Supporting Information

Sherwood and Bibb 10.1073/pnas.1305392110

SI Experimental Procedures

Heterologous Expression of the Planosporicin Biosynthetic Gene Cluster in *Streptomyces coelicolor* After Deletion of *pspW*. pIJ12328 containing *orf-11* to *pspV* (1) was PCR-targeted to replace *pspW* with the P1-flippase (FLP) recognition target (FRT)-hygromycin^R-*oriT*-FRT-P2 cassette amplified from pIJ10700 using the primer pairs listed in Table S1. The disruption cassette was subsequently eliminated by FLP-recombinase–mediated site-specific recombination (2). This was achieved by streaking single colonies of *Escherichia coli* DH5α/BT340 containing the targeted cosmid on LB agar and growing at 42 °C overnight. Clones sensitive to hygromycin (originally used to select for the cassette used for mutagenesis) but carbenicillin and kanamycin resistant (conferred by the cosmid backbone) were identified and removal of the cassette confirmed by PCR using flanking primers; the PCR products were Sanger-sequenced to confirm the presence of the 81-bp scar. The scarred cosmid was targeted once more to introduce the *oriT-attP-int-aac(3)IV* fragment from pIJ10702 into the SuperCos backbone as described previously (1, 2). Successful targeting was confirmed by NotI restriction digest, and the cosmid was introduced into *E. coli* ET12567/pUZ8002 by transformation and then conjugated into *S. coelicolor*; exconjugants were selected with 50 μg/mL apramycin and confirmed by colony PCR.

Detection of Planosporicin Production in *S. coelicolor*. Spores from M1146 and M1152 derivatives containing the *psp* gene cluster integrated at the ΦC31 *attB* site were grown as a lawn on R5 agar. To assay for the presence of antimicrobial compounds, 2 × 2 cm squares of agar were placed on a lawn of *Micrococcus luteus*. Alternatively, agar plugs were excised using a cork borer and embedded in LB agar containing *M. luteus*. Plates were photographed after incubating overnight at 30 °C. To identify the antimicrobial compounds, a ∼4 × ∼1 cm section of agar was extracted with 3–5 mL of 5% formic acid and ∼0.8 μL of the extract spotted onto a MALDI-TOF plate and analyzed as described previously (1).

Construction of *pspR* and *pspX* Expression Plasmids and Transfer to *S. coelicolor*. *pspR* and *pspX* were PCR-amplified to yield 5′ NdeI and 3′ PacI sites, cloned into pGEM-T (Promega) and confirmed by PCR and sequencing. Error-free clones were digested with NdeI and PacI and ligated into similarly digested pIJ10257 to create pIJ12715 (P_{ermE*}-*pspX*) and pIJ12716 (P_{ermE*}-*pspR*), (Table S1). These plasmids were integrated independently at the ΦBT1 site of the M1152 derivative containing the *psp* gene cluster integrated at the ΦC31 *attB* site. Exconjugants were selected with 40 μg/mL hygromycin and confirmed by colony PCR.

Construction of a Minimal *psp* Gene Set Lacking *pspEF*. The *pspR-pspV* gene set was created by replacement of flanking DNA as described previously (1). A 39bp-XbaI-P1-FRT-apramycin^R-*ori*T-FRT-P2-XbaI-39bp–extended resistance cassette was used to replace the region from *PCS-19* to *pspF* in pIJ12327 [containing *PCS-19* to *pspV* (1)], digested with XbaI to excise the cassette, religated, and then targeted once again to replace *neo* on the SuperCosI backbone with the *oriT-attP-int-aac(3)IV* cassette to yield pIJ12720 (Table S1). At each stage, the cosmid derivative was confirmed by PCR with primers flanking the deleted region (Table S2), as well as by diagnostic restriction digests.

