

Eliciting the silent lucensomycin biosynthetic pathway in *Streptomyces cyanogenus* S136 via manipulation of the global regulatory gene *adpA*

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Non-cropped versions of agarose gels from main text Fig. 4

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Supplementary Materials and Methods

Media used in this work

MS, SMMS - Kieser et al. 2000

ISP2 (g/L):

yeast extract – 4;
malt extract (Difco) – 10;
dextrose – 4;
agar – 20;
pH 7.2.

ISP3 (g/L):

finely ground whole oats (tolokno; Kozub®, Ukraine) – 30;
agar – 18;
tapped water – to 1L, pH 8.0 prior to autoclaving (with NaOH).

ISP4 (g/L):

Soluble starch – 10;
CaCO₃ – 2;
K₂HPO₄ (anhydrous) – 1;
MgSO₄ × 7 H₂O – 1;
NaCl – 1;
(NH₄)₂SO₄ – 2;
FeSO₄ × 7 H₂O – 0.001;
MnCl₂ × 4 H₂O – 0.001;
ZnSO₄ × 7 H₂O – 0.001;
Agar – 20;
pH 7.0 – 7.4.

ISP5 (g/L):

L-Asparagine – 1;
K₂HPO₄ – 1;
glycerol – 10;
FeSO₄ × 7 H₂O – 0.001;
MnCl₂ × 4 H₂O – 0.001;
ZnSO₄ × 7 H₂O – 0.001;
Agar – 20;
pH 7.0.

TSB from Himedia (g/L):

pancreatic digest of casein – 17;
papaic digest of soybean meal – 3;
NaCl – 5;
K₂HPO₄ – 2.5;
glucose – 2.5.

SG (g/L):

glucose – 20;
soytone (Difco) – 10;
CaCO₃ – 2;
CoCl₂ – 0.001;
pH 7.2.

YMPG (g/L):

yeast extract – 4;
bacto peptone – 1;
malt extract – 10;
glucose – 10;
MgCl₂ × 6 H₂O – 2;
pH 7.0.
Add 20 g of agar to obtain solid YMPG.

Bennet's agar (g/L):

glucose – 10;
bacto peptone – 1;
yeast extract – 1;
tryptone – 2;
agar – 20;
pH 7.2.

MYM (g/L):

yeast extract – 4;
malt extract – 10;
maltose – 4;
agar – 20;
pH 7.2.

Table S1. Strains and plasmids used in the work

Name	Description (Am ^r , Sp ^r , Ap ^r are marker genes for apramycin, spectinomycin and ampicillin resistance respectively)	Source or reference
<i>E. coli</i> DH5α	General cloning host	MBI Fermentas
<i>E. coli</i> ET12567 (pUZ8002)	(<i>dam-13::Tn9 dcm-6</i>), pUZ8002* (<i>ΔoriT</i>), used for conjugative transfer of DNA	Kieser et al., 2000
<i>D. hansenii</i> VKM Y-9	<i>Saccharomycetales</i> yeasts, test culture	VKM
<i>B. cereus</i> ATCC19637	Gram-positive bacterium, test culture	ATCC
<i>S. cyanogenus</i> S136	Wild type, producer of landomycin A	DSMZ
<i>S. cyanogenus</i> pGM4181 ⁺	<i>S. cyanogenus</i> S136 derivative carrying pGM4181	Yushchuk et al., 2018
<i>S. cyanogenus</i> ΔlanI7	<i>S. cyanogenus</i> S136 with the knockout of pathway-specific regulatory gene <i>lanI</i>	Rebets et al., 2008
<i>S. cyanogenus</i> ΔlanI7 pGM4181 ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGM4181	Yushchuk et al., 2018
<i>S. cyanogenus</i> ΔlanI7 pGM4181d ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGM4181d	This work
<i>S. cyanogenus</i> ΔlanI7 pGM4181i ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGM4181i	This work
<i>S. cyanogenus</i> ΔlanI7 pGM4181 _{ttta} ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGM4181 _{ttta} -	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCY	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCY	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCYd	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCYd	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCLA	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCLA	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCLA _d	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCLA _d	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCO	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCO	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSCOd	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSCOd	This work
<i>S. cyanogenus</i> ΔlanI7 pOOb95d	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pOOb95d	This work
<i>S. cyanogenus</i> ΔlanI7 pGMSGHd	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pGMSGHd	This work
<i>S. cyanogenus</i> ΔlanI7 <i>lcmRI</i> ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pTES22	This work
<i>S. cyanogenus</i> ΔlanI7 <i>lcmRII</i> ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pTES23	This work
<i>S. cyanogenus</i> ΔlanI7 <i>lcmRIII</i> ⁺	<i>S. cyanogenus</i> ΔlanI7 derivative carrying pTES25	This work
pTES	φC31-based integrative expression vector containing <i>ermEp</i> (Am ^r)	Herrman et al., 2012
pmoeE5script	pGUS derivative containing moeE5p cloned upstream of <i>gusA</i> (Am ^r Sp ^r)*	Makitrynsky et al. 2013
pGM4181	pmoeE5script with <i>XNR_4181</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pGM4181d	pmoeE5script with <i>XNR_4181dbd</i> cloned instead of <i>gusA</i>	This work
pGMSCLA	pmoeE5script with <i>adpA_{scf}</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pGMSCLA _d	pmoeE5script with <i>adpA_{scf}dbd</i> cloned instead of <i>gusA</i>	This work
pGMSCO	pmoeE5script with <i>adpA_{scf}</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pGMSCOd	pmoeE5script with <i>adpA_{scf}dbd</i> cloned instead of <i>gusA</i>	This work
pOOb95d	pmoeE5script with <i>adpA_{gh}</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pGMSGHd	pmoeE5script with <i>adpA_{gh}dbd</i> cloned instead of <i>gusA</i>	This work
pGMSCY	pmoeE5script with <i>adpA_{scf}</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pGMSCYd	pmoeE5script with <i>adpA_{scf}dbd</i> cloned instead of <i>gusA</i>	Yushchuk et al. 2018
pTES22	pTES derivative carrying <i>lcmRI</i> under the control of <i>ermEp</i>	This work
pTES23	pTES derivative carrying <i>lcmRII</i> under the control of <i>ermEp</i>	This work
pTES25	pTES derivative carrying <i>lcmRIII</i> under the control of <i>ermEp</i>	This work
pUCXNR	pUC19 carrying the synthetic <i>XNR_4181_{ttta}</i> , Ap ^r	Koshla et al. 2019
pGM4181i	pmoeE5script with <i>XNR_4181i</i> cloned instead of <i>gusA</i>	This work
pGM4181 _{ttta} -	pmoeE5script with <i>XNR_4181_{ttta}</i> - cloned instead of <i>gusA</i>	This work

