

Phyl.	Strain	Acc. Nr.	Cons.	Reference
A	<i>Ajellomyces dermatitidis</i> ER-3	EEQ88796 ¹	FGI	BDSP
A	<i>Ajellomyces dermatitidis</i> SLH14081	EEQ72680 ¹	FGI	BDSP
A	<i>Arthroderma benhamiae</i> CBS112371	EFE34296	HKI	DCSP
A	<i>Arxula adenivorans</i> LS3	CAQ77148		Boer <i>et al.</i> 2009
A	<i>Aspergillus clavatus</i> NRRL 1	EAW07550	JCVI	Fedorova <i>et al.</i> 2008
A	<i>Aspergillus flavus</i> NRRL3357	EED54629	JCVI	ACSP
A	<i>Aspergillus fumigatus</i>	AAL85636		
A	<i>Aspergillus fumigatus</i> A1163	EAW07550	JCVI	Fedorova <i>et al.</i> 2008
A	<i>Aspergillus fumigatus</i> Af293	EAL90615	TIGR	Nierman <i>et al.</i> 2005
A	<i>Aspergillus nidulans</i> FGSC A4	EAA65574	FGI	Galagan <i>et al.</i> 2005
A	<i>Aspergillus niger</i>	P36858		Unkles <i>et al.</i> 1992
A	<i>Aspergillus niger</i> ATCC 1015	208048 (JGI)	JGI	Baker 2006
A	<i>Aspergillus niger</i> CBS513.88	CAK45533	DSM	Pel <i>et al.</i> 2007
A	<i>Aspergillus oryzae</i> KBN616	BAA08551		Kitamoto <i>et al.</i> 1995
A	<i>Aspergillus parasiticus</i> ATCC56775	AAC49605 ¹		Chang <i>et al.</i> 1996
A	<i>Aspergillus terreus</i> NIH2624	EAU39120	FGI	ACSP
A	<i>Botryotinia fuckeliana</i> B05.10	EDN30738	FGI	
A	<i>Botryotinia fuckeliana</i> T4	AAC02633		Levis <i>et al.</i> 1997
A	<i>Chaetomium globosum</i> CBS148.51	EAQ88219 ¹	FGI	
A	<i>Cochliobolus heterostrophus</i> C5	113444 (JGI)	JGI	
A	<i>Cordyceps bassiana</i> Bb147	CAA59336		Maurer <i>et al.</i> 1997
A	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	FOXG_04181.2 (FGI)	FGI	Ma <i>et al.</i> 2010
A	<i>Fusarium oxysporum</i> Fom24	CAA80270		Diolez <i>et al.</i> 1993
A	<i>Fusarium oxysporum</i> JCM11502	BAG12905		Fujii and Takaya 2008
A	<i>Fusarium oxysporum</i> M10	BAG12906		
A	<i>Fusarium verticilloides</i>	FVEG_07298.3 (FGI) ¹		Ma <i>et al.</i> 2010
A	<i>Gibberella fujikuroi</i> m567	CAA62232		Tudzynski <i>et al.</i> 1996
A	<i>Gibberella zeae</i> PH-1	XP_382123 ¹	FGI	Ma <i>et al.</i> 2010
A	<i>Leptosphaeria maculans</i>	P36842		Williams <i>et al.</i> 1995
A	<i>Magnaporthe grisea</i> 70-15	EDJ96311	FGI	Dean <i>et al.</i> 2005
A	<i>Metarhizium anisopliae</i> ME1	CAA04554		Screen <i>et al.</i> 1998
A	<i>Microsporum canis</i> CBS 113480	EEQ28849	FGI	DCSP
A	<i>Microsporum gypseum</i> CBS 118893	MGYG_04402.1 (FGI)	FGI	DCSP
A	<i>Monascus purpureus</i>	BAB84515		
A	<i>Myceliophthora thermophila</i> ATCC42464	97565 (JGI)	JGI	
A	<i>Mycosphaerella fijiensis</i>	56303 (JGI)	JGI	
A	<i>Mycosphaerella graminicola</i>	111003 (JGI)	JGI	
A	<i>Nectria haematococca</i> mpVI 77-13-4	EEU34169	JGI	Coleman <i>et al.</i> 2009
A	<i>Neosartorya fischeri</i> NRRL181	EAW22584	JCVI	Fedorova <i>et al.</i> 2008
A	<i>Neurospora crassa</i> 74OR23-1A	CAA43600		Okamoto <i>et al.</i> 1991
A	<i>Neurospora crassa</i> OR74A	EAA32833	FGI	Galagan <i>et al.</i> 2003
A	<i>Neurospora discreta</i> FGSC8579 mat A	128588 (JGI) ¹	JGI	
A	<i>Neurospora tetrasperma</i> FGSC2508 matA	124300 (JGI) ¹	JGI	
A	<i>Paracoccidioides brasiliensis</i> Pb03	EEH17834	FGI	PbSP
A	<i>Paracoccidioides brasiliensis</i> Pb18	EEH46709 ¹	FGI	PbSP
A	<i>Penicillium camemberti</i> CECT2267	ACI29313		Navarrete <i>et al.</i> 2009
A	<i>Penicillium chrysogenum</i>	AAB03900		Haas and Marzluf 1995
A	<i>Penicillium chrysogenum</i> Wisconsin 54-1255	CAP92210		van den Berg <i>et al.</i> 2008
A	<i>Penicillium griseoroseum</i>	AAP12556		Pereira <i>et al.</i> 2004

