The Biosynthetic Gene Cluster of Zorbamycin, a Member of the Bleomycin Family of Antitumor Antibiotics, from *Streptomyces flavoviridis* ATCC 21892

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Fig. S1. S2
Fig. S2. S3
Fig. S3. S6

Fig. S1. Schematic representation of the arrangement of the cyclization (Cy) and oxidation (Ox) domains within the thiazole forming NRPS modules of the ZBM biosynthetic machinery and approximate locations of the primer pairs used for their amplification. Abbrevations for NRPS domains are: A, adenylation; C, condensation; Cy, cyclization; Ox, oxidation; PCP, peptidyl carrier protein.

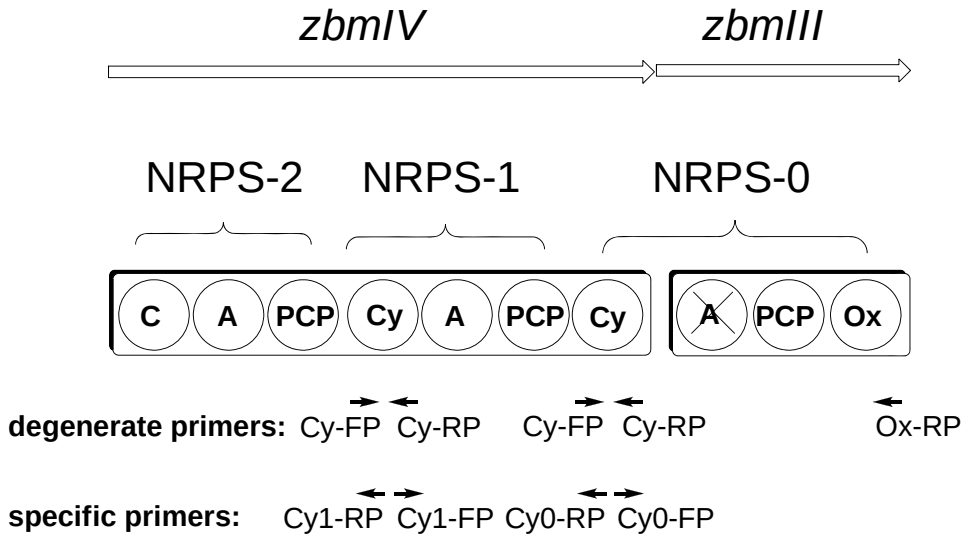
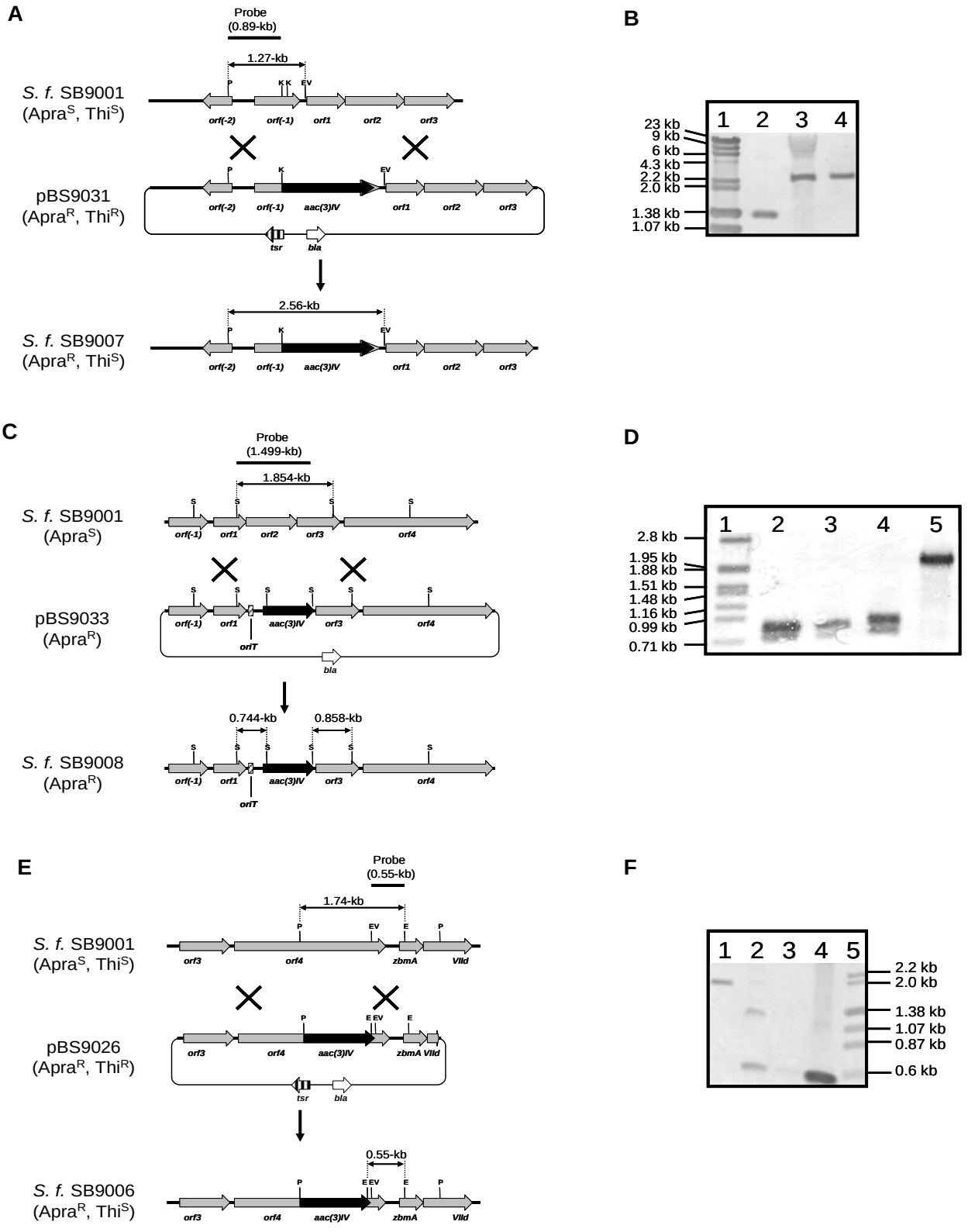


