Additional file for:

Title: Rational engineering of *Streptomyces albus* J1074 for the overexpression of secondary metabolite gene clusters

Authors: Dimitris Kallifidas[1,2*], Guangde Jiang[1,2], Yousong Ding[1,2], Hendrik Luesch[1,2*]

[1] Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610, USA
[2] Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville, Florida 32610, USA
[*]Corresponding authors: dkallifidas@ufl.edu, luesch@cop.ufl.edu

Figure S1: Confirmation of upregulation of paulomycin gene cluster (*plm*) by CRP_{SC} overexpression and WblA_{SA} deletion by LC-MS analysis of organic extracts prepared from *Streptomyces albus* J1074 engineered strains grown in R5A media for 4 days. 1, paulomycin B; 2, paulomycin A; 3, paulomenol B; 4, paulomenol A.

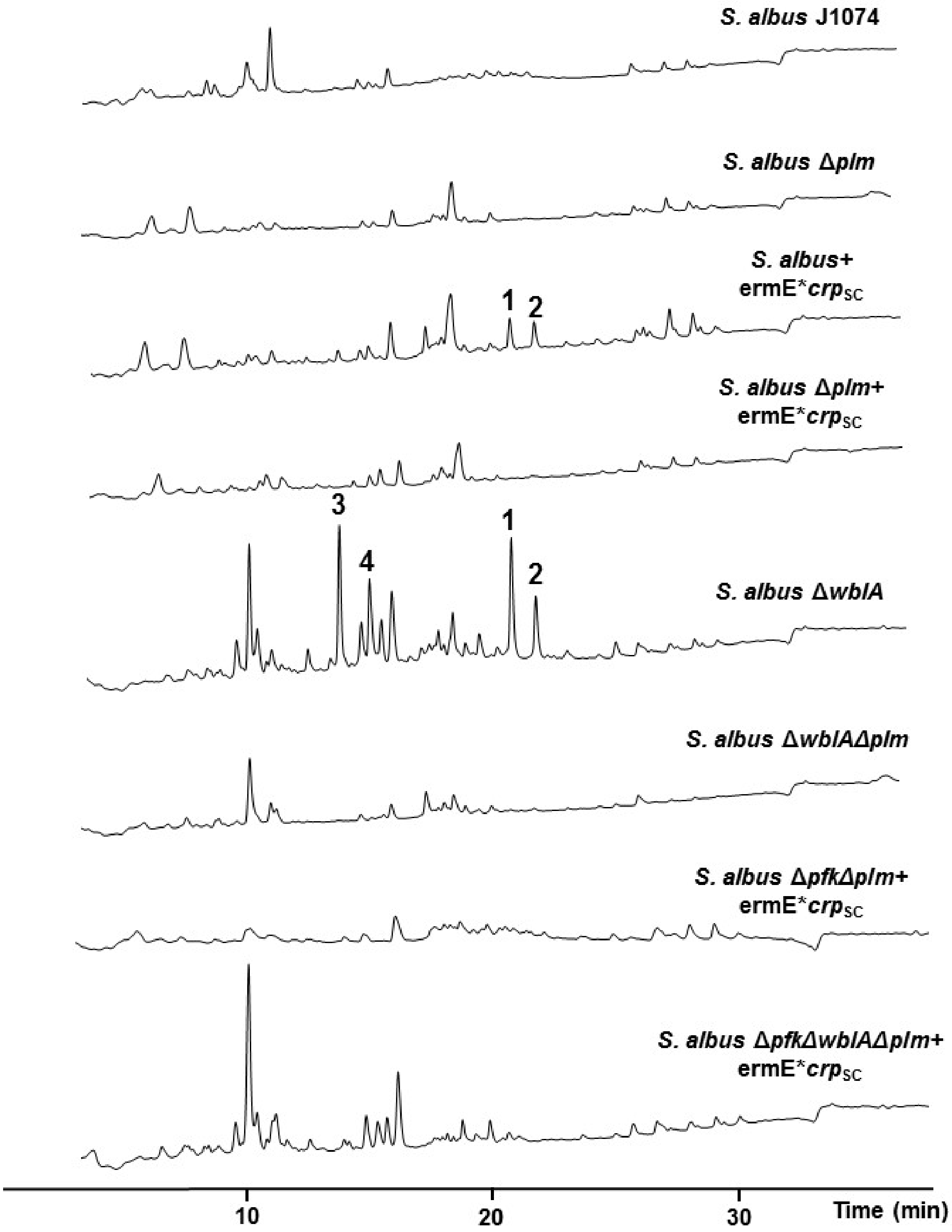

Figure S2: LC-MS analysis of *S. albus* Δ*wblA* culture extracts collected daily for 6 days from R5A fermentation broths. 1, paulomycin B; 2, paulomycin A; 3, paulomenol B; 4, paulomenol A.

