

## **Supplementary material**

### **Title**

A carrier fusion significantly induces unfolded protein response in heterologous protein production by *Aspergillus oryzae*

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## Supplementary figure legends

**Fig. S1** Southern analysis for confirming single copy integration of the plasmids at *niaD* locus. The genomic DNAs were digested with *SalI*, and hybridized with the probe of the 3' end of *niaD* gene.

**Fig. S2** Quantitative RT-PCR analysis of the *CHY* gene. The relative quantity of the *CHY* transcripts was estimated using the  $\beta$ -actin gene transcripts as a reference in each strain. The mean values and standard errors based on three independent experiments are represented.

**Table S1** Primers used in this study

Primer (Purpose)	Sequence
(Plasmid construction)	
pAmyB-cEC-F	GGGG <u>ACAAGTTTGTACAAAAAAGCAGGCT</u> CCAGTGAATTCATGGTGTTTTGATCATTTTAAATTTTAT
sAmyB-prochymosin-R	CCTGGTGATCTCAGCAGCCAAAGCAGGTGCCGC
sAmyB-prochymosin-F	GCACCTGCTTTGGCTGCTGAGATCACCAGGATCCC
prochymosin-cEC-R	GGGG <u>ACCACTTTGTACAAGAAAGCTGGGT</u> TTCAGATGGCTTTGGCCAGCC
pAmyB-ORFst-cEC-R	GGGG <u>ACCACTTTGTACAAGAAAGCTGGGT</u> TTCACGAGCTACTACAGATCTTGCTAC
(Quantitative RT-PCR)	
RealAobipA-F	ACTGGCGGTGTCATGACTAA
RealAobipA-R	GCCTCGATCTTGCTCTTGAT
RealAoclxA-F	TGCTCCTATGATCGACAACC
RealAoclxA-R	GACCTCAGGAGCTTGCTTAA
Aopdi(1681)-F	CACTGGCGTACATTTTCGC
Aopdi(-2093)-R	CCATGGCGCATAAAATTCGAGAA
RealActin-F	CCGTGATTTGACCGATTACC
RealActin-R	TCTGCATACGATCGGAGATG
RealCHY-F	TACATGGACAGGAATGGCCA
RealCHY-R	TGGAAGCCACTGGTACAGAA
(RT-PCR)	
RTAohacA5'	TCCTCCGACGTGGCACAACA
RTAohacA3'	CACGGTGCATTCATCCGTTGAACC

*attB* sites required for the BP clonase reaction are underlined.

**Table S2** Up-regulated genes ( $\geq 1.5$  fold) in the strain expressing the carrier-fused CHY (categories [O][U])

Gene ID	Category	cfCHY7 (fold change)	nfCHY3-1 (fold change)	Product (with similarity)
<b>Protein folding</b>				
AO080505000236	O	5.6 ± 0.6	1.6 ± 0.2	Thioredoxin protein disulfide isomerase, PrpA
AO080511000173	O	3.7 ± 0.5	1.1 ± 0.2	ER Hsp70, Lhs1p
AO080523000842	O	3.3 ± 0.5	1.2 ± 0.3	Molecular chaperone (DnaJ superfamily), Scj1p
AO080509000043	UO	3.2 ± 0.6	1.4 ± 0.2	ER oxidising protein, Ero1p
AO080503000248	O	2.5 ± 0.4	1.2 ± 0.2	ER chaperone BiP, BipA
AO080515000303	O	2.1 ± 0.3	1.1 ± 0.1	Calnexin, ClxA
AO080503000037	O	2.1 ± 0.2	1.1 ± 0.1	Zuotin and related molecular chaperones, contains DNA-binding domains
AO080546000193	O	1.9 ± 0.2	1.2 ± 0.2	Peptidyl prolyl isomerases, CypB
AO080554000328	O	1.9 ± 0.1	1.2 ± 0.1	Protein disulfide isomerase, TigA
AO080569000121	O	1.7 ± 0.3	1.2 ± 0.1	Protein disulfide isomerase, PdiA
AO080536000177	O	1.7 ± 0.2	1.2 ± 0.1	DnaJ-class molecular chaperone with C-terminal Zn finger domain
<b>Glycosylation</b>				
AO080546000311	O	2.6 ± 0.1	1.3 ± 0.1	Oligosaccharyltransferase, delta subunit (ribophorin II), Swp1p
AO080559000057	MOG	2.5 ± 0.5	1.2 ± 0.3	Glucosidase II catalytic (alpha) subunit and related enzymes, Rot2p
AO080541000070	O	2.5 ± 0.2	1.2 ± 0.0	Oligosaccharyltransferase subunit, Stt3p
AO080527000460	O	2.4 ± 0.3	1.2 ± 0.1	Oligosaccharyltransferase, beta subunit Wbp1p
AO080569000083	O	2.3 ± 0.2	1.2 ± 0.1	Oligosaccharyltransferase, alpha subunit (ribophorin I), Ost1p
AO080569000135	O	2.1 ± 0.1	1.2 ± 0.1	Oligosaccharyltransferase, gamma subunit, Ost3p
AO080527000100	O	1.7 ± 0.2	1.0 ± 0.0	Dolichyl-phosphate-mannose:protein O-mannosyl transferase, PmtB
AO080531000224	O	1.7 ± 0.3	1.1 ± 0.1	Dolichyl-phosphate-mannose:protein O-mannosyl transferase, PmtC
<b>Translocation/Signal peptidase complex</b>				
AO080559000021	U	3.0 ± 0.7	1.3 ± 0.3	Signal peptidase complex subunit, Spc3p
AO080541000046	U	2.7 ± 0.2	1.2 ± 0.1	ER protein-translocation complex subunit, Sec66p
AO080511000187	O	2.6 ± 0.4	1.2 ± 0.2	ER protein-translocation complex subunit, Sec63p
AO080532000162	U	2.3 ± 0.2	1.2 ± 0.1	Membrane component of ER protein translocation complex, Sec62p
AO080555000180	U	2.3 ± 0.0	1.2 ± 0.1	Signal peptidase I, Sec11p
AO080551000052	UO	2.0 ± 0.3	1.1 ± 0.1	Transport protein, alpha subunit, Sec61p
AO080559000093	U	1.5 ± 0.1	1.1 ± 0.0	Signal recognition particle, subunit Srp54p
<b>Proteolytic degradation</b>				
AO080509000069	O	2.7 ± 0.2	1.1 ± 0.1	E3 ubiquitin ligase, Hrd1p
AO080538000111	O	1.9 ± 0.3	1.2 ± 0.1	Non-canonical ubiquitin conjugating enzyme 1, Ubc6p
<b>Vesicle trafficking/transport</b>				
AO080551000113	U	2.8 ± 0.5	1.2 ± 0.3	COPII vesicle protein, Erv41p
AO080559000147	U	2.6 ± 0.2	1.2 ± 0.1	ER lumen protein retaining receptor, Erd2p
AO080533000237	U	2.6 ± 0.2	1.2 ± 0.1	Putative cargo transport protein, Erv29p

(continued)

