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 *Ase*I 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] (μg/mL)	[FMOC-ACL] (μg/mL)	Sample	[L-Lys] (μg/mL)	[FMOC-ACL] (μg/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

[a]Abbreviations are LM, liquid media; FMOC-ACL, L-α-amino-ε-caprolactam derivatized with FMOC.

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

	Reaction[a]									
Variable	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

[a]All 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.
[b]Corresponds to Fig. S6B, trace *b.*
[c]Corresponds to Fig. S6B, trace *c.*
[d]Corresponds to Fig. S6B, trace *d.*
[e]Based 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]
capA	924	0.521	0.464	77.6
capB	1671	0.597	0.473	84.0
capC	951	0.646	0.491	88.3
capD	975	0.779	0.650	92.9
capE	1404	0.690	0.598	90.4
capF	951	0.592	0.469	85.2
orf15	1200	0.697	0.533	88.3
capG	1158	0.717	0.594	89.9
capH	1239	0.654	0.548	87.4
capI	264	0.529	0.478	77.3
capJ	2286	0.730	0.601	91.2
capK	738	0.595	0.476	82.9
capL	507	0.644	0.508	85.8
capM	870	0.709	0.549	89.7
capN	2034	0.649	0.502	87.9
capO	1839	0.717	0.524	91.0
capP	915	0.503	0.411	79.0
capS	780	0.559	0.442	81.2
capT	1890	0.655	0.535	87.0
orf16	402	0.487	0.336	79.1
capU	1410	0.495	0.337	77.3
capV	3312	0.401	0.414	69.1
capW	1209	0.617	0.544	86.4
orf17	546	0.635	0.527	85.2

[a]Genes 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**.
[b]Referenced against the codon usage table for *Streptomyces griseus* sups. griseus.
[c]Referenced against the glucokinase gene from *Streptomyces griseus* sups. griseus (Genbank accession no. AP009493).
[d]G+C content at the third position of the codon.