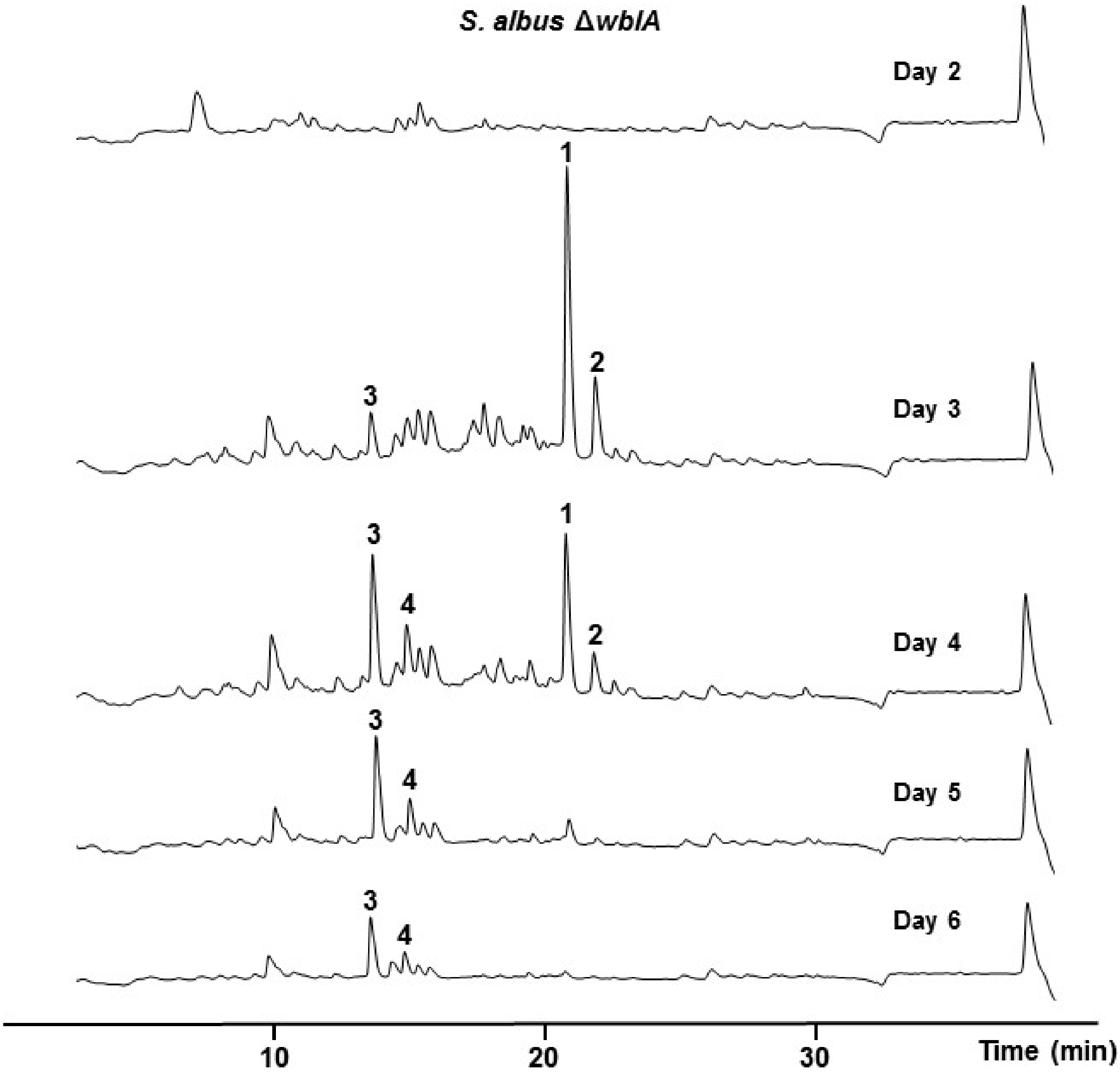

Figure S3: MS fragmentation pattern of paulomenol A. Negative mode.