AO080511000222	U	2.3 ± 0.3	1.0 ± 0.2	Vesicle coat complex COPII subunit, Sfb3p
AO080538000044	U	2.2 ± 0.2	1.1 ± 0.2	Lectin VIP36, involved in the transport of glycoproteins carrying high mannose-type glycans, Emp47p
AO080532000140	U	2.1 ± 0.1	1.0 ± 0.2	Vesicle coat complex COPII subunit, Sec31p
AO080549000318	U	2.1 ± 0.2	1.0 ± 0.1	Vesicular amine transporter, Dtr1p
AO080532000225	U	2.1 ± 0.1	1.2 ± 0.0	Lectin VIP36, involved in the transport of glycoproteins carrying high mannose-type glycans, Uip5p
AO080549000116	U	1.9 ± 0.1	1.1 ± 0.2	R-SNARE protein, Sec22p
AO080508000439	U	1.9 ± 0.2	1.2 ± 0.1	Guanine nucleotide exchange factor, Sec7p
AO080503000388	U	1.9 ± 0.2	1.0 ± 0.1	Vesicle coat complex COPII subunit, Sec24p
AO080508000442	U	1.9 ± 0.1	1.1 ± 0.0	Golgi SNAP receptor complex member, Bos1p
AO080541000400	U	1.9 ± 0.1	1.2 ± 0.1	Endosomal membrane proteins, Emp70p
AO080525000488	U	1.8 ± 0.3	1.0 ± 0.2	ER-Golgi vesicle-tethering protein, Uso1p
AO080523000551	U	1.7 ± 0.1	1.0 ± 0.1	Vesicle coat complex COPI, beta subunit, Sec27p
AO080503000458	U	1.7 ± 0.1	1.0 ± 0.1	Endosomal membrane protein, Emp70p
AO080509000173	U	1.7 ± 0.2	1.1 ± 0.0	Vesicle coat complex COPII subunit, Sec23p
AO080511000079	U	1.7 ± 0.2	1.1 ± 0.2	Medium subunit of clathrin adaptor complex, Ret2p
AO080559000026	U	1.7 ± 0.2	1.1 ± 0.1	p24 family of membrane trafficking proteins, Emp24p
AO080532000390	U	1.7 ± 0.1	1.0 ± 0.1	Vesicle coat complex COPI, gamma subunit, Sec21p
AO080533000268	U	1.7 ± 0.1	1.2 ± 0.2	Putative cargo transport protein (p24 protein family) Emp24p
AO080546000006	U	1.6 ± 0.2	1.0 ± 0.3	Vesicle coat complex COPII subunit, Sec13p
AO080533000298	U	1.6 ± 0.1	1.0 ± 0.2	Vesicle coat complex COPI, beta subunit, Sec26p
AO080546000164	U	1.6 ± 0.1	1.1 ± 0.1	p24 family of membrane trafficking proteins, Emp24p
AO080551000043	U	1.6 ± 0.1	1.0 ± 0.0	Exocyst complex subunit, Sec8p
AO080550000139	U	1.5 ± 0.3	1.0 ± 0.2	Vesicle trafficking protein, Sec1 family, Sly1p
AO080509000134	O	1.5 ± 0.1	1.1 ± 0.1	AAA+-type ATPase, NsfA
Others				
AO080511000433	O	2.0 ± 0.4	1.3 ± 0.2	Dipeptidyl aminopeptidase, Ste13p
AO080554000284	U	1.8 ± 0.2	1.0 ± 0.1	Pattern-formation protein guanine nucleotide exchange factor, Gea2p
AO080515000279	O	1.7 ± 0.1	1.1 ± 0.2	Kexin-like processing protease, KexB
AO080512000033	OE	1.7 ± 0.1	1.2 ± 0.1	Serine carboxypeptidases, Kex1p
AO080555000236	O	1.7 ± 0.4	1.2 ± 0.3	Membrane protease subunits, stomatin prohibitin homologs

Values with fold changes ( $\geq 1.5$ ) are indicated by a shadow.

Gene IDs are referred in the *A. oryzae* genome database (<http://nribf21.nrib.go.jp/CFGD/gnm.cgi?prj=02201&gnm=ao0-1>), in which each gene ID can be converted to another ID accessible in other databases DOGAN (<http://www.bio.nite.go.jp/dogan/project/view/AO/>) / NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucore&itool=toolbar>).

**Table S3** Up-regulated genes ( $\geq 1.5$  fold) in the strain expressing the carrier-fused CHY (except for categories [O][U])

Gene/EST ID	Category	Fold change	Product (with similarity)
B, Chromatin structure and dynamics (1 gene)			
AO080508000293	KB	2.2 $\pm$ 0.6	NAD-dependent histone deacetylases and class I sirtuins (SIR2 family)
J, Translation, ribosomal structure, and biogenesis (4 genes)			
AO080525000379	J	2.8 $\pm$ 0.8	Cytoplasmic tryptophanyl-tRNA synthetase
AO080527000011	J	2.4 $\pm$ 0.3	Mitochondrial polypeptide chain release factor
AO080503000311	J	2.1 $\pm$ 0.3	Predicted translation elongation factor
AO080555000204	J	1.8 $\pm$ 0.2	Putative translation initiation inhibitor, yjgF family
K, Transcription (3 genes)			
AO080508000293	KB	2.2 $\pm$ 0.6	NAD-dependent histone deacetylases and class I sirtuins (SIR2 family)
AO080527000512	K	1.7 $\pm$ 0.3	Transcriptional coactivator p100
AO080531000200	KTI	1.5 $\pm$ 0.1	Serine threonine protein kinase TGF-beta stimulated factor
L, Replication, recombination, and repair (1 gene)			
D, Cell cycle control, cell division, chromosome partitioning (1 gene)			
AO080515000316	LD	1.8 $\pm$ 0.2	Checkpoint 9-1-1 complex, RAD1 component
M, Cell wall/membrane/envelope biogenesis (3 genes)			
AO080559000057	MOG	2.5 $\pm$ 0.7	Glucosidase II catalytic (alpha) subunit and related enzymes, glycosyl hydrolase family 31, Rot2p
AO080568000078	M	2.1 $\pm$ 0.2	Glycosyltransferase, Alg11p
AO080546000336	M	1.5 $\pm$ 0.1	Ankyrin
T, Signal transduction mechanisms (6 genes)			
AO080511000443	T	2.5 $\pm$ 0.2	Phosphoinositide-specific phospholipase C
AO080508000102	T	2.4 $\pm$ 0.5	Protein kinase C substrate, 80 kDa protein, heavy chain
AO080525000619	TZ	1.7 $\pm$ 0.2	Adenylate cyclase-associated protein, Srv2p/CapA
AO080532000630	T	1.7 $\pm$ 0.1	Phosphatidylinositol 4-kinase, Pik1p
AO080550000176	T	1.6 $\pm$ 0.2	RhoGEF GTPase
AO080531000200	KTI	1.5 $\pm$ 0.1	Serine threonine protein kinase TGF-beta stimulated factor
V, Defense mechanisms (2 genes)			
AO080527000010	V	3.6 $\pm$ 0.3	dsRNA-activated protein kinase inhibitor P58, contains TPR and DnaJ domains
AO080523000686	V	1.6 $\pm$ 0.3	Pathogenicity protein in <i>M. grisea</i> , HVA22 DP1 gene product-related proteins
Z, Cytoskeleton (4 genes)			

AO080530000106	Z	1.9 ± 0.2
AO080525000619	TZ	1.7 ± 0.2
AO080518000140	Z	1.7 ± 0.2
AO080508000114	Z	1.5 ± 0.2

Actin-binding protein Coronin, contains WD40 repeats  
 Adenylate cyclase-associated protein, Srv2p/CapA  
 Type V myosin motors involved in actin-based transport of cargos, Myo2p  
 Cyclopropane fatty acid synthase and related methyltransferase

C, Energy production and conversion (2 genes)

AO080508000028	CH	3.8 ± 0.6
AO080568000072	CE	1.6 ± 0.3

2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase  
 Aconitase homoaconitase (aconitase superfamily)

E, Amino acid transport and metabolism (14 genes)

AO080529000045	E	51.9 ± 51.4
AO080502000018	E	6.4 ± 2.5
AO080527000070	E	2.9 ± 0.6
AO080503000294	E	2.8 ± 0.2
AO080511000298	E	1.9 ± 0.2
AO080511000257	E	1.9 ± 0.3
AO080512000033	OE	1.7 ± 0.1
AO080506000265	E	1.7 ± 0.3
AO080510000033	E	1.7 ± 0.2
AO080527000019	E	1.7 ± 0.4
AO080523000568	E	1.7 ± 0.3
AO080568000072	CE	1.6 ± 0.3
AO080511000245	E	1.6 ± 0.1
AO080503000469	E	1.5 ± 0.2

