tritici</i>]	163	1e-44
EGG01975.1	[<i>Melampsora larici-populina</i>]	158	1e-43
XP_002910369.1	[<i>Coprinopsis cinerea</i> okayama]	155	1e-40
XP_756191.1	[<i>Ustilago maydis</i>]	147	4e-38

Figure S1. Similarity of *A. fumigatus* SidI with proven and putative Acyl-CoA synthases from *A. nidulans* (Reiser et al., 2010). FaaB, FatA, FatB, FatC and FatD have been shown to, while PcsA is predicted to, localize to peroxisomes (A) BlastP search results (<http://www.ncbi.nlm.nih.gov/blast/>) using *A. fumigatus* SidI as bait; functionally proven acyl-CoA synthases are in bold; (B) Phylogenetic tree (<http://www.genome.jp/tools-bin/clustalw>); (C) Alignment of *A. fumigatus* SidI and the peroxisomal *A. nidulans* acyl-CoA synthase FatD.

A

Protein	Score (Bits)	E- Value
SidI-AN0609 (XP_658213.1)	804	0.0
FatD-AN4397 (XP_662001.1)	164	3e-44
FatB-AN5877 (XP_663481.1)	97.1	2e-21
FacA-AN5626 (XP_663230.1)	86.7	5e-18
FatA-AN5192 (XP_662796.1)	87.4	3e-18
PcsA-AN5833 (XP_663437.1)	80.9	3e-16
FaaB-AN8280 (XP_681549.1)	57.8	6e-09
FaaA-AN6014 (XP_663618.1)	56.6	1e-08
FatC-AN6649 (XP_664253.1)	50.4	9e-07

B



C

Identities = 147/523 (28%), Positives = 236/523 (45%), Gaps = 55/523 (11%)

AfSidI	66	TYTDLKDEADRVARGLLAMGIQKGRIGIMAGNCEQYISVFFAAARVGGAILVVLNNTYTP	125
		TY L L +G+ G + + N ++I F AA+ AI LN Y	
AnFatD	28	TYAQLHSHISAFQEKLAKLGVGHGAAVNLALINSYEFIVGFLLAASWQRAIAAPLNPAYKQ	87
AfSidI	126	SELYYALGHGTDCRLLFLTPRIGRHSLEEVLAQLGPRPKEQGTSSALEEIIILRGQYSGFS	185
		E + + L+ L PR E A+ GP + + I ++G	
AnFatD	88	DEFEFYIDDLSSSTLV-LIPR-----ESYAQNGPAV----LAGRKYQAAIAECYWNGTE	135
AfSidI	186	TYEHVIQRGLPLPSHALQDREAELHSTDVCNLQFTSGSTGNPKAAMLTHHNLVNNRSRFIG	245
		V + G + + A+ D+ + TSG+TG PKA LTH NL R I	
AnFatD	136	VVLVDVKELGKLGKGDIGVQTAQ--PDDIALVLHTSGTTGRPKAVPLTHKNLTTTMRNIQ	193
AfSidI	246	DRMNLTSFDILCCPPPLFHCFLVGLMLAVVTHGSKIIFPSETFDPTAVLHAISDEKCTA	305
		LT D PLFH GL+ LA + G +I P++ F + K	
AnFatD	194	ATYKLTPODRTYLVMLPLFHVHGLLAFLAPLASGGSVIVPTK-FSAHQFWSDFIEYKANW	252
AfSidI	306	LHGVPMTFEAILSLPKP-PNFDCSNLRTGIIAGAPVPRPLMKRLLLEELNMT--EYTSSY	361
		VPT+ + +L P P P +R+ PL + ++L T +Y	
AnFatD	253	YSAVPTIHQILLKSLPNPIQIRFIRS-----CSSPLSPKTFQDLEKTLNAPVLEAY	305
AfSidI	362	GLTEASPTCFNALTTDSI---ERRLTVGKVMPHAKAKIIDTQGHIVPIGQRGELCIAGY	418
		+TEA+ + +T++ + +R+ +VG + + KI+D G+ VP G E+C+ G	
AnFatD	306	AMTEAA----HQMTSNLPLPAKRQPGSVG-IGQGVEIKILDQSGNEVPQGHEAECVIRGE	360
AfSidI	419	QLTKGYWNNPEKTAELITDSGVTWLKTGDEAIFDEEGYCSITGRFKDIIIRGENIYP	478
		+TKGY NNP + D + +TGD+ D +GY ITGR K++I +GGE I P	
AnFatD	361	NVTKGYLNNPAANKSFTKDG----FFRTGDQGGKDPDGYVITGRIKELINKGGEKISP	416
AfSidI	479	LEIEERLAAHPAIEVASVIGIPDQ-KYGEVVGAFLLAADVARSDEELRAWTRETTLGR	537
		+E++ L +P + A IPD YGE +GA + L + +A +++EL++W +E L +	
AnFatD	417	IELDNTLLQNPVGEAVCFaipDPGHYGEDIGAAVVLKSGQNA--TEDELKSWVQEKLAK	474
AfSidI	538	HKAPQYFFVFGEEGVDRITIPVTGSGKVRKVDLRKIAASVLERR	580
		K P+ + IP T +GK+++ RK+A ++L+ +	
AnFatD	475	FKTPK-----QQIPKTATGKIQR---RKVAEAMLPKPK	503