Supplementary Figures and Tables

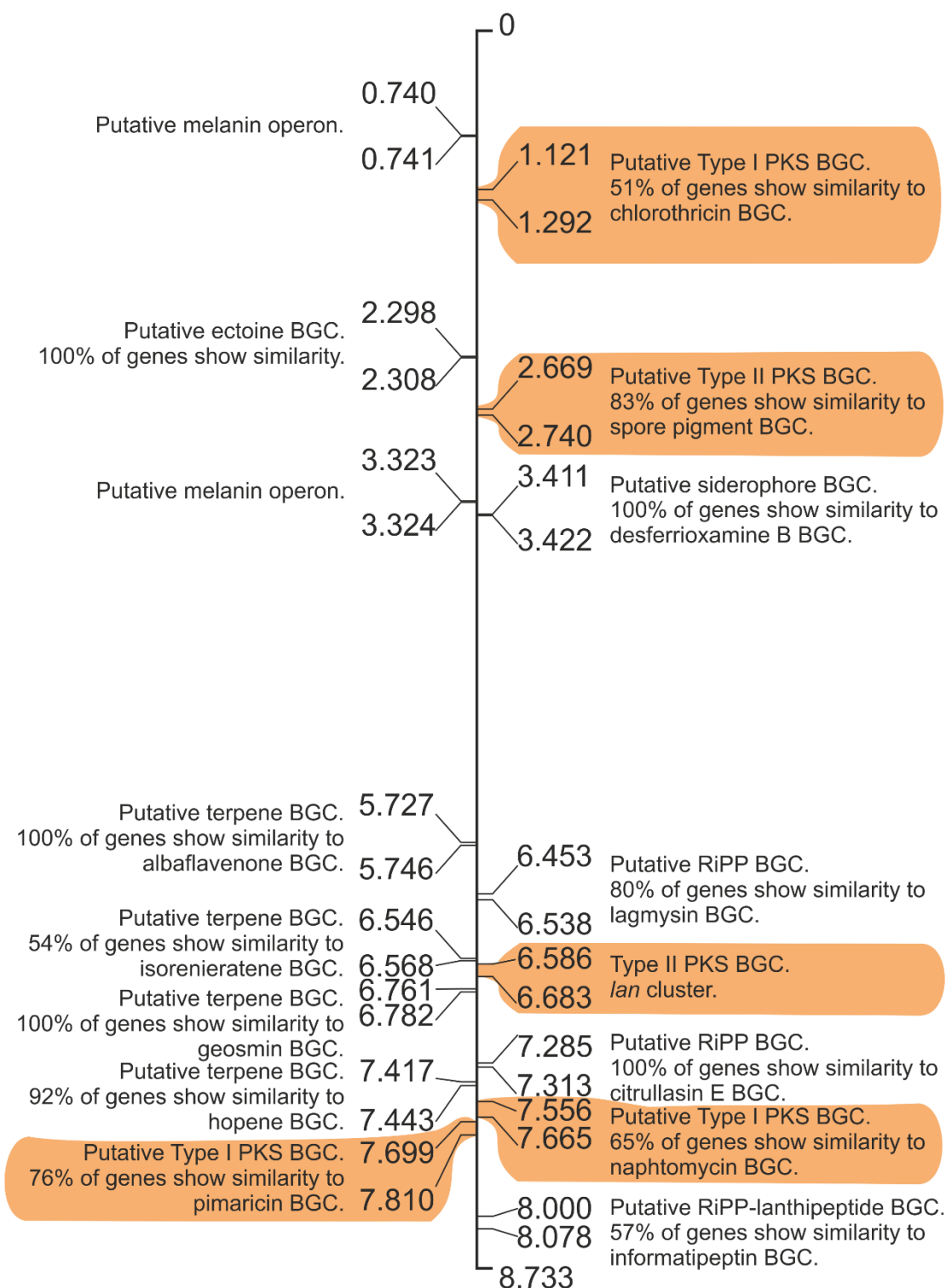


Fig. S1. Schematic representation of secondary metabolite biosynthetic gene clusters (BGCs) revealed on the chromosome of *S. cyanogenus* S136 using antiSMASH. The polyketide BGCs are highlighted (light-brown background).