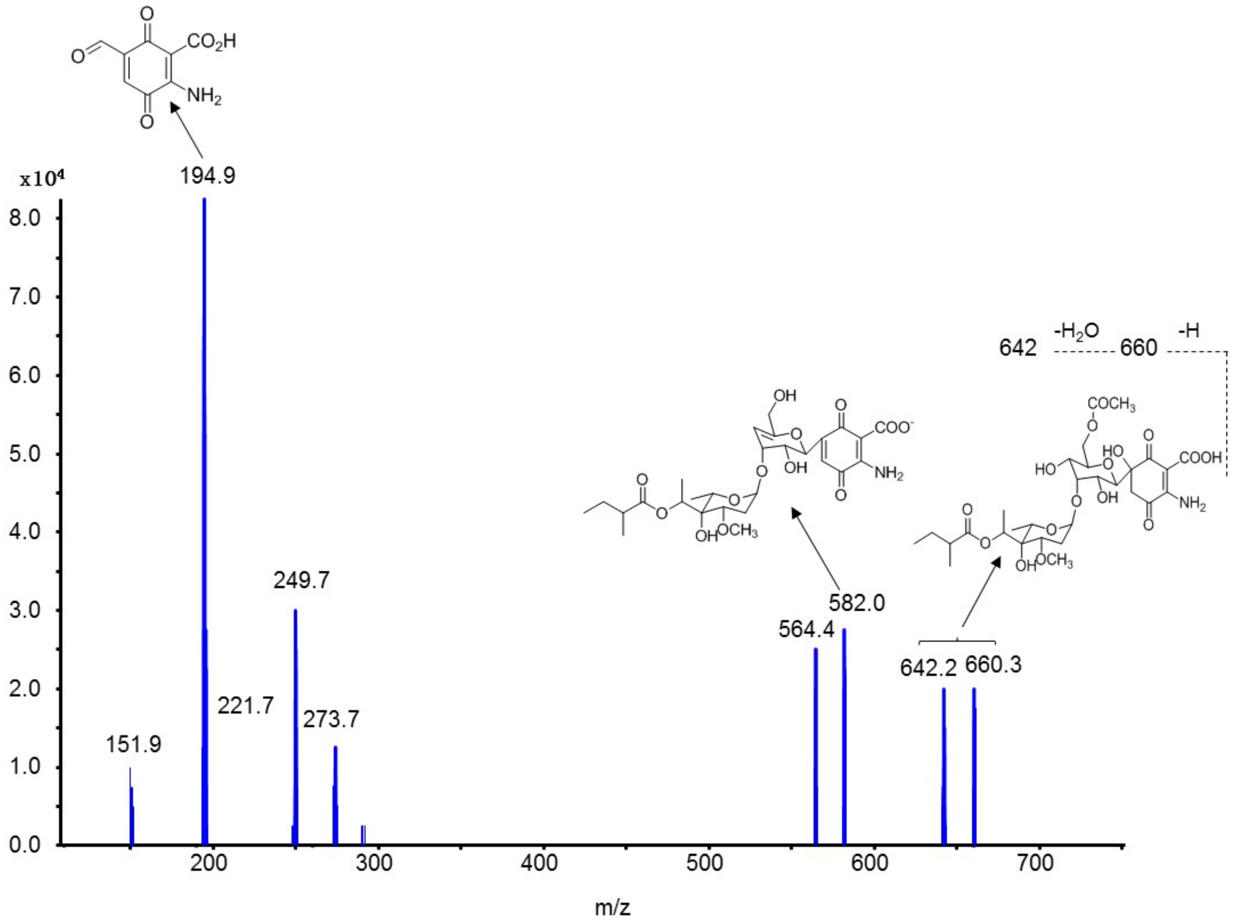

Figure S4: MS fragmentation pattern of paulomenol B. Negative mode.

