Supplemental Information

Functional analysis of a biosynthetic cluster essential for production of 4-formylaminooxyvinylglycine, a germination-arrest factor from *Pseudomonas fluorescens* WH6

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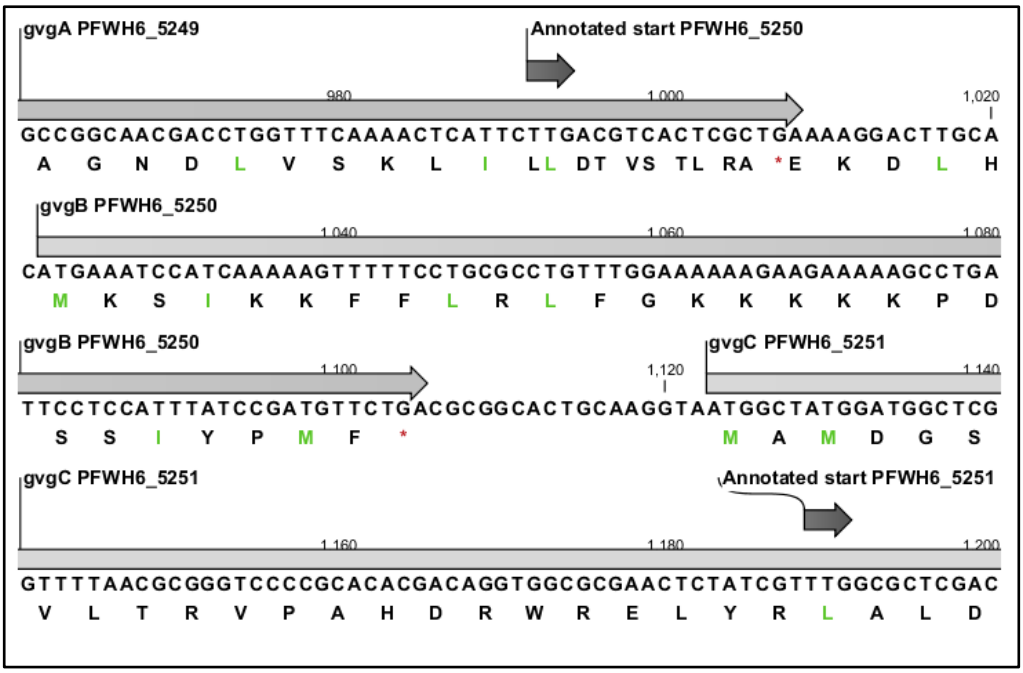
Table of Contents:

Supplemental figures:

Supplemental tables:

(a)



(b)

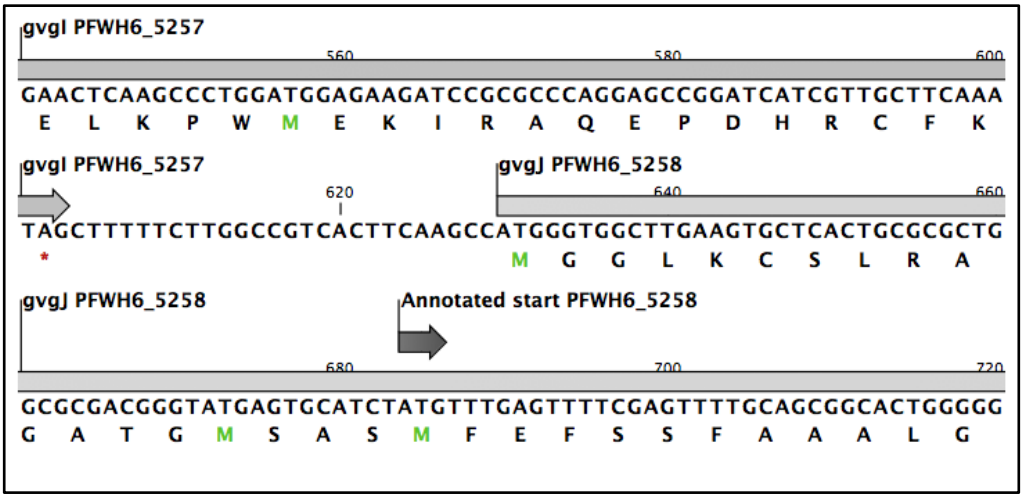


Fig. S1. Diagram of sequence discrepancies between annotated ORFs in the *Pseudomonas fluorescens* WH6 genome (NCBI CM001025, start sites indicated with dark gray arrows) and ORFs utilized for constructing deletion mutations and plasmids for complements (light gray arrows). The translations are shown under the gene diagrams. (a) Comparison of annotated start sites and utilized start sites for *gvgB* and *gvgC*. In the translation for the overlapping region between *gvgA* and the annotated *gvgB*, the first letter is the translation for *gvgA* and the second for *gvgB*. (b) Comparison of annotated start site and the start site used for constructing deletions mutations and complements of *gvgJ*.

