

Supporting Information

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1. Supporting Results

The development of a genetic system within the 1-producing strain. We had previously prepared several high-, low-, or non-producing strains by random chemical mutagenesis, and expression analysis with these strains revealed a set of genes (*orfs* 7-28) within the 65-kb sequenced region that are only expressed in low- or high-producing strains at the onset of **1** production.^[S1] Strains representing each production type were subjected to PCR analysis for amplification of every predicted *orf* within the sequenced locus to reveal that *orfs* 1-28 are intact in both producing and non-producing strains alike (Supporting Information, Figure S1). This includes the NRPS-encoding genes, *orf26* and *orf27*. In contrast *orfs* 29-38 were only present in the producing strains. The genomic DNA for each strain type was subsequently digested with *AseI* and subjected to pulse field gel electrophoresis, yielding two large DNA fragments (610 and 680-kb DNA) that each hybridized with a DIG-labelled *orf8* probe for all of the producing strains. In contrast while the 680-kb DNA fragment was observed, the 610-kb DNA fragment was not detected in representative nonproducing strains. In a similar manner, *orf37* was only present in the 610-kb DNA fragment of the producing strains. These results are consistent with the **1**-producing strain harboring multiple copies for several if not all of the required structural genes. The reason for complete loss of production in the non-producing strain is still unclear but is proposed to be a result of regulation malfunction.

2. Supporting Tables

Table S1. Biotransformation of L-Lys to L-ACL.

Sample ^a	[L-Lys] ($\mu\text{g/mL}$)	[FMOC-ACL] ($\mu\text{g/mL}$)	Sample	[L-Lys] ($\mu\text{g/mL}$)	[FMOC-ACL] ($\mu\text{g/mL}$)
500 mL LM culture	100	2.7	150 mL LM culture	100	4.9
	200	5.5		200	5.7
	300	2.4		300	2.3
	400	2.0		400	1.7
	500	2.0		500	3.3

^aAbbreviations are LM, liquid media; FMOC-ACL, L- α -amino- ϵ -caprolactam derivatized with FMOC.

Table S2. Enzymatic formation of **2** from **12**.

Variable	Reaction ^a									
	1 ^b	2 ^c	3 ^d	4	5	6	7	10	11	12
MgCl ₂	-	+	-	-	+	+	+	+	+	-
ATP	+	+	-	-	+	+	+	+	-	+
CoA	-	+	-	-	+	-	+	+	+	+
L-Lys	+	+	-	+	+	+	+	+	+	+
Svp	-	+	-	-	+	-	+	+	+	+
CapU	-	+	-	-	+	+	-	+	+	+
CapV	-	+	-	-	-	-	-	-	-	-
CapW	+	+	+	+	+	+	+	-	+	+
L-ACL	-	-	+	-	-	-	-	-	-	-
Yield (%) ^e	nd	37	95	nd	32	5	nd	nd	nd	nd

^aAll reactions contained 50 mM Tris-HCl (pH = 7.5) and substrate **12** with (+) or without (-) indicated variable. Concentrations of each component are provided within the experimental procedures.

^bCorresponds to Fig. S6B, trace *b*.

^cCorresponds to Fig. S6B, trace *c*.

^dCorresponds to Fig. S6B, trace *d*.

^eBased on amount of **12** converted to product **2**; nd, not detected.

Table S3. Codon adaptation index for genes involved in **2** biosynthesis.