Cysteine dioxygenase Cdo1p  
 Lysine-ketoglutarate reductase saccharopine dehydrogenase  
 Lactoylglutathione lyase and related lyases  
 Glutamate decarboxylase and related PLP-dependent protein  
 Vesicle coat complex COPI, alpha subunit  
 Tryptophan synthase beta chain  
 Serine carboxypeptidases, Kex1p  
 Alpha-isopropylmalate synthase homocitrate synthase  
 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase  
 Cystathionine beta-lyases cystathionine gamma-synthases  
 Branched chain aminotransferase BCAT1, pyridoxal phosphate enzymes type IV superfamily  
 Aconitase homoaconitase (aconitase superfamily)  
 Glycine serine hydroxymethyltransferase  
 Histidinol dehydrogenase

F, Nucleotide transport and metabolism (1 gene)

AO080508000261	F	4.5 ± 1.0
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Thiamine pyrophosphokinase

G, Carbohydrate transport and metabolism (10 genes)

AO080533000229	G	9.1 ± 3.0
AO080515000391	G	5.3 ± 0.9
AO080551000042	G	4.9 ± 2.1
AO080503000057	G	3.2 ± 0.9
AO080532000321	G	2.7 ± 0.6
AO080523000493	G	2.7 ± 0.5
AO080559000057	MOG	2.5 ± 0.7
AO080549000120	G	2.2 ± 0.2
AO080536000198	G	2.0 ± 0.2

Maltase, MalT  
 UDP-galactose transporter related protein, Hut1p  
 Endoglucanase, Expansin 45  
 1, 2-alpha-mannosidase, Mns1p  
 Glycosyltransferase, Alg7p  
 1,6-glucan synthesis, Kre5p  
 Glucosidase II catalytic (alpha) subunit and related enzymes, glycosyl hydrolase family 31, Rot2p  
 Glucosidase I, Glc1p/Der7p/Cwh41p  
 Glycosyl hydrolase, family 47, Htm1p

AO080513000194	G	1.9 ± 0.6	Permease of the major facilitator superfamily
H, Coenzyme transport and metabolism (7 genes)			
AO080515000091	H	6.3 ± 1.8	Geranylgeranyl pyrophosphate synthase Polyprenyl synthetase
AO080530000036	H	5.9 ± 1.3	Pyridoxamine-phosphate oxidase
AO080508000028	CH	3.8 ± 0.6	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase
AO080508000470	H	1.7 ± 0.1	5-formyltetrahydrofolate cyclo-ligase
AO080549000204	H	1.7 ± 0.3	Geranylgeranyl pyrophosphate synthase Polyprenyl synthetase
AO080536000001	H	1.5 ± 0.3	S-adenosylhomocysteine hydrolase
AO080555000165	H	1.5 ± 0.2	Protoporphyrinogen oxidase
I, Lipid transport and metabolism (7 genes)			
AO080527000319	I	2.0 ± 0.5	sn-1,2-diacylglycerol ethanolamine- and cholinephosphotranferase
AO080561000013	I	2.0 ± 0.1	C-8,7 sterol isomerase
AO080541000276	I	2.0 ± 0.6	Uncharacterized conserved protein
AO080522000083	I	1.9 ± 0.1	Predicted undecaprenyl diphosphate synthase
AO080503000260	I	1.6 ± 0.2	Vigilin
AO080550000059	IQR	1.6 ± 0.1	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
AO080531000200	KTI	1.5 ± 0.1	Serine threonine protein kinase TGF-beta stimulated factor
P, Inorganic ion transport and metabolism (6 genes)			
AO080527000184	P	3.0 ± 0.6	Acetyl-CoA transporter
AO080503000051	P	2.4 ± 0.2	Ca <sup>2+</sup> transporting ATPase
AO080569000091	P	2.2 ± 0.4	Ca <sup>2+</sup> transporting ATPase, Pmr1p/Ssc1p
AO080555000247	P	2.1 ± 0.2	P-type ATPase
AO080532000231	P	1.7 ± 0.0	Cl <sup>-</sup> channel CLC-3 and related proteins (CLC superfamily), Gef1p
AO080513000234	P	1.7 ± 0.2	Protein involved in inorganic phosphate transport
Q, Secondary metabolites biosynthesis, transport, and catabolism (6 genes)			
AO080513000324	Q	2.4 ± 0.6	Multidrug resistance-associated protein mitoxantrone resistance protein, ABC superfamily
AO080538000067	Q	2.2 ± 0.2	Polyketide synthase modules and related proteins
AO080551000144	Q	2.1 ± 0.4	Polyketide synthase modules and related protein
AO080527000049	Q	1.9 ± 0.3	Amidases related to nicotinamidase
AO080541000209	Q	1.7 ± 0.1	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
AO080550000059	IQR	1.6 ± 0.1	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
R, General function prediction only (20 genes)			
AO080515000008	R	6.6 ± 0.4	Predicted dehydrogenase and related protein



AO080521000329	R	5.7 ± 2.4	Predicted oxidoreductase related to nitroreductase
AO080550000112	R	4.9 ± 0.3	Predicted epimerase, PhzC PhzF homolog
AO080531000026	R	4.2 ± 2.0	Zn-dependent hydrolase, including glyoxylase
AO080511000118	R	3.2 ± 0.8	Permease of the major facilitator superfamily
AO080561000011	R	2.4 ± 0.5	Predicted Rossmann fold nucleotide-binding protein
AO080533000323	R	2.4 ± 0.8	FOG: WD40 repeat
AO080522000080	R	2.2 ± 0.3	Sortilin and related receptor
AO080549000113	R	2.2 ± 0.2	Transmembrane protein
AO080521000222	R	2.1 ± 0.6	Predicted oxidoreductase related to nitroreductase
AO080551000060	R	2.1 ± 0.1	Glycosyltransferase, Alg5p
AO080513000133*	R	2.0 ± 0.2	Predicted hydrolases or acyltransferase (alpha beta hydrolase superfamily)
AO080554000369	R	2.0 ± 0.4	GTP-binding protein GP-1
AO080511000027	R	2.0 ± 0.3	Putative steroid membrane receptor Hpr6.6 25-Dx
AO080570000046	R	2.0 ± 0.4	Leucine-rich repeat protein
AO080505000001	R	1.8 ± 0.2	Dolichol phosphate mannose synthase, DpmA
AO080536000025	R	1.6 ± 0.2	Aldo keto reductase family proteins, Gcy-3
AO080532000324	R	1.6 ± 0.1	PHD Zn-finger protein
AO080550000059	IQR	1.6 ± 0.1	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) Type I PKS cluster
AO080523000602	R	1.6 ± 0.3	Multifunctional pyrimidine synthesis protein CAD (includes carbamoyl-phosphate synthetase, aspartate transcarbamylase, and glutamine amidotransferase)
S, Function unknown (10 genes)			
AO080511000193	S	2.3 ± 0.1	Uncharacterized membrane protein
AO080549000053	S	2.2 ± 0.1	Predicted membrane protein
AO080506000107	S	2.2 ± 0.4	Uncharacterized conserved protein
AO080549000147	S	2.1 ± 0.2	Predicted membrane protein
AO080523000460	S	2.1 ± 0.2	Uncharacterized conserved protein
AO080506000053	S	1.8 ± 0.1	Uncharacterized conserved protein
AO080523000524	S	1.7 ± 0.2	Uncharacterized conserved protein
AO080511000404	S	1.7 ± 0.2	Uncharacterized conserved protein
AO080549000009	S	1.6 ± 0.3	Uncharacterized conserved protein
AO080549000031	S	1.5 ± 0.1	Predicted membrane protein
Unclassified for COG (53 genes)			
AO080536000096		24.4 ± 3.5	Predicted protein
AO080536000047		12.6 ± 2.8	Predicted protein
AO080550000107		12.4 ± 1.8	Predicted protein
AO080515000103		9.9 ± 3.7	SAM-dependent methyltransferase