3. Supporting Figures

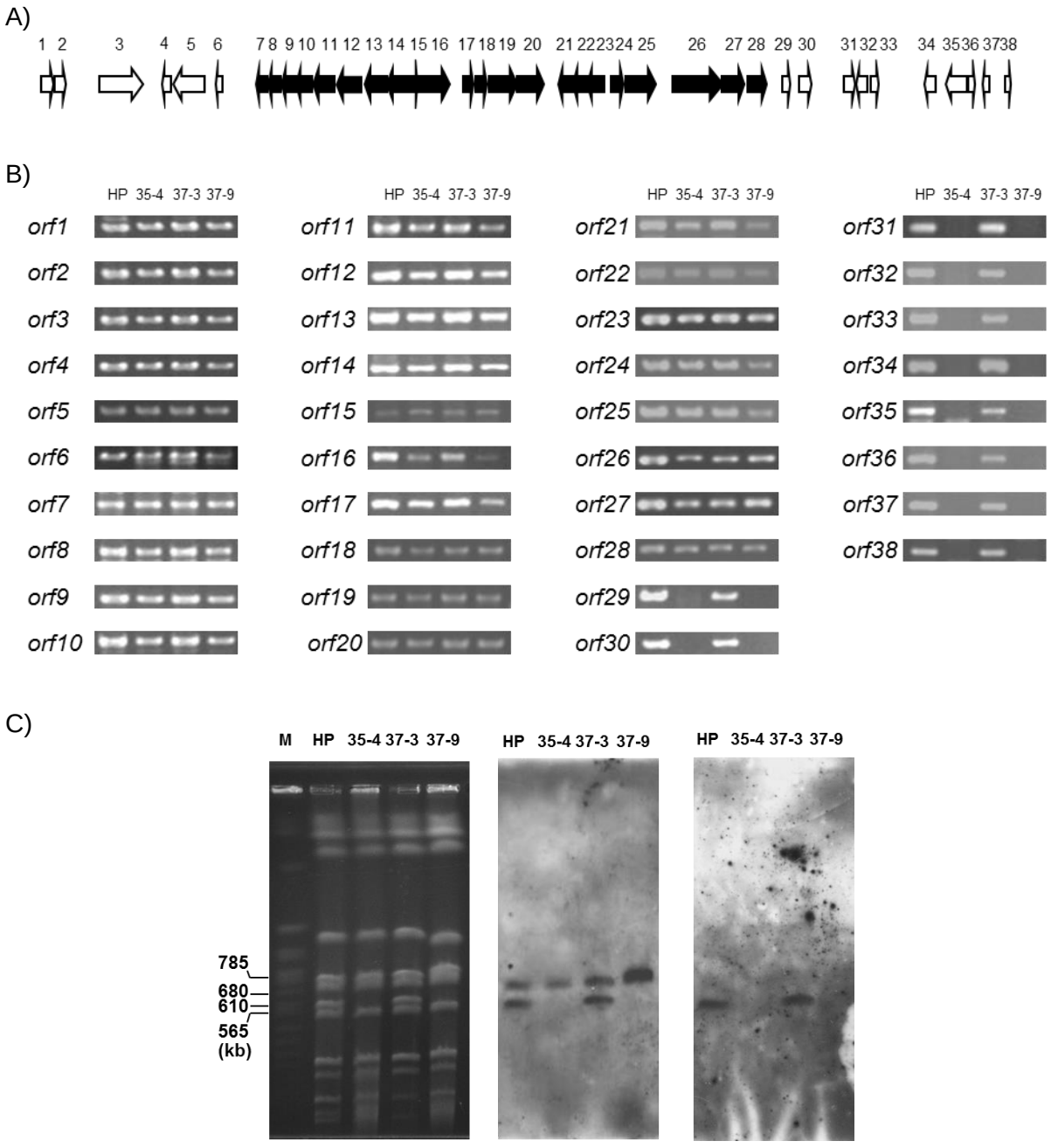

Figure S1. Analysis of the **1** biosynthetic gene cluster. A) Genetic architecture of the open reading frames (*orfs*) from the sequenced region encompassing 65-kb DNA. Filled *orfs* (7-28) are highly expressed during the onset of **1** production and hence are probably required for biosynthesis. B) 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).

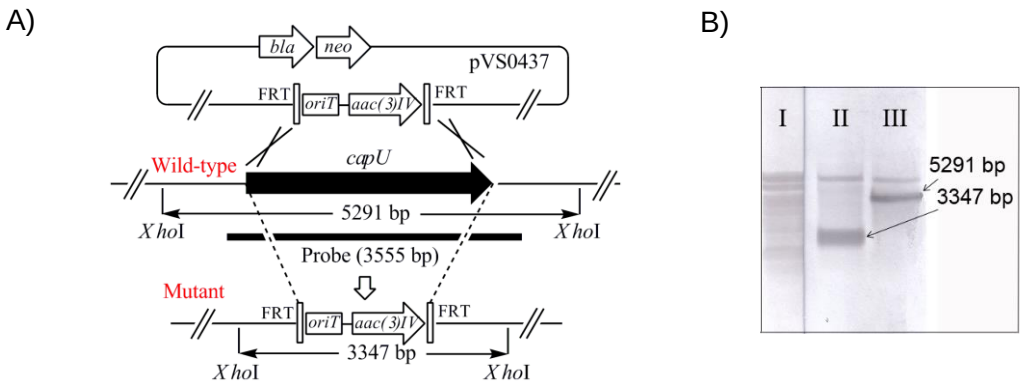

Figure S2. Development of a genetic system within the producing strain of **2**. A) Strategy to prepare an in-frame deletion of *capU*. B) Southern blot analysis of genomic DNA isolated from the *ΔcapU* mutant strain (II) or wild-type strain (III) and digested with *XhoI*. Lane I, DNA marker.

