

## SUPPLEMENTARY MATERIALS

### FIGURE LEGENDS

**Supplementary Figure 1.** Alignment of AMP domain amino acid sequences encoded by all *C. heterostrophus* *NPS* and *NPS*-like genes.

Bold letters above alignment indicate positions of 10 conserved motifs named A1—A10 (1). Asterisks indicate the 10 positions that constitute the amino acid specificity-conferring code of the adenylation domains (2). The AMP consensus sequences were mapped onto the *C. heterostrophus* alignment based on the alignment of all sequences represented in Fig. 7 and Supplementary Table 2 and thus vary from the *ChAMP* consensus.

**Supplementary Figure 2.** Alignment of Condensation (C)-Epimerization (E) domain amino acid sequences encoded by all *C. heterostrophus* *NPS* and *NPS*-like genes.

Bold letters above and below alignment show five conserved C motifs and two conserved E motifs, respectively (1). The C and E consensus sequences were mapped onto the *C. heterostrophus* alignment based on the alignment of all sequences represented in Supplementary Fig. 1. and Supplementary Table 2 and thus vary from the *ChC-E* consensus.

**Supplementary Figure 3.** *NPS* deletion construct design and evaluation of integration events.

In transformants, primer pair up/2 and primer pair 3/dn should yield PCR products with DNA from strains with homologous integration of the transforming DNA, while those with ectopic integrations would not, nor would wild type. With homologous integrants, primer pair

up/dn or primer pair 1/4 would yield predicted-sized PCR products that are different in size (in all cases here) from those of wild type. There would be no wild-type bands. If disruption were unsuccessful (i.e., ectopic integrant), primer pair up/dn or primer pair 1/4 would yield wild type bands of predicted size plus a band of predicted size corresponding to the input DNA.

**Supplementary Figure 4.** NPS6 confers resistance to hydrogen peroxide.

Colony growth after three days on non-amended MM (A) and 7mM H<sub>2</sub>O<sub>2</sub> amended MM (B) of wild type strain C5 (top left); *nps6* mutants N6-R-3 (top right), N26-R-9 (middle left), N26-R-10 (middle right) and wild-type progeny, N26-R-11 and N26-R-12 (bottom).

**REFERENCES**

1. **Stachelhaus, T., and M. A. Marahiel.** 1995. Modular structure of peptide synthetases revealed by dissection of the multifunctional enzyme GrsA. *J. Biol. Chem.* **270**:6163-9.
2. **Stachelhaus, T., H. D. Mootz, and M. A. Marahiel.** 1999. The specificity-conferring code of adenylation domains in nonribosomal peptide synthetases. *Chem. Biol.* **6**:493-505.
3. **Kim, M. K., J. H. Jeon, M. Fujita, L. B. Davin, and N. G. Lewis.** 2002. The western red cedar (*Thuja plicata*) 8-8' DIRIGENT family displays diverse expression patterns and conserved monolignol coupling specificity. *Plant Mol. Biol.* **49**:199-214.

**Supplementary Table 1. Real Time PCR: *NPS* gene expression.**

<i>Actin</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )*	
6 hrs CM	18.57 ± 0.29	100	
20 hrs CM	19.81 ± 0.66	100	
72 hrs CMX	22.06 ± 0.21	100	
120 hrs Fries	18.57 ± 0.29	100	
24 hrs – nitrogen	20.83 ± 1.12	100	

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<i>NPS1</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	28.82 ± 0.55	0.06200	1.30
20 hrs CM	30.85 ± 0.53	0.04760	1.00
72 hrs CMX	35.39 ± 0.91	0.00969	0.20
120 hrs Fries	29.54 ± 0.26	0.04980	1.05
24 hrs – nitrogen	28.35 ± 0.42	0.54620	11.5