AO080546000314	8.0 ± 6.0	Predicted protein
AO080549000080	8.0 ± 2.2	Predicted protein
AO080529000092	7.6 ± 5.3	Predicted protein
AO080521000219	5.7 ± 3.1	Predicted protein
AO080536000168**	5.6 ± 1.2	Predicted protein
AO080541000476	5.2 ± 1.8	Predicted protein
AO080511000123	4.4 ± 1.0	Transcription factor MEIS1 and related HOX domain protein
AO080542000151	4.2 ± 0.4	Predicted protein
AO080530000061	3.5 ± 1.2	Predicted protein
AO080513000091	3.0 ± 0.6	Predicted protein
AO080509000025	2.8 ± 0.3	Predicted protein
AO080550000041	2.6 ± 0.8	Predicted protein
AO080509000009	2.6 ± 0.3	Predicted protein
AO080511000223	2.5 ± 0.6	Predicted protein
AO080506000048	2.4 ± 0.2	Predicted protein
AO080531000356*	2.4 ± 0.6	Predicted protein
AO080542000060	2.3 ± 0.5	Predicted protein
AO080511000091	2.0 ± 0.1	Predicted protein
AO080546000196	2.0 ± 0.5	Predicted protein
AO080533000236	1.9 ± 0.5	Predicted protein
AO080503000018**	1.9 ± 0.5	Predicted protein
AO080523000670	1.9 ± 0.2	Predicted hydrolase or acyltransferase (alpha beta hydrolase superfamily)
AO080541000064	1.9 ± 0.6	Predicted protein
AO080525000184	1.9 ± 0.2	Predicted protein
AO080503000039	1.8 ± 0.2	Predicted protein
AO080510000034	1.8 ± 0.1	Predicted protein
AO080523000739	1.8 ± 0.1	Predicted protein
AO080523000690	1.8 ± 0.4	Predicted protein
AO080551000112	1.8 ± 0.3	Predicted protein
AO080523000449	1.8 ± 0.2	Predicted protein
AO080538000068	1.8 ± 0.4	Predicted protein
AO080510000088	1.8 ± 0.2	Predicted protein
AO080550000091**	1.8 ± 0.2	Molecular chaperone (small heat shock protein), Hsp30
AO080536000180	1.8 ± 0.4	Predicted protein
AO080528000010	1.7 ± 0.2	Predicted protein
AO080541000063*	1.7 ± 0.4	Predicted protein
AO080523000629	1.7 ± 0.2	Predicted protein
AO080528000075	1.7 ± 0.2	Predicted protein

AO080542000195	1.7 ± 0.4	Predicted protein
AO080559000144	1.7 ± 0.1	Predicted protein
AO080551000097	1.6 ± 0.2	Predicted protein
AO080532000295	1.6 ± 0.1	Predicted protein
AO080518000083	1.6 ± 0.1	Predicted protein
AO080512000002	1.6 ± 0.1	Predicted protein
AO080531000315	1.6 ± 0.1	Predicted protein
AO080533000290	1.6 ± 0.1	Predicted protein
AO080536000211	1.6 ± 0.2	Predicted protein
AO080506000267	1.5 ± 0.2	Predicted protein
AO080515000445	1.5 ± 0.3	Transcriptional activator of the JUN family
Unregistered for COG (18 genes)		
AO080560000038	18.5 ± 4.6	Glutathione S-transferase
AO080529000005	4.7 ± 2.7	Predicted protein
AO080570000057	4.6 ± 1.3	Predicted protein
AO09M000000006	3.4 ± 1.6	Mitochondrial ribosomal protein s5
AO080505000213	2.6 ± 0.3	COPII vesicle protein, Erv46p
AO080531000350	2.6 ± 0.2	Predicted protein
AO080549000153	2.5 ± 0.5	Predicted protein
AO080531000222	2.5 ± 0.2	Protein OS-9, Yos9p
AO080523000808	2.2 ± 0.4	Predicted protein
AO080506000072	2.0 ± 0.3	Defender against cell death protein oligosaccharyltransferase, epsilon subunit
AO080156000007	1.9 ± 0.5	Predicted protein
AO080531000376	1.8 ± 0.2	Predicted protein
AO070329000042	1.7 ± 0.2	Probable protein transport protein SEC61 gamma subunit, Sss1p
AO080522000064	1.6 ± 0.2	Predicted protein
AO080515000444	1.6 ± 0.3	Predicted protein
AO080525000525	1.6 ± 0.3	Predicted protein
AO080525000526	1.6 ± 0.2	Predicted protein
AO080528000058	1.5 ± 0.2	Cysteinyl-tRNA synthetase
Expressed Sequence Tag (20 ESTs)		
AB227117	17.3 ± 4.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3979
AB225486	12.3 ± 6.9	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2339
AB224963	6.7 ± 4.9	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1814
AB223761	3.5 ± 1.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0602
AB226737*	3.4 ± 0.4	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3599

AB224653	3.4 ± 0.7	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1502
AB226737	3.1 ± 0.2	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3599
AB224929	2.8 ± 0.2	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1780
AB226814	2.3 ± 0.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3676
AB229896	2.3 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST6781
AB224800	2.2 ± 0.5	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1650
AB230226	2.1 ± 0.7	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST7114
AB223621	2.1 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0459
AB227435*	2.0 ± 0.7	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4302
AB227494	2.0 ± 0.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4362
AB225433	1.8 ± 0.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2286
AB230786	1.8 ± 0.4	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST7676
AB228228	1.8 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5101
AB228611	1.8 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5484
AB227481	1.7 ± 0.2	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4348

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Gene IDs are referred in the *A. oryzae* genome database (<http://nrif21.nrib.go.jp/CFGD/gnm.cgi?prj=02201&gnm=aor0-1>), in which each gene ID can be converted to another ID accessible in other databases DOGAN (<http://www.bio.nite.go.jp/dogan/project/view/AO>) / NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>).

EST IDs are referred in NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>) / *A. oryzae* EST database (<http://nrif21.nrib.go.jp/EST2/index.html>).

\* Genes and ESTs up-regulated in the strain expressing the non-carrier-fused CHY (Table S5).

\*\* Genes down-regulated in the strain expressing the non-carrier-fused CHY (Table S6)

**Table S4** Down-regulated genes ( $\geq 1.5$  fold) in the strain expressing the carrier-fused CHY

Gene/EST ID	Category	Fold change	Product (with similarity)
A, RNA processing and modification (1 gene)			
AO080511000009	A	-1.5 $\pm$ 0.3	Ribonuclease T2
B, Chromatin structure and dynamics (1 gene)			
AO080525000127	B	-1.5 $\pm$ 0.2	Histone deacetylase complex, catalytic component, Rpd3p
J, Translation, ribosomal structure, and biogenesis (1 gene)			
AO080509000251	J	-1.7 $\pm$ 0.4	Aspartyl-tRNA synthetase
D, Cell cycle control, cell division, chromosome partitioning (1 gene)			
AO080518000101	D	-1.6 $\pm$ 0.1	Checkpoint kinase and related serine threonine protein kinase
M, Cell wall/membrane/envelope biogenesis (2 genes)			
AO080511000439*	M	-9.3 $\pm$ 2.4	UDP-glucose 4-epimerase
AO080505000181	M	-1.5 $\pm$ 0.2	Glycosyltransferase
O, Posttranslational modification, protein turnover, chaperones (10 genes)			
AO080527000336	OE	-3.6 $\pm$ 0.4	Serine carboxypeptidases (lysosomal cathepsin A)
AO080505000284	OR	-2.9 $\pm$ 0.8	Hydrolytic enzyme of the alpha beta hydrolase fold
AO080532000098	OG	-2.6 $\pm$ 1.2	beta-1,6-N-acetylglucosaminyltransferase, contains WSC domain, Wsc1p
AO080523000224	O	-2.6 $\pm$ 1.1	Tripeptidyl peptidase, TppA
AO080515000148	O	-2.2 $\pm$ 0.1	Secreted aspartic protease, alpha-factor protease, OpsA
AO080546000129	O	-2.2 $\pm$ 0.8	Aspartyl protease
AO080570000070	OPR	-2.1 $\pm$ 0.9	Aminopeptidase
AO080555000108	UO	-1.8 $\pm$ 0.3	Component of vacuolar transporter chaperone (Vtc) involved in vacuole fusion
AO080521000323	O	-1.8 $\pm$ 0.6	Aspartyl protease
AO080554000466	O	-1.6 $\pm$ 0.2	Cyclophilin type peptidyl-prolyl cis-trans isomerase, Cpr1p
T, Signal transduction mechanisms (5 genes)			
AO080546000277	TI	-4.0 $\pm$ 1.3	Sterol reductase lamin B receptor
AO080515000353	TI	-3.0 $\pm$ 1.2	Sterol reductase lamin B receptor, Erg24p
AO080504000033	T	-1.7 $\pm$ 0.2	PKA regulatory subunit, PkaR
AO080527000119	T	-1.6 $\pm$ 0.3	Predicted Rho GTPase-activating protein
AO080501000086	T	-1.5 $\pm$ 0.1	Peripheral-type benzodiazepine receptor and related protein
U, Intracellular trafficking, secretion, and vesicular transport (1 gene)			