***gusA* Transcriptional Fusions.** The four *psp* promoters were amplified to yield 5′ XbaI and 3′ SpeI sites and ligated into pGUS digested with the same two enzymes to place *gusA* under the control of each promoter, thus creating pIJ12724 (P_{pspA}-*gusA*), pIJ12726 (P_{pspX}-*gusA*), pIJ12727 (P_{pspJ}-*gusA*), and pIJ12728 (P_{pspE}-*gusA*). The plasmids were integrated into the ΦBT1 *attB* site of *S. coelicolor* M1152 after conjugation via the donor strain *E. coli* ET12567/pUZ8002. Exconjugants were selected using apramycin and confirmed by PCR. These strains were used as negative controls in GUS assays and as conjugation recipients for constructs in which *pspX*, *pspR*, or *pspXW* was expressed from P_{ermE*}. The latter constructs were made by PCR-amplifying each gene to yield 5′ NdeI and 3′ PacI sites followed by cloning in pIJ10257 digested with the same two enzymes (Table S2). Exconjugants were selected using hygromycin and confirmed by PCR.

GUS Assays. For agar plate-based assays, *S. coelicolor* strains were streaked from spore stocks onto 25-mL plates of R2 agar containing 100 μL of 40 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-glucoronide and photographed after 3 d at 30 °C. For spectrophotometric GUS assays, *S. coelicolor* strains were grown in biological triplicates in TSB (30 g/L Oxoid Tryptone Soya Broth powder dissolved in distilled water and autoclaved) liquid medium. The positive control strain expressed *gusA* from the constitutive P_{ermE*}. Negative control strains contained *gusA* under the control of each of the *psp* promoters (integrated at the ΦC31 *attB* site) in the absence of a transcriptional activator gene; 10-mL aliquots of TSB were inoculated with $\sim 10^8$ spores of each strain and incubated in a universal containing a steel spring for 1–2 d at 30 °C and 250 rpm. Strains were subcultured into 10 mL TSB to equivalent densities (measured at OD_{450}) in triplicate. After 24 h, 100 μL was used to measure cell density at 450 nm, whereas mycelium harvested from 1 mL was washed in distilled water, resuspended in 1 mL of lysis buffer (50 mM phosphate buffer at pH 7, 0.1% triton X-100, 0.27% β-mercaptoethanol, and 4 mg/mL lysozyme) and incubated for 30 min at 37 °C. Cell debris was removed by centrifugation; 100 μL of cell lysate was assayed in triplicate in a 96-well assay plate, using phosphate buffer (8 g/L Na_2HPO_4 $7H_2O$, 2.35 g/L NaH_2PO_4 H_2O, adjusted to pH 7) as a blank; 100 μL of Z-buffer (16.1 g/L Na_2HPO_4 $7H_2O$, 5.5 g/L NaH_2PO_4 H_2O, 0.75 g/L KCl, 0.246 g/L $MgSO_4$ $7H_2O$, adjusted to pH 7 and supplemented with 2.7 mL/L β-mercaptoethanol immediately before use) containing 20 μL of 4 mg/mL *p*-nitrophenyl-β-D-glucuronide was added to each well. OD_{420} was measured every minute for 1 h at 30 °C on a Powerwave 340 plate reader. If the kinetic reaction proceeded too quickly for accurate measurement of product accumulation, the assay was repeated using 25 μL or 10 μL of lysate. The remaining cell lysate was used to calculate the amount of protein released from the mycelium using Bradford reagent (BioRad). Miller units were calculated as 1,000 × (OD_{420} of sample − OD_{420} of blank)/(time of reaction in minutes × volume of culture assayed in milliliters × OD_{450} of culture) and expressed per milligram of protein.

1. Sherwood EJ, Hesketh AR, Bibb MJ (2013) Cloning and analysis of the planosporicin lantibiotic biosynthetic gene cluster of *Planomonospora alba*. *J Bacteriol* 195(10):2309–2321, 10.1128/JB.02291-12.
2. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci USA* 100(4):1541–1546.
3. Gust B, et al. (2004) Lambda red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. *Adv Appl Microbiol* 54:107–128.
4. Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttner MJ (1999) Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). *J Bacteriol* 181(1):204–211.

5. Yanai K, Murakami T, Bibb M (2006) Amplification of the entire kanamycin biosynthetic gene cluster during empirical strain improvement of *Streptomyces kanamyceticus*. *Proc Natl Acad Sci USA* 103(25):9661–9666.
6. Bierman M, et al. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* 116(1):43–49.
7. Hong HJ, Hutchings MI, Hill LM, Buttner MJ (2005) The role of the novel Fem protein VanK in vancomycin resistance in *Streptomyces coelicolor*. *J Biol Chem* 280(13):13055–13061.
8. Myronovskyi M, Welle E, Fedorenko V, Luzhetskyy A (2011) Beta-glucuronidase as a sensitive and versatile reporter in actinomycetes. *Appl Environ Microbiol* 77(15): 5370–5383.

