Supplemental Material

Product-Mediated Regulation of Pentalenolactone Biosynthesis in *Streptomyces* by the MarR/SlyA Family Activators PenR and PntR

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Table S1. Strains, plasmids and cosmids used in this study.

Strain or plasmid	**Relevant phenotype and/or characteristics**	**Source**
S. exfoliatus strains		
UC5319	Wild-type, pentalenolactone producer	Upjohn Co (Pfizer) (1)
ZD20	*penD* in-frame deletion mutant	(2)
ZD22	*penM* in-frame deletion mutant	(3)
ZD27	*penR* mutant, $Apra^+$	This work
ZD27::pDQ90	Complementation of ZD27 with *penR*, $Apra^+$, $Thio^+$	This work
ZD27::pDQ91	Complementation of ZD27 with *pntR*, $Apra^+$, $Thio^+$	This work
ZD28	*penM-penR* double mutant, $Apra^+$	This work
ZD28::pDQ90	Complementation of ZD28 with *penR*, $Apra^+$, $Thio^+$	This work
ZD28::pDQ91	Complementation of ZD28 with *pntR*, $Apra^+$, $Thio^+$	This work
S. arenae strains		
TÜ469	Wild-type, pentalenolactone producer	DSM 40734, DMSZ Braunschweig, DE (4)
E. coli strains		
DH10B	F^- *mcrA, Δ(mrr-hsdRMS-mcrBC),* ϕ80d*lacZΔM15, ΔlacX74, recA1, endA1, araD139, Δ(ara, leu)7697, galU, galK, rpsL, nupG*	Gibco BRL
BL21(DE3)	F^- *dcm*, *ompT*, *hsdS* ($r_B^-m_B^-$), *gal*	Invitrogen
ET12567/pUZ8002	F^- *ara-14, leuB6, fhuA13, lacY1, tsx-78, supE44, glnV44, galK2, galT22, mcrA, dcm-6, hisG4, rfbD1, rpsL136, dam-13::*Tn*9, xyl-5, mtl-1, recF143, thi-1, mcrB, hsdR2, hsdS::*Tn*10*pUZ8002*:* (derivative of pUB307, *tra*)	(5, 6)

BW25113/pIJ790	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), rph-1, Δ(rhaD-rhaB)568, hsdR514, λ*$^{-}$; pIJ790: λ-RED (P*araBAD*, *gam, bet, exo*), *cat, araC, rep101*^{ts}	(7, 8)
Plasmids		
pET-26b	Kan^{+}, *ori*^{f1}, *lacI, ori*^{pBR322}, T7 promoter	Novagen
pHL133	*aac(3)IV, oriT, int*, *xylE*	(9)
pHZ1358	pIJ101 derivative, *bla*, *tsr*, *cos*, *oriT, sti*	(10)
pIB139	*aac(3)IV, oriT*, *int*, *ermE** promoter	(11)
pIJ773	*aac(3)IV, rep*^{pUC}*, oriT*	(7)
pIJ2925	*bla, ori, lacZα*	(12)
1E2	pHZ1357 derivative, cosmid harboring *pnt* gene cluster	(2)
G21	pHZ1357 derivative, cosmid harboring *pen* gene cluster	(2)
pDQ20	pSET152 derivative, *orf-1, pntR, gapR,* and partial *pntM*	(2)
pDQ44	pJTU1278 derivative, without XbaI and SpeI sites	(2)
pDQ71	Gene *pntR* amplified with primer DQ108F/R, digested with NdeI/HindIII and inserted into the corresponding site of pET-26b	This work
pDQ72	Gene *penR* amplified with primer DQ109F/R, digested with NdeI/HindIII and inserted into the corresponding site of pET-26b	This work
pDQ75	473-bp DNA fragment harboring partial *penM* and the intergenic region of *penM* and *penH* amplified with primer pair DQ103F2 and DQ103R2 inserted into SmaI site of pIJ2925	This work
pDQ77	Gene *tsr* from pHZ1358 amplified with primer DQ113F/R, digested with EcoRI and inserted into the corresponding site of pIB139	This work
pDQ78	Gene *tsr* from pHZ1358 amplified with primer DQ113F/R, digested with EcoRI and inserted into the corresponding site of pHL133	This work