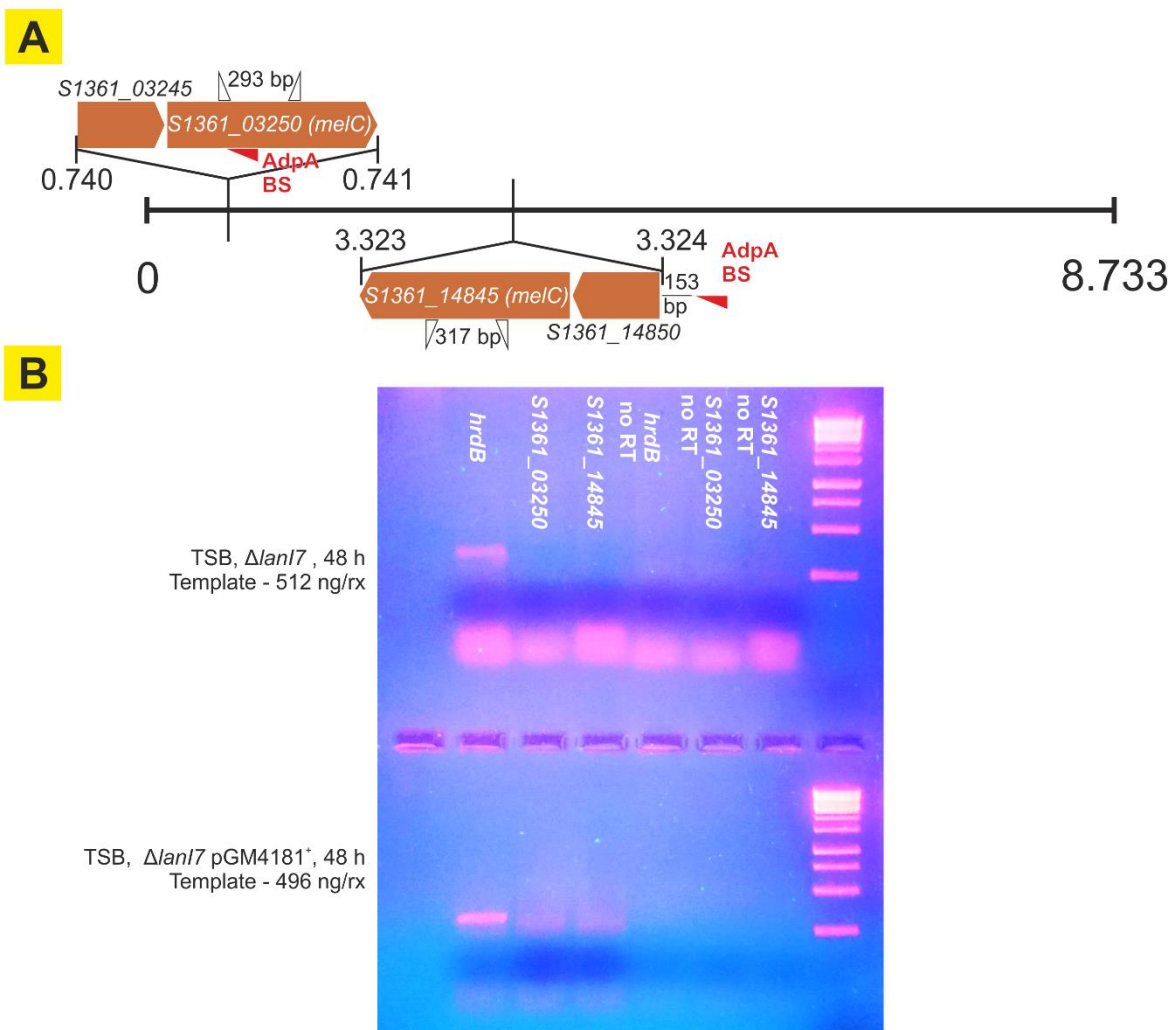


Fig. S2. Allocation of the genes, putatively involved in the biosynthesis of melanoid pigments, on the chromosome of *S. cyanogenus* (**A**). Red triangles label the location and orientation of putative AdpA binding sites (for S1361_03250: TGGCCGGTC, for S1361_14845: TGGCCCGAT). Expression of S1361_03250 and S1361_14845 genes is activated in *XNR_4181* (*adpA* gene from *S. albus*) overexpressing strain (Δ lanI7 pGM4181⁺, **B**), as measured by semi-quantitative RT-PCR. Fragments amplified during RT-PCR are marked with open triangles. The photo was uniformly contrasted (PowerPoint drawing settings) to emphasize the bands in pGM4181-expressing strain; original photo is shown on the last page of this ESM file. Positive control is *hrdB* gene for major vegetative sigma factor in *Streptomyces* (e.g. SCO5820 in *S. coelicolor* M145)