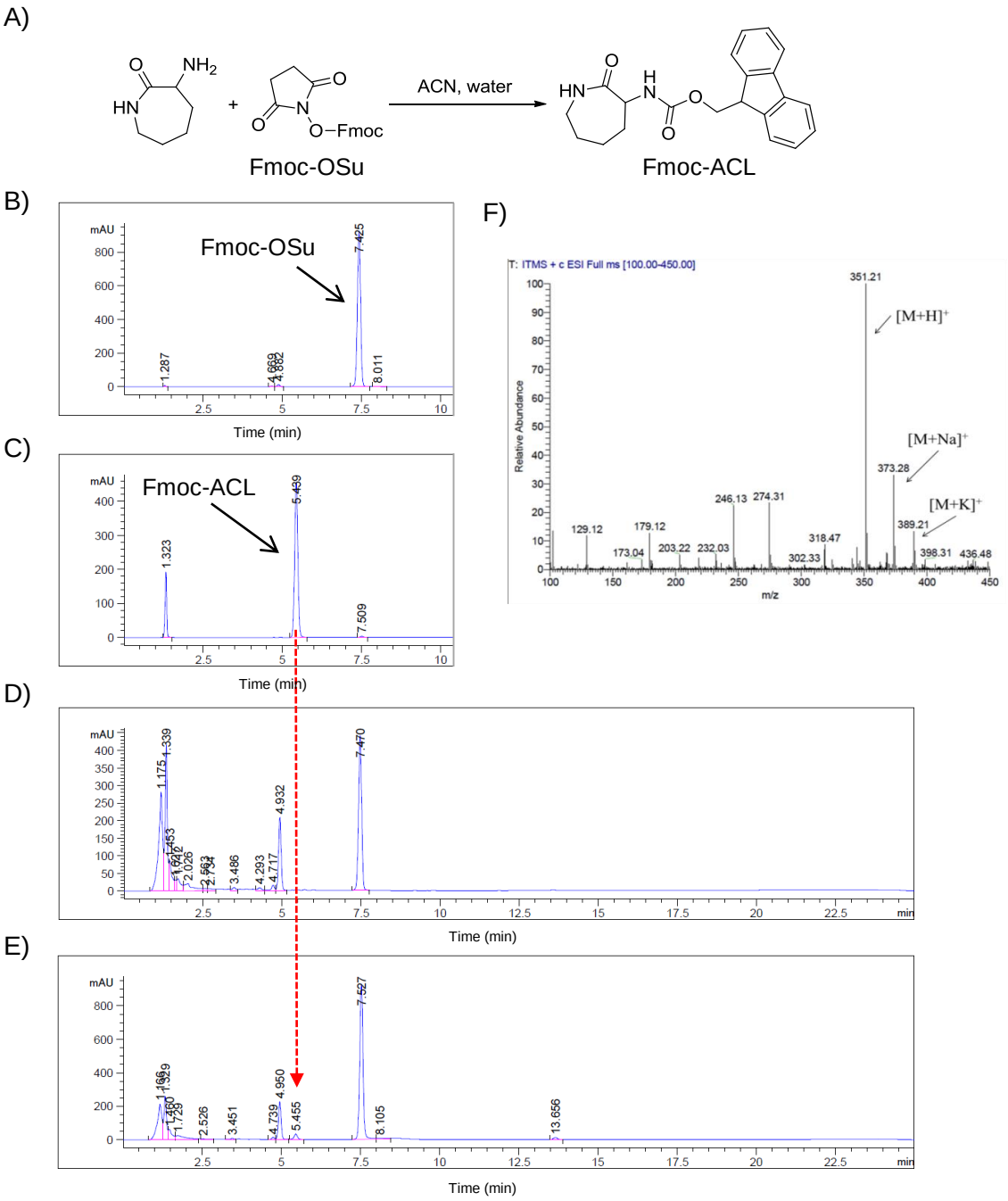

Figure S3. Biotransformation of L-Lys upon heterologous expression of *capV* and *capU*. (A) Modification of ACL with Fmoc. Fmoc-OSu, Fmoc *N*-hydroxysuccinimide ester. HPLC analysis of Fmoc-ACL production including (B) Fmoc-OSu control, (C) L-ACL standard modified by Fmoc-OSu, (D) Fmoc-OSu modified 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 $C_{21}H_{22}N_2O_3$).

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).

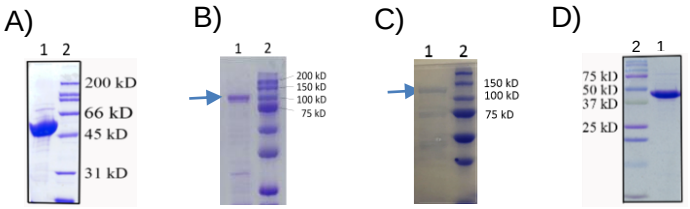

Figure S5. Recombinant CapV, CapU, CapU_AT, and CapW. (A) SDS-PAGE analysis of partially purified His_6-CapV (lane 1, expected MW of 48.2 kD); (B) SDS-PAGE analysis of partially purified His_6-CapU (lane 1, expected MW of 114.4 kD) expressed from pET30Xa in *E. coli* BL21(DE3). In both cases the engineered N-terminal His_6-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 His_6-CapW (lane 1) expressed from pXY200 in *S. lividans* TK24 (expected MW of ~44 kD; the engineered N-terminal His_6-tag contributes ~1 kD to the predicted native molecular weight).