pDQ80	ca. 0.5-kb BglII DNA fragment harboring partial *penM* and the intergenic region of *penM* and *penH* recycled from pDQ75 and inserted into BglII site of vector pDQ78	This work
pDQ88	ca. 823 bp DNA fragment carrying *penR* and its promoter amplified with primer DQ109R and DQ112R, inserted into SmaI site of pIJ2925	This work
pDQ89	A 1728-bp SmaI DNA fragment harboring partial *orf -1*, complete *pntR* and partial *gapR* recycled from pDQ20 and inserted SmaI site of pIJ2925	This work
pDQ90	an 823-bp BglII and DNA fragment carrying *penR* and its promoter from pDQ88 recycled and inserted into BamHI site of pDQ77	This work
pDQ91	ca. 1.8-kb BglII DNA fragment harboring partial *orf -1*, complete *pntR* and partial *gapR* recycled from pDQ89 and inserted into BamHI site of pDQ77	This work
pDQ93	ca. 6.7-kb BamHI DNA fragment harboring the upstream of *pen* gene cluster, *penR*, *gapN* and partial *pntM* recycled from cosmid G21 inserted into the corresponding site of pDQ44	This work
pDQ94	198-bp DNA fragment of *penR* from nt 223 to nt 420 of pDQ93 replaced by 1369-bp *oriT* and *aac(3)IV* using PCR targeting system	This work
pDQ108	548-bp DNA fragment carrying partial *penR* and the intergenic region of *penR* and *gapN* amplified with primer pair DQ84F and DQ112R, and inserted into SmaI site of vector pIJ2925	This work
pDQ109	ca. 540-bp BamHI DNA fragment fragment carrying partial *penR* and the intergenic region of *penR* and *gapN* recycled from pDQ108 and inserted into BglII site of pDQ78 with the direction along *penR*	This work
pDQ110	ca. 540-bp BamHI DNA fragment fragment carrying partial *penR* and the intergenic region of *penR* and *gapN* recycled from pDQ108 and inserted into BglII site of pDQ78 with the direction along *gapN*	This work

Table S2. Primers used in this study.

Primer	**Sequence (5′—3′), (restriction enzyme site underlined)**	**Purpose**
DQ92F2	AAGACCGAGCAGATCAGCCAGCCGGGGCTCA CTCAGCTGATTCCGGGGAT CCGTCGACC	*penR* deletion
DQ92R	CCGTCCGGCTTCGGTCCCGAGTTCCGCCAGGC GGGTCAGTGTAGGCTGGAGCTGCTTC	*penR* deletion
DQ93F	TCCGGCTTCGGTCCC GAGTT	Confirmation of *penR* mutant
DQ93R	TGCTTTGGCTGTCACCTGTCT	Confirmation of *penR* mutant
DQ96F	TGGAGGAGGTGAAGGAGGCG	Intergenic region of *gapR-pntM*
DQ96R2	GAGATCCGTCATATGGTCCGTC	Intergenic region of *gapR-pntM*
DQ97F2	CGGGGACGCGGTGATGCT	Intergenic region of *pntM-pntH*
DQ97R2	GTCCGTCATATGTCCTTGTCCT	Intergenic region of *pntM-pntH*
DQ98F	CGACGGCAGCCACAAGAAG	Intergenic region of *pntH-pntG*
DQ98R2	GTTTCGTCATATGTTGCCTACCAG	Intergenic region of *pntH-pntG*
DQ99F	GCATCGGCTGCTACGGAGTG	Intergenic region of *pntG-pntF*
DQ99R2	GTGAGTGCATATGGCTGTCTCCG	Intergenic region of *pntG-pntF*
DQ100F	ACGACGTGCTCGGCATCTGG	Intergenic region of *pntB-pntA*
DQ100R2	CCTGGGGCATATGGGAATCCTCG	Intergenic region of *pntB-pntA*
DQ101F	GCGTCGCTGGAGAAGGAAGA	Intergenic region of *pntA-pntI*
DQ101R2	GCTCGGTCATATGATCCTCGACT	Intergenic region

		of *pntA-pntI*
DQ102F2	ATTCTCCAACCGCCTCAT	Intergenic region of *gapN-penM*
DQ102R2	CTTCACCTCGTCGTATCG	Intergenic region of *gapN-penM*
DQ103F	CCGTCGGCGAGACAGTGAT	Intergenic region of *penM-penH*
DQ103R	TGAGGGTGTCGAGCGTGGTG	Intergenic region of *penM-penH*
DQ103F2	AAGGCGCACATGGCATTC	Intergenic region of *penM-penH*
DQ103R2	GCTGAGAGTGTCCGTCAT	Intergenic region of *penM-penH*
DQ103F3	TTCGCCCGGCGCCATCGA	Intergenic region of *penM-penH*
DQ103R3	GCGGAGGTGACGAGGTCA	Intergenic region of *penM-penH*
DQ105F2	GCGGAACCCTCTATGTCTC	Intergenic region of *penG-penF*
DQ105R	AGGACCCCGGCGTTGTTG	Intergenic region of *penG-penF*
DQ107F	CGAGCACGGCTGGTCCAAGA	Intergenic region of *penA-penI*
DQ107R2	TCTTGATCTCGACCAGGTT	Intergenic region of *penA-penI*
DQ108F	CATTTCCCATATGACTCCTACTCAGCG (NdeI)	*pntR*
DQ108R	CGTGATCAAAGCTTCCGTGCGGCTT (HindIII)	*pntR*
DQ109F	TATTTCCCATATGACCTTCCACCACCG (NdeI)	*penR*
DQ109R	TTTGATCAAAGCTTCCGTCCGGCTTCG (HindIII)	*penR*
DQ110F	CATGCTCTTCCTGGACCT	*hrdB*
DQ110R	GGACCTCGATGACCTTCT	*hrdB*

