Characterization of the tautomycin biosynthetic gene cluster from *Streptomyces spiroverticillatus* unveiling new insights into dialkylmaleic anhydride and polyketide biosynthesis*

Wenli Li,[†] Jianhua Ju,[†] Scott R. Rajski,[†] Hiroyuki Osada,[¥] and Ben Shen[†,‡,§,*]

From the [†]Division of Pharmaceutical Sciences, [‡]University of Wisconsin National Cooperative Drug Discovery Group, and [§]Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin, 53705, and [¥]Antibiotics Laboratory, Discovery Research Institute, RIKEN, Wako 351-0198, Japan

Running title: Tautomycin biosynthetic gene cluster from *S. spiroverticillatus*

Address correspondence to: Ben Shen, Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, Tel: (608) 263-2673; Fax: (608) 262-5245; Email: bshen@pharmacy.wisc.edu

SUPPLEMENTAL DATA

TABLE S1. Targeted gene inactivation by the REDIRECT technology in *S. spiroverticillatus* (1)

gene	primers[a]	cosmid[b]	strain
ΔttmJ	*ttmJ*F1: 5'GCCGGTCACGAACCGGACCGGCTCACGTCCGCCACCGAGATTCCGGGGATCCGTCGACC-3' *ttmJ*R1: 5'–CACCGCAACGGAATCGGACGGCGCCTCGGGCGTCGCGTCTGTAGGCTGGAGCTGCTTC-3'	pBS6018	SB6003
ΔttmK	*ttmK*F1:5'-GTGGTGGAGACGTCGTCCGGCTCGATTCGGGGCTTCCTGATTCCGGGGATCCGTCGACC-3' *ttmK*R1: 5'–TCAGTCCGCCGGTACGCCGATCCGGTGCGGGGCCGGTGATGTAGGCTGGAGCTGCTTC-3'	pBS6019	SB6004
ΔttmP	*ttmP*F1:5'-ATGGCGATTCCGCGCGGCAGAACCGAATGTGGCAGGCCGA-TTCCGGGGATCCGTCGACC-3' *ttmP*R1:5'-TCATGCCCCGGCCTCCCCGGCCGTCGAGCGGGCGGGCGCTGTAGGCTGGAGCTGCTTC-3'	pBS6021	SB6006
ΔttmR	*ttmR*F1:5'-GTGACCCCGGCCGAACGGCTGGGCGCGCTGGCCGAACTCATTCCGGGGATCCGTCGACC-3' *ttmR*R1: 5'–TCAGGTCGCCGAACGCAGCAGGCAGGTGATCGCCGCCGGTGTAGGCTGGAGCTGCTTC-3'	pBS6022	SB6007
ΔttmS	*ttmS*F1:5'-ATGACGG-ACGCGCGAACCGGTGCGCTGCTGGCCGTGCTGATTCCGGGGATCCGTCGACC-3' *ttmS*R1: 5'-TCACGCGCCCACCACCGCGATCGGCCGGATCGGGGAACCTGTAGGCTGGAGCTGCTTC-3'	pBS6023	SB6008

[a]Underlined letters represent the 39 nt homologous to the DNA regions internal to targeted genes.
[b]pBS6018 and pBS6019 are based on pBS6016, and pBS6021, pBS6022 and pBS6023 are based on pBS6017.

TABLE S2. Southern Analysis Confirming the Genotypes of Mutant Strains[a]