Fig. S2. Determination of the *zbm* gene cluster boundaries by gene replacements of *zbm-orf(-1)*, *zbm-orf2*, *zbm-orf4*, *zbmVIId*, *zbmL*, and *zbm-orf41*.
(A) Construction of the *zbm-orf(-1)* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9007 mutant strains showing fragment sizes upon *Eco*RV-*Pst*I digestion.
(B) Southern analysis of SB9001 (lane 2) and SB9007 (lanes 3+4 are two individual isolates) genomic DNA digested with *Eco*RV and *Pst*I using a 0.89-kb *Pst*I-*Kpn*I fragment as a probe. Lane 1, molecular weight marker.
(C) Construction of the *zbm-orf2* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9008 mutant strains showing fragment sizes upon *Sst*I digestion.
(D) Southern analysis of SB9001 (lane 5) and SB9008 (lanes 3+4 are two individual isolates) genomic DNA and inactivation plasmid harbouring the mutated *zbm-orf2* (lane 2) locus as control digested with *Sst*I using a 1.499-kb PCR-amplified fragment as a probe (primer pair C-zorf1-f1 and C-zorf1-r1). Lane 1, molecular weight marker.
(E) Construction of the *zbm-orf4* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9006 mutant strains showing fragment sizes upon *Eco*RI-*Pst*I digestion.
(F) Southern analysis of SB9001 (lane 1) and SB9006 (lanes 2+3 are two individual isolates) genomic DNA and inactivation plasmid harbouring the mutated *zbm-orf4* (lane 4) locus as control digested with *Eco*RI and *Pst*I using a 0.55-kb *Eco*RV-*Eco*RI fragment as a probe. Lane 5, molecular weight marker.
(G) Construction of the *zbmVIId* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9005 mutant strains showing fragment sizes upon *Bam*HI digestion.
(H) Southern analysis of SB9001 (lane 5) and SB9005 (lanes 3+4 are two individual isolates) genomic DNA and inactivation plasmid harbouring the mutated *zbmVIId* (lane 2) locus as control digested with *Bam*HI using a 1.585-kb PCR-amplified fragment as a probe (primer pair C-zorf5-f1 and C-zorf5-r1). Lane 1, molecular weight marker.
(I) Construction of the *zbmL* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9003 mutant strains showing fragment sizes upon *Bam*HI-*Kpn*I digestion.
(J) Southern analysis of SB9001 (lane 2) and SB9003 (lanes 3+4 are two individual isolates) genomic DNA digested with *Bam*HI and *Kpn*I using a 1.28-kb *Bam*HI-*Bgl*II fragment as a probe. Lane 1, molecular weight marker.
(K) Construction of the *zbm-orf41* gene replacement mutant and restriction map of *S. flavoviridis* SB9001 wild-type and SB9004 mutant strains showing fragment sizes upon *Eco*RV-*Bgl*II digestion.
(L) Southern analysis of SB9001 (lane 2) and SB9004 (lane 3) genomic DNA digested with *Eco*RV and *Bgl*II using a 1.09-kb *Bam*HI-*Nco*I fragment as a probe. Lane 1, molecular weight marker.
B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; EV, *Eco*RV; K, *Kpn*I; N, *Nco*I; P, *Pst*I; S, *Sst*I; ApraR, apramycin resistant; ApraS, apramycin sensitive; ThiR, thiostrepton resistant; ThiS, thiostrepton sensitive.