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<i>NPS2</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	40.00 ± 0.00	0.00003	N/A
20 hrs CM	40.00 ± 0.00	0.00008	N/A
72 hrs CMX	40.00 ± 0.00	0.00039	N/A
120 hrs Fries	40.00 ± 0.00	0.00004	N/A
24 hrs – nitrogen	40.00 ± 0.00	0.00017	N/A

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<i>NPS3</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	28.76 ± 0.44	0.06490	1.80
20 hrs CM	31.25 ± 0.81	0.03600	1.00
72 hrs CMX	35.05 ± 0.25	0.01230	0.34
120 hrs Fries	30.13 ± 0.28	0.03320	0.90
24 hrs – nitrogen	28.37 ± 0.60	0.53800	14.9

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<i>NPS4</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	29.63 ± 0.54	0.03550	1.60
20 hrs CM	31.96 ± 0.45	0.02210	1.00
72 hrs CMX	36.75 ± 0.72	0.00374	0.17
120 hrs Fries	32.00 ± 0.80	0.00905	0.40
24 hrs – nitrogen	29.61 ± 0.38	0.22760	10.3

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<i>NPS5</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	30.18 ± 0.99	0.02430	0.15
20 hrs CM	34.52 ± 2.18	0.00374	1.00
72 hrs CMX	40.00 ± 0.00	0.00040	N/A
120 hrs Fries	32.48 ± 2.30	0.00648	1.73
24 hrs – nitrogen	30.44 ± 0.64	0.12760	34.11

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<i>NPS6</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	25.64 ± 0.45	0.56140	0.47
20 hrs CM	26.23 ± 1.39	1.18100	1.00
72 hrs CMX	30.32 ± 0.58	0.32530	0.28
120 hrs Fries	24.24 ± 0.13	1.96050	1.66
24 hrs – nitrogen	26.90 ± 0.74	1.48780	1.26

<i>NPS7</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	29.64 ± 1.95	0.03520	2.32
20 hrs CM	29.17 ± 0.78	0.15190	1.00
72 hrs CMX	31.19 ± 0.79	0.17780	1.17
120 hrs Fries	31.42 ± 4.64	0.01350	0.09
24 hrs – nitrogen	27.10 ± 1.04	1.29380	8.52

<i>NPS8</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	31.02 ± 1.61	0.01350	1.11
20 hrs CM	32.51 ± 0.84	0.01500	1.00
72 hrs CMX	36.42 ± 0.17	0.00473	0.35
120 hrs Fries	31.48 ± 0.88	0.01300	0.86
24 hrs – nitrogen	29.51 ± 2.02	0.24450	16.3

<i>NPS9</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	30.74 ± 0.58	0.01640	3.88
20 hrs CM	30.43 ± 1.01	0.06370	1.00
72 hrs CMX	34.75 ± 0.12	0.01510	0.23
120 hrs Fries	31.72 ± 1.84	0.01100	0.17
24 hrs – nitrogen	30.17 ± 0.37	0.15390	2.41

<i>NPS10</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	40.00 ± 0.00	0.00003	N/A
20 hrs CM	33.00 ± 4.38	0.01070	1.00
72 hrs CMX	40.00 ± 0.00	0.00040	N/A
120 hrs Fries	39.76 ± 0.62	0.00004	3.74
24 hrs – nitrogen	36.74 ± 0.45	0.00162	0.15

<i>NPS11</i>	Ave. Ct	Relative to actin 2 $2^{-\Delta Ct}$ (n x 10 <sup>-2</sup> )	Fold change relative to 20h
6 hrs CM	27.72 ± 0.90	0.13270	0.80
20 hrs CM	29.05 ± 0.53	0.16540	1.00
72 hrs CMX	34.09 ± 0.82	0.02390	0.14
120 hrs Fries	28.86 ± 0.31	0.08010	0.48
24 hrs – nitrogen	27.89 ± 0.34	0.74870	4.53

Notes:

6hrs CM: conidia germinated 6 hrs in shaken liquid CM.

20 hrs CM: mycelium grown 20 hrs in shaken liquid CM.