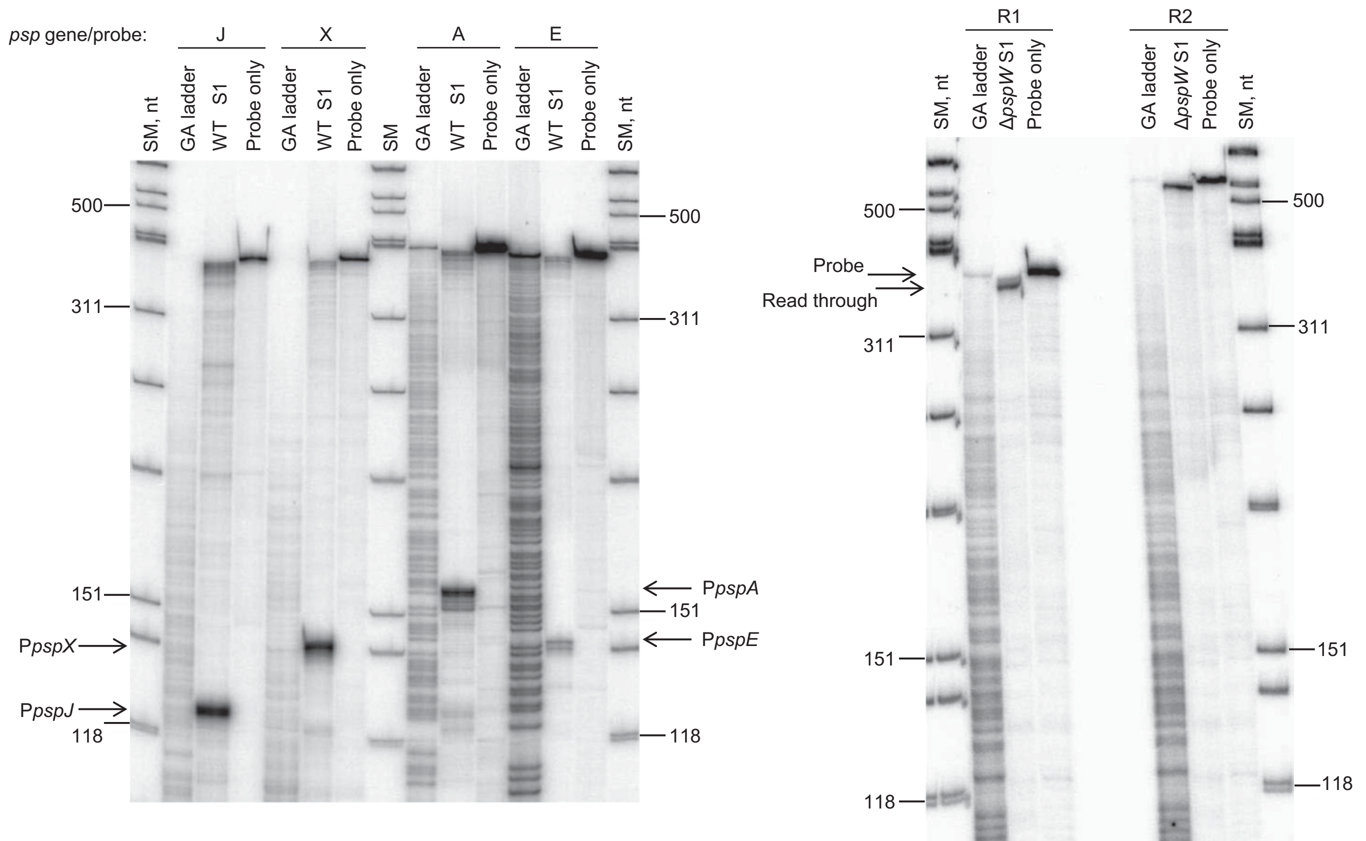

Fig. S1. High-resolution S1 nuclease mapping of the 5′ ends of transcripts reading into *pspJ*, *pspX*, *pspA*, *pspE*, and *pspR* using PCR-generated probes and RNA from *Planomonospora alba* wild type (WT) or the *pspW* mutant. Protected fragments were subjected to polyacrylamide gel electrophoresis adjacent to GA-sequencing ladders derived from the corresponding 5′ end-labeled probe to determine precise transcriptional start sites. Size markers (SM) were a heat-denatured γ-^{32}P–labeled HinfI digest of phiX174 DNA.