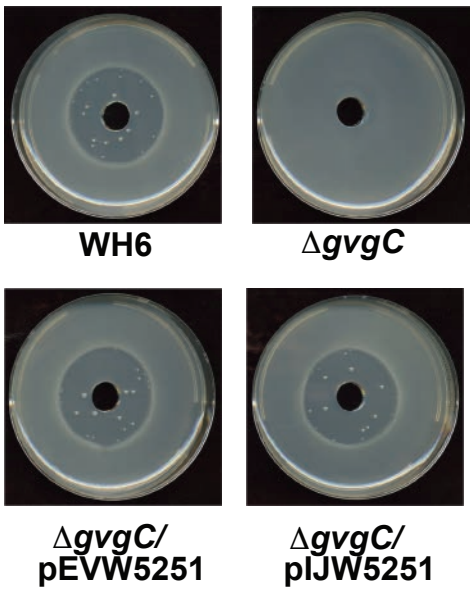
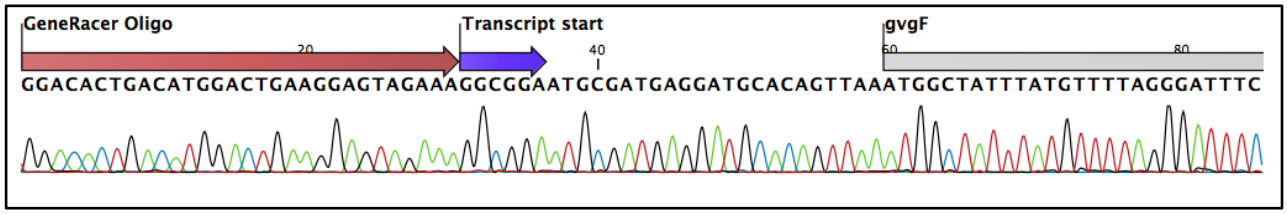


Fig. S2. Confirmation that pIJW5251 (*gvgC* under an Ara-inducible promoter) complements the Δ*gvgC* mutation. Strains tested in the agar diffusion assays for anti-*Erwinia* activity were wild-type WH6, WH6-25G (Δ*gvgC*), WH6-25G/ pEVW5251, and WH6-25G/ pIJW5251.

(a)



(b)

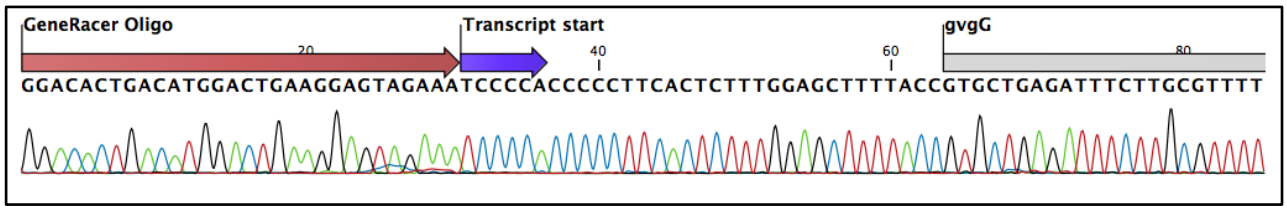


Fig. S3. Transcriptional start sites determined from longest sequenced transcripts from 5′ RACE analysis for (a) *gvgF,* with reverse primer within *gvgF* and (b) *gvgG-H,* with reverse primer within *gvgH*.

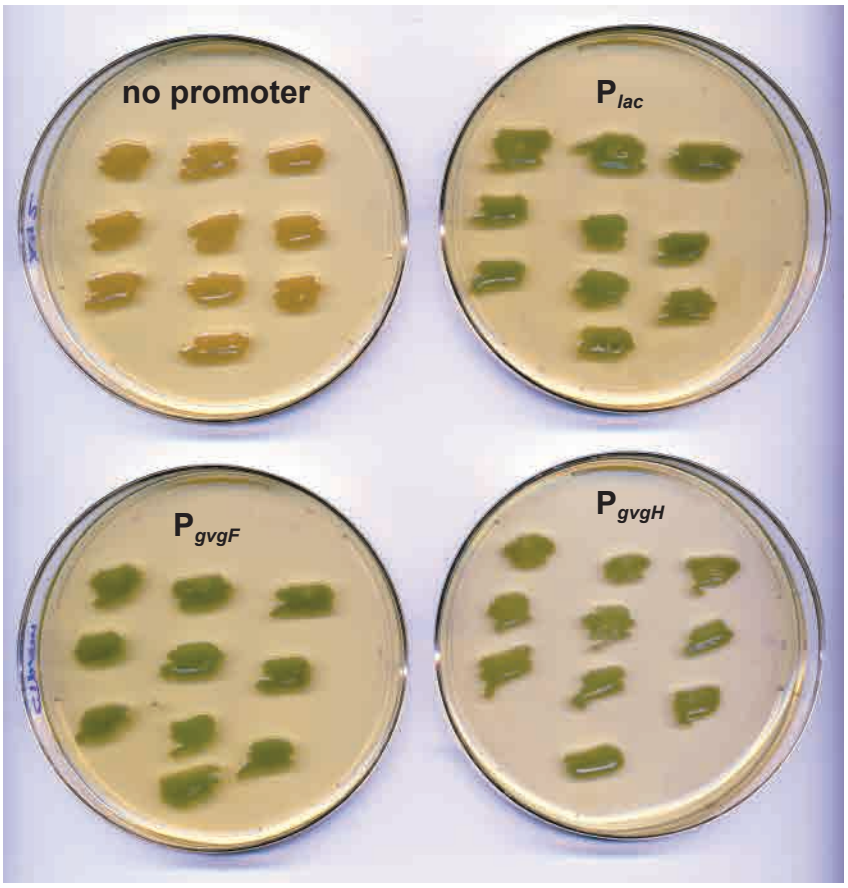


Fig. S4. β-galactosidase activity was qualitatively assessed on LB agar supplemented with X-gal for *lacZ* promoter fusions P_{lac}-*lacZ,* P_{gvgF}-*lacZ* and P_{gvgH}-*lacZ* along with a promoter-less *lacZ* negative control in the *P. fluorescens* WH6 background. Ten clones were tested from each transformation of *P. fluorescens* WH6.