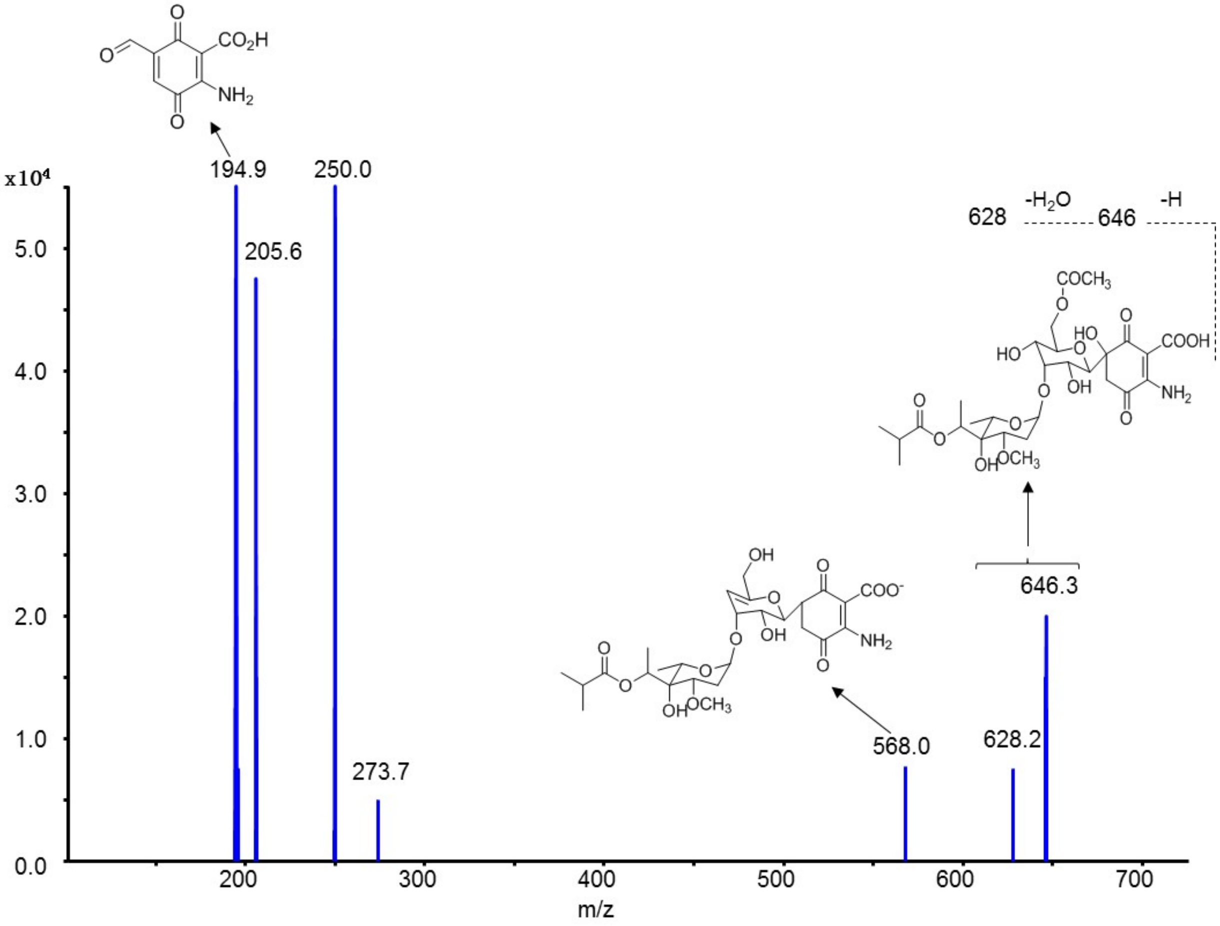

Figure S5: MS fragmentation pattern of paulomycin A. Negative mode.