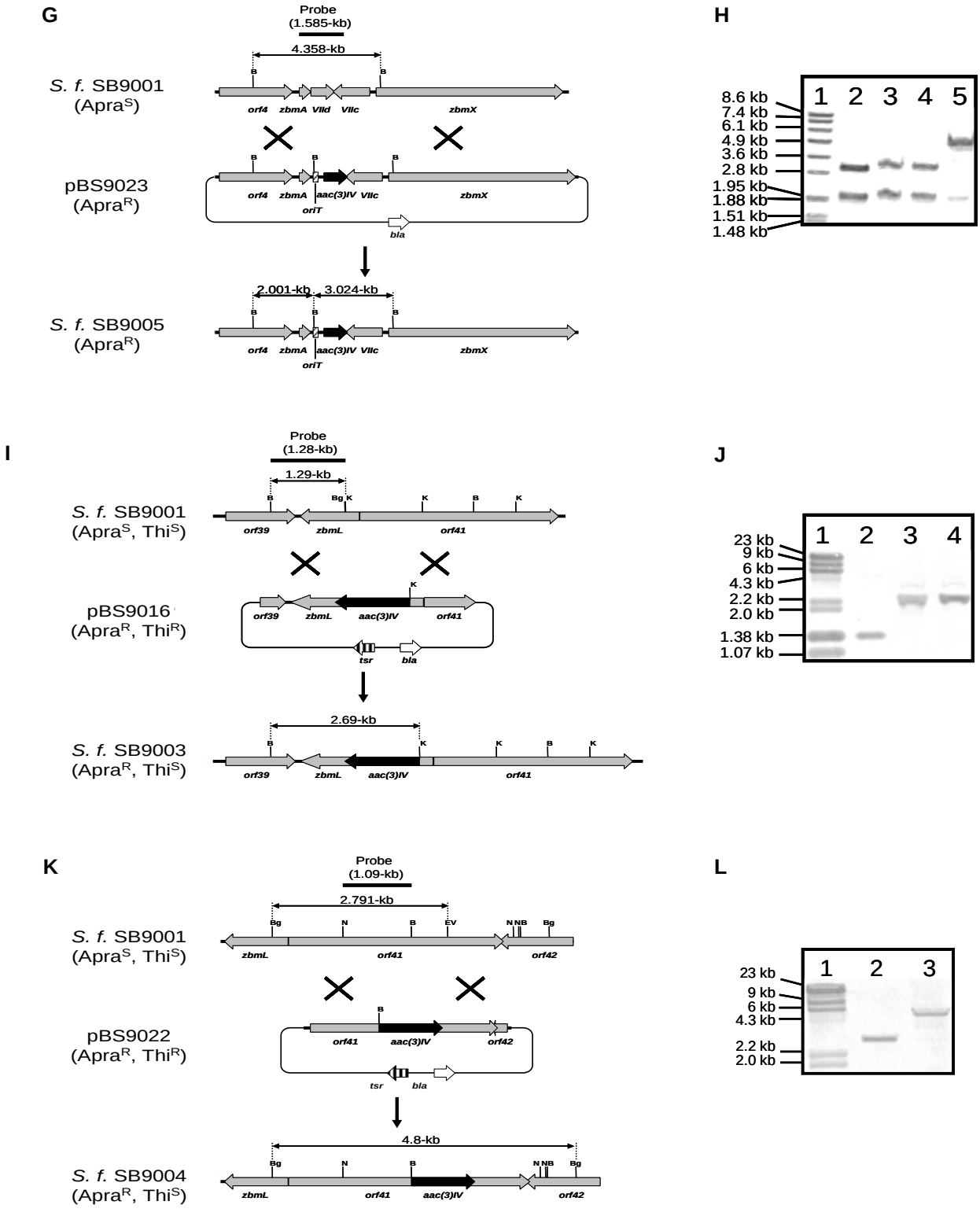
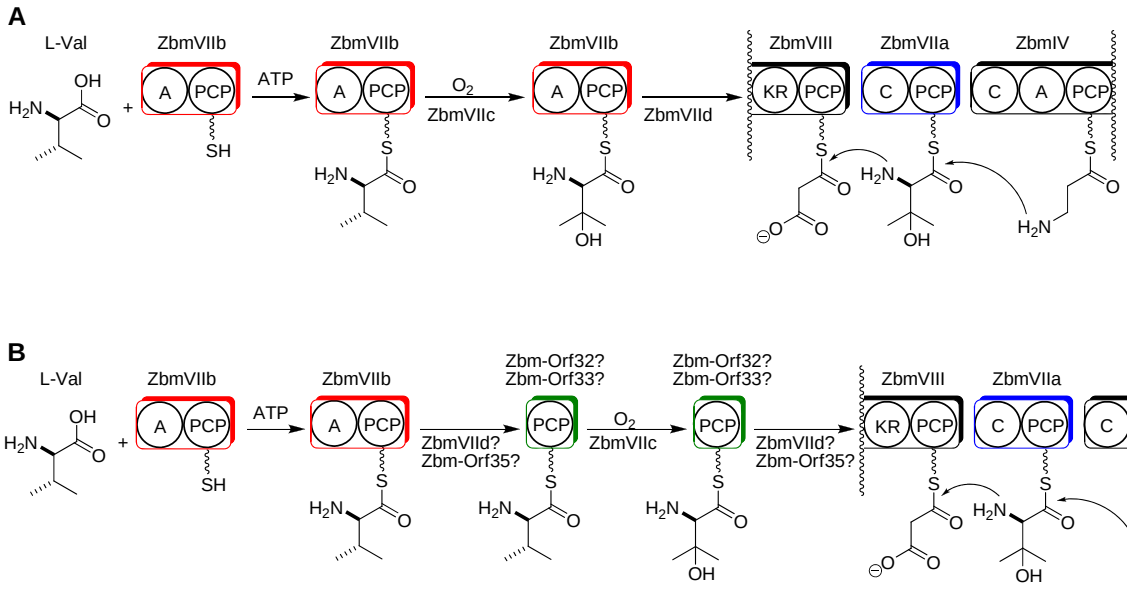


Fig S3. Proposed mechanisms for the biosynthesis and incorporation of L-hydroxyvaline in analogy to the (A) syringomycin and (B) coronatine biosynthetic pathways. (C) Alignment of the active site regions of ZbmVIId and Zbm-Orf35 with the ones of the reported acyltransferases SyrC, CmaE, and BarC. The conserved catalytic Cys/Ser residues are boxed.



C

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                  201                                                250
      SyrC  (198) DTDAQVADMISVMNHFGLSTAHLMGICGGAVIALSAAAAHAERV---NSL
      CmaE   (79) EGVADFIRQFNAALPEPVRVDALVGYCSSAPLALLAANQGACR-----TL
    ZbmIId   (66) ERFAD--GLIEDLRPWMDRPFALLGHCSGALAAYETAVELRRRGLPAPVL
      BarC   (71) EDINL--VLAQELSPFLSKPFAFMGHSMGALIAFDLIRLLRQQELGQPQF
 Zbm-Orf35   (68) ALDEV-------VRKTGRGPDRLIGYSYGALVAVEMAHQAKARGLPAPRL
 Consensus  (201) E IAD   L   L PF  RP ALMGHS GALIALEAA  LR R L AP L
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