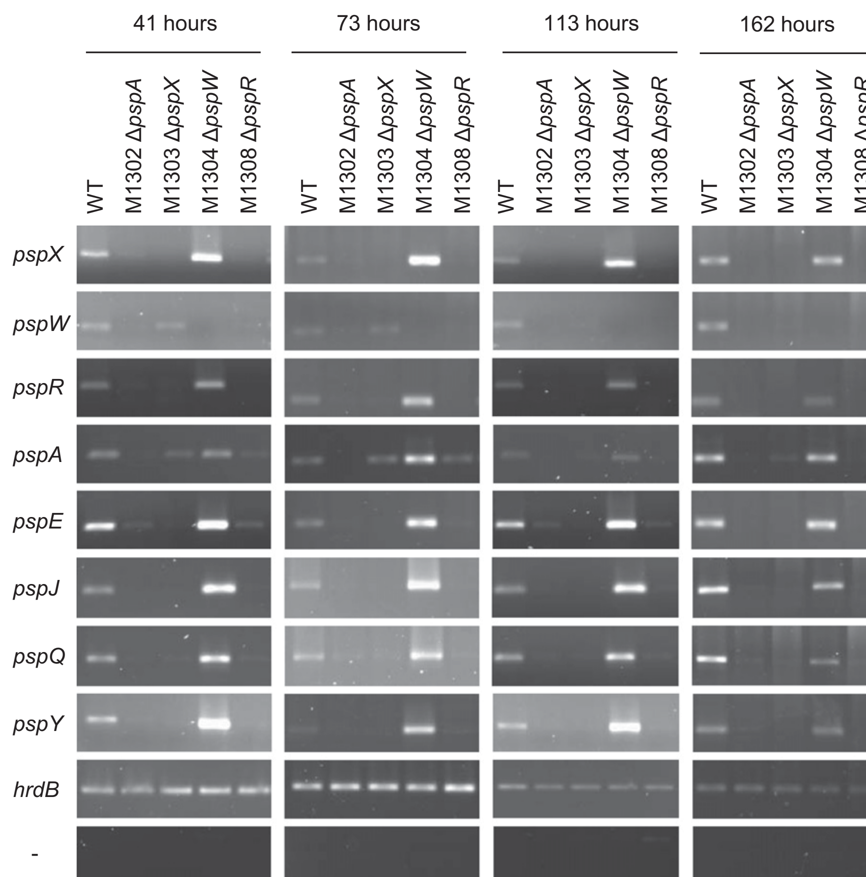

Fig. S2. RT-PCR analysis of *P. alba* strains WT, M1302 (Δ*pspA*), M1303 (Δ*pspX*), M1304 (Δ*pspW*), and M1308 (Δ*pspR*). RNA extracted from each strain at four different time points was used as a template for cDNA synthesis. Primers annealing within *pspX*, *pspW*, *pspR*, *pspA*, *pspE*, *pspJ*, *pspQ*, *pspY*, and *hrdB* (positive control) were used to assess the level of transcription of these genes at each time point. Attempts to synthesize *hrdB* cDNA without pretreatment with RT served as negative controls (marked as –). PCR products were subjected to electrophoresis on a 2% agarose gel.