AO080555000108	UO	-1.8 ± 0.3	Component of vacuolar transporter chaperone (Vtc) involved in vacuole fusion
V, Defense mechanisms (1 gene)			
AO080539000026	V	-1.7 ± 0.4	Beta-lactamase class C and other penicillin binding protein
C, Energy production and conversion (6 genes)			
AO080506000113	C	-5.6 ± 3.3	Glycolate oxidase
AO080532000236	C	-3.4 ± 1.9	Mitochondrial phosphate carrier protein
AO080549000025	C	-2.6 ± 1.2	Glyoxylate hydroxypyruvate reductase (D-isomer-specific 2-hydroxy acid dehydrogenase superfamily)
AO080513000340	C	-2.0 ± 0.3	NADP FAD dependent oxidoreductase
AO080546000416	CHR	-1.7 ± 0.1	Lactate dehydrogenase and related dehydrogenase
AO080549000155	C	-1.6 ± 0.2	Betaine aldehyde dehydrogenase
E, Amino acid transport and metabolism (16 genes)			
AO070312000108	E	-13.7 ± 4.2	carboxypeptidase C (cathepsin A)
AO080527000336	OE	-3.6 ± 0.4	Serine carboxypeptidase (lysosomal cathepsin A)
AO080513000010	E	-3.4 ± 1.6	H <sup>+</sup> oligopeptide symporter
AO080523000545	GEPR	-3.4 ± 0.8	Permeases of the major facilitator superfamily
AO080562000011	E	-2.8 ± 0.0	Amino acid transporter
AO080511000164	E	-2.6 ± 0.2	Amino acid transporter
AO080536000075	E	-2.6 ± 0.2	Amino acid transporter
AO080541000336*	ER	-2.6 ± 0.7	Threonine dehydrogenase and related Zn-dependent dehydrogenase, AdhB
AO080549000203	E	-2.4 ± 0.3	carboxypeptidase S1, Cpl
AO080515000304	GE	-2.2 ± 0.2	Methylmalonate semialdehyde dehydrogenase
AO080523000335	E	-2.1 ± 0.1	Glycine dehydrogenase (decarboxylating)
AO080522000003	E	-1.7 ± 0.4	Amino acid transporter
AO080568000140	E	-1.6 ± 0.3	Delta-1-pyrroline-5-carboxylate dehydrogenase, AoprnC
AO080567000045	E	-1.6 ± 0.5	Glycine cleavage system H protein (lipoate-binding)
AO080523000198	E	-1.6 ± 0.1	Amino acid transporter
AO080523000884	E	-1.5 ± 0.4	Glycine serine hydroxymethyltransferase
F, Nucleotide transport and metabolism (1 gene)			
AO080562000027	FH	-2.0 ± 0.2	Uridine permease thiamine transporter allantoin transport
G, Carbohydrate transport and metabolism (17 genes)			
AO080525000664	G	-8.8 ± 2.8	Permease of the major facilitator superfamily
AO080551000064	G	-7.9 ± 5.0	Permease of the major facilitator superfamily
AO080523000545	GEPR	-3.4 ± 0.8	Permeases of the major facilitator superfamily

AO080506000171	G	-3.1 ± 0.6	Ribulose kinase and related carbohydrate kinases
AO080554000052*	G	-2.7 ± 0.7	Acid trehalase, vacuolar, TreA/Ath1p
AO080532000098	OG	-2.6 ± 1.2	beta-1,6-N-acetylglucosaminyltransferase, contains WSC domain, Wsc1p
AO080508000451	G	-2.3 ± 1.1	Fructose-6-phosphate 2-kinase fructose-2,6-biphosphatase
AO080515000304	GE	-2.2 ± 0.2	Methylmalonate semialdehyde dehydrogenase
AO080525000288	G	-2.2 ± 0.6	D-ribulose-5-phosphate 3-epimerase
AO080525000586	G	-2.1 ± 0.2	Glycogen phosphorylase
AO080554000143	G	-2.1 ± 0.3	Phosphoglycerate mutase
AO080511000373	G	-1.9 ± 0.4	Sucrose transporter and related protein
AO080541000221	G	-1.7 ± 0.3	Chitinase
AO080523000596	G	-1.7 ± 0.2	Predicted sugar kinase
AO080532000175	G	-1.6 ± 0.2	Glucosidase I
AO080521000163	G	-1.5 ± 0.2	Glycogen branching enzyme, GbeA
AO080523000549	G	-1.5 ± 0.1	Mannitol-1-phosphate altronate dehydrogenase, MpdA

#### H, Coenzyme transport and metabolism (7 genes)

AO080503000412	H	-5.2 ± 0.8	3-polyprenyl-4-hydroxybenzoate decarboxylase and related decarboxylase
AO080562000027	FH	-2.0 ± 0.2	Uridine permease thiamine transporter allantoin transport
AO080569000086	H	-1.9 ± 0.6	Riboflavin kinase
AO080546000088	H	-1.9 ± 0.2	Folylpolyglutamate synthase
AO080546000416	CHR	-1.7 ± 0.1	Lactate dehydrogenase and related dehydrogenase
AO080503000384	H	-1.6 ± 0.2	Methyltransferase
AO080527000104	H	-1.5 ± 0.1	Protein involved in ubiquinone biosynthesis

#### I, Lipid transport and metabolism (13 genes)

AO080523000390	IQ	-58.4 ± 63.7	Cytochrome P450 CYP4 CYP19 CYP26 subfamily
AO080508000153	IQR	-7.0 ± 3.7	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
AO080546000277	TI	-4.0 ± 1.3	Sterol reductase lamin B receptor
AO080523000408	IQR	-3.7 ± 1.2	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
AO080521000167	I	-3.4 ± 1.4	Hydroxymethylglutaryl-CoA synthase, Erg13p
AO080515000353	TI	-3.0 ± 1.2	Sterol reductase lamin B receptor, Erg24p
AO080541000139	I	-2.6 ± 1.0	Sterol C5 desaturase, Erg3p
AO080511000072	I	-2.5 ± 0.4	Sterol C5 desaturase, Erg3p
AO080521000348	I	-2.4 ± 0.6	C-4 sterol methyl oxidase, Erg25p
AO080539000063	I	-2.1 ± 0.5	Fatty acid desaturase
AO080548000024	I	-2.1 ± 0.3	Sterol C5 desaturase, Erg3
AO080570000001	I	-1.8 ± 0.2	C-4 sterol methyl oxidase, Erg25p
AO080532000288	I	-1.5 ± 0.1	17 beta-hydroxysteroid dehydrogenase type 3, HSD17B3

P, Inorganic ion transport and metabolism (10 genes)