strains	gene targeted	probe		fragment replaced (bp)	Restriction digestion	Signal Size (kp)	
		primers used to amplify the probe	Size (bp)			WT	mutant
SB6003	*ΔttmJ*	*ttmJ*FP2: 5'-GGACGCCGAATACTGGTGC-3' *ttmJ*RP2: 5'-GGGCGAGATGCCGAAGAA-3'	918	288	*Bam*HI	4.0	3.5
SB6004	*ΔttmK*	*ttmK*FP2: 5'-CGGTCAGGGCCGCCACGAGCTCTG-3' *ttmK*RP2: 5'-CCCGAGTGGAGGTGTTCG-3'	853	1292	*Bam*HI	3.7	1.3
SB6006	*ΔttmP*	*ttmP*FP2:5'-GGC-TGGTGTCCATGGTCGGC-3' *ttmP*RP2: 5'-GAACTTCAGGCCGGGCACCC-3'	1405	1156	*Pvu*I	3.3	1.7 1.4
SB6007	*ΔttmR*	*ttmR*FP2:5'-GAACCGGTCGCCGCAGAACG-3' *ttmR*RP2: 5'-GGCATGCCCTGCTCCCCG-3'	1490	1324	*Sma*I	1.6	2.3
SB6008	*ΔttmS*	*ttmS*FP2:5'-ACGCGTCGGTCTGTTCCGCG-3' *ttmS*RP2: 5'-TACCTGGGGCACGCGACC-3'	659	747	*Apa*I	1.6	2.2

[a]See Figures S1, S2, S3, S4, S5 for details.

FIGURE S1. Inactivation of *ttmJ* by gene replacement. (A) Construction of the *ΔttmJ* gene replacement mutant and restriction maps of *S. spiroverticillatus* wild-type and SB6003 mutant strains showing predicted fragment sizes upon *Bam*HI digestion. (B) Southern analysis of the wild-type (lane 5) and SB6003 (lanes 2, 3 and 4 are three individual isolates) genomic DNAs digested with *Bam*HI using the 918-bp amplified DNA fragment as a probe. Lane 1, molecular weight standard.

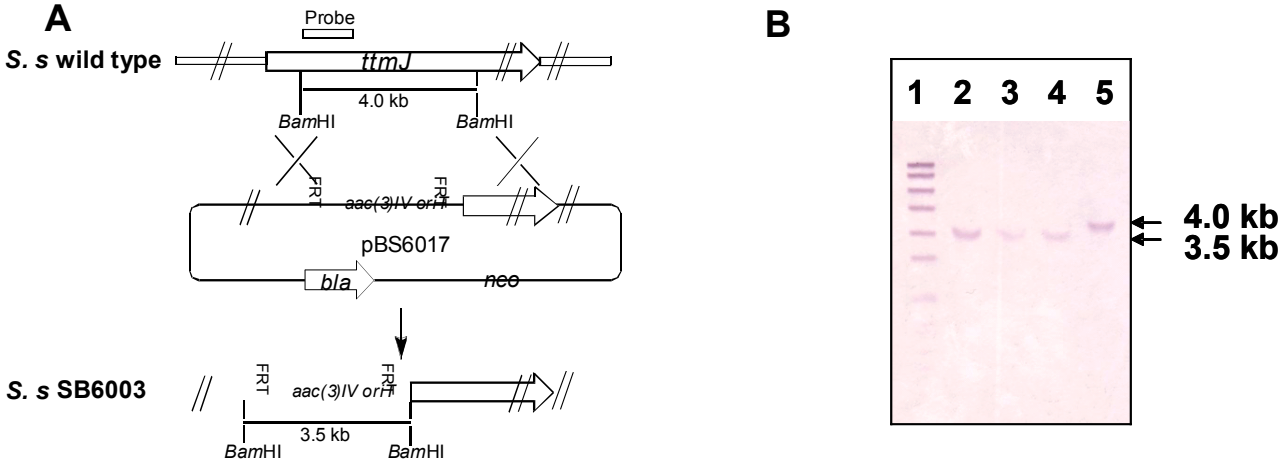

FIGURE S2. Inactivation of *ttmK* by gene replacement. (A) Construction of the *ΔttmK* gene replacement mutant and restriction maps of *S. spiroverticillatus* wild-type and SB6004 mutant strains showing predicted fragment sizes upon *Bam*HI digestion. (B) Southern analysis of the wild-type (lane 1) and SB6004 (lanes 2, 3 and 4 are three individual isolates) genomic DNAs digested with *Bam*HI using the 853-bp amplified DNA fragment as a probe. Lane 5, molecular weight standard.