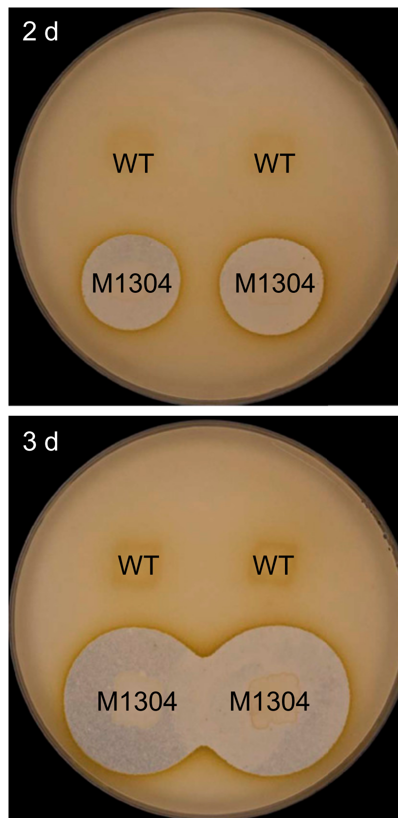

Fig. S3. Planosporicin production by *P. alba* WT and M1304 (Δ*pspW*). Approximately 10^6 *P. alba* spores of each strain were patched on AF/MS agar plates and incubated at 30 °C. After 2 or 3 d, plates were overlaid with *M. luteus* to reveal halos of growth inhibition.

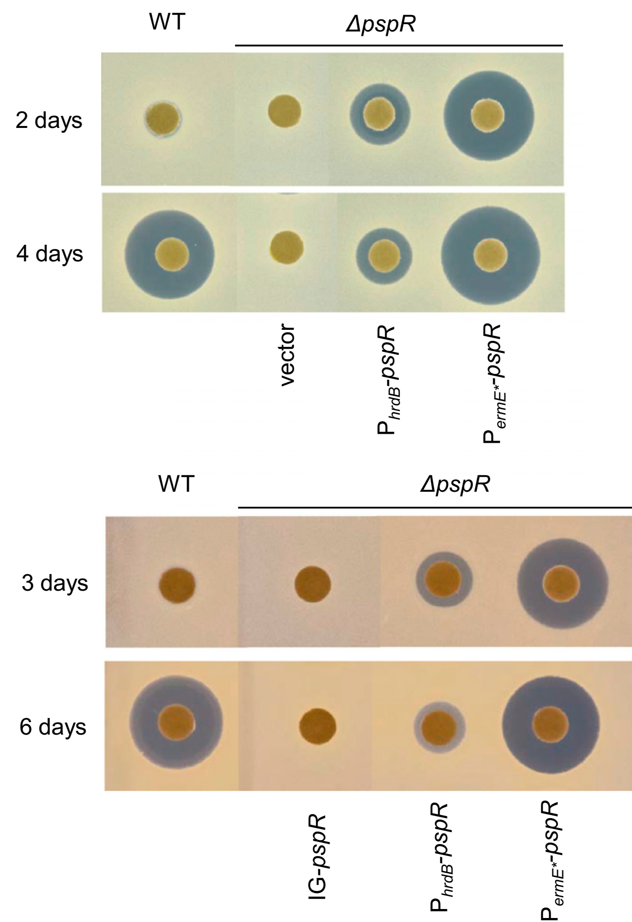

Fig. S4. Time course of complementation of *P. alba ΔpspR* mutant (M1308) with *pspR* expressed from the *hrdB* or *ermE** promoters. In two independent experiments (*Upper* and *Lower*), the four strains were cultured in parallel in AF/MS liquid medium, and culture supernatants were assayed for activity against *Micrococcus luteus* after 2 and 4 d (*Upper*) or 3 and 6 d (*Lower*). IG-*pspR*, vector containing *pspR* preceded by the *pspQ-pspR* intergenic region that lacks promoter activity; Vector, empty vector with no *pspR* present (Fig. S1).