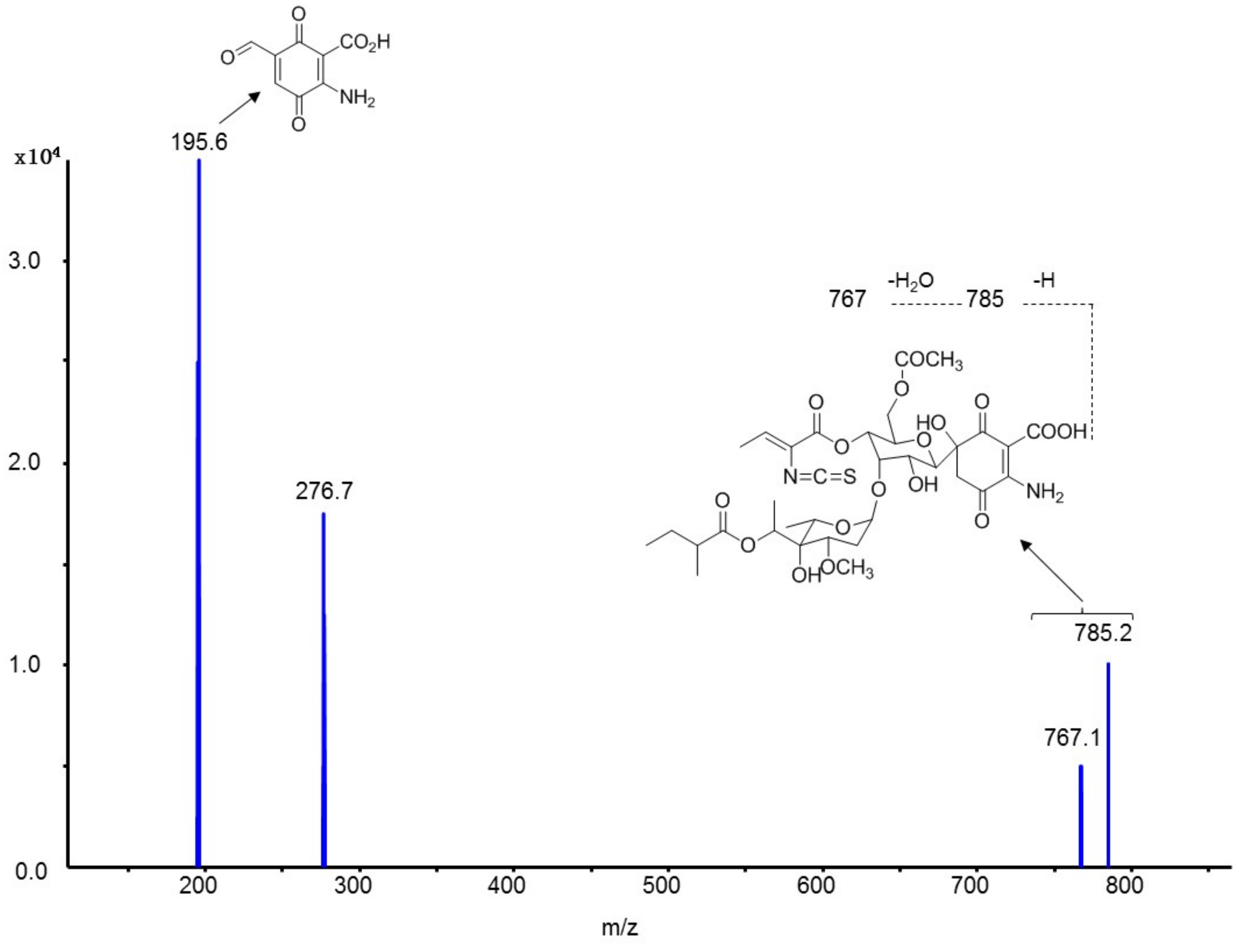

Figure S6: MS fragmentation pattern of paulomycin B. Negative mode.