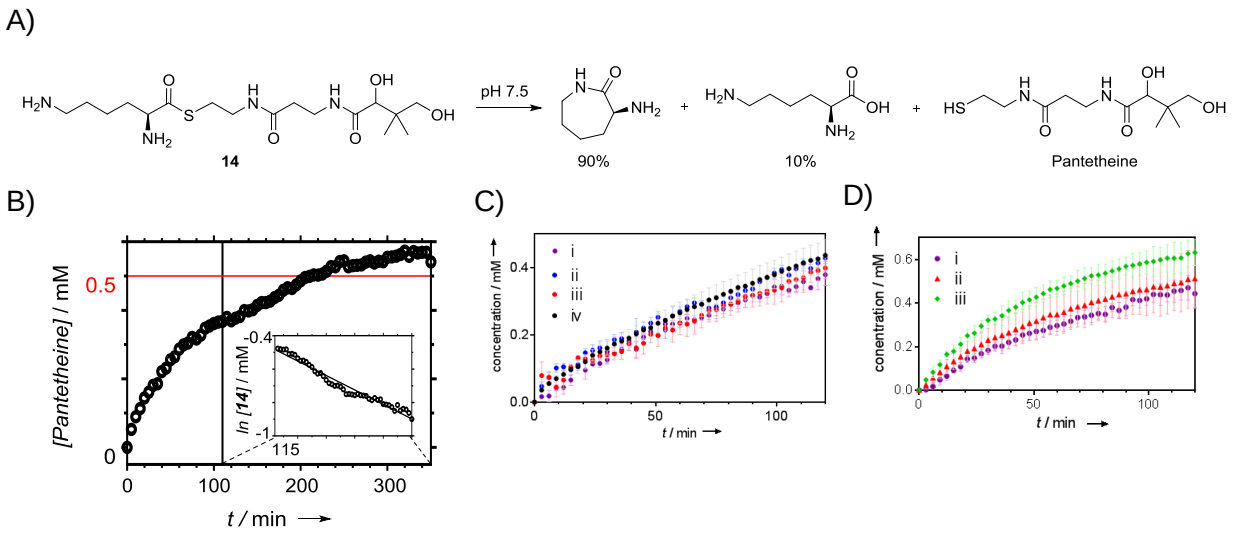

Figure S6. Nonenzymatic lactamization/hydrolysis of **14**. (A) Structure of the surrogate substrate **14** and the nonezymatic formation of L-ACL, L-Lys, and pantetheine. (B) Time dependence of the nonenzymatic reaction of **14**. The inset depicts a secondary plot for later time points during the reaction ([**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**.

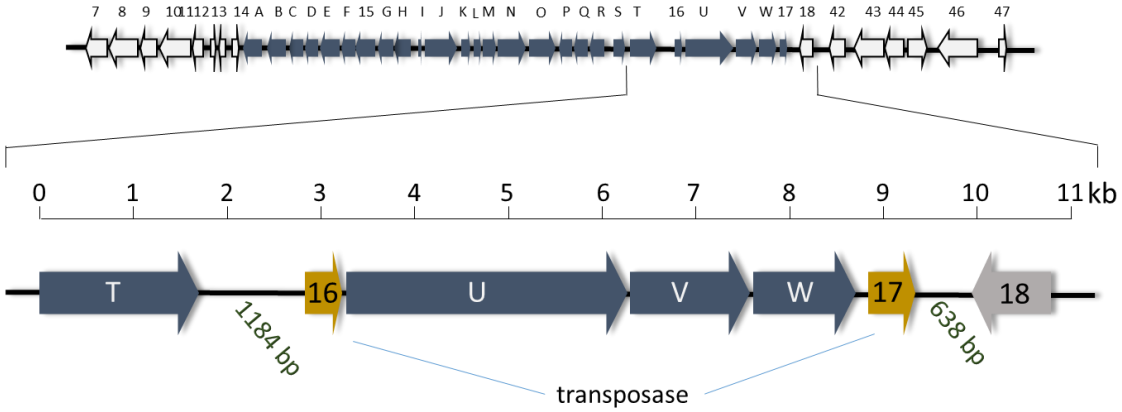

Figure S7. Organization of the genetic locus encompassing the **2** biosynthetic gene cluster. A close-up view of the region between *capT* and *orf18* demonstrating that the amide bond forming catalysts (*capU* and *capW*) are flanked by two *orf*s encoding putative transposases and long intergenic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