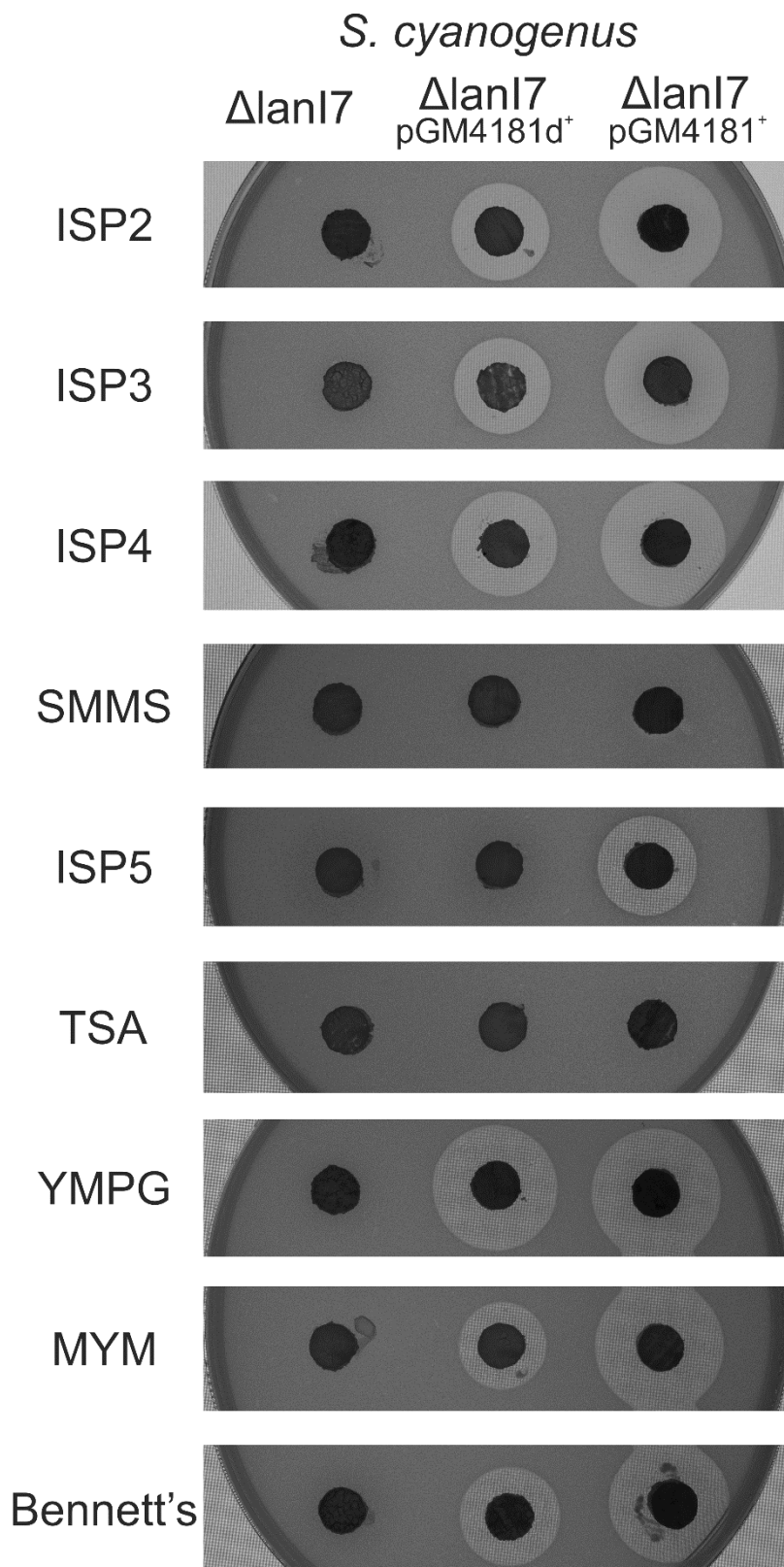


Fig. S3. Induction of antifungal activity in *S. cyanogenus* upon introduction of *XNR_4181* (pGM4181) and *XNR_4181dbd* (pGM4181d) is medium-dependent. *D. hansenii* was used as a fungal culture in agar plug assay, as described in Methods of the main text. Conditions of *S. cyanogenus* cultivation: 120 h of growth, 30 °C, type of the medium is noted to the left of the photos. Photos represent typical result of four biological replicates.