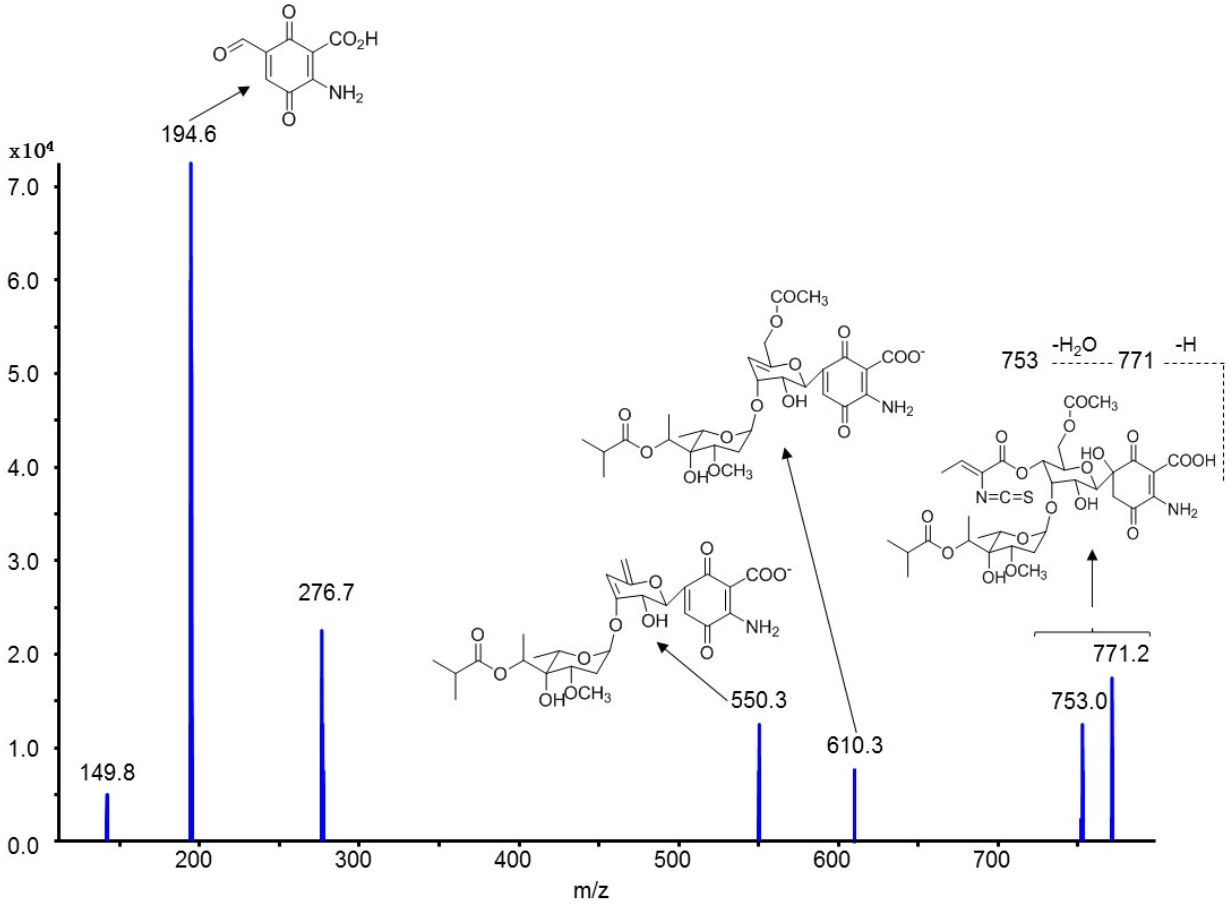

Table S1: Sequence scanning of paulomycin (*plm*) gene cluster for the detection of putative $\mathrm{CRP_{SC}}$ binding sites using the $\mathrm{GTG(N)_6GNCAC}$ motif. Distance from the beginning of the motif to the start codon of the corresponding genes is reported. Negative values indicate sites upstream of start codons while positive values indicate sites within open reading frames.

***plm* gene**	**Function**	**distance**	**Binding site**
plm 12	Glycosyltransferase	+130	cctcGTGGACACCGCCACcggc
plm 28	Putative sulfotransferase	+628	ggcgGTGGCCATGGCCACggag
plm 29	Aminotransferase	+91	cctgGTGCCGCTCGTCACcggc
plm 35	Ribulose-5-phosphate-4-epimerase	+29	acgcGTGAGCGAGGGCACcccg
plm 37	Acyl-CoA dehydrogenase	+763	cgcgGTGGGGCTCGCCACcgcg
plm 40	dTDP-4-keto-6-deoxy-L-hexose 2,3-reductase	+301	ccggGTGGTGCTGGCCACcaag

Table S2: Sequence scanning of paulomycin (*plm*) gene cluster for the detection of putative $\mathrm{CRP_{SC}}$ binding sites using the $\mathrm{GTG(N)_6GNGAN}$ motif. Distance from the beginning of the motif to the start codon of the corresponding genes is reported. Negative values indicate sites upstream of start codons while positive values indicate sites within open reading frames.