DQ111F	GTGGATCCCACCCTGGAAATGTATCG (BamHI)	Intergenic region of *pntR-gapR*
DQ111R	CGGGATCCCATTTGGTGTGTTCCTCA (BamHI)	Intergenic region of *pntR-gapR*
DQ111F2	GCAMCTCGGCGCAACG (M=A or C)	Intergenic region of *pntR-gapR*
DQ111R2	AACGCGGGCCGTACGCCT	Intergenic region of *pntR-gapR*
DQ112F	TGGGATCCCATACTGGAAATATATCG (BamHI)	Intergenic region of *penR-gapN*
DQ112R	GTGGATCCCATGATTTCCGTCCTGAT (BamHI)	Intergenic region of *penR-gapN*
DQ112F2	TGCGGGGCAGGCCGACCA	Intergenic region of *penR-gapN*
DQ112R2	GTTCACACCACGCGCCCA	Intergenic region of *penR-gapN*
DQ112R3	CAGCGGTCGCGGCCGTCA	Intergenic region of *penR-gapN*
DQ113F	GGGAATTC TGATCAAGGCGAATACTT (EcoRI, BclI)	*tsr*
DQ113R	TCGAATTC TGATCATCACTGACGAAT (EcoRI, BclI)	*tsr*
WYP7F	CCCGAGGGCGACTTGAAC	*penR*
WYP7R	CCGGCTGGCTGATCTGCT	*penR*
WYP9F	GACCATCACTGTGGGAAT	*gapN*
WYP9R	ACACGCTGTCGTACTTCA	*gapN*
WYP10F	GCACGGCTACGTCTATCT	*penH*
WYP10R	CTTCTCCCAGACCTTGAG	*penH*

Table S3. TTA codons in pentalenolactone (*pen*, *pnt*, *SBI*) and neopentalenolactone (*ptl*) biosynthetic gene clusters.

	R	***gap***	***M***	***H***	***G***	***F***	***E***	***D***	***B***	***A***	***I***
pen	-	-	7	-	-	3	-	-	-	-	-
pnt	-	-	7	-	-	-	69	44	-	-	-
SBI		-	7	-	-	-	-	-	-	-	-
ptl	-	-		31	30	-	591	-	-	-	-

ptl, *S. avermitilis ptl* gene cluster for neopentalenolactone biosynthesis;
pnt, *S. arenae pnt* gene cluster for pentalenolactone biosynthesis;
pen, *S. exfoliatus pen* gene cluster for pentalenolactone biosynthesis;
SBI, proposed *S. bingchenggensis* pentalenolactone biosynthetic gene cluster;
Rows, genes organized by original location in gene cluster;
Columns, homologous genes;
Numbers correspond to amino acid residue encoded by TTA codons;
Minuses indicate that no amino acids are encoded by TTA codons;
The blank entry indicates that there is no homologue of *penM* in the *ptl* cluster.

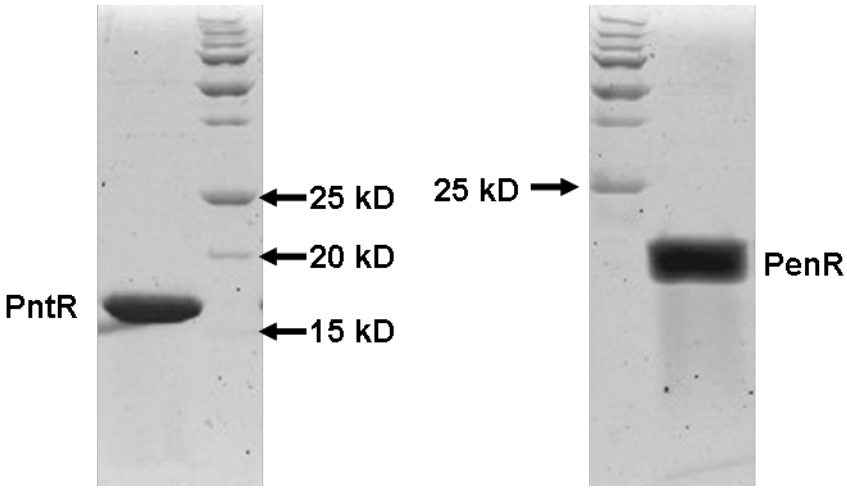

Fig. S1. SDS-PAGE analysis of purified recombinant protein PntR-His$_6$-tag and PenR-His$_6$-tag