Table S1. Bacterial strains used in this study

Strains	Relevant Characteristics	Reference
Pseudomonas fluorescens		
WH6	Wild type; from *Triticum aestivum* L. roots; Ap^r	(Elliott *et al.*, 1998)
WH6-21G	Δ*gvgR*; PFWH6_5248 deleted (lacking nt 1 – 1455); Ap^r	This study
WH6-22G	Δ*tam;* PFWH6_5247 deleted (lacking nt 1 – 762); Ap^r	This study
WH6-23G	Δ*gvgA;* PFWH6_5249 deleted (lacking nt 1 – 1008); Ap^r	This study
WH6-24G	Δ*gvgB;*PFWH6_5250 deleted (lacking nt 4 – 82); Ap^r	This study
WH6-25G	Δ*gvgC;* PFWH6_5251 deleted (lacking nt 1 – 2211); Ap^r	This study
WH6-26G	Δ*gvgD;* PFWH6_5252 deleted (lacking nt 4 – 1041); Ap^r	This study
WH6-27G	Δ*gvgE;* PFWH6_5253 deleted (lacking nt 4 – 615); Ap^r	This study
WH6-28G	Δ*gvgF;* PFWH6_5254 deleted (lacking nt 4 – 1833); Ap^r	This study
WH6-29G	Δ*gvgG;* PFWH6_5255 deleted (lacking nt 6 – 135); Ap^r	This study
WH6-30G	Δ*gvgH;* PFWH6_5256 deleted (lacking nt 4 – 1317); Ap^r	This study
WH6-31G	Δ*gvgI;* PFWH6_5257 deleted (lacking nt 4 – 594); Ap^r	This study
WH6-32G	Δ*gvgJ;* PFWH6_5258 and 51 additional nt upstream deleted; Ap^r	This study
WH6-33G	Δ*gvgK;* PFWH6_5259 deleted (lacking nt 4 – 609); Ap^r	This study
WH6-34G	Δ*gvgCJK;* PFWH6_5251, PFWH6_5258 and PFWH6_5259 deleted; Ap^r	This study
Escherichia coli DH5α	F^- *recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 Δ*(*arg-lacZYA*)*1169* (*Φ80lacZ ΔM15*)	Life Technologies
Erwinia amylovora 153	Wild type; originally isolated from a fire blight canker on Gala apple in Eastern Oregon (Obtained from Dr. Joyce Loper, USDA-ARS, Corvallis, OR)	(Halgren *et al.*, 2011)

Table S2. Bacterial plasmids used in this study

Plasmid	Relevant Characteristics	Source
pEX-18Tc	Mob$^+$ *sacB* gene replacement vector; Tc^r	(Hoang *et al.*, 1998)
pEX-18Km	Modified version of pEX-18Tc with *tet* replaced by *kan* from pKD13; Km^r	This study
pRK2013	Mob$^+$ RK2 *tra* ColE1 plasmid; Km^r	(Figurski & Helinski, 1979)
pBH474	Suc^s derivative of pTH474 with *flp* expressed constitutively; Gm^r	(House *et al.*, 2004)
pKD13	Source of FRT-*kan*-FRT for pJET-KanR-FRT plasmid and *kan* for pEX-18Km plasmid*;* Km^r	(Datsenko & Wanner, 2000)
pJet1.2/blunt™	Cloning vector for PCR products; Ap^r	Life Technologies
pCR4Blunt-TOPO™	Cloning vector for PCR products; Ap^r, Km^r	Life Technologies
pJET-KanR-FRT	Plasmid with FRT-*kan*-FRT sequence from pKD13 flanked by BamHI and XhoI sites; Ap^r; Km^r	(Okrent *et al.*, 2014)
pBBR1MCS-5	Broad host range cloning vector for use in complementation; Gm^r	(Kovach *et al.*, 1995)
pBBR1EVM	Derivative of the pBBR1MCS-5 cloning vector for constitutive expression; Gm^r.	(Okrent *et al.*, 2014)
pJN105	Derivative of the pBBR1MCS-5 cloning vector for inducible expression using L-arabinose (*araC*-P_{BAD}); Gm^r	(Newman & Fuqua, 1999)
pXY2	Mini-Tn*7* vector for constructing translational fusions with *lacZ*, inserts region of interest at conserved attTn*7* site; Gm^r	(Liu *et al.*, 2014)
pTNS3	Helper plasmid for insertion of Mini-Tn*7* containing *tnsABCD*; Ap^r	(Choi *et al.*, 2008)
pEXW21	pEX-18Km- Δ*gvgR*; Km^r	This study
pEXW22	pEX-18Km-Δ*tam*; Km^r	This study
pEXW23	pEX-18Km-Δ*gvgA*; Km^r	This study
pEXW24	pEX-18Tc-Δ*gvgB*; Tc^r, Km^r	This study
pEXW25	pEX-18Km-Δ*gvgC*; Km^r	This study
pEXW26	pEX-18Tc-Δ*gvgD*; Tc^r, Km^r	This study
pEXW27	pEX-18Tc-Δ*gvgE;* Tc^r, Km^r	This study
pEXW28	pEX-18Tc-Δ*gvgF*; Tc^r, Km^r	This study
pEXW29	pEX-18Tc-Δ*gvgG*; Tc^r, Km^r	This study
pEXW30	pEX-18Tc-Δ*gvgH*; Tc^r, Km^r	This study
pEXW31	pEX-18Tc-Δ*gvgI*; Tc^r, Km^r	This study
pEXW32	pEX-18Tc-Δ*gvgJ*; Tc^r, Km^r	This study
pEXW33	pEX-18Tc-Δ*gvgK*; Tc^r, Km^r	This study
pEXW34	pEX-18Tc-Δ(*gvgJ-gvgK)*; Tc^r, Km^r	This study