Gene ^a	Length	CAI ^b	CAI ^c	%G+C ^d
<i>capA</i>	924	0.521	0.464	77.6
<i>capB</i>	1671	0.597	0.473	84.0
<i>capC</i>	951	0.646	0.491	88.3
<i>capD</i>	975	0.779	0.650	92.9
<i>capE</i>	1404	0.690	0.598	90.4
<i>capF</i>	951	0.592	0.469	85.2
<i>orf15</i>	1200	0.697	0.533	88.3
<i>capG</i>	1158	0.717	0.594	89.9
<i>capH</i>	1239	0.654	0.548	87.4
<i>capI</i>	264	0.529	0.478	77.3
<i>capJ</i>	2286	0.730	0.601	91.2
<i>capK</i>	738	0.595	0.476	82.9
<i>capL</i>	507	0.644	0.508	85.8
<i>capM</i>	870	0.709	0.549	89.7
<i>capN</i>	2034	0.649	0.502	87.9
<i>capO</i>	1839	0.717	0.524	91.0
<i>capP</i>	915	0.503	0.411	79.0
<i>capS</i>	780	0.559	0.442	81.2
<i>capT</i>	1890	0.655	0.535	87.0
<i>orf16</i>	402	0.487	0.336	79.1
<i>capU</i>	1410	0.495	0.337	77.3
<i>capV</i>	3312	0.401	0.414	69.1
<i>capW</i>	1209	0.617	0.544	86.4
<i>orf17</i>	546	0.635	0.527	85.2

^aGenes required for **12** biosynthesis are highlighted in red. The *capP* gene encodes an ATP:2 phosphotransferase that confers self-resistance. The gene *capT* encodes a putative C-methyltransferase that modifies the L-ACL following incorporation into **12**.

^bReferenced against the codon usage table for *Streptomyces griseus* sups. griseus.

^cReferenced against the glucokinase gene from *Streptomyces griseus* sups. griseus (Genbank accession no. AP009493).

^dG+C content at the third position of the codon.

3. Supporting Figures

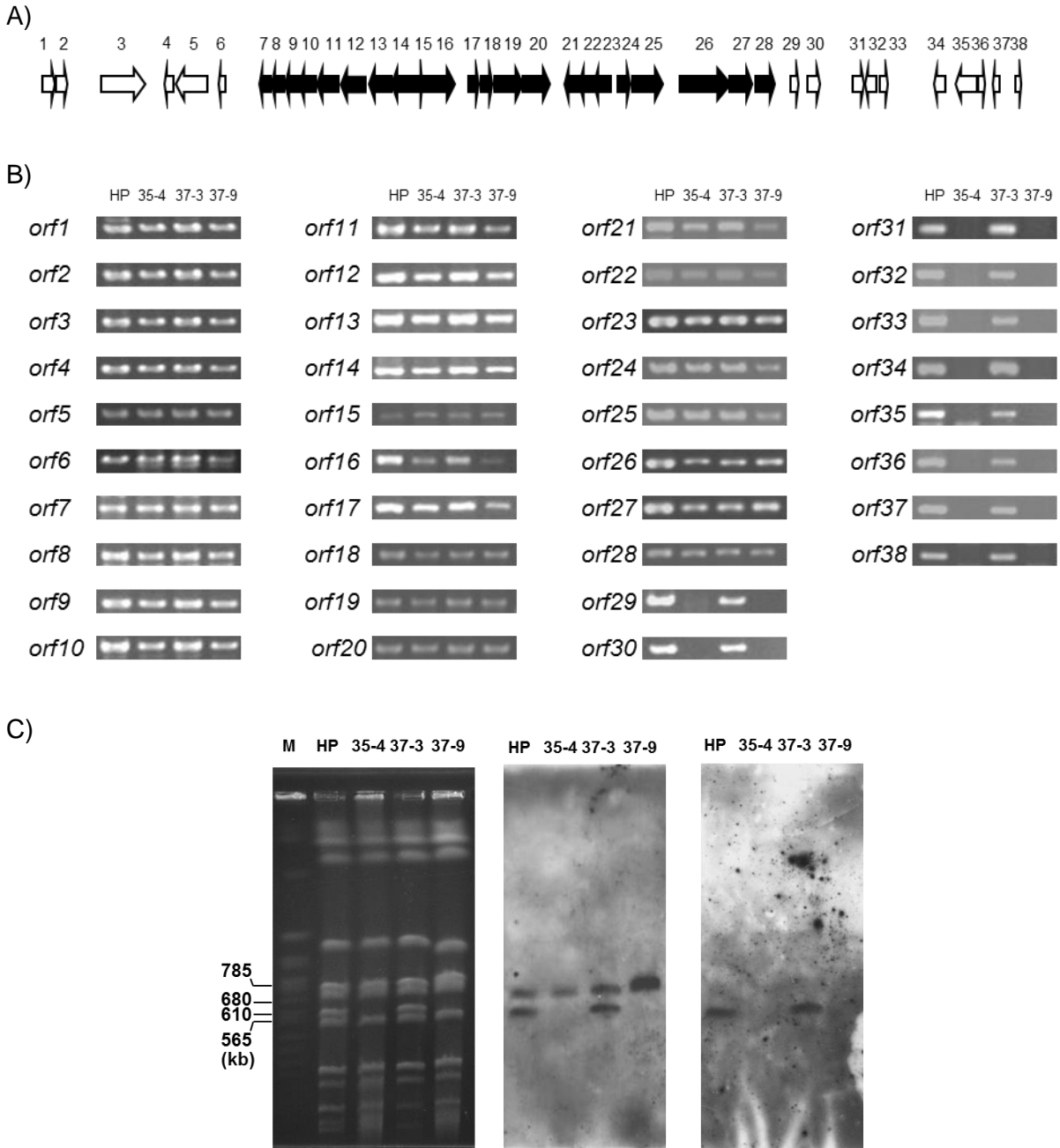
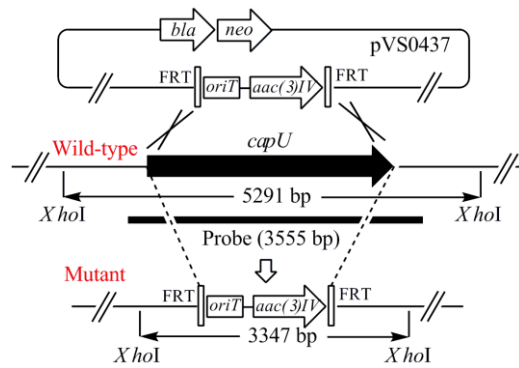


