Supplementary Information

Gene Deletion Leads to Improved Valinomycin Production by *Streptomyces* sp. CBMAI 2042

Bruno S. Paulo,[a] Renata Sigrist,[a] Célio F. A. Angolini,[b] Marcos N. Eberlin[c] and Luciana G. de Oliveira *,[a]

[a]*Instituto de Química, Universidade Estadual de Campinas, CP 6154, 13083-970 Campinas-SP, Brazil*

[b]*Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, 09210-580 Santo André-SP, Brazil*

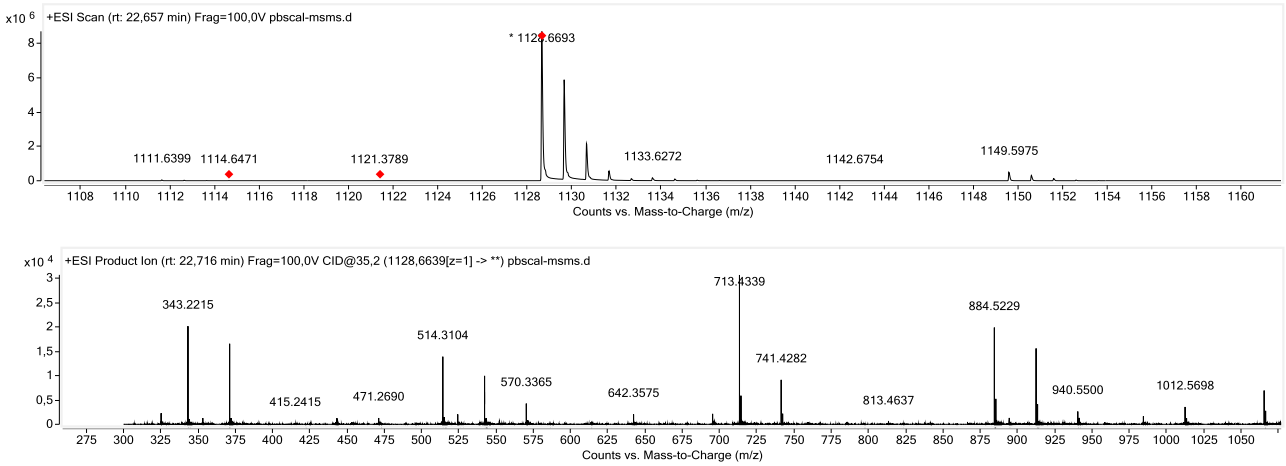
[c]*Universidade Presbiteriana Mackenzie, 01301-100 São Paulo-SP, Brazil*

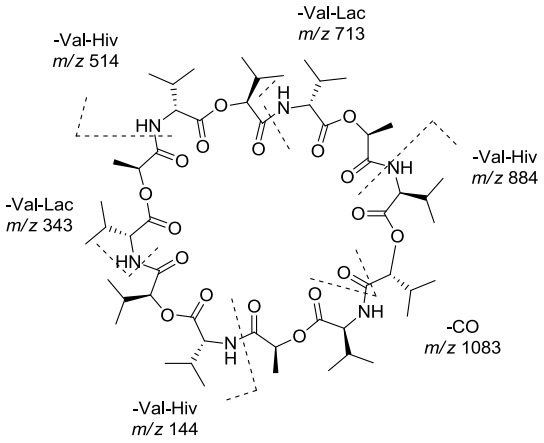
*e-mail: luciana@iqm.unicamp.br

Table S1. antiSmash output of the biosynthetic gene clusters from *Streptomyces* sp. CBMAI 2042