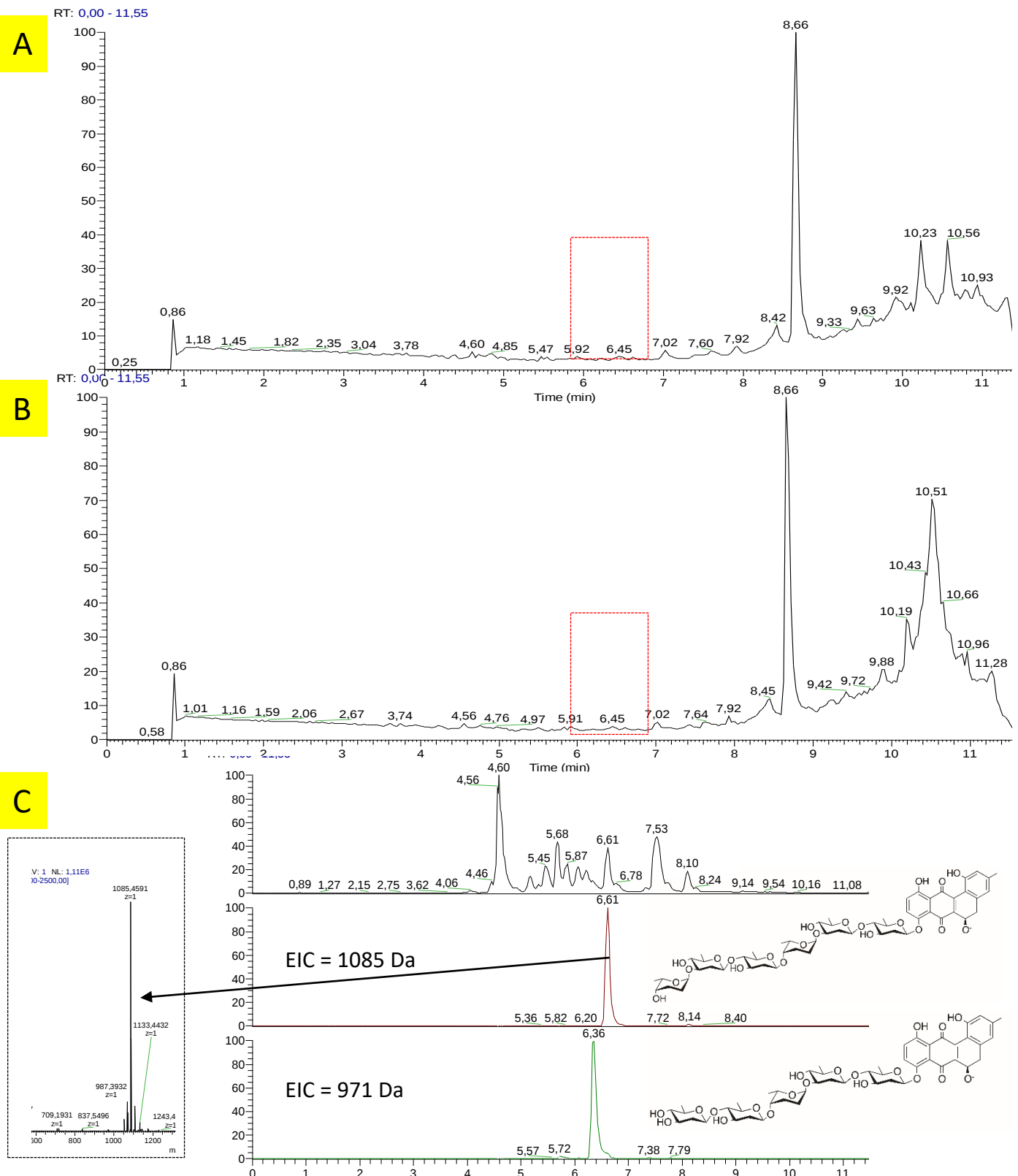


Fig. S4. *S. cyanogenus* S136 and S136 pGM4181⁺ show qualitatively identical secondary metabolomes in YMPG. Total ion chromatograms (positive ionization) of ethyl-acetate-methanol extracts from the biomass of *S. cyanogenus* S136 (**A**) and S136 pGM4181⁺ (**B**) grown in YMPG for 120 h. Red dashed rectangle marks the retention time window where lucensomycin and landomycins (La) appear; please see for comparison also Fig. 1 and the TIC traces from YMPG in negative ionization mode (**C**) for S136, where La become detectable. Accumulation of La was increased in S136 pGM4181⁺ $((1.6 \pm 0.2) \times 10^4$ au) as compared to control strain $((0.7 \pm 0.3) \times 10^4$ au); these titers are ~100-fold less than those observed in SG (see Suppl. Ref 2).

Table S2. Lcm activity against different bacterial and fungal species.

Test-culture	Zone of growth inhibition*, mm
<i>Escherichia coli</i> DH5 α	No growth inhibition zone
<i>Bacillus cereus</i>	No growth inhibition zone
<i>Staphylococcus aureus</i>	No growth inhibition zone
<i>Debaryomyces hansenii</i>	11 $\pm$ 2
<i>Aspergillus niger</i>	16 $\pm$ 2
<i>Fusarium oxisporum</i>	24 $\pm$ 3

*Paper discs (Whatman 3MM, $\varnothing$ 5mm) were impregnated with 15 μ l of methanol solution containing 10 μ g of Lcm