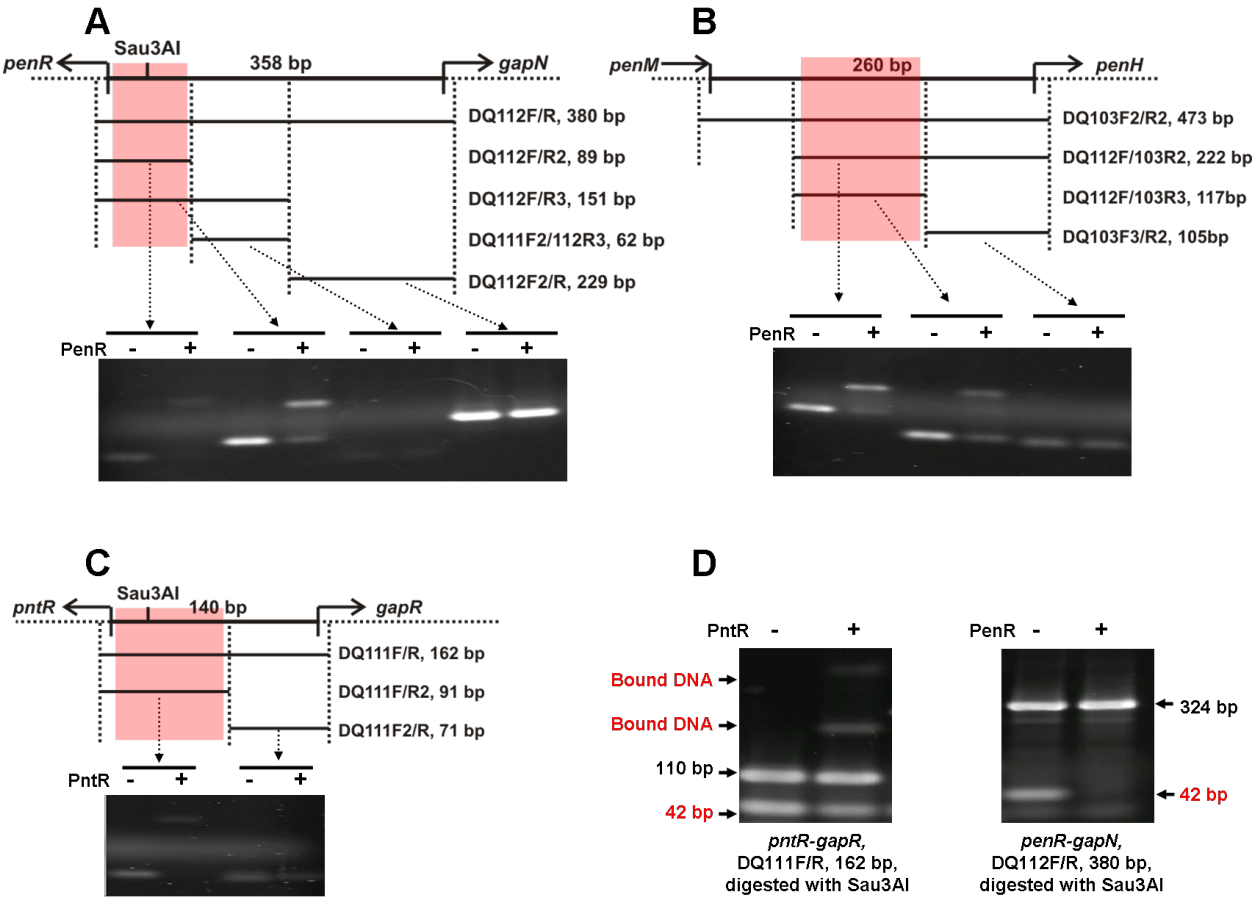

Fig. S2. EMSA of binding of PenR and PntR with their target DNA segments. A, PenR with DNA fragments located within the *penR-gapN* intergenic region. B, PenR with DNA fragments located within the *penM-penH* intergenic region. C, PntR with DNA fragments located within the *pntR-gapR* intergenic region. D, PntR and PenR with conserved ~42-bp DNA segments prepared by Sau3AI digestion of the *pntR-gapR* and *penR-gapN* intergenic regions, respectively.