pEVW5248	pBBR1EVM with *gvgR* flanked by XhoI and BamHI sites; Gm^r	This study
pNBW5248N2	pBBR1MCS-5 with *gvgR, gvgA* and the intergenic region between (containing putative native promoters for both genes) flanked by BamHI sites; Gm^r	This study
pEVW5249	pBBR1EVM with *gvgA* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5249N2	pBBR1EVM with *gvgA-gvgB* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5250	pBBR1EVM with *gvgB* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5251	pBBR1EVM with *gvgC* flanked by XhoI and BamHI sites; Gm^r	This study
pIJW5251	pJN105 with *gvgC* flanked by EcoRI and XbaI sites; Gm^r	This study
pEVW5254	pBBR1EVM with *gvgF* flanked by XhoI and SacI sites; Gm^r	This study
pEVW5255	pBBR1EVM with *gvgG* flanked by XhoI and SacI sites; Gm^r	This study
pEVW5256	pBBR1EVM with *gvgH* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5257	pBBR1EVM with *gvgI* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5258	pBBR1EVM with *gvgJ* flanked by XhoI and BamHI sites; Gm^r	This study
pEVW5259	pBBR1EVM with *gvgK* flanked by XhoI and SacI sites; Gm^r	This study
pNZWp5254	pXY2 with putative promoter of *gvgF* flanked by SpeI and HindIII sites; Gm^r	This study
pNZWp5256	pXY2 with putative promoter of *gvgH* flanked by SpeI and HindIII sites; Gm^r	This study
pXY2-plac	pXY2 with lac promoter flanked by SpeI and HindIII sites; Gm^r	This study

Table S3. Primers* used in this study

Primer ID	Primer sequence (5' to 3')
AH024	GCAGTTTCAAGGAGCACACC
AH100	CAGAACCCCAGGGGCTTGCCTTT
AH103	CCAGCACAACGACCCGGAAATCA
AH106	ACTTTTCAGGCAAGCCGCGTTCG
AH107	CCTCCGAGCCGCCGGATAAAAAC
AH108	CCACCGCTGGCAAACGAGGAAAT
AH109	CCTGCAGGTCAGCCAGGGTGATG
AH137	GTTTTTATCCGGCGGCTCGGAGG
KT12	TTACGGGTTGGATCCCTCGAGCACGGTAAAAGCTCCAAAGAGTGAAGG
KT13	GCTTTTACCGTGCTCGAGGGATCCAACCCGTAA GGCCAATCGCCCAAGG
KT14	gctataGAATTCGGTCGCTGATCAACAGTGCC
KT18	gctataAAGCTTTGGCGATGAAGAAATCG
lacZ6	CTTTCCGGCACCGCTTCTGG
M13F	GTAAAACGACGGCCAGT
MM005	GGATCATCGTTGCTTCAAATAG
MM006	GACGAGTCTGCGCTTGCCGT
MM007	gatcaGCGGCCGCGAGCTCCATGGATATTTCCTGGAAACGG
MM008	ccatgGAGCTCGCGGCCGCTGATCCCAGGCACGCAGTTGT
MM009	gccgccAAGCTTGCTGGCGCGACGGGTATGAG
MM010	gccgccGAATTCCTTGGCCGAGCGCCTCAAGG
MM011	TGGCAGGTAAGCGCGAAGGC
MM012	GGCGCCGGATTTCGACAGCT
MM014	GCCGATTCGATCTGGGGCG
MM017	gccgccCTCGAGTCAAAAGTGATTTACAAAAATC
MM018	aaagccGGATCCCTAGTACGCGACAGCC
MM019	CCACGCTTGGGGCGTTGAT
MM021	GCCGACGGCTTGGGATCAC
MM022	CCTTGTCCTTGCATGGCTCGA
MM023	gccgccAAGCTTGGGCCGGGTTTTGGCGATGA
MM024	gccgccGAGCTCGAAATCACCCCGGCCGCCAA
MM025	GCTAAAGGATCCCTCGAG CATGCGCGCGGTTCCTTGG
MM026	CATGCTCGAGGGATCCTTTAGCGACAGCGCGGGCTTG
MM027	TCATCGCCAAAACCCGGCC
MM028	GTGCAGCAACGTCAAGCCGC
MM029	TACCGGCCACATGGCGCAAG
MM030	gccgccAAGCTTGAGCCTGCGCGAGGCCTATT
MM031	ggcggcGAATTCCCCTCGGCGTCCAGGCAGTA
MM032	TCCTGCTCAGCGGCCGCGGATCCCATAATTGTTCCTCGTGGCGTTAAGG
MM033	CAATTATGGGATCCGCGGCCGCTGAGCAGGACGTGAGCATGCAG
MM034	CAGACTTCGCCCTGCGTTTTAAAGGC
MM035	CAGGGCGAAGTCTGACTGTAGCTGCTC