Results represent mean values $\pm$ SD of five independent experiments

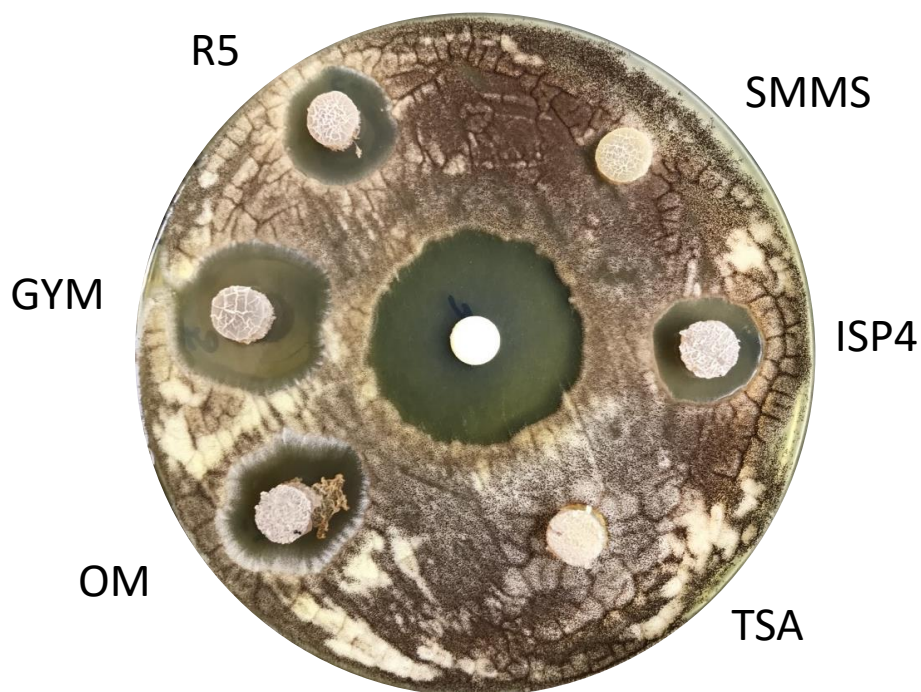


Fig. S5. Agar plugs of *S. cyanogenus* Δ lanI7 pGM4181⁺ exhibit activity against *Fusarium oxisporum*. Strains were grown for 120 h on the agar media and stacked on top of freshly seeded lawn of *F. oxisporum*. In the center of the plate a paper disc carrying 10 μ g of Lcm is placed.

