TABLE S1. Sequences of the primers used for RT-PCR and PCR

Primer	Sequence (5′ to 3′)	Purpose
Cloning: pOSUPrtId-1 & pOSUPrtId-2 deletion plasmids		
DM01	AGCTCCAGCGGGTCGCCGGGGTTGGCC	OE-PCR
DM02	CCAGCGGGTCGCCGGGGTTGGCC	OE-PCR
DM03	TAGATGCGGGATCCCTCGAGCATGGGGTGTCT GGCTGGTCACTAC	OE-PCR
DM04	CACCCCATGCTCGAGGGATCCCGCATCTAAGG CGACCTGCGCGCC	OE-PCR
DM05	AATTCTAGAACTGCAACAGGTGTGCGGGC	OE-PCR
DM06	CTAGAACTGCAACAGGTGTGCGGGC	OE-PCR
DM13	GATCCTGTAGGCTGGAGCTGCTTCG	FRT-KanR-FRT
DM14	CTGTAGGCTGGAGCTGCTTCG	FRT-KanR-FRT
JHC327	TCGAGATTCCGGGGATCCGTCGACC	FRT-KanR-FRT
JHC328	GATTCCGGGGATCCGTCGACC	FRT-KanR-FRT
AH05	AATTAATCAGCGCCGGGTCAAGGTT	OE-PCR
AH06	AATCAGCGCCGGGTCAAGGTT	OE-PCR
Cloning: pOSUAprXd deletion plasmid		
DaprX-1	CGATCGAAGCTTCGGGATGATTTGCCATGAGT TCAAA	OE-PCR
DaprX-2	CGGGATGATTTGCCATGAGTTCAAA	OE-PCR
DaprX-3	TCAGGTCGTTACCGGGATCCCTCGAGCATAAA CGTACTTCCTTGTGTGC	OE-PCR
DaprX-4	ACGTTTATGCTCGAGGGATCCCGGTAACGACC TGATCATCGGCAACAAC	OE-PCR
DaprX-5	GCATCGAATTCATCAAGGCGGTGAAGCAGCCA ATAC	OE-PCR
DaprX-6	ATCAAGGCGGTGAAGCAGCCAATAC	OE-PCR
KanR-F-B1	ACGGATCCTGTAGGCTGGAGCTGCTTCG	pJET-KanR-FRT
KanR-R-X1	ACCTCGAGATTCCGGGGATCGGTCGACC	pJET-KanR-FRT
Cloning: Complements		
PrtI-357F	CTCGGTCGAAGGCTTGAG	pOSU2000
ATPase-R	GCCTGGTTCTCAAAGAATGC	pOSU2000, pOSU1900
prtR-rbs-F	CGAAATTGCCAGCCCTTC	pOSU1900
aprX-comp-X1-F	AATGGACTCGAGTCCAAAGTAAAAGACAAGG	pEVW2895
aprX-comp-B1-R	GTGGTGGGATCCTCACGCTACGATGT	pEVW2895
5′ F PvuI	CGATCGGTGCGGGCCT	pBBR1EVM
5′ R MCS	CTGCAGGAGCTCGGATCCGCTAGCTCGCCCTA TAGTGAGTCGTATTACGC	pBBR1EVM
3′ F MCS	AAGCTTCCCGGGGAATTCGTCGACCTCGAGCA TAGCTGTTTCCTGTGTG	pBBR1EVM
3′ R NcoI	CCATGG GCAAATATTATACG	pBBR1EVM
MCS LIG F	GAGCTCCTGCAGAAGCTTCCCGGGG	pBBR1EVM
MCS LIG R	GAAGCTTCTGCAGGAGCTCGGATCCG	pBBR1EVM
prtI-comp-X1-F2	AATGGACTCGAGCATGAACTCGACGAAC	pEVW3686
prtI-comp-B1-R2	GTGGTGGGATCCTTATTTGAGTATCCGCA	pEVW3686