Figure S1. Analysis of the **1** biosynthetic gene cluster. A) Genetic architecture of the open reading frames (*orfs*) from the sequenced region encompassing 65-kb DNA. Filled *orfs* (7-28) are highly expressed during the onset of **1** production and hence are probably required for biosynthesis. B) PCR amplification of each of the 38 *orfs* using template DNA from a high-producing strain (HP), a low-producing mutant strain (37-3), and two non-producing strains (35-4 and 37-9). C) Pulse-field gel electrophoresis analysis of total genomic DNA isolated from the indicated strains and subjected to *AseI* digestion. Lane M (marker) is *Saccharomyces cerevisiae* chromosomes (Bio Rad), and Southern blot analysis was performed using *orf8* as a probe (middle panel) or *orf37* (right panel).

A)



B)

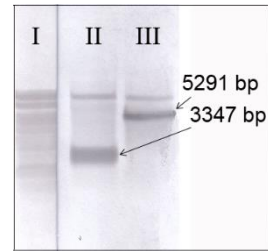


Figure S2. Development of a genetic system within the producing strain of **2**. A) Strategy to prepare an in-frame deletion of *capU*. B) Southern blot analysis of genomic DNA isolated from the $\Delta capU$ mutant strain (II) or wild-type strain (III) and digested with *XhoI*. Lane I, DNA marker.