A	<i>Penicillium marneffei</i> ATCC 18224	EEA25715	JCVI	
A	<i>Phaeosphaeria nodorum</i> BS171	CAA08857		Cutler <i>et al.</i> 1998
A	<i>Phaeosphaeria nodorum</i> BSm300	CAA74005		Cutler <i>et al.</i> 1998
A	<i>Phaeosphaeria nodorum</i> SN15	EAT87086	FGI	
A	<i>Pichia angusta</i> NCYC495	CAA88925		Avila <i>et al.</i> 1995
A	<i>Podospira anserina</i> DSM980	CAP70343	GS	Espagne <i>et al.</i> 2008
A	<i>Sclerotinia sclerotiorum</i> 1980	EDN96957	FGI	
A	<i>Sordaria macrospora</i> k-hell	CBI54944	RUB	Nowrousian <i>et al.</i> 2010
A	<i>Talaromyces stipitatus</i> ATCC 10500	EED21598	JCVI	
A	<i>Thielavia terrestris</i>	36169 (JGI)	JGI	
A	<i>Trichoderma atroviride</i>	128887 (JGI) ¹	JGI	
A	<i>Trichoderma reesei</i>	81955 (JGI) ¹	JGI	Martinez <i>et al.</i> 2008
A	<i>Trichoderma virens</i> Gv29-8	33018 (JGI)	JGI	
A	<i>Trichophyton equinum</i> CBS127.97	TEQG_08349.1 (FGI)	FGI	DCSP
A	<i>Trichophyton rubrum</i> CBS118892	TERG_07816.2 (FGI)	FGI	DCSP
A	<i>Trichophyton tonsurans</i> CBS112818	TESG_00729.1 (FGI)	FGI	DCSP
A	<i>Trichophyton verrucosum</i> HKI 0517	EFE41244	HKI	DCSP
A	<i>Tuber borchii</i>	AAN64993		Guescini <i>et al.</i> 2003
A	<i>Tuber melanosporum</i> Mel28	CAZ85382 ¹	GS	Martin <i>et al.</i> 2010
A	<i>Verticillium albo-atrum</i> VaMs.102	EEY14658	FGI	
A	<i>Verticillium dahliae</i> VdLs.17	VDAG_00378 (FGI)	FGI	
A	<i>Verticillium fungicola</i> 150-1	AAO63560		Amey <i>et al.</i> 2007
A	<i>Wickerhamomyces anomalus</i> CECT1112	AAF28059		Garcia-Lugo <i>et al.</i> 2000
B	<i>Coprinopsis cinerea</i> okayama 7*130	EAU81300	FGI	Stajich <i>et al.</i> 2010
B	<i>Hebeloma cylindrosporum</i> h1	CAB60010		Jargeat <i>et al.</i> 2000
B	<i>Laccaria bicolor</i> S238N-H82	EDR02368	JGI	Martin <i>et al.</i> 2008
B	<i>Melampsora larici-populina</i> 98AG31_300	33678 (JGI)	JGI	
B	<i>Oryza sativa</i> EST1	translated from AK110249		Kikuchi <i>et al.</i> 2003
B	<i>Phanerochaete chrysosporium</i>	136276 (JGI) ¹	JGI	Martinez <i>et al.</i> 2004
B	<i>Postia placenta</i> MAD-698	47370 (JGI) ¹	JGI	Martinez <i>et al.</i> 2009
B	<i>Sporobolomyces roseus</i>	34275 (JGI)	JGI	
B	<i>Ustilago maydis</i> 521	EAK84753	FGI	Kamper <i>et al.</i> 2006
M	<i>Mucor circinelloides</i> CBS277.49	81522 (JGI)	JGI	
O	<i>Phytophthora infestans</i>	P39864		Pieterse <i>et al.</i> 1995
O	<i>Phytophthora ramorum</i>	71442 (JGI)	JGI	Tyler <i>et al.</i> 2006
O	<i>Phytophthora sojae</i>	140563 (JGI)	JGI	Tyler <i>et al.</i> 2006
Bp	<i>Cylindrotheca fusiformis</i>	AAV59538	JGI	Poulsen and Kroger 2005
Bp	<i>Phaeodactylum tricorutum</i> CCAP 1055/1	EEC44781	JGI	Bowler <i>et al.</i> 2008
Bp	<i>Thalassiosira pseudonana</i> CCMP1335	EED88244	JGI	Armbrust <i>et al.</i> 2004

