

Supplementary information

The cysteine-rich virulence factor NipA of *Arthrobotrys flagrans* interferes with cuticle integrity of *Caenorhabditis elegans*

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running head: *C. elegans* infection with *A. flagrans*

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Suppl. Information Table 1: *A. flagrans* and *C. elegans* strains used in this study.

Strain	Genotype	Reference
<i>A. flagrans</i>		
sJM01	$\Delta nipA$, pJM06	This work
sJM02	$gpdA(p)::nipA::lccC^{\Delta AS1-18}$ pJM07	This work
sJM42	$gpdA(p)::nipA^{\Delta AS1-16}lcc^{\Delta AS1-18}$ pJM68	This work
sVW10	$h2b(p)::h2b::GFP::h2b(t)$ pJW04	15
sJM15	sVW10 x $nipA(p)::h2b::mCherry::tubA(t)$ pJM10	This work
sJM05	$nipA(p)::nipA::gfp$ pJM01	This work
sVWZ	$tubA(p)::lifeact::gfp::gluC(t)$	15
sNH30	$cyrA(p)::cyrA::GFP::gluC(t)$	20
sJM08	sVW07 x $nipA(p)::nipA::mCherry$ pJM16	This work
sJM09	sNH30 x $nipA(p)::nipA::mCherry$ pJM16	This work
sJM43	WT x $cyrA(p)::cyrA::mCherry$ pNH94	This work
sJM44	$\Delta nipA$ x $cyrA(p)::cyrA::mCherry$ pNH94	This work
sJM50	sJM44 x $nipA(p)::nipA::GFP$ pJM102	This work
sJM51	sJM44 x $nipA(p)::nipA^{Cys23/Ala}::GFP$ pJM100	This work
sJM52	sJM44 x $nipA(p)::nipA$ pJM103	This work
<i>C. elegans</i>		
N2	Wild type	University of Freiburg
GS2478	$arls37\ I$; $dpy-20(e1282)\ IV$; $cup-8(ar466)\ V$	<i>C. elegans</i> center, University of Minnesota
GOU2043	$arls37\ I$; $dpy-20(e1282)\ IV$; $cup-8(ar466)$	<i>C. elegans</i> center, University of Minnesota
KIT39	N2 x $eft-3(p)::mScarlet$ pJM86	This work
KIT40	N2 x $eft-3(p)::nipA::mScarlet$ pJM85	This work
KIT41	GS2478 x $eft-3(p)::mScarlet$ pJM86	This work
KIT42	GS2478 x $eft-3(p)::nipA::mScarlet$ pJM85	This work
KIT44	N2 x $dpy-7(p)::nipA::mScarlet$ pJM81	This work

KIT45	N2 x <i>col-19(p)::nipA::mScarlet</i> pJM46	This work
KIT46	N2 x <i>col-19(p)::mScarlet</i> pJM72	This work
KIT48	N2 x <i>col-19(p)::nipA^{ΔSP}::mScarlet</i> pJM36	This work
KIT49	N2 x <i>col-19(p)::nipA</i> pJM32	This work
KIT50	GS2478 x <i>col-19(p)::mScarlet</i> pJM72	This work
KIT51	GS2478 x <i>col-19(p)::nipA::mScarlet</i> pJM46	This work
KIT52	N2 x <i>dpy-7(p)::mScarlet</i> pJM91	This work
KIT53	N2 x <i>dpy-7(p)::mScarlet; col-19(p)::nipA</i> pJM91; pJM32	This work
KIT54	GOU2043 x <i>col-19(p)::nipA::mScarlet</i> pJM46	This work
KIT55	GOU2043 x <i>col-19(p)::nipA</i> pJM32	This work
KIT57	N2 x <i>col-19(p)::nipA^{Cys/Ala}::mScarlet</i> pJM92	This work
KIT58	N2 <i>eft-3(p)::nipA::mScarlet; unc-122(p)::GFP</i> pJM85; pNH79	This work

Suppl. Information Table 2: Plasmids used in this study.