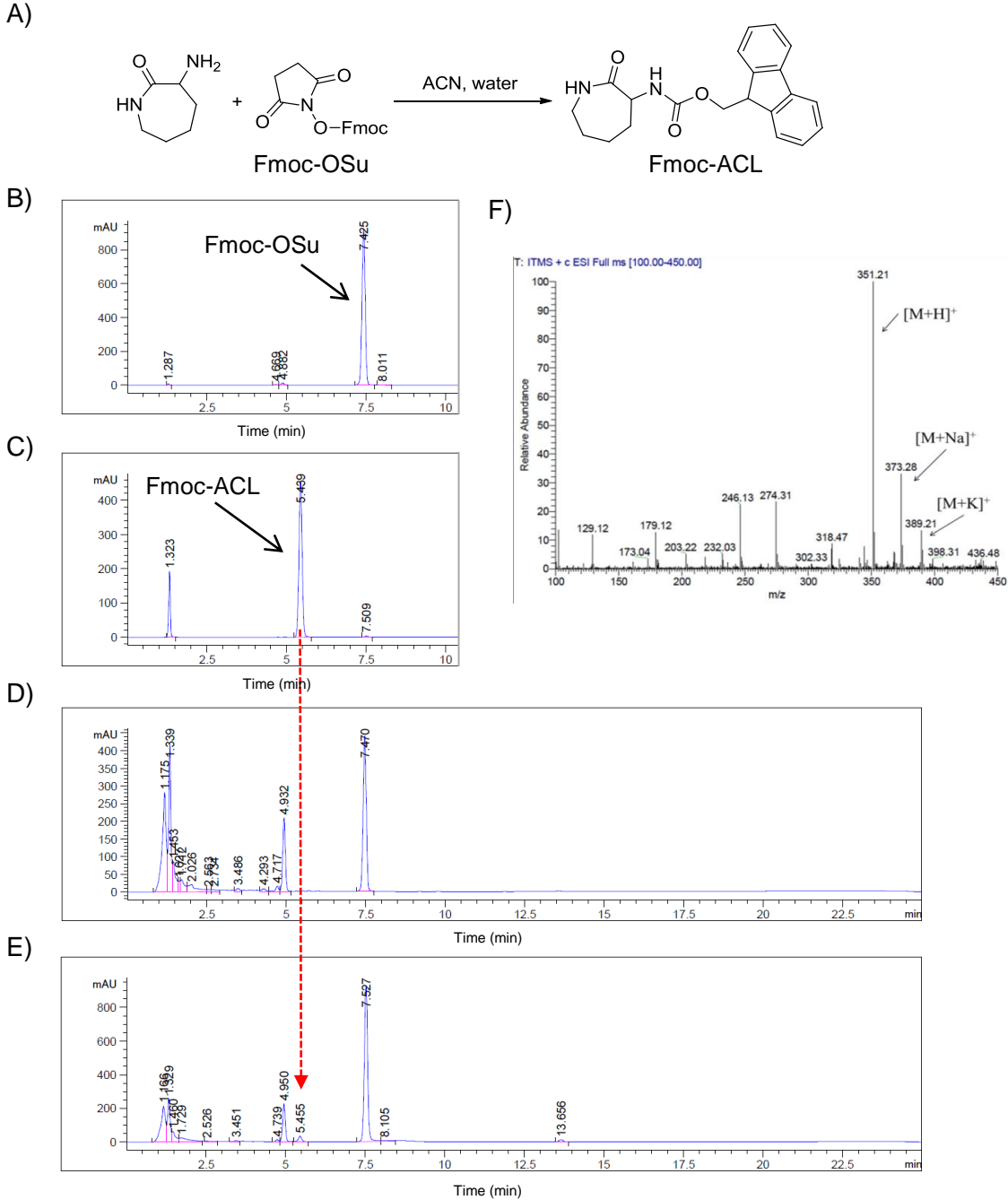


Figure S3. Biotransformation of L-Lys upon heterologous expression of *capV* and *capU*. (A) Modification of ACL with Fmoc. Fmoc-OSu, Fmoc *N*-hydroxysuccinimide ester. HPLC analysis of Fmoc-ACL production including (B) Fmoc-OSu control, (C) L-ACL standard modified by Fmoc-OSu, (D) Fmoc-OSu modified extract from *S. lividans* TK64 with empty vector (pUWL201pw), (E) Fmoc-OSu modified extract from *S. lividans* TK64 pUWL201pw-*capUV* revealing a peak with retention time 5.455 min that co-elutes with Fmoc-OSu modified L-ACL. (F) The observed mass spectrum for the product at retention time = 5.455 min (expected $m/z = 350.16$ for $C_{21}H_{22}N_2O_3$).