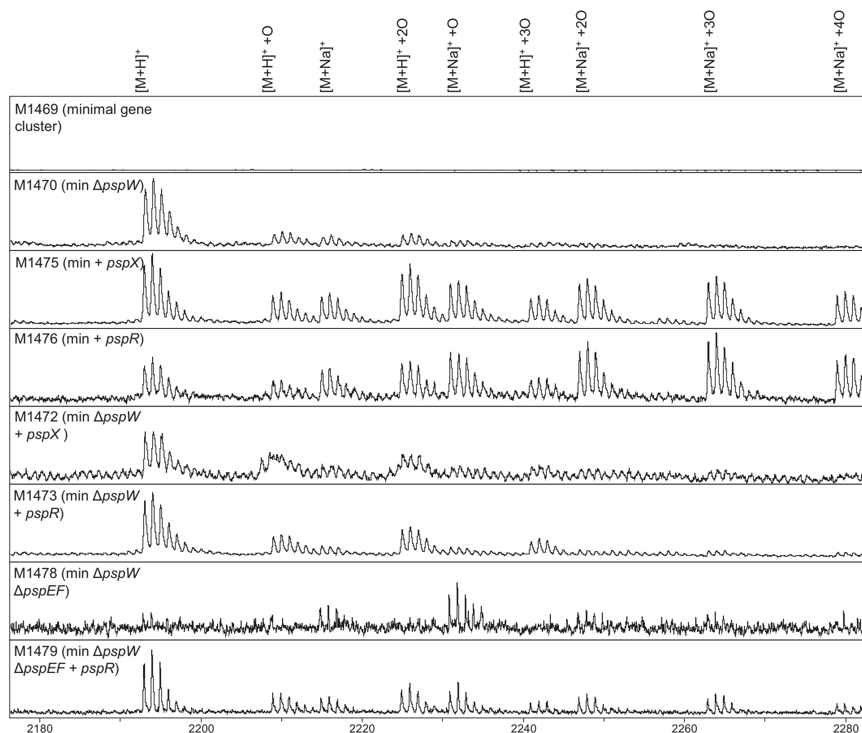

Fig. S5. MALDI-TOF mass spectrometry of culture supernatants of *S. coelicolor* M1152 derivatives with the *psp* WT and Δ*pspW* gene clusters integrated at the phiC31 *attB* site and overexpressing either *pspX* or *pspR* integrated at the phiBT1 *attB* site. *S. coelicolor* strains M1469 [M1152 containing the minimal *psp* gene cluster (min)], M1470 (M1152 with min Δ*pspW*), M1475 (M1152 with min plus P_{ermE*}-*pspX*), M1476 (M1152 with min plus P_{ermE*}-*pspR*), M1472 (M1152 with min Δ*pspW* plus P_{ermE*}-*pspX*), M1473 (M1152 with min Δ*pspW* plus P_{ermE*}-*pspR*), M1478 (M1152 with min Δ*pspW* Δ*pspEF*), and M1479 (M1152 with min Δ*pspW* Δ*pspEF* plus P_{ermE*}-*pspR*) were grown as confluent lawns on R5 agar for 6 d. Agar samples were extracted with 5% formic acid and analyzed by MALDI-TOF mass spectrometry. Intensity is given on the *y* axis in arbitrary units (au) and the mass/charge ratio (*m/z*) on the *x* axis. The identities of each monoisotopic *m/z* peak for planosporicin and its adducts are indicated. $[M+H]^+$: 2,192 Da; $[M+H]^+$ + O: 2,208 Da; $[M+Na]^+$: 2,214 Da; $[M+H]^+$ + 2O: 2,224 Da; $[M+Na]^+$ + O: 2,230 Da; $[M+H]^+$ + 3O: 2,240 Da; $[M+Na]^+$ + 2O: 2,246 Da; $[M+Na]^+$ + 3O: 2,262 Da; and $[M+Na]^+$ + 4O: 2,278 Da.