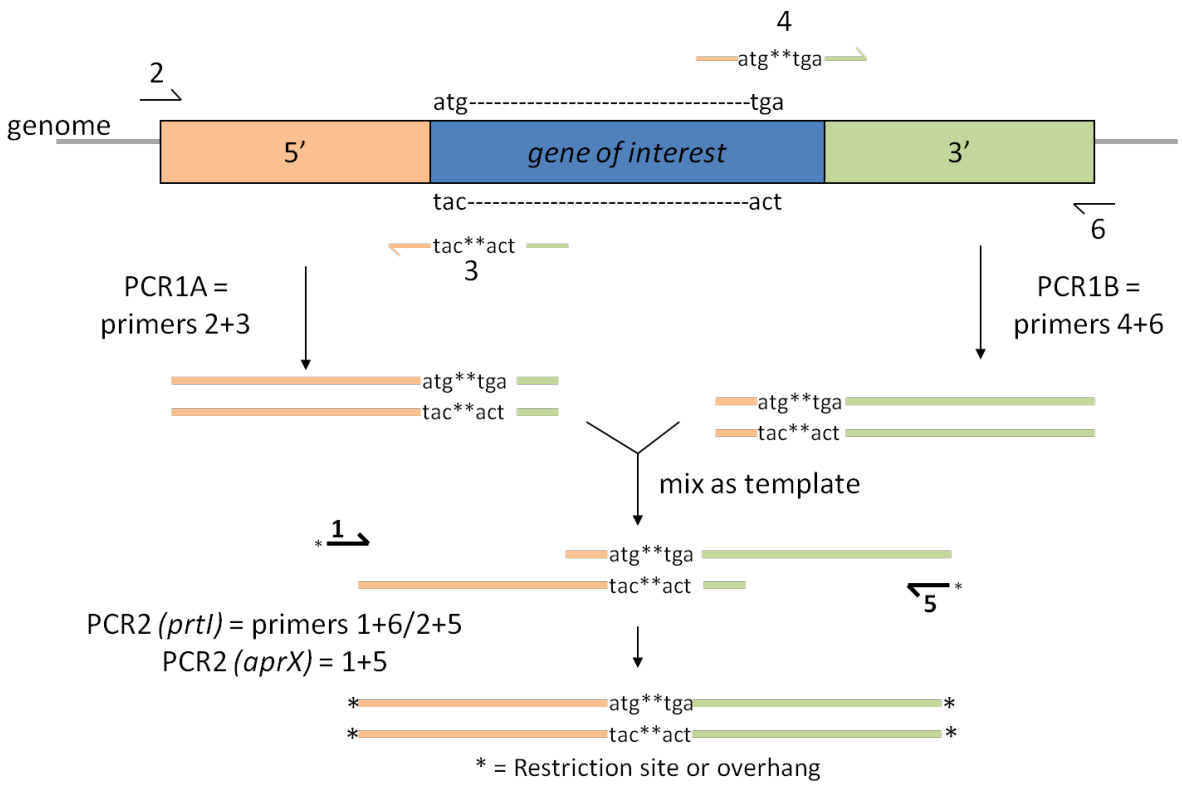
RT-PCR	
Promoter-upper-F	CAAACTCCAGGGCCAGTG
Right-201	TTTGAGTATCCGCAGGGAAG
prtI-RT-F	ATGCATGAACTCGACGAACC
prtR-RT-R	GGACCAGTATTGCGCTTGC
5′ RACE	
PrtI-5′ RACE-1R	AGGAACTGGCGGTAGAGGAT
PrtI-5′ RACE-2R	TTTGAGTATCCGCAGGGAAG
PrtR-5′ RACE-1R	GTGCCGCTGTCCTGGGCGTTGTAAT
PrtR-5′ RACE-2R	TGACGACCGCATAGTTGT

The sequences of restriction sites are underlined. OE-PCR is overlap extension PCR.