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1      10     20     30     40     50     60
TycC6  KQAYYPVSSAQRMYIILDQFEGVGI SYNMPSTMLIEGKLERTRVEAAAFQRLIARHESLRTSF
SrfA-C VQDMYYLSPMQEGMLFHAILNPGQSFYLEQITMKVIGSLNIKCLEESMNVIIMDRYDVFRITVF
VibH   MLLAQKPFWRHLAYPHINLDTVAHSLRLTGLPLDTLLLEPALHLTVSEIDLFRAPF
CapV   ILEVAPAHPHSRCAGIHQLFVLRGVPVEPARLRAAWRNLQYRHDALRTIF
CapU   VPPGQLRWSAQQEQMWFREQTGGDPSEHNLVYRFL LHGQVDDRLVAALPATVSRQSVLSAPQ

70     80     90     100    110    120
TycC6  AVVNGEP-----VQNIH-EDVVPFALAYSEVTEEEARELVS-SLVQPFDDLEVAPLIRVSLLK
SrfA-C IHEKVKRPVQVVLKRPQHIEEIDLTLTGLSEQTAKINEYKEQDKIRGFDLTRDIPMRAAIFK
VibH   SAQGELY-----WHPPSPPIDYQDLSIHLEAEP LAWRIEQDLQRSSTLIDAPITSHQVYR
CapV   SEQGSQW-----RQRVTRMTSELVIRGAAIDERTMRETGVRHRVEGFDLAGGALARAEVLV
CapU   GRHATVP-----RADFH---VRTVDATG SAPDSVLSVLESLRTESAHPLEENQLLARATLVH

130    140    150    160    170    180
TycC6  IGEDRYVLFDTMHHISIDGVSSGILLAEWVQLYQGDVL-PELRIQYKDFAVWQDEFSSQSAAFH
SrfA-C KAEESFEVWWSYHHIILDFWCFGI VVQDLFKVYNALREKPYSLPPVKPYKDYIKWLEKQDRQ
VibH   LSHSEHLIYTRAHHIVLDGFGYGMMLFEQRLSQHYQSLLS---GQTPTAAPKPYQSYLEEEAAIY
CapV   GREATHILVLSVHHSVVDGYSGLVLDLDCQAYNDPGD-APLPTQGEFAQQQ----SARRIV
CapU   CSEDRRLLVVTVHQAARDLRVVAIFMREVAAYQSTTAGRDLELSYSDFSHWQYEMLSRRGDA

190    200    210    220    230    240    250
TycC6  KQEAYWLQTFADDIPVNLNPTDFTRPSTQSFAGDQCTIGAGKALT---EGLHQLAQATGTTLY
SrfA-C ASLRYWREYLEGFEGQTTFAEQ-RKKQKDGYPEKLELFSPEAET---KAFTELAKSQHTTLS
VibH   TSHRYWQDRQFWQGYLREAPDLTSLATYDPLSHAVSLSTLNSQLNHLLKLANANQIGWFL
CapV   RAKEAALTIRSRYPKALDSPAD-TRPRPHVYDLGGIMLRWIGIVES---GRLAARAGEEGLTLY
CapU   LPKWWSANQYSGVSLPPDRPPDRGDDGPLRGLTSARICADVASSGVTSALAEVGRAVGVVVP