Cluster	Structural type	Predicted product	Similarity to characterized biosynthetic gene clusters / %	MIBiG BGC-ID
1	butyrolactone	coelimycin	coelimycin gene cluster (12)	BGC0000038_c1
2	terpene	–	–	–
3	NRPS-transAT-T1-PKS	griseobactin	griseobactin gene cluster (94)	BGC0000368_c2
4	NRPS	coelichelin	coelichelin gene cluster (81)	BGC0000325_c2
5	T1-PKS-NRPS	arsenopolyketides	arsenopolyketides gene cluster (50)	BGC0001283_c2
6	T3-PKS-NRPS	herboxidiene	herboxidiene gene cluster (6)	BGC0001065_c1
7	–	–	roseoflavin gene cluster (100)	BGC0000927_c1
8	terpene		steffimycin gene cluster (19)	BGC0000273_c1
9	ectoine		ectoine gene cluster (100)	BGC0000853_c1
10	lantipeptide	–	–	–
11	siderophore	desferrioxamine B	deferrioxamine gene cluster (100)	BGC0000941_c1
12	–	indigoidine	indigoidine gene cluster (84)	BGC0000469_c1
13	thiopeptide	–	–	–
14	melanin	–	grixazone gene cluster (76)	BGC0000662_c1
15	T1-PKS	–	coelymicin P1 gene cluster (25)	BGC0000038_c1
16	lantipeptide	–	–	–
17	lassopeptide	–	SRO15-2005 gene cluster (100)	BGC0000578_c1
18	bacteriocin	–	–	–
19	lantipeptide	–	amfs gene cluster (100)	BGC0000496_c1
20	terpene	–	–	–
21	siderophore	–	–	–
22	bacteriocin	–	–	–
23	T3-PKS-NRPS	feglymycin	feglymycin gene cluster	BGC0001233_c1
24	butyrolactone	–	kanamycin gene cluster (5)	BGC0000705_c1
25	lantipeptide	–	SF2575 gene cluster (4)	BGC0000269_c1
26	terpene	–	hopene gene cluster (69)	BGC0000663_c1
27	T1-PKS-NRPS	–	SGR PTMs gene cluster (100)	BGC0001043_c1
28	bacteriocin	–	tetronasin gene cluster (3)	BGC0000163_c1
29	NRPS	–	WS9326 gene cluster	BGC0001297_c1
30	NRPS	valinomycin	valinomycin gene cluster (22)	BGC0000453_c1
31	melanin	–	melanin gene cluster (100)	BGC0000911_c1
32	T3-PKS	alkylresorcinol	alkylresorcinol gene cluster (100)	BGC0000282_c1
33	terpene	isorenieratene	isorenieratene gene cluster (100)	BGC0000664_c1
34	thiopeptide-T1-PKS-NRPS	lactazoles	lactazole gene cluster (33)	BGC0000606_c1
35	T2-PKS	–	rabelomycin gene cluster (52)	BGC0000262_c1

NRPS: nonribosomal peptide synthetases; PKS: polyketides.





valinomycin (**1**)

Figure S1. ESI (+)-MS/MS spectrum of valinomycin. The neutral loss represents the sequential loss of amino and hydroxyl acid backbone from valinomycin.

Table S2. High resolution ESI (+)-MS/MS data of valinomycin identified in *Streptomyces* sp. CBMAI 2042

Compound	*m/z* (experimental)	*m/z* (calcd.)	Error / ppm	Molecular formula
Valinomycin	1111.6399	1111.6390	0.8	$[C_{54}H_{90}N_6O_{18} + H]^+$
	1128.6693	1128.6655	3.4	$[C_{54}H_{90}N_6O_{18} + NH_4]^+$
	1133.6272	1133.6209	5.5	$[C_{54}H_{90}N_6O_{18} + Na]^+$
	1149.5975	1149.5949	2.3	$[C_{54}H_{90}N_6O_{18} + K]^+$

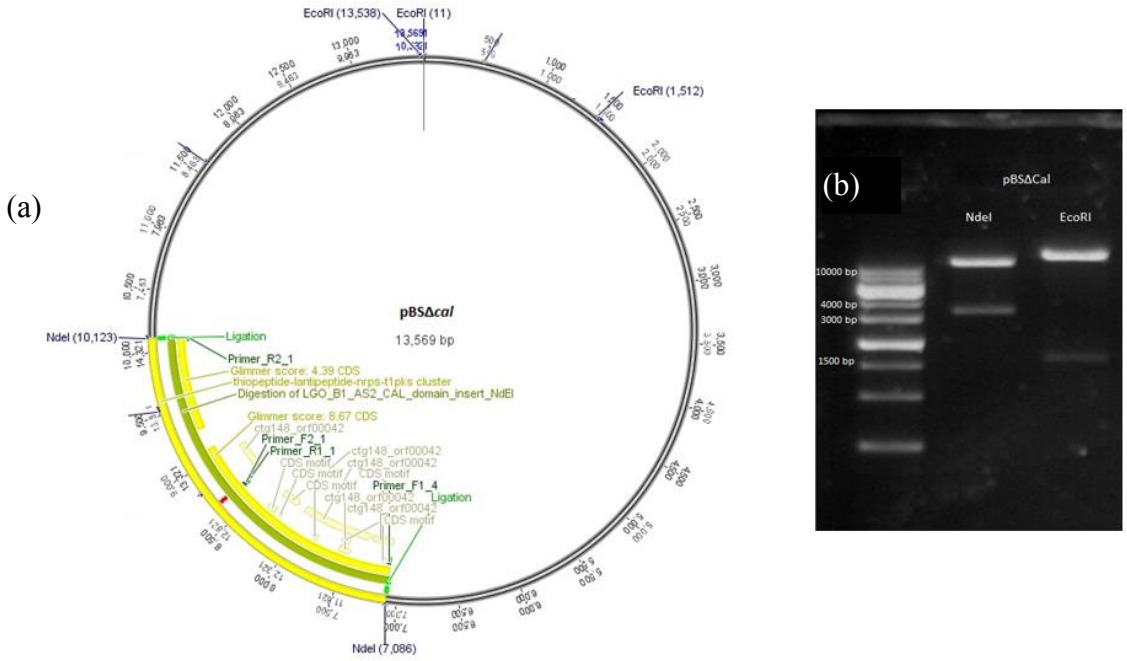


Figure S2. (a) Restriction map of pBSΔcal; (b) double restriction analysis with *NdeI* and *EcoRI*.