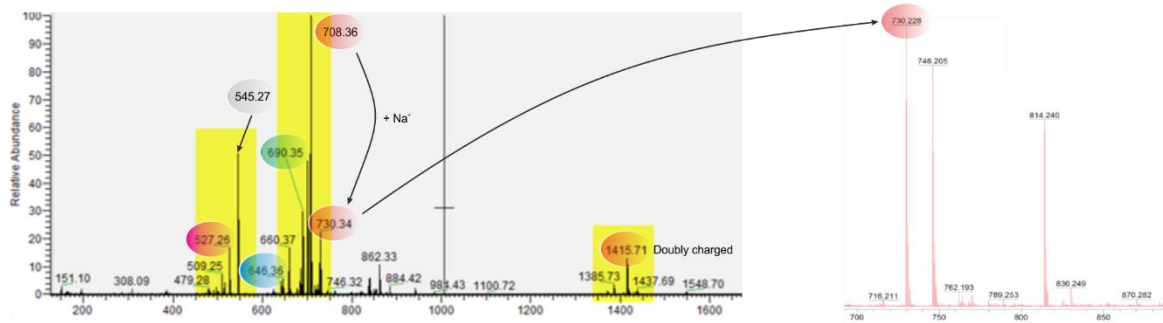
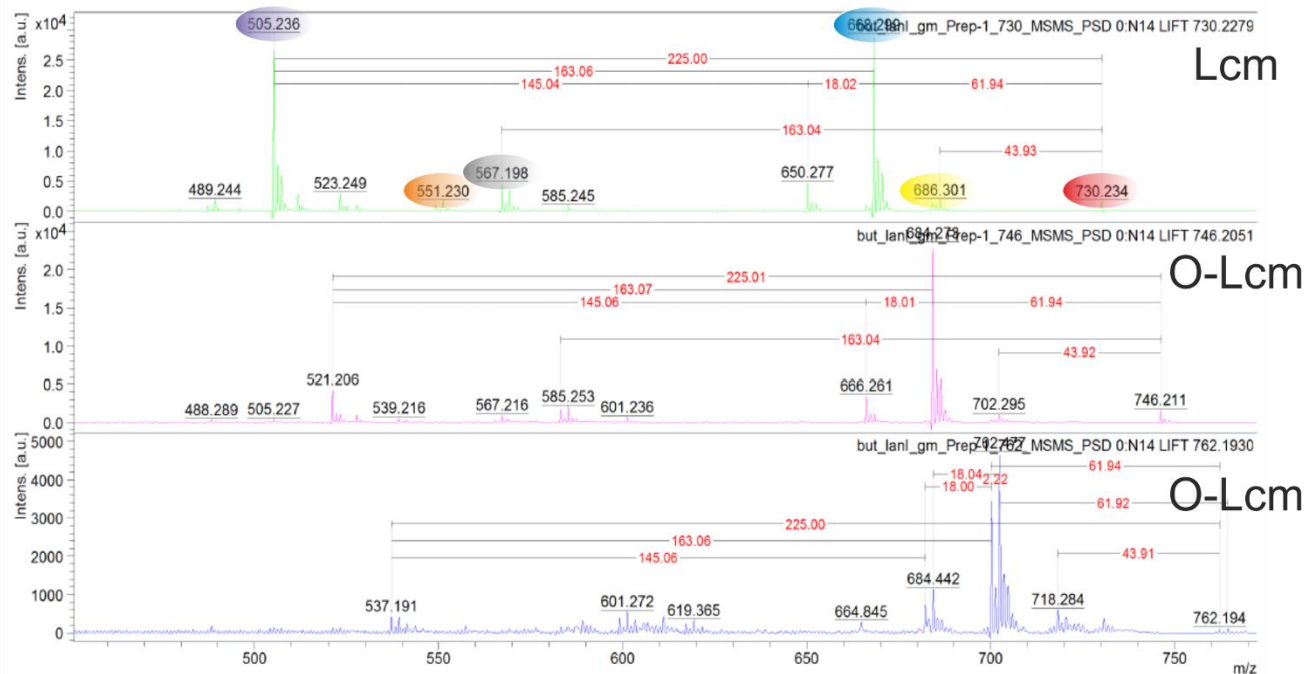
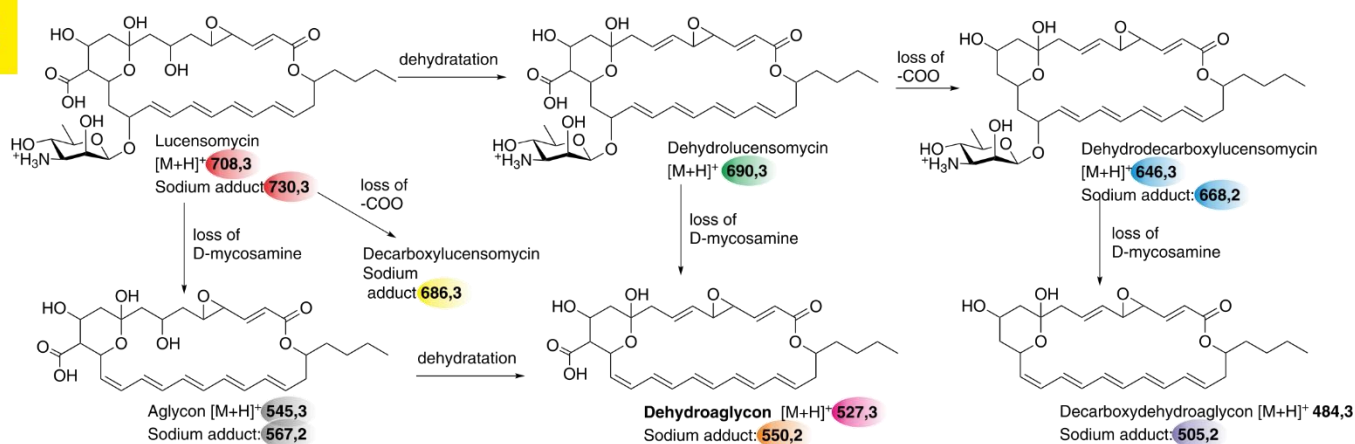
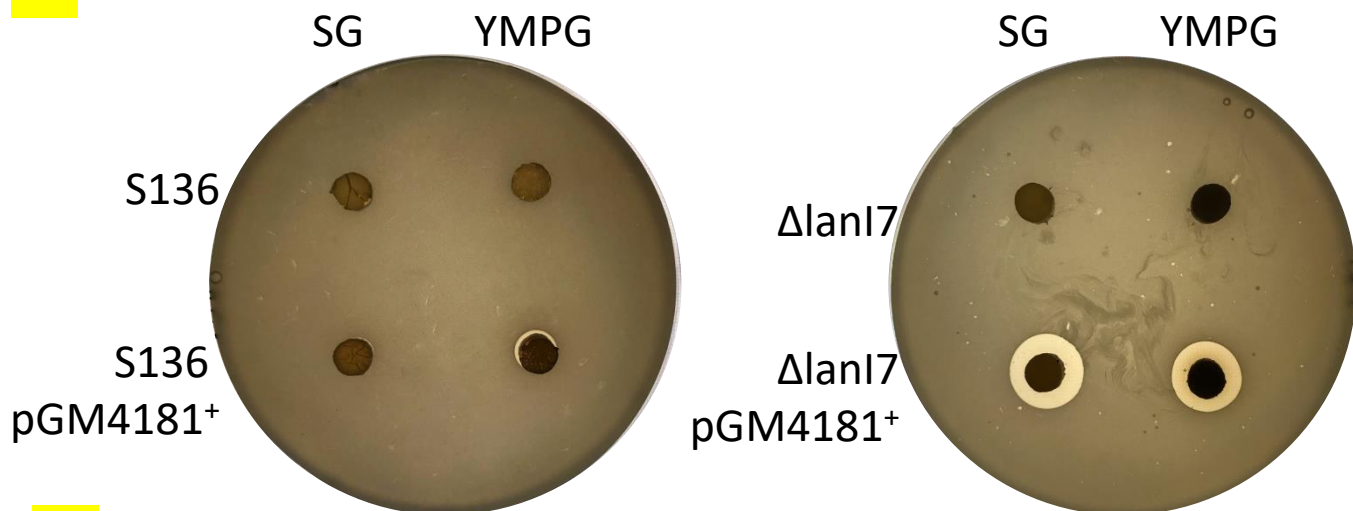
A**B****C**

Fig. S6. Fragmentation pattern of 708.35 Da compound is consistent with the chemical structure of Lcm. In-source (+)-ESI fragmentation of the 708.35 Da compound (**A**) and (+)-ESI-MS² spectrum for 730.34 Da compound (Na⁺ adduct of 708.35 Da compound; **B**) are shown; both agree with the CID pathway depicted at the bottom of the figure (**C**). Under MS conditions the dehydration position was arbitrarily chosen. Note that strain accumulated several hydroxylated derivatives of Lcm (O-Lcm; 746.2 Da, 762.2 Da, Na adducts; see **A**, **B**) whose structures could not be inferred due to low yield.