260    270    280    290    300    310    313
TycC6  MVLLAAAYN-VLLAKYAGQEDIIVGTPITGESHAD--LEPIVGMFVNTLAMRNKPKQR
SrfA-C TALQAVWS-VLISRYQQSGDLAFGTVVSGRPAEIKGVEHMGVLFINVVPPRVKLS
VibH   DALVALCALYLES--AEPDAPWLLPFFMNRWGSV--AANVPLMNVSLPLRLRFAQQTS
CapV   MVLLAAAYR.SALEGKGLLSPDAPVWSPMSGRVSSC--FSRSVGLFMMILVVPVFGSIARAPEGE
CapU   TVVFGLLA-LLVSRWNQDEVTIGWTGDRPGEQ--FGDVIIGPFSNVLVPSISIDPAAEVP

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Figure S4. Amino acid sequence alignment of select condensation domains of nonribosomal peptide synthetases. The alignment includes TycC6 involved in tyrocidine biosynthesis from *Brevibacillus brevis* (amino acids 5206-5496 of accession no. O30409); SrfA-C involved in surfactin biosynthesis from *Bacillus subtilis* (amino acids 9-309 of accession no. CAA51224); VibH involved in vibriobactin biosynthesis from *Vibrio cholera* (amino acids 1-290 of accession no. AAD48879); CapV (amino acids 1-281 of accession no. BAJ19067); and CapU (amino acids 55-357 of accession no. BAJ19067).

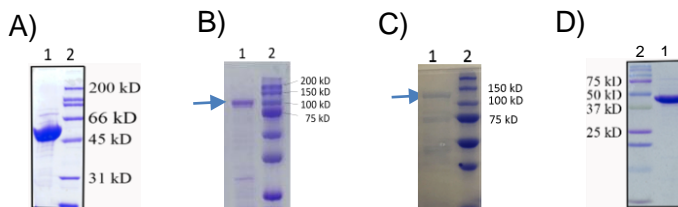


Figure S5. Recombinant CapV, CapU, CapU_AT, and CapW. (A) SDS-PAGE analysis of partially purified His₆-CapV (lane 1, expected MW of 48.2 kD); (B) SDS-PAGE analysis of partially purified His₆-CapU (lane 1, expected MW of 114.4 kD) expressed from pET30Xa in *E. coli* BL21(DE3). In both cases the engineered N-terminal His₆-tag contributes ~5 kD to the predicted native molecular weight. (C) SDS-PAGE analysis of partially purified MBP-CapU_AT (lane 1, expected MW of 125.1 kD) expressed from Pdb.His.MBP in *E. coli* BL21(DE3). The engineered N-terminal MBP-tag contributes ~42.5 kD to the predicted native molecular weight. (D) SDS-PAGE analysis of partially purified His₆-CapW (lane 1) expressed from pXY200 in *S. lividans* TK24 (expected MW of ~44 kD; the engineered N-terminal His₆-tag contributes ~1 kD to the predicted native molecular weight).