Table S3. Quantification of valinomycin production by wild type and *cal*$^-$ mutant in A liquid medium for 7 days

Streptomyces sp. CBMAI 2042	Sample	Concentration / (µg mL^{-1})	Average / (µg mL^{-1})
	n_1	0.357	
Wild type	n_2	0.372	0.491 ± 0.221
	n_3	0.746	
	n_1	4.631	
cal$^-$ mutant[a]	n_2	4.502	5.171 ± 1.048
	n_3	6.379	

[a]All points belonging to *cal*$^-$ mutants in A liquid medium were additionally diluted 2× to be within the limits of quantification curve.

Table S4. Quantification of valinomycin production by wild type and *cal*$^-$ mutant in GYM liquid medium for 7 days

Streptomyces sp. CBMAI 2042	Sample	Concentration / (µg mL^{-1})	Average / (µg mL^{-1})
	n_1	0.135	
Wild type	n_2	0.306	0.231 ± 0.087
	n_3	0.253	
	n_1	0.826	
cal$^-$ mutant	n_2	1.164	1.290 ± 0.538
	n_3	1.880	

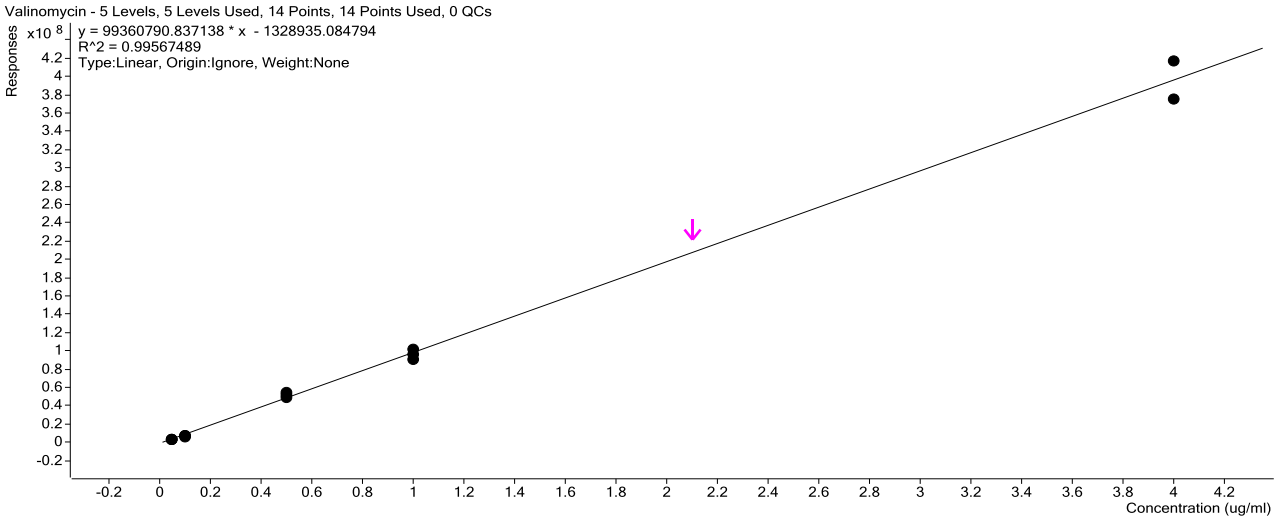


Figure S3. Calibration curve constructed to quantify valinomycin in *Streptomyces* sp. CBMAI 2042 extracts. The curve was evaluated in a range of 0.05-4.00 µg mL^{-1} showing a linear regression coefficient of 0.99.

Conjugation of *Streptomyces* sp. CBMAI 2042 with the deletion plasmid pBSΔcal

E. coli ET12567/pUZ8002 (methylase deficient) electrocompetent cells were transformed with pBSΔcal plasmid. This strain is used to avoid the defensive mechanism of *Streptomyces* strain, which identify the methylated DNA and degrade it. The non-conjugative plasmid pUZ8002 was used to perform the intergeneric transference of pBSΔcal plasmid.