***plm* gene**	**Function**	**distance**	**Binding site**
plm 2	TetR-family transcriptional regulator	-5	ggggGTGCGATGCGGGACgcgc
plm 4	Oxidoreductase	+162	ttcgGTGGGGACGGGGACacgg
plm 6	EmrB/QacA subfamily transporter	+1230	cagcGTGCCCGCCGCGACcacg
plm 7	Elongation factor G1	+1620	gttcGTGAACAAGGTGACcggt
plm 8	Dehydrogenase E1 alpha subunit	-299	gggaGTGACCGACGCGACagcg
*plm*8	Dehydrogenase E1 alpha subunit	+900	gctgGTGGCGGAGGCGAGggac
plm 9	Dehydrogenase E1 beta subunit	+333	cggcGTGCCCGTGGTGACccgg
plm 9	Dehydrogenase E1 beta subunit	+507	ggtgGTGCTCATCGAGAAccgc
plm 9	Dehydrogenase E1 beta subunit	+825	cgcgGTGGTGGCCGAGAAcgta
plm 9	Dehydrogenase E1 beta subunit	+864	cccgGTGCGGCGGGTGACcctg
*plm*10	SARP-family transcriptional regulator	+153	ccagGTGCTCCCGGCGACaacg
plm 12	Glycosyltransferase	+672	ggacGTGGCCGGGGAGACgctc
plm 23	C-glycosyltransferase	+204	gggcGTGCCCCTGGTGAGgtcc
plm 28	Putative sulfotransferase	+390	cctcGTGGTGTGCGCGAGcgag
plm 32	Acyl-CoA synthase	+1221	cgtgGTGCTCGAAGTGACcgac
plm 42	dTDP-4-keto-6-deoxyhexose 3,5-epimerase	-9	tgaaGTGGGGAGAGTGAGcccc