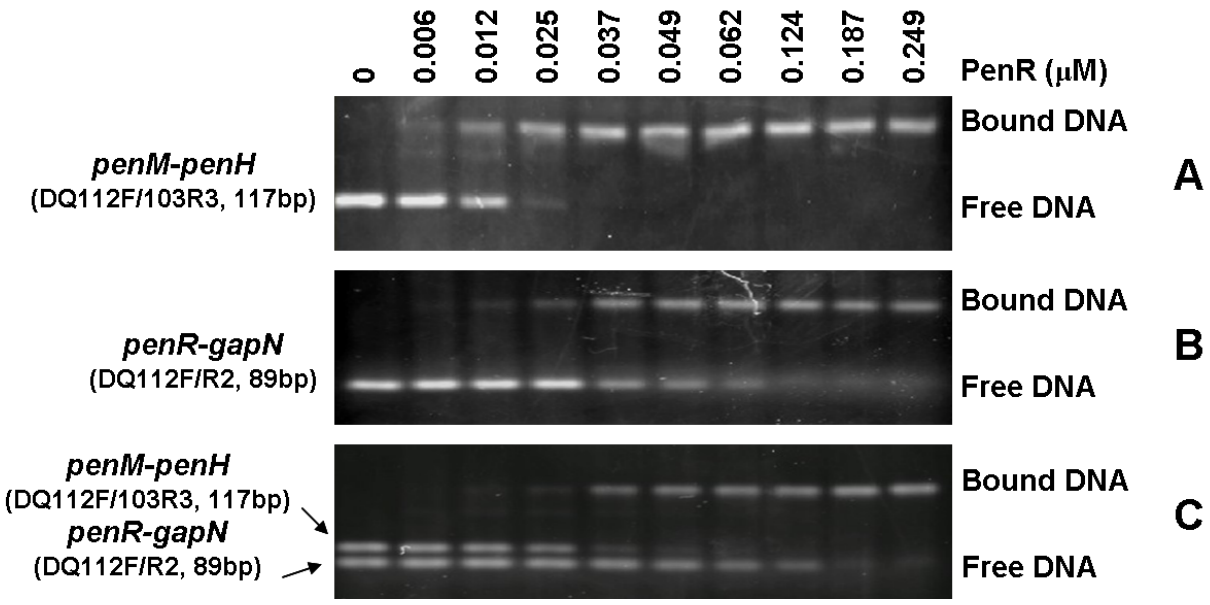

Fig. S3. EMSA comparison of binding strength of PenR with DNA from two *pen* intergenic regions. A, *penM-penH* intergenic region. B, *penR-gapN* intergenic region DNA. C. Mixture of DNA from both *penM-penH* and *penR-gapN* intergenic regions.