Table S1. Oligonucleotides used in this study

Primer name	Sequence, 5′–3′	Application/target
Overexpression and complementation in *P. alba*		
PpspXF_BamHI	ATGGATCCGTCGTCTGGGCAGTGTTCC	*pspX* with upstream region
PspXR_XbaI	ATTCTAGAACGAGACGCTGGTGATGG	
pspRF_Xba	ATATTCTAGAGAAGGTCGGCACGTACTCC	*pspR* with upstream region
PpspRR_BamHI	ATATGGATCCCTCAGCAGGACCATGAAGC	
pspR_F	CAGGACCATGAAGCTCTGC	Fuse P_{hrdB} to *pspR* flanked by XbaI sites
XbaIpspR_R	ATTCTAGATCCGATGCCGGTGTCC	
XbaIPhrdB_F	ATTCTAGAGAACCCCCATATGTTGTTGG	
PhrdB-pspR_R	ACCGGCAGAGCTTCATGGTCCTGGAAAGCGT GACGAGAATGG	
Deletion of *pspW* from minimal gene set		
−2 disruptF	TCGGCGTACGGACCCCGCCGCCGGCCGGGGCTCGC GTCAATTCCGGGGATCCGTCGACC	Δ*pspW*
−2 disruptR	CGCTGCGCGGTTGGAAGGATCGGGACGACGATGAC GGAATGTAGGCTGGAGCTGCTTC	
−2 confirmF	GTCATGTCGTGGCCTTCC	Δ*pspW* confirmation
−2 confirmR	GTCGAGGAACTCGCCTACC	
Deletion of *pspEF* from minimal gene set		
updisruptF	TCTACCTGCCCGCCTTCGAACGCGTCGTCACGCAGG CCTCTAGAATTCCGGGGATCCGTCGACC	Δ*orf-19-pspF* with XbaI sites
updisruptR4	AGGCCTCCGGCCGCCCGTACGCCCCTCCTCGCGGGTACG TCTAGATGTAGGCTGGAGCTGCTTC	
upconfirm	GCGGCGGCCGTGGTGTTCAC	confirm Δ*orf-19-pspF*
PspF confirmR	GTGCTCAATCTCGCCATACC	
Cloning *psp* genes downstream of P_{ermE^*} in pIJ10257		
NdeI_pspXF	ATCATATGTTCCGCCTCATCCGCAGGCT	*pspX*
pspXR_PacI	ATTTAATTAAGTCGTCTGGGCAGTGTTCC	
NdeI_pspRF	ATCATATGCGCCGGCACGCTTCGCAGGC	*pspR*
pspRR_PacI	ATTTAATTAAGCTGACCCCCGCAGAG	
NdeI_pspXW_F	ATCATATGTTCCGCCTCATCCGCAGG	*pspXW*
HindIII_pspXW_R	ATAAGCCTATCCGGGCGAGAAATCC	
NdeI_pspEFF	ATCATATGAACACCACGGCCGCCGCACC	*pspEF*
pspEFR_ HindIII	ATAAGCTTCACGTCCATGACCATCTCC	
RT-PCR primers		
pspE_RTF	GTGACGCTCGAGTTCTGG	*pspE*
pspE_RTR	ACCTTGGCGACGAAGTAGC	
pspR_RTF2	CCGTTCGGAGCAGTAGTAGC	*pspR*
pspR_RTR2	GGAGTACGACAGGGAGCTGA	
pspQ_RTF	CGGAACTTCGGCTACACC	*pspQ*
pspQ_RTF	GGCCGAGACTCACCTTGC	
pspZ_RTF	GGGAGTTCCTCGACTACATCG	*pspZ*
pspZ_RTR	GACGGATCTCCCATCAGC	
pspY_RTF2	AGGTACGAGGCCCTGATGG	*pspY*
pspY_RTR	GGTGCAGAAGGTGTTGACG	
pspJ_RTF	CTGGAGCTGCACCTGTCG	*pspJ*
pspJ_RTF	GCCATCTCCAGGAACACG	
pspW_RTF	ACTGCCGCAGGAGACTGG	*pspW*
pspW_RTR	CCAGCAACAGGTCGAAGG	
ECF_RTF	GTCGAGGAACTCGCCTACC	*pspX*
ECF_RTR	GTCCCGATCCTTCCAACC	
pspA_RTF	CCTGCCGCAGAACACC	*pspA*
pspA_RTR	CGCCGCCTTCACTGG	
hrdB_RTF	CCAAGGGCTACAAGTTCTCG	*hrdB*
hrdB_RTR	GGCCAGCTTGTTGATGACC	
psp promoter primers		
XbaI_PpspEF	ATTCTAGA TTCCTTCCTGACTGGAGTGG	P_{pspE}
SpeI_PpspER	ATACTAGT CTCGGCCGATCGTGGTC	
XbaI_PpspJF	ATTCTAGA GCACGGGAGGATTTCTCG	P_{pspJ}
SpeI_PpspJR	ATACTAGT TCCCTCGTGGAGGAACC	
XbaI_PpspXF	ATTCTAGA GTGGCAGGACGATGACC	P_{pspX}
SpeI_PpspXR2	ATACTAGT GCCATGTACGTATCCGGAA	

Table S1. Cont.