Figure S2. Alignment of *A. fumigatus* SidH and the mitochondrial localized enoyl-CoA hydratase EchA from *A. nidulans* that is involved in β -oxidation (Maggio-Hall & Keller, 2004). Homologs with PTS1 motifs have been postulated to be involved in peroxisomal β -oxidation (Maggio-Hall & Keller, 2004).

BlastP: Score = 228, E-value = 2e-73

Identities = 123/279(44%), Positives = 167/279(59%), Gaps = 15/279(5%)

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Af SidH 1    MSTEAHPTVQGCLVSFPTPHI-----LLLTLNRPEKRNCSLATS AEIQRLWTFD TQ 53
          + + A P+ + L S P P +      +++TLNRP+ N +S      E+      + +D
An EchA 23   LYSSAAPS YEHI LTSTPKPGVGLNTDFTVIVTLNRPKALNALSSPLFKEVNDALSKYDES 82

Af SidH 54   PALYVAIIITGTGESFCAGADLKEWNDLN-ARGITNEMTAP--GLAGLPRRRGSKPIIAAV 110
          + IITG+ ++F AGAD+KE L A +N AP LA R      KP+IAAV
An EchA 83   KDIGAI IITGSEKAF AAGADIKEMAPLTFASAYSNNFIAPWSHLANSIR----KPVIAAV 138

Af SidH 111  NGYCLGGGFEMVANCDIVVASENATFGLPEVQRGIAAVAGSLPRLVRLV LGQRAAEIALS 170
          +G+ LGGG E+   CDI+ + +ATFG PE++ G+   AG   RL   +GK +A E+ L+
An EchA 139  SGFALGGGCE LALMCDIIYCTASATFGQPEIKLGVIPGAGGSQRLTAAV GSKAMELILT 198

Af SidH 171  GLSF S ASQLERWGLVNRVVE--HDQLLATAVEIATAISRNSPDSVRVTMEGLHYGWEMAS 228
          G +FS +   WG+ +VV+ ++LL AV+ A I+ S +   E ++ ++
An EchA 199  GKNFSGKEAGEWGVAAKVVDGGKEELLEAVKTAETIAGYSRVATVAAKEVVNKSQDLGV 258

Af SidH 229  VE--EASSALV-----DQWYAKLMA-----GENFHEGVRAFVEKRKPQW 265
          E E   L       DQ   + ++       G + +G+ AF EK+KPQW
An EchA 259  REGVEYERRLFHGLFGSQDQKIGRFLSLYHYSKNGTDGEKGMTAF AEKKKPQW 311

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