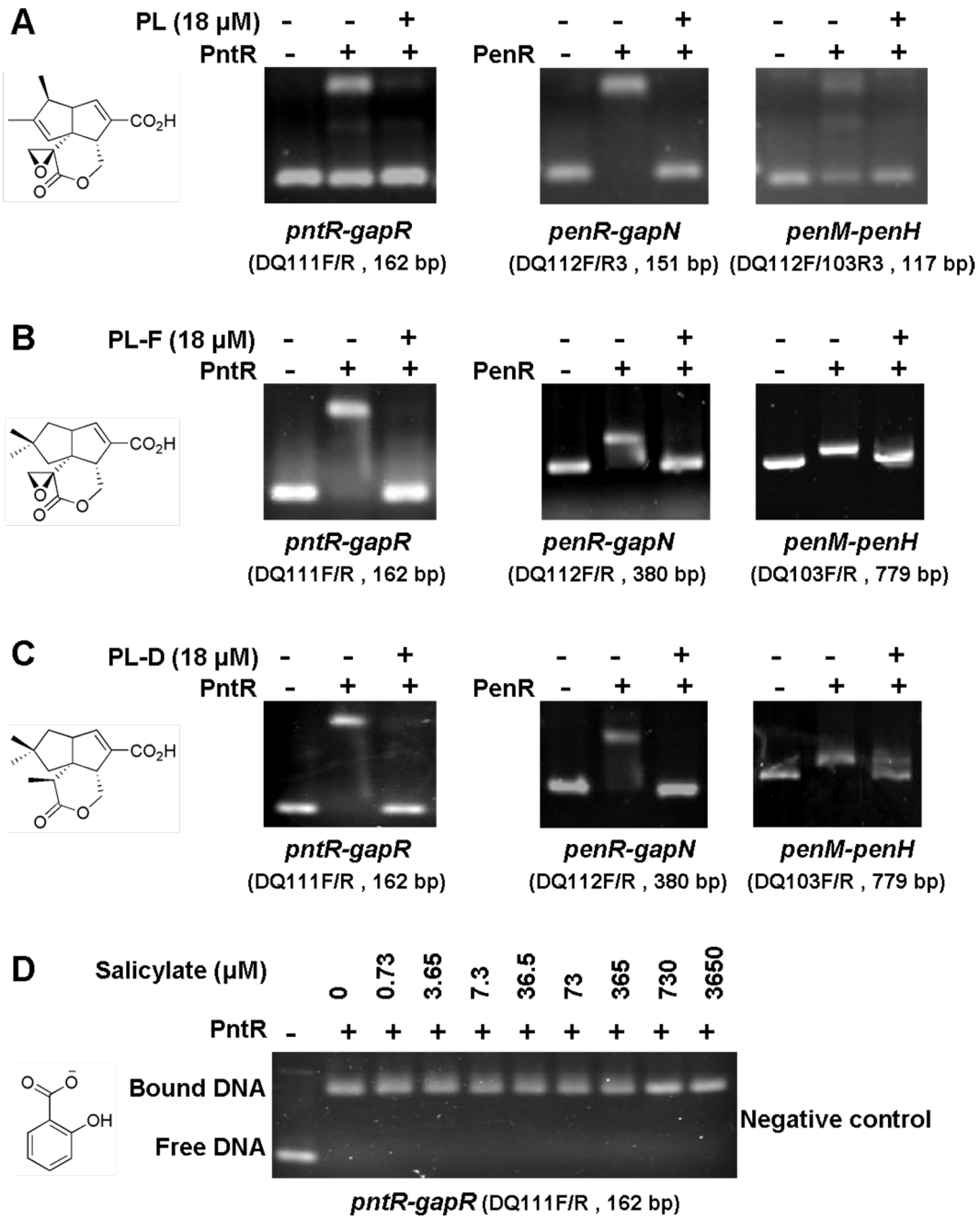

Fig. S4. EMSA analysis of release of bound DNA from binding to PenR PenR proteins by added pentalenolactones. A, Release of bound DNA in presence of 18 μM pentalenolactone: PntR bound to DNA from *pntR-gapR* intergenic region; PenR bound to DNA from *penR-gapN* intergenic region; PenR bound to DNA from *penM-penH* intergenic region. B, Release of bound DNA from PntR and PenR in presence of pentalenolactone F (18 μM). C, Release of bound DNA in presence of in presence of pentalenolactone D (18 μM). D. Negative control, addition of salicylate (0 – 3.7 mM) to PntR bound to DNA from *pntR-gapR* intergenic region.