72 hrs CMX: mycelium grown in 20 hrs shaken liquid CM then shifted to solid CMX for 72 hrs conidiation.

120 hrs Fries: mycelium grown in 120 hrs in high iron, still liquid Fries.

24 hrs – Nitrogen: mycelium grown in 20 hrs in shaken liquid CM shifted to MM minus nitrogen.

Ct = cycle time

N/A = no calculation done

\* based on reference 3

**Supplementary Table 2.** Gene names and GI or Accession numbers for 48 *NPS* and *NPS*-like genes included in the phylogenetic analyses, and DNA sequence source for new GenBank accessions.

<b>Species</b>	<b>NPS protein</b>	<b>GI or Acc. number</b>	<b>DNA source for new Accessions</b>
<i>Acremonium chrysogenum</i>	PCBAB	113315	
<i>Alternaria alternata</i>	AMT1	6018082	
<i>Alternaria brassicae</i>	PSY1	32264582	
<i>Aspergillus fumigatus</i>	NPS6	AY928087	Af293
<i>Aspergillus fumigatus</i>	NPS10	AY928090	Af293
<i>Aspergillus oryzae</i>	NPS6	32400320	
<i>Aspergillus nidulans</i>	ACVA	113314	
<i>Aspergillus nidulans</i>	NPS6	49097826	
<i>Aspergillus nidulans</i>	NPS10	49095988	
<i>Aspergillus nidulans</i>	SIDC	32709395	
<i>Aureobasidium pullulans</i>	SID2	17511288	
<i>Botrytis cinerea</i>	NPS6	AY928086	B05.10
<i>Claviceps purpurea</i>	PS1	4499839	
<i>Claviceps purpurea</i>	PS2	27525931	
<i>Cochliobolus carbonum</i>	HTS1	167218	
<i>Cochliobolus heterostrophus</i>	NPS1	AY884186	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS2	AY884187	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS3	AY884188	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS4	AY884189	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS5	AY884190	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS6	AY884191	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS7	AY884192	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS8	AY884193	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS9	AY884194	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS10	AY884195	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS11	AY884196	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS12	AY884197	C4, ATCC 48331
<i>Cochliobolus heterostrophus</i>	NPS13	AY884198	C4, ATCC 48331
<i>Gibberella zeae</i>	NPS6	46115810	PH-1 (NRRL 31084)
<i>Gibberella zeae</i>	NPS10	46124259	PH-1 (NRRL 31084)
<i>Fusarium scirpi</i>	ESYN1	15591913	
<i>Gibberella moniliformis</i>	NPS6	AY928085	ATCC 38932 (FGSC 7600)
<i>Gibberella moniliformis</i>	NPS10	AY928089	ATCC 38932 (FGSC 7600)
<i>Hypocrea virens</i>	TEX1	21902204	
<i>Kallichroma tethys</i>	PCBAB	13398458	
<i>Leptosphaeria maculans</i>	MAA1	28629055	
<i>Leptosphaeria maculans</i>	SIRP	46403055	
<i>Lysobacter lactamgenus</i>	PCBAB	1752727	
<i>Neurospora crassa</i>	NPS1	28918023	
<i>Neurospora crassa</i>	NPS2	28921789	
<i>Neurospora crassa</i>	NPS6	32418018	
<i>Nocardia lactamdurans</i>	PCBAB	45006	
<i>Magnaporthe grisea</i>	NPS6	AY928088	70-15
<i>Magnaporthe grisea</i>	NPS10	38105248	
<i>Metarhizium anisopliae</i>	PESA	2342600	
<i>Penicillium chrysogenum</i>	ACVA	3118	
<i>Schizosaccharomyces pombe</i>	NPS2	6912019	
<i>Tolypocladium inflatum</i>	SIMA	440168	
<i>Ustilago maydis</i>	SID2	2731632	
<i>Ustilago maydis</i>	NPS10	49072928	