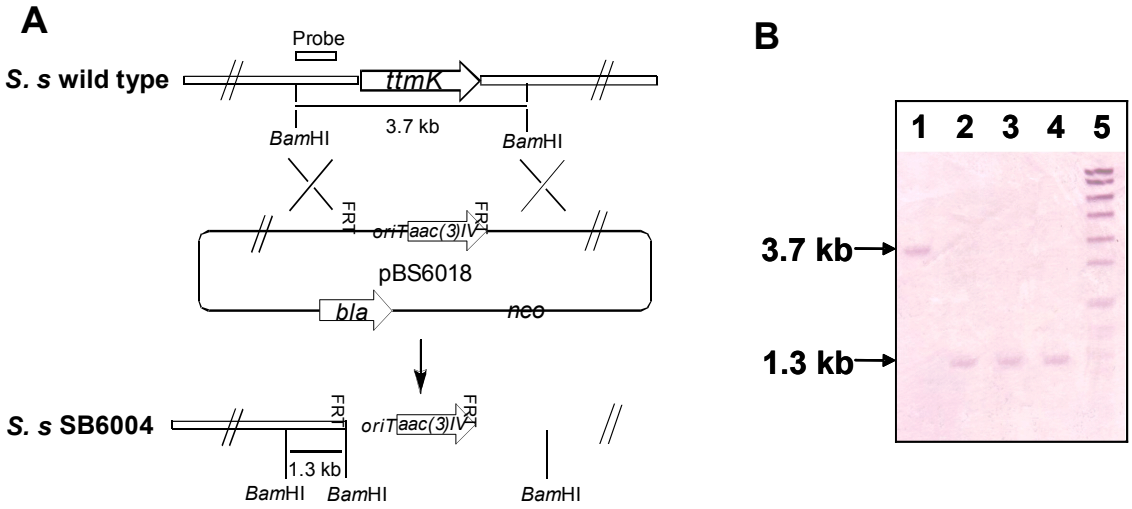

FIGURE S3. Inactivation of *ttmP* by gene replacement. (A) Construction of the *ΔttmP* gene replacement mutant and restriction maps of *S. spiroverticillatus* wild-type and SB6006 mutant strains showing predicted fragment sizes upon *Pvu*I digestion. (B) Southern analysis of the wild-type (lane 2) and SB6006 (lanes 3 and 4 are two individual isolates) genomic DNAs digested with *Pvu*I using the 1405-bp amplified DNA fragment as a probe. Lane 1, molecular weight standard.