Primer name	Sequence, 5′–3′	Application/target
XbaI_PpspAF	ATTCTAGA ATCGGTTCACGGTTCACG	P_{pspA}
SpeI_PpspAR	ATACTAGT AGGTGTTCGCGGATTGC	
S1 mapping primers		
S1pspR2F	*AGGACGTTGAGTTCGACAGC	560 bp P_{pspR}
S1pspR2R	CATCTGCATCTGCATAAAGGACCGACGAATGACC	
S1pspR1F2	*AGGACGTTGAGTTCGACAGC	367 bp P_{pspR}
S1pspR1R	CATCTGCATCTGCATCGGAACTTCGGCTACACC	
S1pspJF	*GCGGACCTCGTGTCTCC	367 bp P_{pspJ}
S1pspJR	CATCTGCATCTGCATAGCCCTGCTGGGATGG	
S1pspXF	*GATCTCCTCGGCCACACC	375 bp P_{pspX}
S1pspXR	CATCTGCATCTGCATCGTAAATCACGCAATCATGG	
S1pspEF	CATCTGCATCTGCATTTCCTTCCTGACTGGAGTGG	386 bp P_{pspE}
S1pspER2	*AGGAACCCGCGTCTGC	
S1pspAF2	CATCTGCATCTGCATCACACGCCATGTACGTATCC	396 bp P_{pspA}
S1pspAR2	*ACGAGACGCTGGTGATGG	

Underlined sequence indicates a nonhomologous tail to distinguish between full-length protection and probe:probe reannealing. ECF, extracytoplasmic function; F, forward; R, reverse.
*Label on the 5′ end of oligonucleotides annealing within the protein coding sequence.

Table S2. Plasmids used in this study

Plasmid	Description	Selectable markers	Source
General plasmids			
pIJ790	λ-RED (*gam, bet, exo*), *cat, araC, rep101ts*	Chl	Ref. 3
pUZ8002	*tra, neo,* RP4	Kan	Ref. 4
pIJ10702	*attP int* (ΦC31) *oriT* pUC*ori* (also known as pMJCos1)	Carb Apra	Ref. 5
pIJ773	pBS SK+ containing cassette P1-FRT-*oriT-aac(3)IV*-FRT-P2	Carb Apra	Ref. 3
pIJ10700	pBS SK+ containing cassette P1-FRT-*oriT-hyg*-FRT-P2	Hyg	Ref. 3
pGEM-T	TA-cloning vector	Carb	Promega
pSET152	*attP int* (ΦC31) *oriT* pUC*ori*	Apra	Ref. 6
Complementation and *P. alba* overexpression constructs			
pIJ12539	pSET152 containing *pspX* and upstream intergenic region	Apra	Ref. 1
pIJ12540	pSET152 containing *pspR* and upstream intergenic region	Apra	This work
pIJ12709	pSET152 P_{hrdB}-*pspR*	Apra	This work
Cosmids for heterologous expression			
pIJ12321	SuperCosI B4-1	Carb Kan	Ref. 1
pIJ12323	pIJ10702 B4-1	Carb Apra	Ref. 1
pIJ12327	pIJ12323 with genes downstream of *pspV* removed	Carb Apra	Ref. 1
pIJ12328	pIJ12327 with genes upstream of *orf-12* removed	Carb Apra	Ref. 1
pIJ12329	pIJ12327 with genes upstream of *pspE* removed	Carb Apra	Ref. 1
pIJ12719	pIJ12328 with Δ*pspW*::(scarred *hyg-oriT*)	Carb Apra	This work
pIJ12721	pIJ12327 with genes upstream of *pspR* removed and Δ*pspW::scar*	Carb Apra	This work
pIJ12722	pIJ12324 with Δ*pspW::scar*	Carb Apra	This work
M1152 *psp* overexpression constructs			
pIJ10257	*attP int* (ΦBT1) *oriT* pUCori P_{ermE^*}	Hyg	Ref. 7
pIJ12715	pIJ10257 *pspX*	Hyg	This work
pIJ12716	pIJ10257 *pspR*	Hyg	This work
pIJ12717	pIJ10257 *pspEF*	Hyg	Ref. 1
pIJ12718	pIJ10257 *pspXW*	Hyg	This work
gusA transcriptional fusions			
pGUS	*gusA* in pSET152	Apra	Ref. 8
pIJ10740	P_{ermE^*}-*gusA*	Apra	Morgan Feeney*
pIJ12724	P_{pspA}-*gusA*	Apra	This work
pIJ12726	P_{pspX}-*gusA*	Apra	This work
pIJ12727	P_{pspJ}-*gusA*	Apra	This work
pIJ12728	P_{pspE}-*gusA*	Apra	This work

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