Table S2. Effect of culture temperature on GAF activity in culture filtrates from *Pseudomonas fluorescens* WH6 and WH6-3. Culture filtrates from 7-day cultures were tested for GAF activity in the *Poa annua* and *Erwinia amylovora* bioassays as described in Materials and Methods.

BACTERIAL STRAIN	CULTURE TEMPERATURE	GAF ACTIVITY IN THE POA BIOASSAY* (Germination Score ± Standard Error of the Mean)** CULTURE FILTRATE CONCENTRATION					ANTI-ERWINIA ACTIVITY** Area of Zone of Inhibition (cm^2) ± Standard Error of the Mean
		0	**0.03X**	**0.1X**	**0.3X**	**1.0X**	
WH6 (Wild type)	**28 ºC**	4.0 ± 0.00	2.2 ± 0.05	1.3 ± 0.10	1.0 ± 0.00	1.0 ± 0.00	17.5 ± 0.15
	20 ºC	4.0 ± 0.0	1.9 ± 0.10	2.0 ± 0.20	1.0 ± 0.00	1.0 ± 0.00	16.4 ± 0.08
WH6-3 (*prtR*::Tn*5* Mutant)	**28 ºC**	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	3.5 ± 0.20	0.0 ± 0.00
	20 ºC	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	4.0 ± 0.00	2.8 ± 0.50	0.0 ± 0.00

*The scoring system for the *Poa* bioassay is described in detail by Banowetz *et al.,* 2008. A score of 4 indicates normal seedling development. A score of 1 indicates germination arrest immediately after emergence of the radicle and coleoptile.
**All values are the average of two replicate experiments that gave identical or nearly identical results. For each filtrate concentration tested in the *Poa* assay, 9 seeds were evaluated in each experiment. For evaluation of anti-*Erwinia* activity, 3 replicate plates were prepared for each experiment.



Primer	pOSUPrtId-1	pOSUPrtId-2	pOSUAprXd
1	DM01	DM01	DaprX-1
2	DM02	DM02	DaprX-2
3	DM03	DM03	DaprX-3
4	DM04	DM04	DaprX-4
5	DM05	AH05	DaprX-5
6	DM06	AH06	DaprX-6

Fig. S1. Overlap Extension PCR for creation of suicide constructs in pEX18-Tc using recombinant/sticky-end PCR and standard restriction digests. To construct pOSUPrtId-1 and 2 using recombinant/sticky-end PCR, the products of the primary PCR reactions (2+3, 4+6) were gel purified, combined at approximately equal amounts and used as template for secondary reactions. The products of the secondary reactions (1+6, 2+5) were mixed in approximately equal ratios, incubated at 95 ºC for 5 minutes, and slowly cooled to room temperature, leading to a subset of the PCR products with overhanging sticky-ends compatible to the pEX18-Tc digested with *Hind*III and *Eco*RI. The FRT-KanR-FRT cassette was amplified from pKD13 using recombinant and sticky-end PCR with primers JHC327 + DM14 and JHC328 +DM13 in separate reactions. The products were combined, denatured and re-annealed as described above and cloned into the *Bam*HI and *Xho*I sites of the pEX18-Tc plasmid containing the mutated *prtI*. To construct pOSUAprXd using standard restriction digests, the products of the primary reactions (2+3, 4+6) were gel purified, combined at approximately equal amounts and used as template for secondary reactions (1+5). The product of the secondary reaction was gel purified, digested with *Hind*III and *Eco*RI, and cloned into pEX18-Tc. The FRT-KanR-FRT cassette was digested with *Xho*I and *Bam*HI from pJET-KanR-FRT, blunted, and then cloned into the *Xho*I site of pEX18-Tc containing the mutated *aprX*.