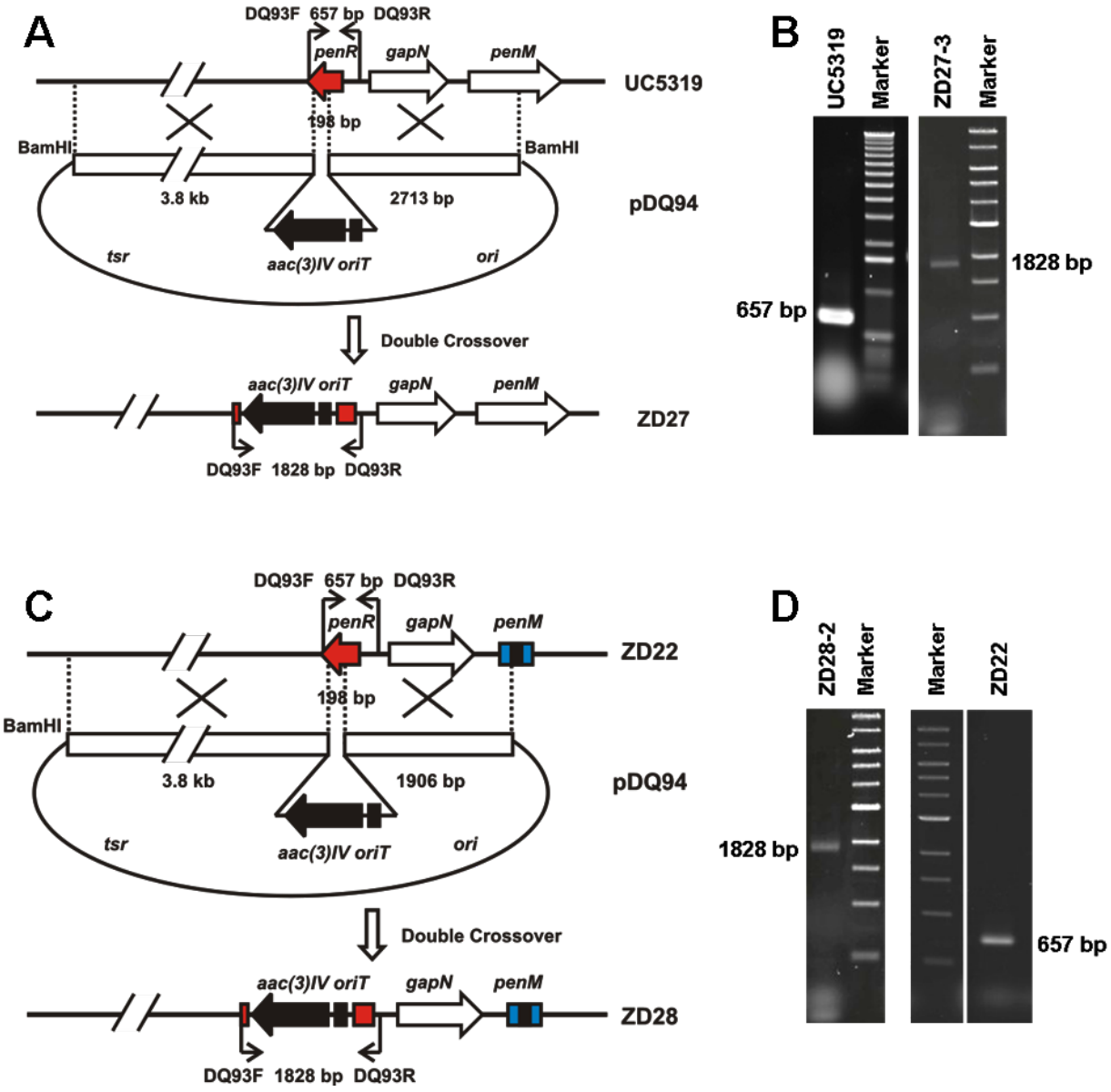

Fig. S5. A, Construction of *penR* deletion mutant *S. exfoliatus* ZD27. B, PCR verification of the *penR* deletion mutant. C, Construction of *penM-penR* deletion mutant *S. exfoliatus* ZD28. D, PCR verification of the *penM-penR* deletion mutant.

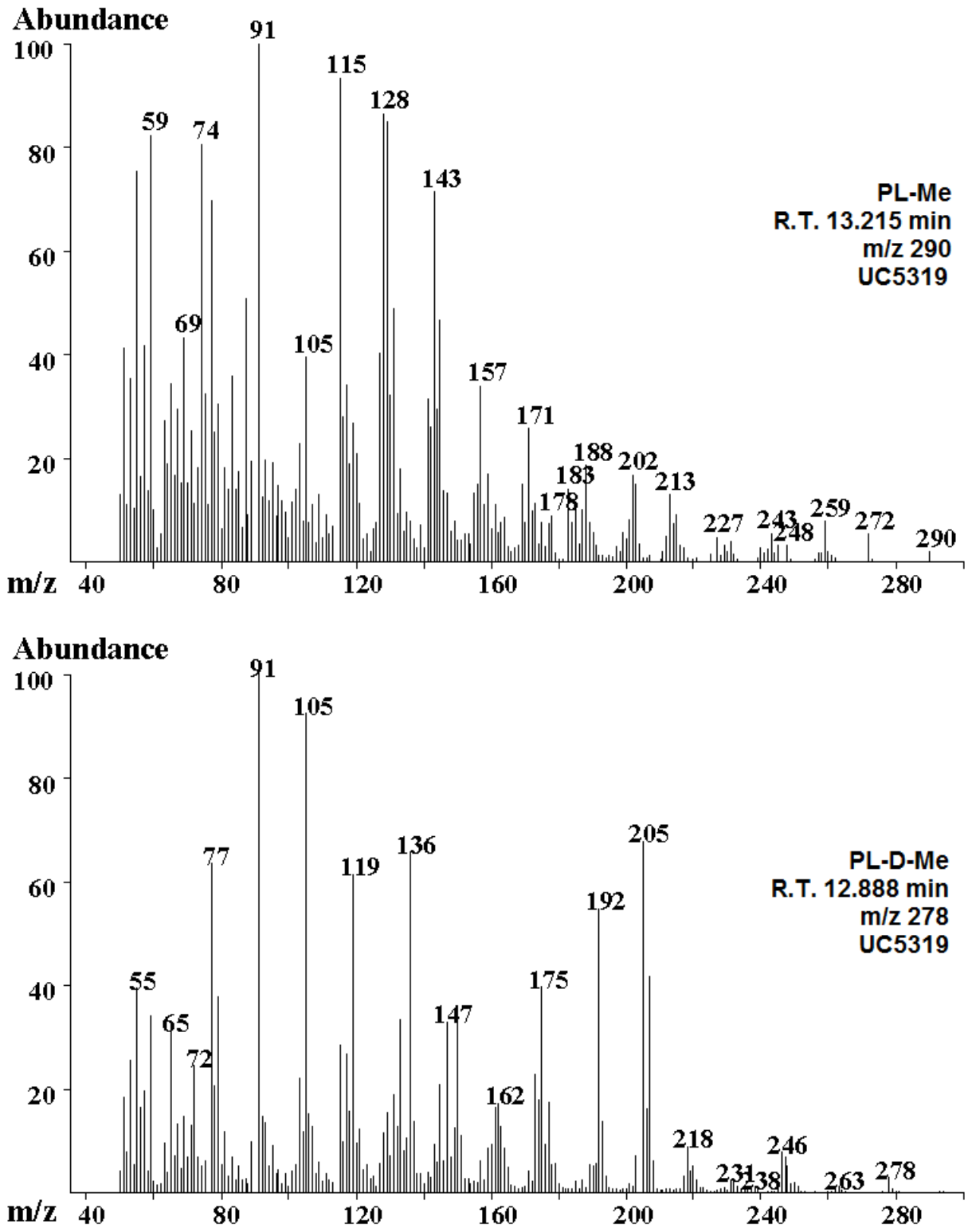

Fig. S6. MS spectra of pentalenolactone methyl ester and pentalenolactone D methyl ester produced by wild-type *S. exfoliatus* UC5319 and the *penR* mutant complemented with *penR, S. exfoliatus ZD27::pDQ90.*