Supplementary Table 3 Published full-length or nearly full-length nitrate reductase sequences used in our study

Phyl.: phylum or subphylum (A: Ascomycota, B: Basidiomycota, M: Mucoromycotina, O: Oomycota, Bp: Bacillariophyta)

Acc.Nr.: Protein ID at NCBI or the webportals of genome sequencing consortia (JGI: DOE Joint Genome Institute; FGI: Fungal Genome Initiative at the Broad Institute)

Cons.: Sequencing consortium when data were obtained from whole genome sequencing projects (DSM: DSM, Delft, The Netherlands; FGI: Fungal Genome Initiative, Broad Institute, MA, USA; GS: Genoscope, France; HKI: Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany; JCVI: J. Craig Venter Institute, MD, USA; JGI: US DOE Joint Genome Initiative, CA, USA; RUB: Ruhr-Universität Bochum, Germany; TIGR: The Institute for Genomic Research, MD, USA)

¹: Protein sequences for multiple alignments were manually edited based on the corresponding genomic sequence.

Supplementary References

- ACSP *Aspergillus* Comparative Sequencing Project, Broad Institute of Harvard and MIT (<http://www.broadinstitute.org/>).
- Amev R, Athey-Pollard A, Mills P, Foster G, Bailey A (2007). Investigations into the taxonomy of the mushroom pathogen *Verticillium fungicola* and its relatives based on sequence analysis of nitrate reductase and ITS regions. *Microbiology* 76: 757-768.
- Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH *et al* (2004). The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* 306: 79-86.
- Avila J, Perez MD, Brito N, Gonzalez C, Siverio JM (1995). Cloning and disruption of the *YNR1* gene encoding the nitrate reductase apoenzyme of the yeast *Hansenula polymorpha*. *FEBS Lett* 366: 137-142.
- Baker SE (2006). *Aspergillus niger* genomics: past, present and into the future. *Med Mycol* 44 Suppl 1: S17-21.
- BDSP *Blastomyces dermatitidis* Sequencing Project, Broad Institute of Harvard and MIT (<http://www.broadinstitute.org/>).
- Boer E, Schroter A, Bode R, Piontek M, Kunze G (2009). Characterization and expression analysis of a gene cluster for nitrate assimilation from the yeast *Arxula adenivorans*. *Yeast* 26: 83-93.
- Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A *et al* (2008). The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456: 239-244.
- Chang PK, Ehrlich KC, Linz JE, Bhatnagar D, Cleveland TE, Bennett JW (1996). Characterization of the *Aspergillus parasiticus niaD* and *niiA* gene cluster. *Curr Genet* 30: 68-75.
- Coleman JJ, Rounsley SD, Rodriguez-Carres M, Kuo A, Wasmann CC, Grimwood J *et al* (2009). The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. *PLoS Genet* 5: e1000618.
- Cutler SB, Cooley RN, Caten CE (1998). Cloning of the nitrate reductase gene of *Stagonospora (Septoria) nodorum* and its use as a selectable marker for targeted transformation. *Curr Genet* 34: 128-137.
- DCSP Dermatophyte Comparative Sequencing Project, Broad Institute of Harvard and MIT (<http://www.broadinstitute.org/>).
- Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ *et al* (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434: 980-986.
- Dioloz A, Langin T, Gerlinger C, Brygoo Y, Daboussi MJ (1993). The *nia* gene of *Fusarium oxysporum*: isolation, sequence and development of a homologous transformation system. *Gene* 131: 61-67.
- Espagne E, Lespinet O, Malagnac F, Da Silva C, Jaillon O, Porcel BM *et al* (2008). The genome sequence of the model ascomycete fungus *Podospira anserina*. *Genome Biol* 9: R77.
- Fedorova ND, Khaldi N, Joardar VS, Maiti R, Amedeo P, Anderson MJ *et al* (2008). Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. *PLoS Genet* 4: e1000046.
- Fujii T, Takaya N (2008). Denitrification by the fungus *Fusarium oxysporum* involves NADH-nitrate reductase. *Biosci Biotechnol Biochem* 72: 412-420.
- Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D *et al* (2003). The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* 422: 859-868.
- Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S *et al* (2005). Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* 438: 1105-1115.
- Garcia-Lugo P, Gonzalez C, Perdomo G, Brito N, Avila J, de La Rosa JM *et al* (2000). Cloning, sequencing, and expression of *H.a.YNR1* and *H.a.YNII*, encoding nitrate and nitrite reductases in the yeast *Hansenula anomala*. *Yeast* 16: 1099-1105.
- Guescini M, Pierleoni R, Palma F, Zeppa S, Vallorani L, Potenza L *et al* (2003). Characterization of the *Tuber borchii* nitrate reductase gene and its role in ectomycorrhizae. *Mol Genet Genomics* 269: 807-816.