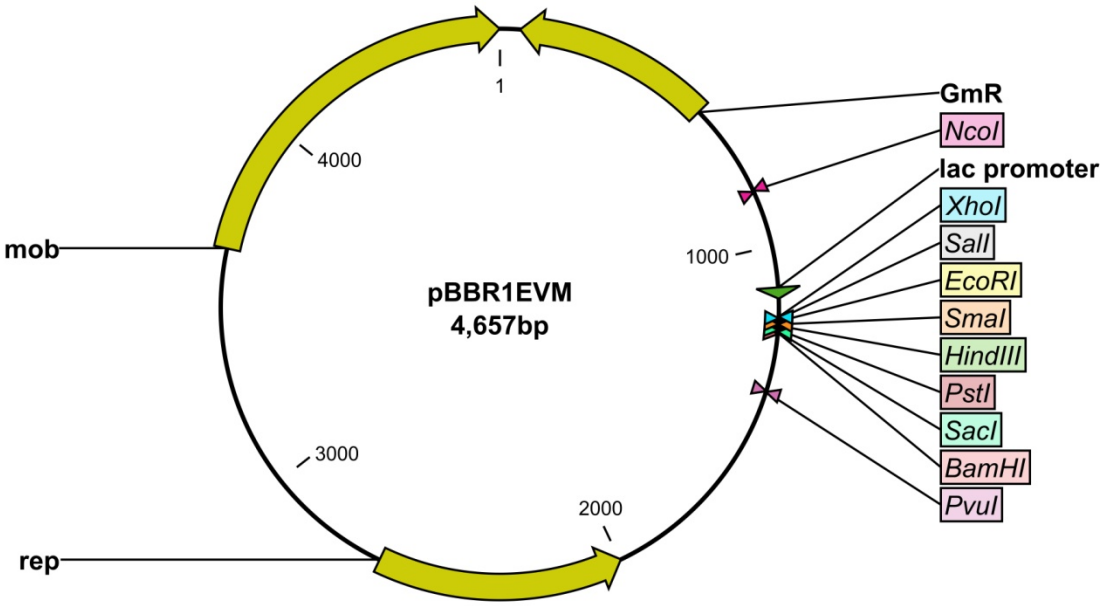


Fig. S2. The constitutive expression vector pBBR1EVM. The vector was created by altering the multiple cloning site of the broad host cloning vector pBBR1MCS-5 (Kovach *et. al*., 1995) and removing extraneous *lacZ*α gene sequence. These modifications were performed using overlap extension PCR to replace 654 nt between the *Pvu*I and *Nco*I sites of pBBR1MCS-5 sequence with 549 nt of new sequence. To generate the overlapping templates for the overlap extension PCR, pBBR1MCS-5 DNA was amplified in a series of reactions using Phusion High Fidelity DNA Polymerase (NEB). All PCR products were purified by gel purification or PCR purification as necessary. In the primary reactions, the 5′ and 3′ ends were amplified using primers 5′ F PvuI + 5′R MCS (**Table S1**) to generate the modified 5′ end, and 3′ F MCS + 3′ R NcoI to generate the modified 3′ end. In the secondary reactions, primers 5′ F PvuI + MCS LIG R were used to add the overlapping region to the product of the 5′ primary reaction, and primers MCS LIG F + 3′ R NcoI were used to add the overlapping region to the 3′ end. These PCR products, consisting of 5′ and 3′ ends with overlapping regions, were then combined and used as the template for overlap extension PCR using primers 5′ F PvuI + 3′ R NcoI. The purified PCR product was digested with *Nco*I and *Pvu*I, cloned into the corresponding restriction sites of pBBR1MCS-5, and the sequence verified. The plasmid map was created using CLC Main Workbench software (CLC bio).