Name	Description	Reference
pNH10	<i>gpdA(p)::cyrA::lccC^{ΔAS1-18} trpC(p)::hph::trpC(t)</i> (<i>hph</i>)	²⁰
pNH79	<i>unc-122::GFP</i>	N. Wernet
pNH94	<i>cyrA(p)::cyrA::mCherry::gluC(t); tub(p)::G418</i>	N. Wernet
pJM06	<i>ΔnipA x pJET1.2, hph</i>	This work
pJM07	<i>gpdA(p)::nipA::lccC^{ΔAS1-18}; hph</i>	This work
pJM68	<i>gpdA(p)::nipA^{ΔAS1-16}lcc^{ΔAS1-18}; hph</i>	This work
pJM10	<i>nipA(p)::h2b::mCherry::tubA(t); hph</i>	This work
pVW23	<i>h2b(p)::h2b::mCherry::h2b(t); hph</i>	²⁰
pJM01	<i>nipA(p)::nipA::gfp; hph</i>	This work
pNH21	<i>oliC(p)::cyrA::gfp::gluC(t); hph</i>	²⁰
pJM08	<i>oliC(p)::nipA::gfp::gluC(t); hph</i>	This work
pJM16	<i>nipA(p)::nipA::mCherry::gluC(t);</i> <i>gpdA(p)::g418::trpC(t) (g418)</i>	This work
pNH53	<i>oliC(p)::broA::GFP; g418</i>	N. Wernet
pNH94	<i>cyrA(p)::cyA::mCherry::gluC(t), g418</i>	N. Wernet
pJM32	<i>col-19(P)::nipA</i>	This work
pJM36	<i>col-19(P)::nipA::mScarlet::unc-54 3'UTR</i>	This work
pJM46	<i>col-19(P)::nipA::mScarlet::unc-54 3'UTR</i>	This work
pJM72	<i>col-19(P)::mScarlet::unc-54 3'UTR</i>	This work
pJM81	<i>dpy-7(p)::nipA::mScarlet::unc-54 3'UTR</i>	This work
pJM85	<i>eft-3(p)::nipA::mScarlet::unc-54 3'UTR</i>	This work
pJM86	<i>eft-3(p)::mScarlet::unc-54 3'UTR</i>	This work
pJM91	<i>dpy-7(p)::mScarlet::unc-54 3'UTR</i>	This work
pJM92	<i>col-19(p)::nipA^{Cys23/Ala}::mScarlet::unc-54 3'UTR</i>	This work
pJM100	<i>nipA(p)::nipA^{Cys23/Ala}::GFP::gluC(t); nat</i>	This work
pJM102	<i>nipA(p)::nipA::GFP::gluC(t); nat</i>	This work
pJM103	<i>nipA(p)::nipA::gluC(t); nat</i>	This work

Suppl. Information Table 3: Oligonucleotides used in this study.

Name	Sequence 5'→ 3'	Description
5407_AscI_for	GGCGCGCCATGAAAGGCGCTATCCTCA TTAT	C-terminal Laccase/GFP fusion
5407_noStop_AgeI_rev	TATACCGGTGGCTTCCTCGTCGGGGAT	C-terminal Laccase fusion
nipA_noSP_gpdA(p)_OL_fw d	agcagacatcacaGGCGCGCCATGCCGAC CGTAAGTGCCCAAG	C-terminal Laccase fusion without SP
SB_5407_RB_fwd	GGATTTCCAGAAATCGCATGC	Southern-Blot analysis
SB_5407_RB_rev	GAGGCAATAGGTCCAATTTATAC	Southern-Blot analysis
nipA_noStopp_Lcc_OL_rev	TGCTCAAAGACCCAAGAACCGGTGGCT TCCTCGTCGGGGAT	C-terminal Laccase fusion without SP
5407_RT_for	ATG AAA GGC GCT ATC CTC ATT AT	qRT PCR
5407_RT_rev	CAA TTG GCG GAA TTC TTG CAT TT	qRT PCR
qpcr_hk_actin2_fwd	TCCAGACTGCCTCCCAGT	qRT PCR; Youssar
qpcr_hk_actin2_rev	AGGTCTTTTCTGACGTCGAC	qRT PCR; Youssar
p5407_H2B_OL_rev	CTTTTCGGCGGCGGCTTTTGGTGGCAT TTTGTTGGGCTTTCTAACCAC	Promoter fusion
p5407_1.7_EfiOL_fwd	TGTAAAACGACGGCCAGTGAATTCGG CACTTTTGTGTAATTGCGCATTGG	Promoter fusion
Backbone_Efi_rev	GAATTCAGTGGCCGTCGTTT	Backbone amplification
H2B-NLS_for	ATGCCACCAAAAGCCGCC	Backbone amplification for promoter fusion
5407_PacI_noStop_rev	gcgttaattaaGGCTTCCTCGTCGGGGAT	C-terminal GFP fusion
p5407_pJM08_OL_fwd	CCCTAAACTCCCCCAGGCACTTTTGTG TAATTGCGC	C-terminal GFP fusion under native promoter
p5407_pJM08_OL_rev	GATAGCGCCTTTCATGGTTTGTGGGC TTTCTAACCACAA	C-terminal GFP fusion under native promoter
pJM08_BB_5407_fwd	CCATGAAAGGCGCTATCCT	C-terminal GFP fusion under native promoter
trpC(t)_rev	TGGGGGGAGTTTAGGGAAA	Backbone amplification
p5407_1.7kb_trpCT_OL_fwd	ATGCTCTTTCCCTAAACTCCCCCAGGC ACTTTTGTGTAATTGCGC	C-terminal mCherry fusion under native promoter