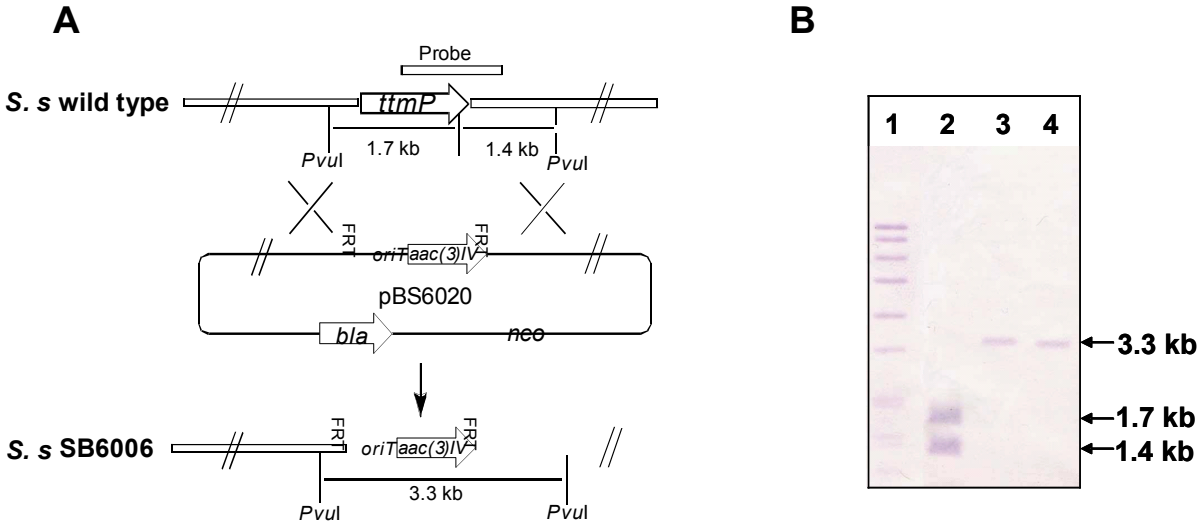

FIGURE S4**.** Inactivation of *ttmR* by gene replacement. (A) Construction of the *ΔttmR* gene replacement mutant and restriction maps of *S. spiroverticillatus* wild-type and SB6007 mutant strains showing predicted fragment sizes upon *Sma*I digestion. (B) Southern analysis of the wild-type (lane 8) and SB6007 (lanes 2-7 are six individual isolates) genomic DNAs digested with *Sma*I using the 1490-bp amplified DNA fragment as a probe. Lane 1, molecular weight standard.

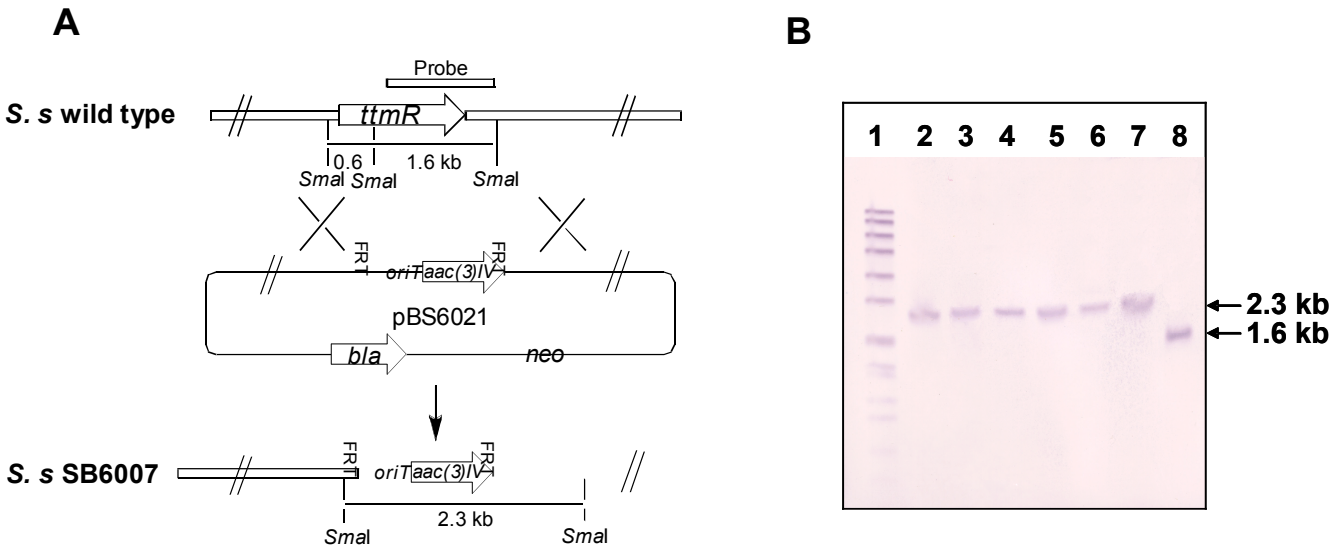

FIGURE S5. Inactivation of *ttmS* by gene replacement. (A) Construction of the *ΔttmS* gene replacement mutant and restriction maps of *S. spiroverticillatus* wild-type and SB6008 mutant strains showing predicted fragment sizes upon *Apa*I digestion. (B) Southern analysis of the wild-type (lane 3) and SB6008 (lanes 1 and 2 are two individual isolates) genomic DNAs digested with *Apa*I using the 659-bp amplified DNA fragment as a probe.