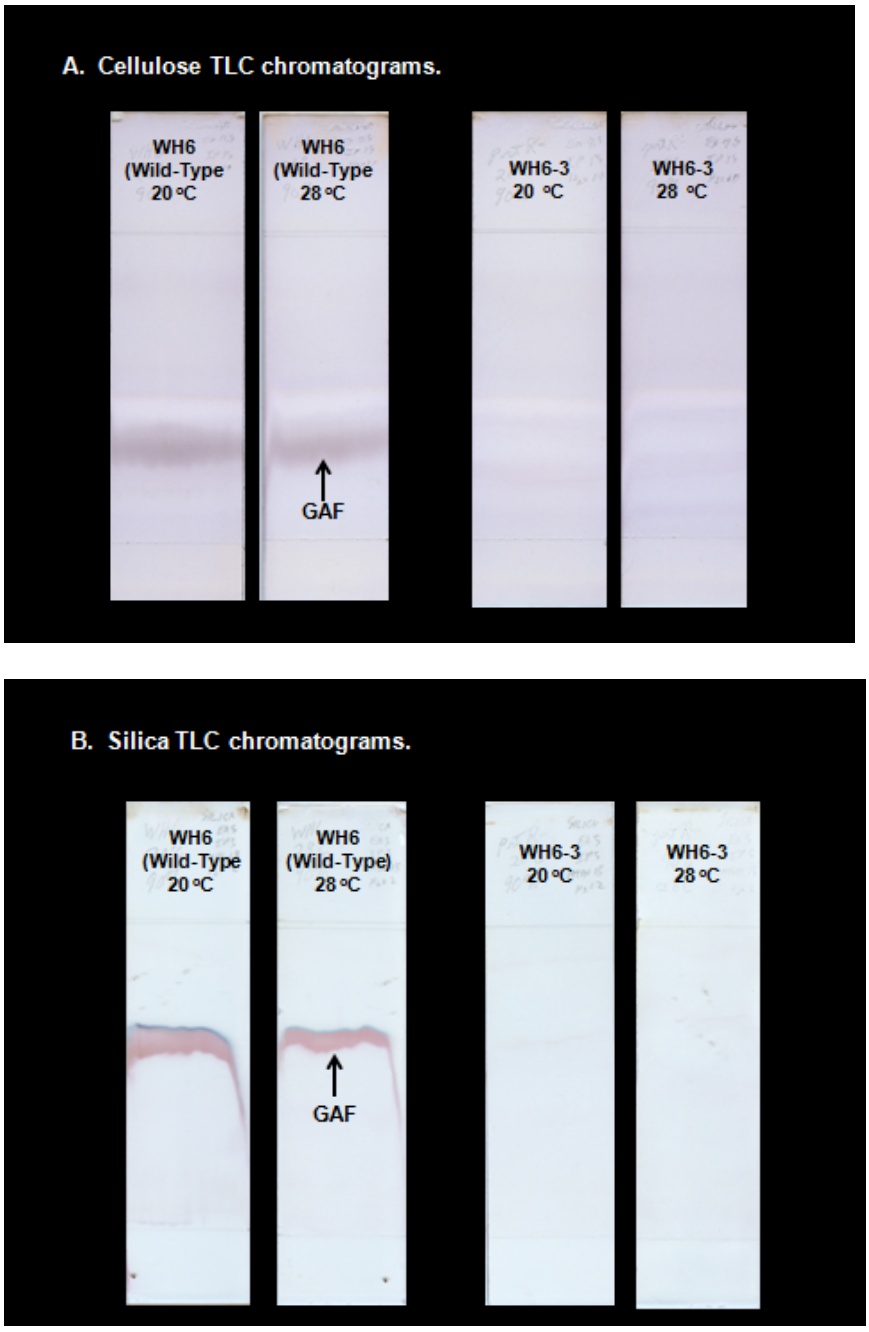


Figure S3. TLC analysis of temperature effects on Germination-Arrest Factor (GAF) production in WH6 (wild type) and WH6-3 (*prtR* mutant). *Pseudomonas fluorescens* WH6 (wild type) and WH6-3 (containing a Tn*5* insertion in *prtR*) were grown for 7 days at the temperatures indicated. The culture filtrates were dried, and the dry solids from the filtrate were extracted with 90% ethanol. The extracts were processed and subjected to TLC analysis as described in Methods. The ninhydrin-stained TLC plates are shown below.