MM036	GCGCCAGATATCCAGATGCT
MM038	ATGTGCGCCATGAATTTACC
MM039	gccgccCTCGAGACTCTTTATCTGAACCTCGCC
MM042	ACCCAGCGCAAAAGGACTTGCACATGAAATCC
MM043	CAAGTCCTTTTGCGCTGGGTCCCCTTGG
MM044	gccgccCTCGAGACGGCAAGGATGACGCTC
MM045	gccgccGGATCCTCAGCGAGTGACGTCAAGAATG
MM046	GCATGATGTAGCCCATGGG
MM047	GGCTCTGGGGCCAGTG
MM048	TCGCAAGGTACGCCACGAGGAACAATTATG
MM049	GTGGCGTACCTTGCAGTGCCGCGTC
MM050	gccgccCTCGAGGCTATGGATGGCTCGGTTT
MM051	gccgccGGATCCTTAAGGTTCGTGGGTTTCCA
MM055	gccgccGGATCCATTCCGGGGATCgGTCGAC
MM056	gccgccGGATCCGTGTAGGCTGGAGCTGCTTCG
MM067	gccgccGCGGCCGCGTGTAGGCTGGAGCTGCTTCG
MM069	gccgccGAGCTCATTCCGGGGATCgGTCGAC
MM072	AGCCCGAAAGCCCGATCTC
MM075	gccgccCTCGAGATTCCGGGGATCgGTCGAC
MM077	CGCTCAAGCAAGAGGCGTTG
MM079	TGGACGATATCGGCGCTTTG
MM080	TGAAGTTGCCGAGCTGGTCG
MM086	ATTGCTGGCCGAGGCAT
MM087	ACTCCGCATCCAGGAAACATTC
MM088	gccgccCTCGAGTTGACGTCACTCGCTGAAAAGG
MM089	gccgccGGATCCTCAGAACATCGGATAAATGGAGGAA
MM095	ACCGATGCCGGTGAAGTTC
MM096	CCACCAGCATCAGTGGAAAG
MM097	gccgccTCTAGACGATCTGTTCCTGCAT
MM098	gccgccGAGCTCCAATGGGCAAGTGATTCCGG
MM099	CCCAAGGCGACGGTGTGTA
MM100	GCTTGGCGGACCAGGTC
MM101	gccgccGGATCC CTAAGGCTGGCGTTGTTG
MM104	gccgcc AAGCTT GGCTTCCAGGTTGATGCCGG
MM105	gccgcc GAGCTC CGGTCAGCGAGGCGTTATA
MM106	GTTGGAAACTGCCCAGCGAA
MM107	CTTGCAGTGCCGCGTCAG
MM108	ATGACGGCAAGGATGACGCTC
MM111	gccgccAAGCTTTAACGGCCCGGTACGCCTGT
MM112	gccgccGAGCTCGACGGACACACAGTGAAAACC
MM113	CGATGTTCTGACGCGGCAC
MM114	GGACTGGCAGTTCGTCGACC
MM119	GGCGAACTTCAGGGTGGTGG

MM120	TCCGCATCTTCAGTGAGGCG
MM123	CGGGTTGACCCACTGGAACAA
MM124	CCTTGTCCTTGCATGGCTCG
MM125	GTGATCGCCACCTGCGTCA
MM126	TTGACCGCAAAATCCAACGC
MM129	TCAACTCGTATGCTGCAGCAGC
MM132	gccgccGAGCTCGCGGTTGATCGAGACCTGGGTG
MM133	gccgccTCTAGAACCTGATTATCACCAGCGGCTGCC
MM135	aagccgcaCGCCGGTAGCGGTCATACGG
MM136	ACCGGCGTGCGGCTTTCCTTTAAAACGCAGC
MM137	GCAGTGACAGCACCAGCCTG
MM138	CTGCCGGCGTAGCCATTC
MM139	CCACCCGCAAGGTCGAGATG
MM140	GTGATCCATGATGCGTGCGC
pJETF	CGACTCACTATAGGGAGAGCGGC
pJETR	AAGAACATCGATTTTCCATGGCAG
RO7	CACGTCAACCATCCGCTGG
RO8	TCAAGAACAGGATCCCTGGAGCATGGCTTGAAGTGACGGCCAAG
RO9	CAAGCCATGCTCGAGGGATCCTGTTCTTGACTCCAACTACAAGTG
RO10	ACGGCATCAAGCGTTACACC
RO11	ACGTAGGTGCCTATTGCCTGC
RO12	CAACAGCATGGCGTTATTGG
RO21	acgtacAAGCTTCACGTCAACCATCCGCTGG
RO25	tcgacGAGCTCACGGCATCAAGCGTTACACC
RO29	GCTGGTGAGTACCTGCGCAAAGG
RO68	aaccggAAGCTTGCTACCACGGCAGCACCCTGG
RO69	GCTACCACGGCAGCACCCTGG
RO70	CAAGAAAAAGCTATTTGAAGGATCCCTCGAGCATCTTCACTTCTCCATTT
RO71	AGAAGTGAAGATGCTCGAGGGATCCTTCAAATAGCTTTTTCTTGGCCGTCA
RO72	cacaggGAATTCGCGATGTTCCATGCCAGGTACAGC
RO73	GCACGGTGTAGAGCACCGGATAGG
RO74	aatggaCTCGAGCAGACAATCGAGCCAT
RO75	gtggttGGATCCCTATTTGAAGCAACGATGA
RO76	aatggaCTCGAGGGTGGCTTGAAGTGC
RO77	gtggtgGGATCCTCAGAACAACGAACTGA
RO78	ccagcaCTCGAGGCTATTTATGTTTTAGGGATTT
RO91	aagttgGAGCTCTCAGTCCAGCGGG
RO92	aaccggAAGCTTCGACTACAGCGAGCACCACGAC
RO93	CGACTACAGCGAGCACCACGAC
RO94	CTCTGCGTTTCAAGACCAGGATCCCTCGAGCATGCTCACGTCCTGCTCAA
RO95	GACGTGAGCATGCTCGAGGGATCCTGGTCTTGAAACGCAGAGAAAGGTT
RO96	cacaggGAATTCCAGTTCAACGCCACGCCACC
RO97	CAGTTCAACGCCACGCCACC