5407_mCherry_OL_rev	TTACTTACCTCGCCCTTGCTTACcttaatt aaGGCTTCCTCGTCG	C-terminal mCherry fusion under native promoter
mCherry_5407_OL_fwd	CCCGACGAGGAAGCCttaagGTAA GCAAGGGCGAGGTAA	C-terminal mCherry fusion under native promoter
mCherry_tgluC_OL_rev	AATCATACATCTTATCTACATACGCTAA GCGGCCGCTTTGTAG	C-terminal mCherry fusion under native promoter
tgluC_for	CGTATGTAGATAAGATGTATGATT	Backbone amplification
pcol-19_pSW49_fwd	CGGGCCTCTTCGCTATTACGCCAGT acgtaccattattcgagacaac	Col-19 promoter
pcol-19_pSW49_rev	TCCGACGTCCCCAGGCAGAATGGCGgt tgatgaactgatgtctttctaa	Col-19 promoter
ppf37_bb_rev	gagcaaagtgtttccaactg	Backbone amplification
ppf37_bb_fwd	TCA GGT GGA TCT GGA GGC T	Backbone amplification
scar_col-19P_OL_fwd	gaaagacatcagttcatcaacATGGTCAGCA AGGGAGAGG	<i>MScarlet under col-19 in C. elegans</i>
scar_Pst_unc54_3UTR_rev	gcggccgatgcggagctcCTGCAGttaCTTGT AGAGCTCGTCCATTC	<i>MScarlet in C. elegans</i>
nipA_Stop_unc-54_OL_rev	tgacagcggccgatgcggagctcCTAGGCTTC CTCGTCGGG	NipA in <i>C. elegans</i>
nipA_unc-54_3'UTR_OL_rev	ccgatgcggagctcGAATTCGGCTTCCTCG TCGGGGAT	NipA in <i>C. elegans</i>
nipA_SP_col-19_OL_fwd	ttagaaagacatcagttcatcaacATGAAAGG CGCTATCCTCATTAT	<i>NipA in C. elegans under col-19</i>
col-19(P)_rev	ggtgatgaactgatgtctttctaaatg	Backbone amplification
nipA_noStopp_rev	GGCTTCCTCGTCGGGGAT	<i>NipA in C. elegans</i>
nipA_SP_fwd	ATGAAAGGCGCTATCCTCATTA	NipA amplification
OL_col19p_nipA-ohneSP_fwd	gaaagacatcagttcatcaacATGCCGACCTC CAAAATATGC	<i>NipA in C. elegans under col-19</i>
peft-3_pPF37_OL_fwd	ggataacaatttcacaGGGCCCgcacctttggt cttttattgtcaac	<i>NipA in C. elegans under eft-3</i>
peft-3_nipA_OL_rev	GATAATGAGGATAGCGCCTTTCATgagc aaagtgtttccaactg	<i>NipA in C. elegans under eft-3</i>
peft-3_mScarlet_OL_rev	ACTGCCTCTCCCTTGCTGACCATgagcaa agtgtttccaactg	<i>NipA in C. elegans under eft-3</i>

dpy-7P_ppf37_OL_fwd	cggataacaatttcacaGGGCCctgaaagtct ctccggtag	<i>NipA in C. elegans under dpy-7</i>
dpy-7P_nipA_OL_rev	CTCCCTTGCTGACCATAACCGGTTTCTCG ATGAAATCTCAACTTATATG	<i>NipA in C. elegans under dpy-7</i>
dpy-7P_scar_OL_rev	GTTGACCTCCACTAGCATTACACTTCTT GAAAGAGTGTCTCGGTTTT	<i>mScarlet expression under dpy-7</i>