The recombinant strain *E. coli* ET12567/pUZ8002/pBSΔcal was used to conjugate with *Streptomyces* sp. CBMAI 2042. The strains were mixed and streaked in a plate without antibiotics and after 16 h overlayed with 2 mL of a solution containing apramycin (25 µg mL^{-1}) and nalidixic acid (50 µg mL^{-1}) to select exconjugants. Exconjugants were transferred to another SFM-agar plate containing apramycin and nalidixic acid to confirm their resistance to apramycin and first crossover event. Those colonies which grew on antibiotic containing-plates were re-streaked on apramycin-free SFM plates to allow the second crossover. Two genotypes could appear, first it is recircularization of PYH7 plasmid without homologous recombination, resulting in wild type genotype. Second, it is deletion of targeted region via homologous recombination, resulting in mutant genotype. Single colonies were replicated on non-apramycin plates and apramycin plates, and those which present the apramycin$^-$ phenotype were inoculated in TSBY liquid medium for genomic DNA purification and polymerase chain reaction (PCR) confirmation.

Purified genomic DNA was subjected to PCR analysis with the pairs scree_pΔcal_F/R. The primers were designed in a way that different lengths of amplicons are amplified for the wild type and *NRPS*$^-$ mutant. The scheme in Figure S4 exemplifies this kind of screen.

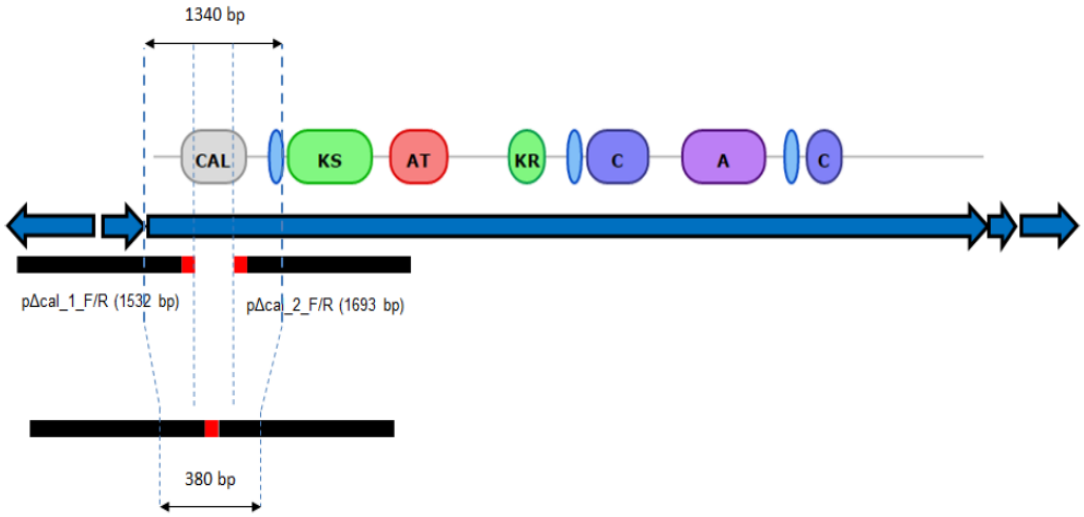


Figure S4. Schematic illustration of PCR screening of potential deletion mutants. In case of deletion, a band of 380 bp is generated indicating the presence of *NRPS*$^{-}$ mutant, whereas the wild type results a band of 1340 bp.

PCR screening of 7 potential mutants were performed (Figure S5) showing that in most cases the second crossover unfrequently occur, observed by presence of both bands in agarose gel indicating only the first crossover. However, some colonies passed through the second crossover event, the 1 and 7 colonies have been returned to the wild type profile, whereas the 2-colony showed exclusively the deleted profile. The DNA from *Streptomyces* sp. CBMAI 2042 was used as negative control indicating the complete construction of the adenylation domain and the plasmid pBSΔcal was used as positive control showing the *cal*$^{-}$ mutant profile.

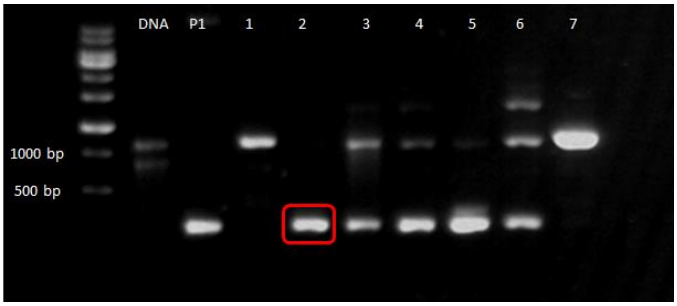


Figure S5. Electrophoresis agarose gel 0.8%. DNA corresponds to the wild type profile. P1 is the expected profile obtained after A domain deletion (pBSΔcal). Exconjugants screening. Lanes 1 and 7: wild type genotype (amplicon 1340 bp). Lanes 3, 4, 5 and 6: First crossover. Lane 2: *cal*$^{-}$ mutant profile and second crossover (amplicon 380 bp).