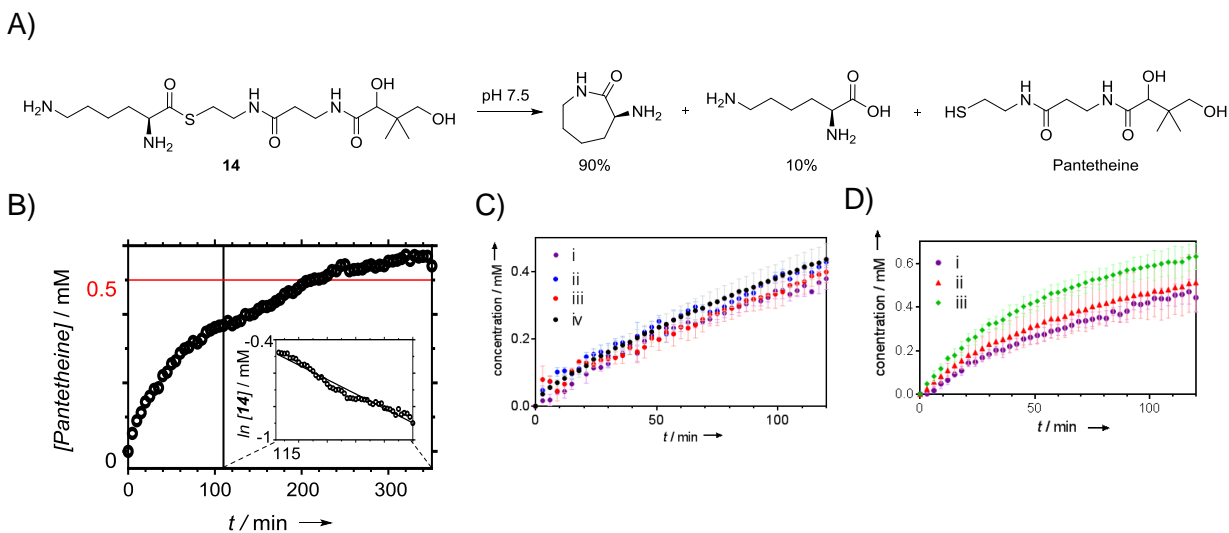


Figure S6. Nonenzymatic lactamization/hydrolysis of **14**. (A) Structure of the surrogate substrate **14** and the nonenzymatic formation of L-ACL, L-Lys, and pantetheine. (B) Time dependence of the nonenzymatic reaction of **14**. The inset depicts a secondary plot for later time points during the reaction ($[14] < 0.65$ mM), which displays a linear relationship that is consistent with a pseudo-first order reaction. (C) Nonenzymatic reaction of **14** detected by sulfhydryl formation including i) control reaction (no enzyme); ii) CapU; iii) CapU and N-terminal His-tagged CapV; and iv) CapU and C-terminal His-tagged CapV. (D) Nonenzymatic reaction of **14** detected by sulfhydryl formation including i) control (no CapW); ii) CapW; and iii) CapW and **12**.

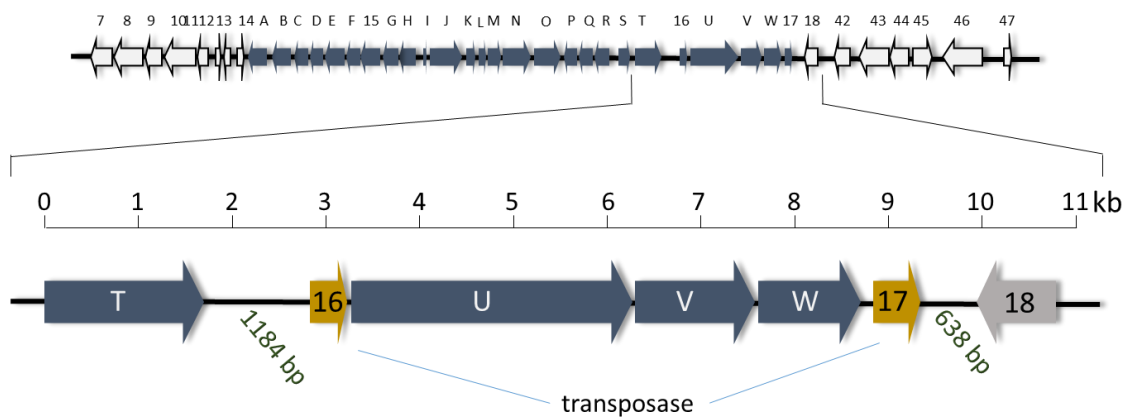


Figure S7. Organization of the genetic locus encompassing the **2** biosynthetic gene cluster. A close-up view of the region between *capT* and *orf18* demonstrating that the amide bond forming catalysts (*capU* and *capW*) are flanked by two *orfs* encoding putative transposases and long intergenic regions.

4. Supporting References

[S1] M. Funabashi, K. Nonaka, C. Yada, T. Suzuki, T. Suzuki, Y. Ogawa, M. Hosobuchi, Y. Fujita, M. Kizuka, T. Shibata, *Actinomycetologica* **2009**, *23*, 46-50.