AO080568000157	P	-3.9 ± 0.5	Predicted flavoprotein involved in K <sup>+</sup> transport
AO080523000545	GEPR	-3.4 ± 0.8	Permeases of the major facilitator superfamily
AO080546000385	P	-2.2 ± 0.9	Predicted flavoprotein involved in K <sup>+</sup> transport
AO080528000068	P	-2.2 ± 0.5	Mitochondrial Fe <sup>2+</sup> transporter MMT1 and related transporters (cation diffusion facilitator superfamily)
AO080523000785	P	-2.2 ± 0.5	High-affinity nickel permease
AO080508000083	P	-2.1 ± 0.3	Zn <sup>2+</sup> transporter ZNT1 and related Cd <sup>2+</sup> Zn <sup>2+</sup> transporters (cation diffusion facilitator superfamily), CzcD
AO080570000070	OPR	-2.1 ± 0.9	Aminopeptidase
AO080523000716	P	-2.1 ± 0.5	Predicted flavoprotein involved in K <sup>+</sup> transport
AO080527000334	P	-1.9 ± 0.6	ATP sulphurylase, sC
AO080523000761	P	-1.7 ± 0.1	Protein involved in vacuolar polyphosphate accumulation, contains SPX domain

Q, Secondary metabolites biosynthesis, transport, and catabolism (15 genes)

AO080523000391	Q	-240.0 ± 333.1	Cytochrome P450 CYP3 CYP5 CYP6 CYP9 subfamily
AO080523000390	IQ	-58.4 ± 63.7	Cytochrome P450 CYP4 CYP19 CYP26 subfamily
AO080523000394	Q	-10.0 ± 9.0	Cytochrome P450
AO080523000894	Q	-9.2 ± 4.9	Ornithine-N5-oxygenase, Sid1/Dffa
AO080508000153	IQR	-7.0 ± 3.7	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
AO080513000129	Q	-4.6 ± 2.1	Cytochrome P450
AO080523000408	IQR	-3.7 ± 1.2	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
AO080541000451	Q	-3.4 ± 1.4	Flavin-containing monooxygenase
AO080533000295	Q	-2.8 ± 0.5	Pleiotropic drug resistance proteins (PDR1-15), ABC superfamily
AO080533000376	Q	-2.7 ± 1.0	Multidrug resistance-associated protein mitoxantrone resistance protein, ABC superfamily
AO080529000067	Q	-2.6 ± 0.8	Cytochrome P450 CYP11 CYP12 CYP24 CYP27 subfamilies
AO080525000666	Q	-2.3 ± 0.7	1-Acyl dihydroxyacetone phosphate reductase and related dehydrogenases
AO080503000195	Q	-1.9 ± 0.6	Cytochrome P450, Erg11p
AO080559000087	Q	-1.9 ± 0.1	Multicopper oxidases
AO080551000152	Q	-1.6 ± 0.1	Multidrug resistance-associated protein mitoxantrone resistance protein, ABC superfamily

R, General function prediction only (24 genes)

AO080508000153	IQR	-7.0 ± 3.7	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
AO080501000148	R	-4.0 ± 1.6	Hydroxyindole-O-methyltransferase and related SAM-dependent methyltransferases
AO080508000180	R	-3.9 ± 0.8	Predicted transporter (major facilitator superfamily)
AO080523000408	IQR	-3.7 ± 1.2	Dehydrogenase with different specificities (related to short-chain alcohol dehydrogenases)
AO080523000168	R	-3.4 ± 2.0	Multiple inositol polyphosphate phosphatase
AO080523000545	GEPR	-3.4 ± 0.8	Permease of the major facilitator superfamily
AO080523000392	R	-3.3 ± 1.7	Synaptic vesicle transporter SVOP and related transporters (major facilitator superfamily)



AO080541000089	R	-2.9 ± 1.1	Predicted hydrolases or acyltransferase (alpha beta hydrolase superfamily)
AO080505000284	OR	-2.9 ± 0.8	Hydrolytic enzymes of the alpha beta hydrolase fold
AO080541000336*	ER	-2.6 ± 0.7	Threonine dehydrogenase and related Zn-dependent dehydrogenase, AdhB
AO080501000145	R	-2.4 ± 0.8	Predicted protein
AO080549000265	R	-2.3 ± 0.8	Predicted dehydrogenases and related protein
AO080511000111	R	-2.3 ± 1.0	Predicted NAD-dependent oxidoreductase
AO080570000070	OPR	-2.1 ± 0.9	Aminopeptidase
AO080513000310	R	-2.0 ± 0.3	Predicted oxidoreductase
AO080508000325	R	-2.0 ± 0.4	Metal-dependent amidase aminoacylase carboxypeptidase
AO080509000223*	R	-1.8 ± 0.6	1,4-benzoquinone reductase-like; Trp repressor binding protein-like protoplast-secreted protein
AO080542000126	R	-1.8 ± 0.4	Predicted transporter (major facilitator superfamily)
AO080570000053	R	-1.7 ± 0.4	HGG motif-containing thioesterase
AO080546000416	CHR	-1.7 ± 0.1	Lactate dehydrogenase and related dehydrogenase
AO080523000435	R	-1.6 ± 0.3	DMQ mono-oxygenase Ubiquinone biosynthesis protein COQ7 CLK-1 CAT5
AO080525000671	R	-1.6 ± 0.5	Putative intracellular protease
AO080542000202	R	-1.5 ± 0.1	Multiple inositol polyphosphate phosphatase
AO080536000007	R	-1.5 ± 0.1	Predicted TIM-barrel enzyme, possibly a dioxygenase
S, Function unknown (5 genes)			
AO080511000061	S	-2.4 ± 0.9	Predicted membrane protein
AO080525000673	S	-1.7 ± 0.4	Uncharacterized conserved protein
AO080551000078	S	-1.6 ± 0.1	Uncharacterized conserved protein
AO080511000485	S	-1.6 ± 0.4	Predicted membrane protein
AO080513000318	S	-1.5 ± 0.0	Uncharacterized conserved protein
Unclassified for COG (93 genes)			
AO080523000387		-455.3 ± 535.7	Predicted protein
AO080541000466		-13.7 ± 11.6	Predicted protein
AO080541000467		-10.6 ± 7.9	Predicted protein
AO080513000205		-8.4 ± 6.2	Predicted protein
AO080513000281		-8.0 ± 6.4	Predicted protein
AO080536000046		-7.3 ± 4.7	Uncharacterized conserved protein
AO080541000077		-7.1 ± 3.7	Predicted protein
AO080515000406		-6.6 ± 2.9	Predicted protein
AO080525000507		-6.1 ± 3.9	Predicted protein
AO080513000035		-5.9 ± 3.1	Predicted protein
AO080523000498		-5.4 ± 4.8	Predicted protein
AO080532000160		-5.0 ± 2.7	Predicted protein

AO08055000114	-4.9 ± 1.9	Predicted protein
AO080525000333	-4.8 ± 1.4	Predicted protein
AO080515000324	-4.4 ± 1.9	Predicted protein
AO080515000013	-4.3 ± 2.7	Predicted protein
AO080511000438	-4.2 ± 2.0	Predicted protein
AO080543000012	-4.2 ± 1.4	Predicted protein
AO080510000043	-4.1 ± 0.7	Predicted protein
AO080539000059	-4.1 ± 2.2	Predicted protein
AO080525000289	-4.0 ± 2.3	Predicted protein
AO080523000786	-3.9 ± 2.0	Predicted protein
AO080553000045	-3.9 ± 0.3	Predicted protein
AO080525000335	-3.8 ± 1.4	Predicted protein
AO080505000008	-3.8 ± 1.7	Predicted protein
AO080508000417	-3.7 ± 2.0	Predicted protein
AO080511000437	-3.7 ± 1.4	Predicted protein
AO080501000147	-3.6 ± 1.2	Predicted protein
AO080527000488	-3.6 ± 0.4	Predicted protein
AO080157000001	-3.2 ± 1.5	Predicted protein
AO080550000130	-3.1 ± 0.6	Predicted protein
AO080557000050	-3.0 ± 0.9	Predicted protein
AO080549000140	-3.0 ± 1.7	Predicted protein
AO080523000237	-2.9 ± 1.5	Predicted protein
AO080525000226	-2.9 ± 1.5	Predicted protein
AO080511000417	-2.9 ± 0.5	von Willebrand factor and related coagulation protein
AO080546000388	-2.9 ± 0.7	Predicted protein
AO080515000203	-2.8 ± 1.5	Predicted protein
AO080511000435	-2.8 ± 1.3	Predicted protein
AO080511000163	-2.7 ± 0.6	Predicted protein
AO080527000406	-2.7 ± 1.4	Predicted protein
AO080506000040	-2.7 ± 1.3	Predicted protein
AO080527000156	-2.6 ± 0.5	Predicted protein
AO080525000647	-2.5 ± 1.1	Predicted protein
AO080562000080	-2.5 ± 0.7	AflJ
AO080560000054	-2.5 ± 1.0	Predicted protein
AO080532000088	-2.5 ± 1.3	Predicted protein
AO080550000132	-2.5 ± 1.2	Predicted protein
AO080511000527	-2.5 ± 1.0	Predicted protein
AO080513000021	-2.4 ± 1.0	Predicted protein