RO98	TCAACGACCAGCAGGTCC
RO99	CCGCACTGTCGTGATAGAAGG
RO102	GTTGGATTTTGCGGTCAA
RO103	CGATGACTTGTTGCACTT
RO106	GAAATGGACGTGCTGGTTCT
RO107	CAGGTGTCGAGGTAGCGTTT
RO108	CACGAACCTTAACGCCAC
RO109	ACTGGGCGATTTCTTCAT
RO116	ccagcaCTCGAGGTGCTGAGATTTCTTGC
RO117	aagttgGAGCTCTTACGGGTTACTTACGGA
RO121	aagttgGAGCTCTCAACTCGTATGCTGC
RO124	aagttgCTCGAGCGCAGCTTTCAGTA
RO141	aatggaCTCGAGTCCTTGTCGTTTGATTTA
RO156	aaccggAAGCTTGAACTGGGACATCATCTGG
RO157	GAACTGGGACATCATCTGG
RO158	GGTGGGGATCAGTCCAGGGATCCCTCGAGCATTTAACTGTGCATCCT
RO159	CACAGTTAAATGCTCGAGGGATCCCTGGACTGATCCCCACC
RO160	cacaggGAATTCCATCGAGGATCAGCAGCA
RO161	ATCGAGGATCAGCAGCA
RO165	ATCGATTGGCACAGGCA
RO166	AACGGGACGAATGAAACACC
RO167	TGTACCTGTTGTTCCTGGC
RO171	ACGCACCTGGACACTAC
RO173	CATAGGCGAACAGCTGC
RO213	gtagcTACTAGTCTTGCAGGTGATCGG
RO215	gtagttACTAGTGAAGATCAAATACCGCGAGTCG
RO217	gtcgttACTAGTCCCAGGCTTTACACTTTATGC
T7	TAATACGACTCACTATAGGG
VM001	TGCCTGGGATCAGCGGCCGCGAGCTCCATGGCTTGAAGTGACGGCCA
VM002	CTTCAAGCCATGGAGCTCGCGGCCGCTGATCCCAGGCACGCAGTT
VM005	CCTGCTATCTGTTTTTCCT
VM006	TTTACCTTCGGTGTCTTCT
VM007	gccgccTCTAGAGACGAGTCTGCGCTTGCCGT
VM008	GCGGTTTGTCGGTTGTGTTCCTG
VM010	TAACAAAGCAGCACAGTACGC
VM011	TGCAGTGCCGCGTCAGGATCCCTCGAGCATGTGCAAGTCCTTTTC
VM012	AAGGACTTGCACATGCTCGAGGGATCCTGACGCGGCACTGCAAGG
VM013	TCACTGAAGATGCGGAACA
VM014	acgtacAAGCTTTAACAAAGCAGCACAGTACGCACC
VM015	gccgccGAATTCTCACTGAAGATGCGGAACA
VM019	AGCCATGGAGCTCGCGGCCGCATTCCGGGGATCGGTCGA
VM020	GTGCCTGGGATCAGCGGCCGCAGTGTAGGCTGGAGCTGCTTC

VM027	CATTCATCCTGCAACCCTT
VM029	TTGACAAAGGGAATCAGG
VM036	caggacGAATTCATGGCTATGGATGGCTCG
VM037	gtcctgTCTAGATTAAGGTTCGTGGGTTTCC
VM047	gtcaccAAGCTTGTGATAGAAGGCAGAAATCCC
VM049	acAAGCTTCGGCGAGGTGTTGAGATTTT

* The purposes of these primers are described in Tables S4–S9. Underlined nucleotides indicate restriction recognition sites and nucleotides in lowercase indicate nucleotides added for efficient cleavage by restriction enzymes.