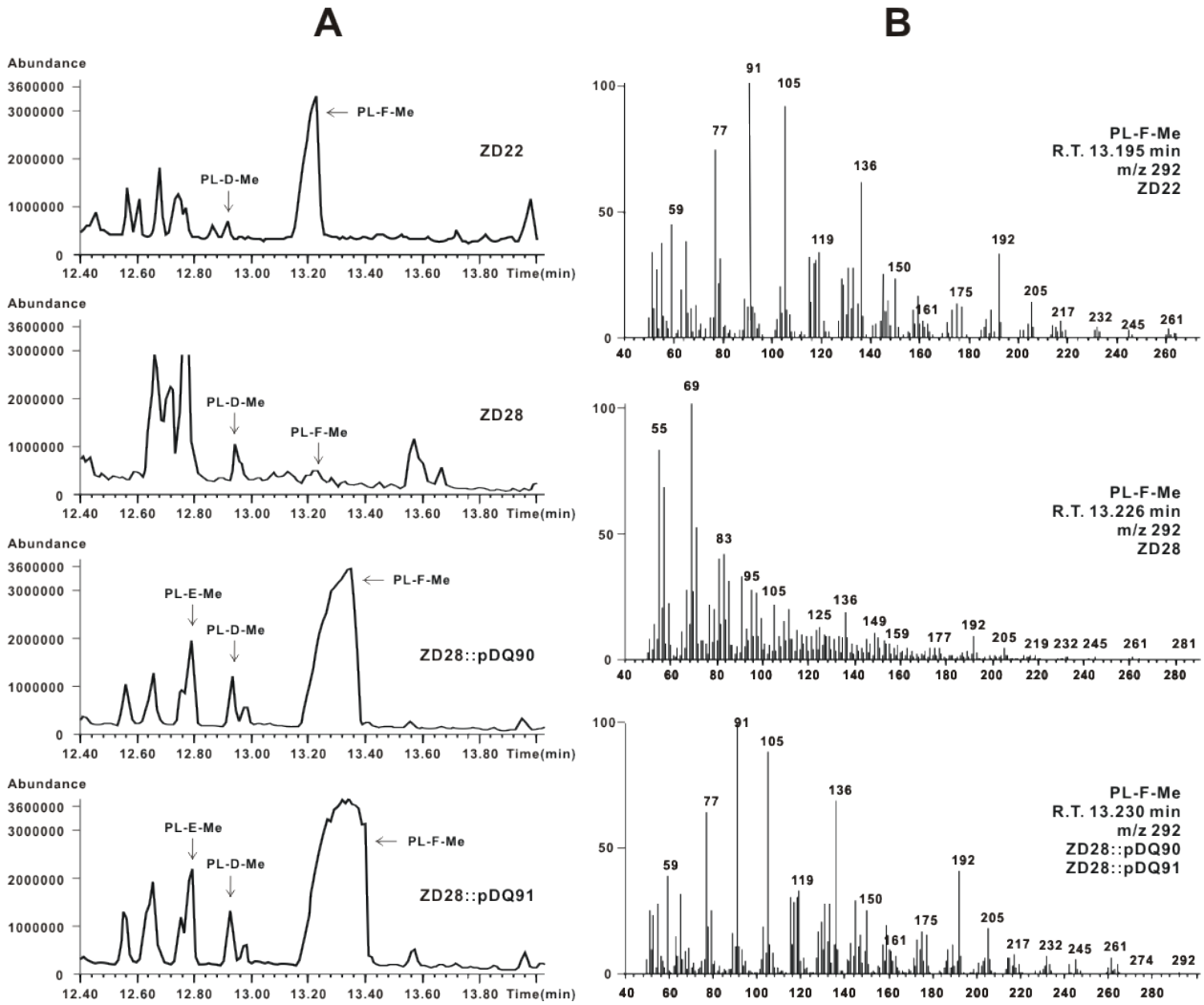

Fig. S7. GC-MS analysis of *S. exfoliatus penR* mutants. A, GC analysis of *penM* mutant *S. exfoliatus* ZD22, *penM-penR* double mutant *S. exfoliatus* ZD28, *S. exfoliatus* ZD28 complemented with *penR* (pDQ90) and *S. exfoliatus* ZD28 complemented with *pntR* (pDQ91). B, MS spectra of PL-F-Me produced by ZD22, ZD28:pDQ90, and ZD28:pDQ:91

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