AO080529000082	-2.4 ± 0.8	Predicted protein
AO080514000009	-2.4 ± 0.9	Galactosyltransferase
AO080541000084*	-2.3 ± 0.3	Predicted protein
AO080508000182	-2.3 ± 0.7	Predicted protein
AO080531000131	-2.3 ± 0.2	Predicted protein
AO080553000044	-2.3 ± 0.5	Predicted protein
AO080503000040	-2.2 ± 0.6	Predicted protein
AO080504000080	-2.2 ± 1.0	Predicted protein
AO080527000412	-2.2 ± 0.6	Predicted protein
AO080549000325	-2.1 ± 0.8	Predicted protein
AO080529000068	-2.1 ± 0.6	Predicted protein
AO080562000021	-2.1 ± 0.5	Predicted protein
AO080511000434	-2.0 ± 0.9	Predicted protein
AO080506000023	-2.0 ± 0.7	Predicted protein
AO080569000064	-2.0 ± 0.7	Predicted protein
AO080503000162	-1.9 ± 0.1	Predicted protein
AO080523000328	-1.9 ± 0.6	Predicted protein
AO080523000537	-1.9 ± 0.6	Predicted protein
AO080537000063	-1.8 ± 0.1	Predicted protein
AO080513000020	-1.8 ± 0.2	Predicted protein
AO080570000002	-1.8 ± 0.2	Predicted protein
AO080536000068	-1.7 ± 0.4	Predicted protein
AO080541000136	-1.7 ± 0.4	Predicted protein
AO080536000216	-1.7 ± 0.3	Predicted protein
AO080567000012	-1.7 ± 0.3	Predicted protein
AO080550000134	-1.7 ± 0.6	Predicted protein
AO080509000063	-1.7 ± 0.1	Predicted protein
AO080551000149	-1.7 ± 0.6	Predicted protein
AO080536000173	-1.7 ± 0.4	Predicted protein
AO080521000127	-1.7 ± 0.1	Predicted protein
AO080536000010	-1.7 ± 0.1	Predicted protein
AO080502000076	-1.6 ± 0.2	Predicted protein
AO080546000099	-1.6 ± 0.3	Predicted protein
AO080521000128	-1.6 ± 0.2	Predicted protein
AO080549000048	-1.6 ± 0.1	Predicted protein
AO080523000663	-1.6 ± 0.2	Predicted protein
AO080533000319	-1.6 ± 0.1	Predicted protein
AO080511000160	-1.6 ± 0.2	Predicted protein

AO080515000128	-1.6 ± 0.2	Predicted protein
AO080515000305	-1.6 ± 0.2	Predicted protein
AO080532000553	-1.5 ± 0.1	FAD FMN-containing dehydrogenase
AO080549000097	-1.5 ± 0.3	Predicted protein
AO080511000004	-1.5 ± 0.2	Predicted protein
Unregistered for COG (27 genes)		
AO070333000246	-13.6 ± 10.8	Predicted protein
AO080525000665	-10.8 ± 2.9	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductase
AO080539000088	-5.5 ± 2.5	Predicted protein
AO080570000082	-5.4 ± 3.5	Amino acid transporter
AO080521000297	-5.3 ± 3.3	Histone acetyltransferase HPA2 and related acetyltransferase
AO080515000166	-5.2 ± 3.4	Predicted protein
AO080506000192	-4.9 ± 3.5	Predicted protein
AO080525000667	-3.7 ± 0.8	Predicted protein
AO080525000335	-3.5 ± 1.0	Predicted protein
AO080542000065	-3.2 ± 0.6	Predicted hydrolases or acyltransferase (alpha beta hydrolase superfamily)
AO080525000335	-3.2 ± 0.9	Predicted protein
AO080508000151	-2.9 ± 1.5	Predicted protein
AO080521000334	-2.7 ± 1.4	Predicted protein
AO080511000208	-2.6 ± 0.6	Predicted protein
AO080510000018	-2.5 ± 1.3	Predicted protein
AO080537000037	-2.4 ± 0.8	Predicted protein
AO080515000390	-2.2 ± 0.2	Parvulin-like peptidyl-prolyl cis-trans isomerase
AO080508000079	-2.1 ± 0.6	Predicted protein
AO080523000056	-2.1 ± 0.4	Amino acid transporter
AO080559000088	-1.9 ± 0.2	Predicted protein
AO080559000088	-1.9 ± 0.1	Predicted protein
AO080513000128	-1.9 ± 0.6	Cytochrome P450 CYP4 CYP19 CYP26 subfamily
AO080506000022	-1.8 ± 0.7	Predicted protein
AO080504000030	-1.8 ± 0.4	Predicted protein
AO080546000019	-1.8 ± 0.5	Transposase and inactivated derivative
AO080505000071	-1.7 ± 0.3	THUMP domain-containing protein
AO080554000051	-1.6 ± 0.3	Predicted protein
Expressed Sequence Tag (25 ESTs)		
AB225221	-12.1 ± 4.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2073
AB229651	-9.8 ± 7.5	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST6533

AB225921	-5.1 ± 3.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2776
AB223691	-4.6 ± 3.5	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0531
AB224431	-4.1 ± 1.9	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1276
AB229289	-3.3 ± 1.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST6168
AB224084	-3.2 ± 1.5	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0926
AB224014	-3.1 ± 1.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0855
AB230171	-2.8 ± 0.7	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST7058
AB225459	-2.7 ± 1.5	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2312
AB224047	-2.6 ± 0.8	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0889
AB228194	-2.5 ± 0.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5067
AB228033	-2.4 ± 0.8	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4906
AB223341	-2.4 ± 1.0	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0172
AB223929	-2.4 ± 0.8	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0770
AB225612	-2.4 ± 1.2	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST2467
AB224429	-2.4 ± 1.2	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1274
AB226317	-2.3 ± 0.9	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3179
AB228194	-2.2 ± 0.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5067
AB226766	-2.2 ± 1.0	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3628
AB230645	-1.8 ± 0.4	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST7535
AB228007	-1.7 ± 0.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4880
AB226179	-1.6 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3037
AB228576	-1.6 ± 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5449
AB224686	-1.6 ± 0.1	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST1535

Gene IDs are referred in the *A. oryzae* genome database (<http://nrribf21.nrib.go.jp/CFGD/gnm.cgi?prj=02201&gnm=aor0-1>), in which each gene ID can be converted to another ID accessible in other databases DOGAN (<http://www.bio.nite.go.jp/dogan/project/view/AO>) / NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>).

EST IDs are referred in NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>) / *A. oryzae* EST database (<http://nrribf21.nrib.go.jp/EST2/index.html>).

\* Genes down-regulated in the strain expressing the non-carrier-fused CHY (Table S6).