Table S4. Primers* for overlap extension PCR to construct deletions and restriction sites for cloning PCR fragments into pEX18-Km or pEX18-Tc vectors

Deletion Construct	Gene(s)	Backbone (pEX18-)	5′ 1º PCR	3′ 1º PCR	2º PCR	Restriction sites†
pEXW21G	*gvgR*	Km	MM097/ MM035	MM034/ MM098	MM097/ MM098	X/S
pEXW22G	*tam*	Km	MM133/ MM136	MM135/ MM132	MM133/ MM132	X/S
pEXW23G	*gvgA*	Km	MM104/ MM43	MM042/ MM105	MM104/ MM105	H/S
pEXW24G	*gvgB*	Tc	VM010/ VM011	VM012/ VM013	VM014/ VM015	H/E
pEXW25G	*gvgC*	Km	MM111/ MM049	MM048/MM112	MM111/ MM112	H/S
pEXW26G	*gvgD*	Tc	MM030/ MM032	MM033/ MM031	MM030/ MM031	H/E
pEXW27G	*gvgE*	Tc	RO93/ RO94	RO95/ RO97	RO92/ RO96	H/E
pEXW28G	*gvgF*	Tc	RO157/ RO158	RO159/ RO161	RO156/ RO160	H/E
pEXW29G	*gvgG*	Tc	KT18/ KT12	KT13/ KT14	KT18/ KT14	H/E
pEXW30G	*gvgH*	Tc	MM023/ MM025	MM026/ MM024	MM023/ MM024	H/S
pEXW31G	*gvgI*	Tc	RO69/ RO70	RO71/ RO73	RO68/ RO72	H/E
pEXW32G	*gvgJ*	Tc	RO7/ RO8	RO9/ RO10	RO21/ RO25	H/E
pEXW33G	*gvgK*	Tc	MM005/ MM007	MM008/ MM006	MM009/ MM010	H/E
pEXW34G	*gvgJK*	Tc	RO7/ VM001	VM002/ MM006	RO21/ VM007	H/X

* Primer sequences are listed in Table S3.

† E = EcoRI, H = HindIII, S = SacI, X = XbaI

Table S5. Primers* for amplification of the FRT-*kan*-FRT fragment for cloning into the deletion constructs

Deletion construct	Gene(s)	*kan* inserted?	Sites	Primers
pEXW21	*gvgR*	N	N/A	N/A
pEWX22	*tam*	N	N/A	N/A
pEXW23	*gvgA*	N	N/A	N/A
pEXW24	*gvgB*	Y	XhoI/ BamHI	digest†
pEXW25	*gvgC*	N	N/A	N/A
pEXW26	*gvgD*	Y	BamHI/ NotI	MM055/ MM067
pEXW27	*gvgE*	Y	XhoI/ BamHI	digest
pEXW28	*gvgF*	Y	XhoI/ BamHI	digest
pEXW29	*gvgG*	Y	XhoI/ BamHI	digest
pEXW30	*gvgH*	Y	XhoI/BamHI	MM075/ MM056
pEXW31	*gvgI*	Y	XhoI/ BamHI	digest
pEXW32	*gvgJ*	Y	XhoI/ BamHI	digest
pEXW33	*gvgK*	Y	SacI/ NotI	MM069/ MM067
pEXW34	*gvgJK*	Y	HiFi Assembly	VM019/ VM020

*Primer sequences are listed in Table S3.

†"Digest" indicates the FRT-*kan*-FRT fragment was excised from the pJET-KanR-FRT plasmid.

Table S6. Primers* used for confirmation of deletion mutants

Strain	Mutation	Distinguish†	Amplification‡	Sequencing§
WH6-21G	Δ*gvgR*	MM099/ MM100	MM036/ MM038	T7, MM099
WH6-22G	*tam*	MM139/MM140	MM137/MM138	MM139
WH6-23G	Δ*gvgA*	MM106/ MM107	MM046/ MM047	T7, MM106
WH6-24G	Δ*gvgB*	MM086/MM087	MM079/ MM119	pJETF, VM010, VM027, pJETR
WH6-25G	Δ*gvgC*	MM113/ MM114	MM095/ MM096	T7, MM114
WH6-26G	Δ*gvgD*	MM028/ MM029	MM028/ MM027	T7, pJETR
WH6-27G	Δ*gvgE*	RO98/ RO99	RO108/ RO109	pJETF, RO98, RO99, pJETR
WH6-28G	Δ*gvgF*	RO165/ RO166	RO171/ RO173	pJETF, RO167, pJETR
WH6-29G	Δ*gvgG*	RO106/ RO107	RO102/ RO103	pJETF, AH024, pJETR
WH6-30G	Δ*gvgH*	MM021/ MM022	MM021/ MM019	T7, pJETR
WH6-31G	Δ*gvgI*	RO7/ AH103	AH137/ AH100	pJETF, RO7, pJETR
WH6-32G	Δ*gvgJ*	RO11/ RO12	AH106/ RO29	pJETF, RO7, RO12, pJETR
WH6-33G	Δ*gvgK*	MM011/ MM012	MM011/ MM014	T7, pJETR
WH6-34G	Δ*gvgJK*	VM005/ VM006	VM008/ AH106	pJETF, pJETR, RO7, RO124, MM012

* Primer sequences are listed in Table S3.

† Distinguish: Primer set used to distinguish between wild-type and mutant copies of an allele.

‡ Amplification: Primer set used to amplify the region flanking the deletion prior to sequencing.

§ Sequencing: Primers used in sequencing reaction to confirm deletion genotype.