- Haas H, Marzluf GA (1995). NRE, the major nitrogen regulatory protein of *Penicillium chrysogenum*, binds specifically to elements in the intergenic promoter regions of nitrate assimilation and penicillin biosynthetic gene clusters. *Curr Genet* 28: 177-183.
- Jargeat P, Gay G, Debaud JC, Marmeisse R (2000). Transcription of a nitrate reductase gene isolated from the symbiotic basidiomycete fungus *Hebeloma cylindrosporium* does not require induction by nitrate. *Mol Gen Genet* 263: 948-956.
- Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, Saville BJ *et al* (2006). Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444: 97-101.
- Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N *et al* (2003). Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. *Science* 301: 376-379.
- Kitamoto N, Kimura T, Kito Y, Ohmiya K, Tsukagoshi N (1995). The nitrate reductase gene from a shoyo koji mold, *Aspergillus oryzae* KBN616. *Biosci Biotechnol Biochem* 59: 1795-1797.
- Levis C, Fortini D, Brygoo Y (1997). Transformation of *Botrytis cinerea* with the nitrate reductase gene (*niaD*) shows a high frequency of homologous recombination. *Curr Genet* 32: 157-162.
- Ma L-J, van der Does HC, Borkovich KA, Coleman JJ, Daboussi M-J, Di Pietro A *et al* (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464: 367-373.
- Martin F, Aerts A, Ahren D, Brun A, Danchin EG, Duchaussoy F *et al* (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* 452: 88-92.
- Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O *et al* (2010). Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* 464: 1033-1038.
- Martinez D, Larrondo LF, Putnam N, Gelpke MD, Huang K, Chapman J *et al* (2004). Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nat Biotechnol* 22: 695-700.
- Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE *et al* (2008). Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nat Biotechnol* 26: 553-560.
- Martinez D, Challacombe J, Morgenstern I, Hibbett D, Schmoll M, Kubicek CP *et al* (2009). Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *PNAS* 106: 1954-1959.
- Maurer P, Rejasse A, Capy P, Langin T, Riba G (1997). Isolation of the transposable element hupfer from the entomopathogenic fungus *Beauveria bassiana* by insertion mutagenesis of the nitrate reductase structural gene. *Mol Gen Genet* 256: 195-202.
- Navarrete K, Roa A, Vaca I, Espinosa Y, Navarro C, Chavez R (2009). Molecular characterization of the *niaD* and *pyrG* genes from *Penicillium camemberti*, and their use as transformation markers. *Cell Mol Biol Lett* 14: 692-702.
- Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J *et al* (2005). Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* 438: 1151-1156.