**Table S5** Up-regulated genes/ESTs ( $\geq 1.5$  fold) in the strain expressing the non-carrier-fused CHY

Gene/EST ID	Category	Fold change	Product (with similarity)
O, Posttranslational modification, protein turnover, chaperones (2 genes)			
AO080505000236*	O	1.6 $\pm$ 0.2	Thioredoxin protein disulfide isomerase, PrpA
AO080527000493	O	1.5 $\pm$ 0.1	Thioredoxin
R, General function prediction only (1 gene)			
AO080513000133*	R	1.7 $\pm$ 0.1	Predicted hydrolases or acyltransferase (alpha beta hydrolase superfamily)
Unclassified for COG (4 genes)			
AO080542000164		4.1 $\pm$ 1.2	Predicted protein
AO080566000067		1.8 $\pm$ 0.3	Uncharacterized protein conserved in bacteria
AO080531000356*		1.8 $\pm$ 0.3	Predicted protein
AO080541000063*		1.7 $\pm$ 0.4	Predicted protein
Expressed Sequence Tag (3 ESTs)			
AB223285		10.1 $\pm$ 2.4	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST0116
AB227435*		1.9 $\pm$ 0.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST4302
AB226737*		1.5 $\pm$ 0.3	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST3599

Gene IDs are referred in the *A. oryzae* genome database (<http://nrif21.nrib.go.jp/CFGD/gnm.cgi?prj=02201&gnm=aor0-1>), in which each gene ID can be converted to another ID accessible in other databases DOGAN (<http://www.bio.nite.go.jp/dogan/project/view/AO/>) / NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>).

EST IDs are referred in NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>) / *A. oryzae* EST database (<http://nrif21.nrib.go.jp/EST2/index.html>).

\* Genes and ESTs up-regulated in the strain expressing the carrier-fused CHY (Tables S2 and S3).

**Table S6** Down-regulated genes/ESTs ( $\geq 1.5$  fold) in the strain expressing the non-carrier-fused CHY

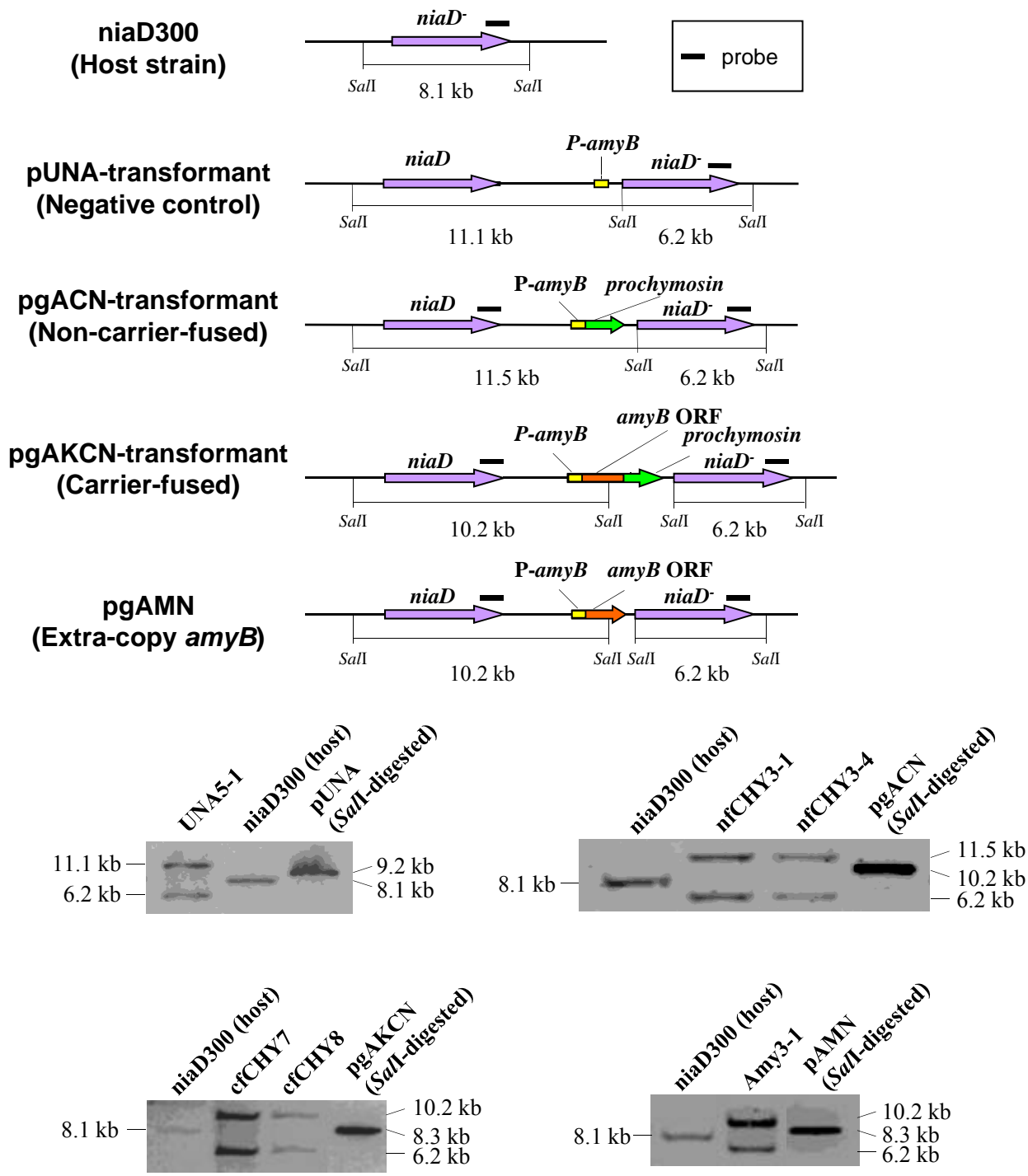
Gene/EST ID	Category	Fold change	Product (with similarity)
M, Cell wall/membrane/envelope biogenesis (1 gene)			
AO080511000439*	M	-1.9 $\pm$ 0.5	UDP-glucose 4-epimerase
E, Amino acid transport and metabolism (2 genes)			
AO080541000336*	ER	-1.7 $\pm$ 0.4	Threonine dehydrogenase and related Zn-dependent dehydrogenase, AdhB
AO080523000651	E	-1.6 $\pm$ 0.4	Aspartate aminotransferase Glutamic oxaloacetic transaminase AAT2 GOT1
G, Carbohydrate transport and metabolism (3 genes)			
AO080533000090	G	-4.1 $\pm$ 3.0	Ribokinase
AO080511000480	G	-1.6 $\pm$ 0.3	Trehalose-6-phosphate synthase component TPS1 and related subunit
AO080554000052*	G	-1.6 $\pm$ 0.3	Acid trehalase, vacuolar, TreA/Ath1p
R, General function prediction only (4 genes)			
AO080549000130	R	-3.4 $\pm$ 1.8	Predicted transporter (major facilitator superfamily)
AO080532000167	R	-1.8 $\pm$ 0.7	Synaptic vesicle transporter SVOP and related transporter (major facilitator superfamily)
AO080541000336*	ER	-1.7 $\pm$ 0.4	Threonine dehydrogenase and related Zn-dependent dehydrogenase, AdhB
AO080509000223*	R	-1.6 $\pm$ 0.6	1,4-benzoquinone reductase-like; Trp repressor binding protein-like protoplast-secreted protein
Unclassified for COG (4 genes)			
AO080503000018**		-4.1 $\pm$ 2.8	Predicted protein
AO080536000168**		-2.5 $\pm$ 0.9	Predicted protein
AO080550000091**		-1.9 $\pm$ 0.8	Molecular chaperone (small heat shock protein)
AO080541000084*		-1.7 $\pm$ 0.4	Predicted protein
EST (1 EST)			
AB228596		-1.9 $\pm$ 0.6	<i>Aspergillus oryzae</i> cDNA, contig sequence: AoEST5469

Gene IDs are referred in the *A. oryzae* genome database (<http://nrif21.nrib.go.jp/CFGD/gnm.cgi?prj=02201&gnm=aor0-1>), in which each gene ID can be converted to another ID accessible in other databases DOGAN (<http://www.bio.nite.go.jp/dogan/project/view/AO/>) / NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>).

EST IDs are referred in NCBI (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nuccore&itool=toolbar>) / *A. oryzae* EST database (<http://nrif21.nrib.go.jp/EST2/index.html>).

\* Genes down-regulated in the strain expressing the carrier-fused CHY (Table S4).

\*\* Genes up-regulated in the strain expressing the carrier-fused CHY (Table S3).



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