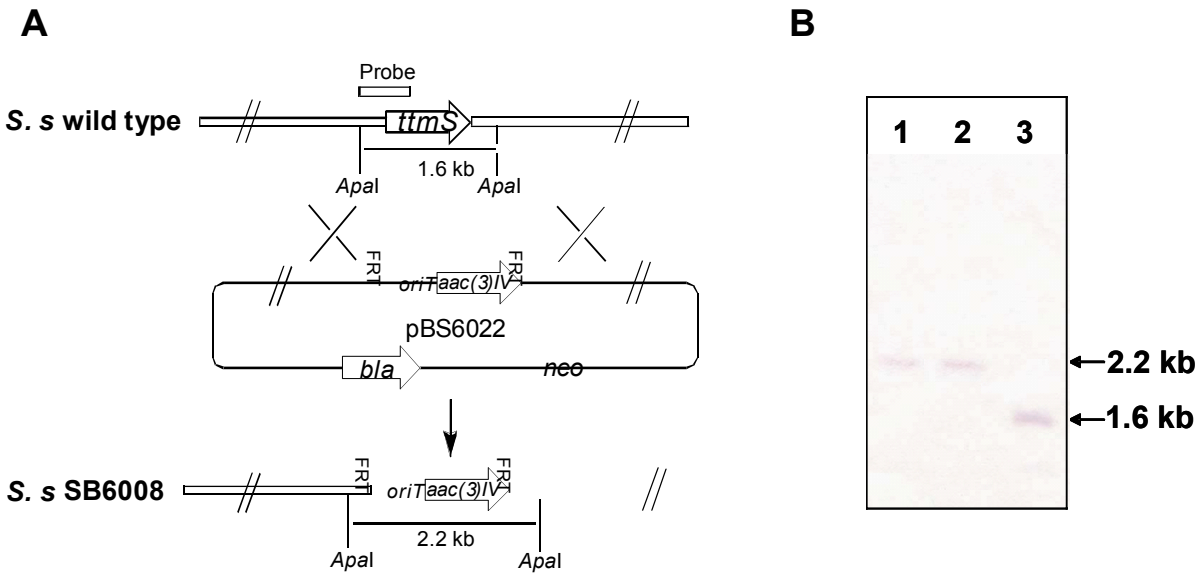

TABLE S3. Expression constructs for complementation to the *ΔttmK* and *ΔttmS* mutants

mutant strain	gene mutated	primers used to make the expression constructs[a]	construct	complemented strain
-	-	*ErmE*p*-FP: 5'-TAGAATTCGTGATGCTAGTCGCGGTTGATC- *ErmE*p*-RP:5'-TAGAATTCGTAATCATGCATTATCTCCTTCTCGCTGGATCCTACCAACCGG-3'	pBS6027[b]	-
SB6004	*ΔttmK*	*ttmK*FP3: 5'-CCAATGCATATGGTGGAGACGTCGTCC-3' *ttmK*RP3: 5'-GCTCTAGATCAGTCCGCCGGTACGC-3'	pBS6024[c]	SB6009
SB6008	*ΔttmS*	*ttmS*FP3: 5'-CCAATGCATGACGGACGCGCGAA-3' *ttmS*RP3: 5'-GCTCTAGATCACGCGCCCACCACCG-3'	pBS6026[c]	SB6011

[a]*Eco*RI, *Nsi*I and *Xba*I restriction sites are underlined.
[b]This construct was generated by inserting the *ErmE** promoter amplified from pWHM79 (2) at the *Eco*RI site of pBS8004 (3).
[c]These constructs were generated by inserting the PCR-amplified *ttmK* or *ttmS* fragment from pBS6004 into *Nsi*I and *Xba*I sites of pBS6027.

References

S1. Gust, B., Challis, G. L., Fowler, K., Kieser, T., and Chater, K. F. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 1541-1546
S2. Shen, B., and Hutchinson, C. R. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 6600-6604
S3. Tao, M., Wang, L., Wendt-Pienkowski, E., George, N. P., Zhang, G., Coughlin, J. M., and Shen, B. (2007) *Mol. Biosyst*. **3**, 60-74