Table S7. Primers* used to make constructs for complementation or *lacZ* reporter studies

Gene(s)	Backbone	Construct	Digestion	Primer Pair
gvgR	pBBR1EVM	pEVW5248	XhoI/ BamHI	MM039/ MM101
gvgR-gvgA	pBBR1MCS5	pNBW5248N2	BamHI/ BamHI	MM101/ MM045
gvgA	pBBR1EVM	pEVW5249	XhoI/ BamHI	MM044/ MM045
gvgA-gvgB	pBBR1EVM	pEVW5249N2	XhoI/ BamHI	MM044/ MM089
gvgB	pBBR1EVM	pEVW5250	XhoI/ BamHI	MM088/ MM089
gvgC	pBBR1EVM	pEVW5251	XhoI/ BamHI	MM050/ MM051
gvgC	pJN105	pIJW5251	EcoRI/ XbaI	VM036/ VM037
gvgF	pBBR1EVM	pEVW5254	XhoI/ SacI	RO78/ RO91
gvgG	pBBR1EVM	pEVW5255	XhoI/ SacI	RO116/ RO117
gvgH	pBBR1EVM	pEVW5256	XhoI/ BamHI	MM017/ MM018
gvgI	pBBR1EVM	pEVW5257	XhoI/ BamHI	RO74/ RO75
gvgJ	pBBR1EVM	pEVW5258	XhoI/ BamHI	RO76/ RO77
gvgK	pBBR1EVM	pEVW5259	XhoI/ SacI	RO141/ RO121
P_{gvgF}	pXY2	pNZWp5254	SpeI/ HindIII	RO213/ VM047
P_{gvgH}	pXY2	pNZWp5256	SpeI/ HindIII	RO215/ VM049
P_{lac}	pXY2	pXY2-plac	SpeI/ HindIII	RO217/ M13F

* Primer sequences are listed in Table S3.

Table S8. Primers* used in RT-PCR for transcript identification

Fragment	Primer Pair	Length (kb)
1	MM079/ MM080	1.4
2	MM108/ MM119	2.4
3	MM120/ MM123	2.6
4	MM125/ MM126	1.7
5	MM072/ MM124	2.7
6	MM077/ MM129	2.3

* Primer sequences are listed in Table S3.

Table S9. Reverse primers* for 5′ RACE analysis

Gene	Primer
gvgF	AH108
gvgF nested	AH109
gvgH	AH106
gvgH nested	AH107

* Primer sequences are listed in Table S3.

Table S10. Germination arrest activity of filtrates from mutant strains of *P. fluorescens* WH6*

Strain	Genotype	0.03X	0.1X	0.3X	1.0X
WT	WT	1.8 ± 0.09	1.1 ± 0.07	1.0 ± 0.00	1.0 ± 0.00
WH6-21G	Δ*gvgR*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.7 ± 0.12
WH6-23G	Δ*gvgA*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.5 ± 0.14
WH6-24G	Δ*gvgB*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.3 ± 0.09
WH6-25G	Δ*gvgC*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00
WH6-26G	Δ*gvgD*	1.8 ± 0.09	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
WH6-27G	Δ*gvgE*	1.6 ± 0.07	1.1 ± 0.07	1.0 ± 0.00	1.0 ± 0.00
WH6-28G	Δ*gvgF*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.6 ± 0.10
WH6-29G	Δ*gvgG*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.1 ± 0.11
WH6-30G	Δ*gvgH*	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00
WH6-31G	Δ*gvgI*	4.0 ± 0.00	3.8 ± 0.12	1.3 ± 0.12	1.0 ± 0.00
WH6-32G	Δ*gvgJ*	4.0 ± 0.00	4.0 ± 0.00	2.8 ± 0.35	1.9 ± 0.29
WH6-33G	Δ*gvgK*	2.1 ± 0.15	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00

* In this semi-quantitative assay, a score of 4.0 indicated normal germination and a score of 1.0 indicates that the germination is arrested. Annual bluegrass seeds were challenged with a dilution series of filtrate of supernatants from bacterial cultures of the indicated *P. fluorescens* WH6 mutant strain. A concentration of 1.0X represents undiluted filtrate. Results are shown as mean values ± SEM.

Table S11. Germination arrest activity of filtrates from complemented mutant strains of *P. fluorescens* WH6*

Genotype	Strain	0.03X	0.1X	0.3X	1.0X
WT	WH6	1.8 ± 0.09	1.1 ± 0.07	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgR*	WH6-21G/ pEVW5248	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.7 ± 0.17
Δ*gvgR*	WH6-21G/ pNBW5248N2	2.1 ± 0.06	1.2 ± 0.09	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgA*	WH6-23G/ pEVW5249N2	3.5 ± 0.17	2.2 ± 0.08	1.2 ± 0.08	1.0 ± 0.00
Δ*gvgB*	WH6-24G/ pEVW5250	2.4 ± 0.11	1.6 ± 0.07	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgC*	WH6-25G/ pEVW5251	1.7 ±0.08	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgF*	WH6-28G/ pEVW5254	2.3 ± 0.09	1.5 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgG*	WH6-29G/ pEVW5255	2.6 ± 0.14	1.3 ± 0.08	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgH*	WH6-30G/ pEVW5256	2.0 ± 0.12	1.1 ± 0.07	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgI*	WH6-31G/ pEVW5257	1.9 ± 0.11	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgJ*	WH6-32G/ pEVW5258	2.4 ± 0.07	1.3 ± 0.09	1.0 ± 0.00	1.0 ± 0.00
Δ*gvgK*	WH6-33G/ pEVW5259	2.6 ± 0.07	1.9 ± 0.10	1.0 ± 0.00	1.0 ± 0.00

* In this semi-quantitative assay, a score of 4.0 indicated normal germination and a score of 1.0 indicates that the germination is arrested. Annual bluegrass seeds were challenged with a dilution series of filtrate of supernatants from bacterial cultures of the indicated *P. fluorescens* WH6 complemented mutant strain. A concentration of 1.0X represents undiluted filtrate. Results are shown as mean values ± SEM.

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