- Nowrousian M, Stajich JE, Chu M, Engh I, Espagne E, Halliday K *et al* (2010). *De novo* assembly of a 40 Mb eukaryotic genome from short sequence reads: *Sordaria macrospora*, a model organism for fungal morphogenesis. *PLoS Genet* 6: e1000891.
- Okamoto PM, Fu YH, Marzluf GA (1991). *Nit-3*, the structural gene of nitrate reductase in *Neurospora crassa*: nucleotide sequence and regulation of mRNA synthesis and turnover. *Mol Gen Genet* 227: 213-223.
- PbSP *Paracoccidioides brasiliensis* Sequencing Project, Broad Institute of Harvard and MIT (<http://www.broadinstitute.org/>).
- Pel HJ, de Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ *et al* (2007). Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nat Biotechnol* 25: 221-231.

Pereira JF, de Queiroz MV, Lopes FJ, Rocha RB, Daboussi MJ, de Araujo EF (2004). Characterization, regulation, and phylogenetic analyses of the *Penicillium griseoroseum* nitrate reductase gene and its use as selection marker for homologous transformation. *Can J Microbiol* 50: 891-900.

Pieterse CM, van't Klooster J, van den Berg-Velthuis GC, Govers F (1995). *NiaA*, the structural nitrate reductase gene of *Phytophthora infestans*: isolation, characterization and expression analysis in *Aspergillus nidulans*. *Curr Genet* 27: 359-366.

Poulsen N, Kroger N (2005). A new molecular tool for transgenic diatoms: control of mRNA and protein biosynthesis by an inducible promoter-terminator cassette. *FEBS J* 272: 3413-3423.

Screen S, Bailey A, Charnley K, Cooper R, Clarkson J (1998). Isolation of a nitrogen response regulator gene (*nrr1*) from *Metarhizium anisopliae*. *Gene* 221: 17-24.

Stajich JE, Wilke SK, Ahren D, Au CH, Birren BW, Borodovsky M *et al* (2010). Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). *PNAS* 107: 11889-11894.

Tudzynski B, Mende K, Weltring KM, Kinghorn JR, Unkles SE (1996). The *Gibberella fujikuroi niaD* gene encoding nitrate reductase: isolation, sequence, homologous transformation and electrophoretic karyotype location. *Microbiology* 142 (Pt 3): 533-539.

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A *et al* (2006). *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313: 1261-1266.

Unkles SE, Campbell EI, Punt PJ, Hawker KL, Contreras R, Hawkins AR *et al* (1992). The *Aspergillus niger niaD* gene encoding nitrate reductase: upstream nucleotide and amino acid sequence comparisons. *Gene* 111: 149-155.

van den Berg MA, Albang R, Albermann K, Badger JH, Daran JM, Driessen AJ *et al* (2008). Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. *Nat Biotechnol* 26: 1161-1168.

Williams RS, Davis MA, Howlett BJ (1995). The nitrate and nitrite reductase-encoding genes of *Leptosphaeria maculans* are closely linked and transcribed in the same direction. *Gene* 158: 153-154.