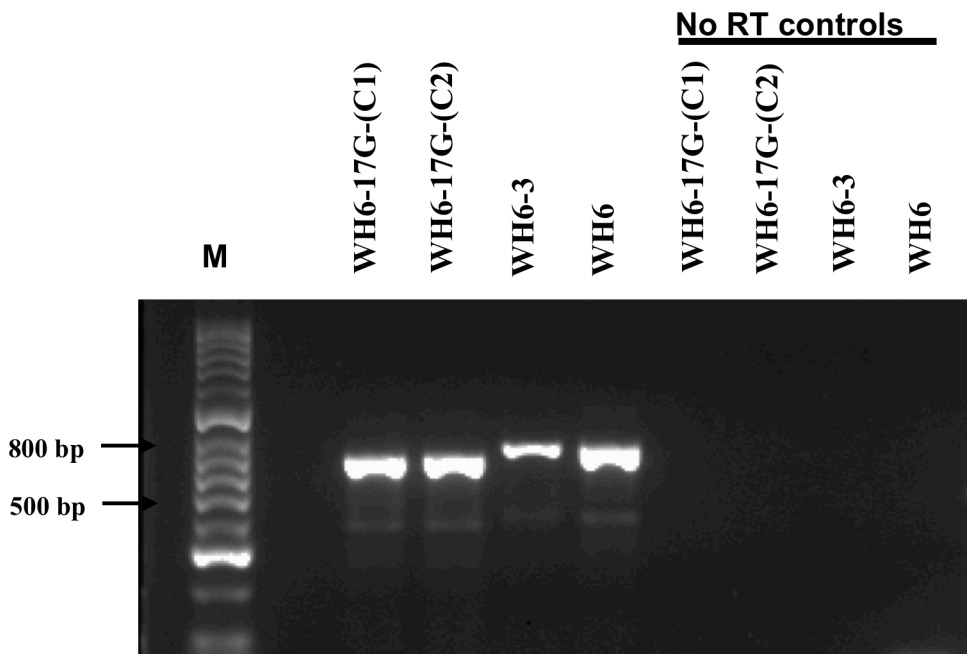


Fig. S4. RT-PCR analysis of *prtI* transcription in WH6, WH6-17G (carrying a deletion mutation in *prtI*), and WH6-3 (carrying the Tn*5* insertion in *prtR*). PCR primers (Promoter-upper-F and Right-201) were selected to span the region of deletion mutagenesis. Expected transcript sizes are 670 nt (*prtI*) and 625 nt (mutated *prtI*). WH6-17G-(C1) and WH6-17G-(C2) are multiple clones of the same strain. Lanes M contain a 100 nt ladder (Bioneer).

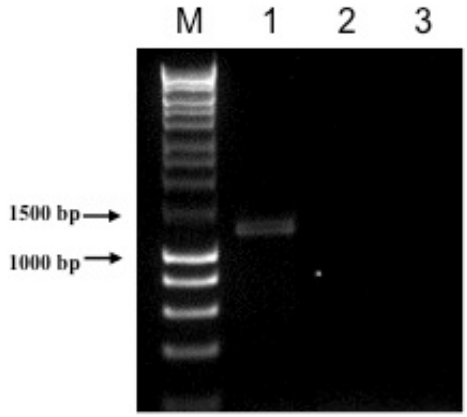


Fig. S5. RT-PCR analysis of a full-length *prtI* and *prtR* transcript in WH6. Primers (prtI-RT-F and prtR-RT-R) anneal to 5′ end of *prtI* and within 100 nt of the 3′ end of *prtR*. Expected size is 1172 nt. Lane M HyperLadder I (Bioneer), lane 1 WH6 cDNA, lane 2 No Reverse-Transcriptase control, and lane 3 No Template control.