Supplemental material

FIG S1 Sequence alignment of TvaF with AviCys forming enzymes. MibD (Accession number: ADK32557), EpiD (P30197) and CypD (ADR72965) are involved in the biosynthesis of microbisporicin, epidermin and cypemycin, respectively. Amino acid sequence alignment was carried out using CLUSTAL W multiple sequence alignment program (version 2.1) (2) and edited with GeneDoc (version 2.7) (1).

Isolation of thioviridamide. The fermentation broth (1 litter) was centrifuged and the mycelium was extracted with acetone. The extract was evaporated and then extracted with EtOAc at pH 3. The extract was applied to a silica gel column, which was washed with $CHCl_3$ - MeOH (10:1) and eluted with $CHCl_3$ - MeOH (5:1). The eluate was subjected to HPLC (YMC-Pack D-ODS-7; YMC, Kyoto, Japan) with 85% MeOH - 5 mM disodium hydrogen citrate. The corresponding peak fraction was concentrated and then partitioned between EtOAc and water. The organic layer was washed with 0.01 M HCl and water. The EtOAc solution was dried over anhydrous sodium sulfate and evaporated to dryness to give a pure powder of thioviridamide.

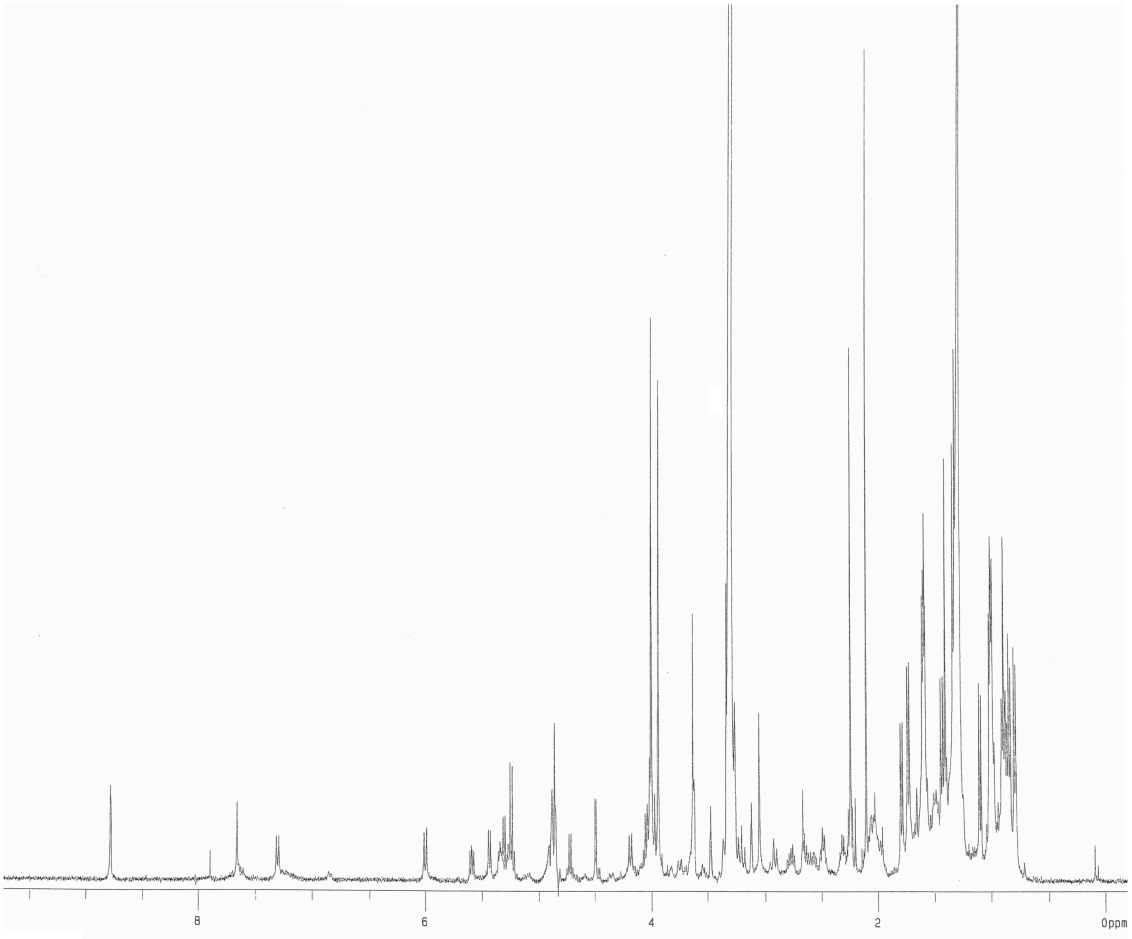

FIG S2 ^{1}H NMR spectrum of thioviridamide produced by *S. lividans* expressing the *tva* gene cluster. ^{1}H NMR spectrum was obtained in CD_3OD on a JEOL JNM-LA400 spectrometer at 400 MHz.

TABLE S1 Bacterial strains and plasmids used in this study

Strain and plasmid	Relevant characteristics
Streptomyces strains	
S. olivoviridis NA05001	Thioviridamide producer
S. lividans TK23	Heterologous host for thioviridamide production
Escherichia coli strains	
XL1-blue MR	Strain for cosmid library construction; Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*
XL1-blue MRF'	Cloning host; Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F' *proAB lacI*^{q}*Z*ΔM15 Tn*10* (*Tet*^{r})]
JM110	*dam*/*dcm* methylases deficient strain; *dam dcm supE*44 *hsdR*17 *thi leu rpsL*1 *lacY galK galT ara tonA thr tsx* Δ(*lac-proAB*) [F' *traD*36 *proAB lacI*^{q}*Z*ΔM15]
Plasmids	
pWE15	General cosmid vector for a genomic library of *S. olivoviridis* NA05001.
pGEM-11Z	General cloning vector with multiple cloning sites for cloning of a 0.8-kbp *tvaJ* fragment into the HindIII site.
pBluescript II SK (+)	General cloning vector with multiple cloning sites for cloning of a 2.0-kbp *tvaA* fragment into the EcoRI/XbaI sites and cloning of the *tva* gene cluster (16.6-kbp) into the XbaI/HindIII sites.
pWHM3	*E. coli*/*Streptomyces* shuttle vector for cloning and expression of the *tva* gene cluster.

Supplemental References

1. **Nicholas, K. B., H. B. Nicholas, and D. W. Derrfield.** 1997. GeneDoc: analysis and visualization of genetic variation. EMBNEW NEWS **4**:14.
2. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22:**4673-80.