A**B**

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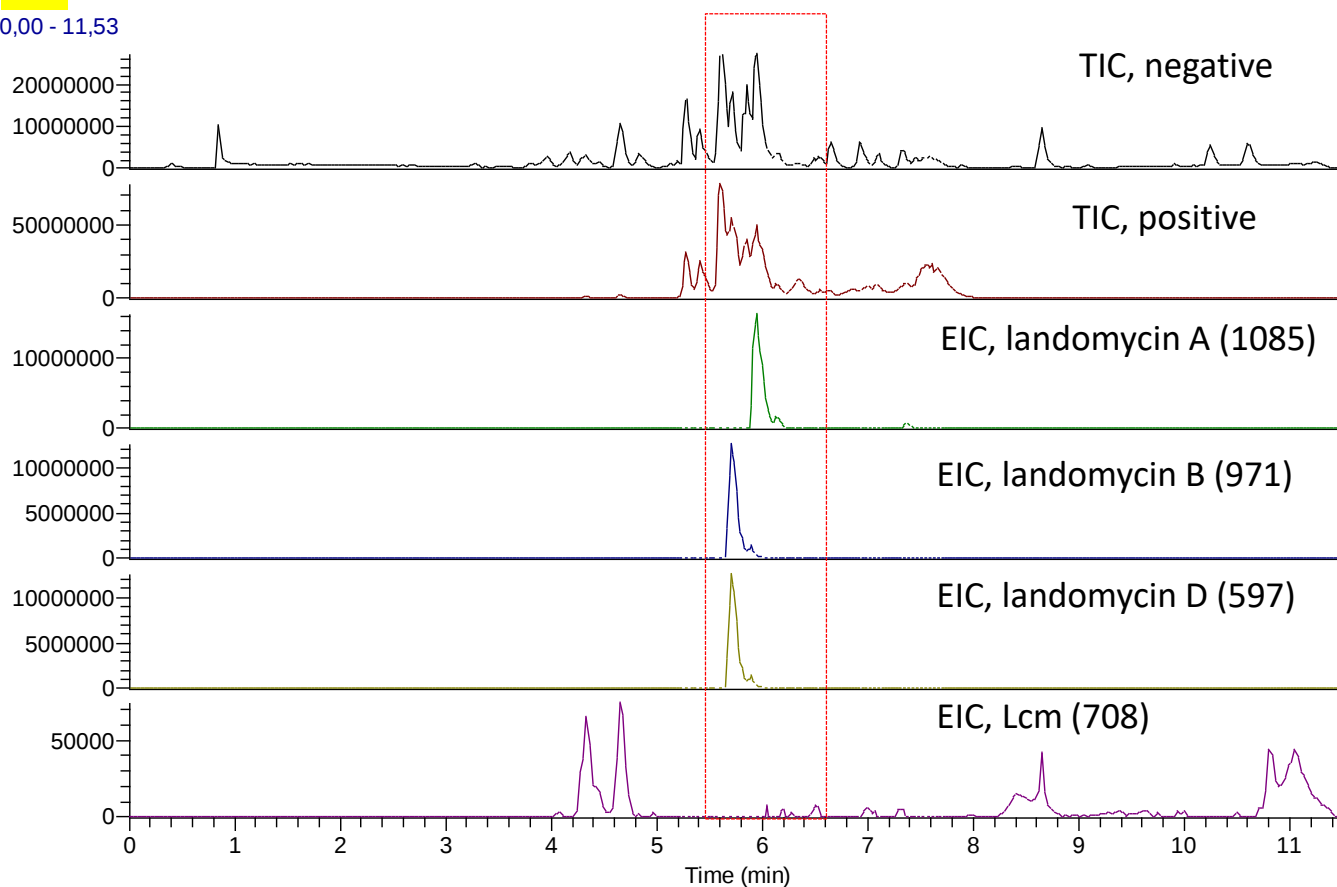


Fig. S7. The AdpA-induced production of Lcm is barely detectable or completely arrested under conditions where landomycin biosynthesis occurs. **(A)** Agar plug assay of *S. cyanogenus* S136 and S136 pGM4181⁺ strain grown on SG and YMPG agars against *D. hansenii*. Strains were grown for 120 h. Photos represent typical result of four biological replicates. **(B)** LC-MS traces of methanol-acetone extracts of the biomass of *S. cyanogenus* S136 pGM4181⁺ strain grown for 72 h in SG. Extracted ion chromatograms (EIC) show the presence of landomycins A, B and D, and absence of Lcm. See also Fig. S4 for landomycin structures. Red dashed rectangle marks the retention time window where the analyzed compounds occur.

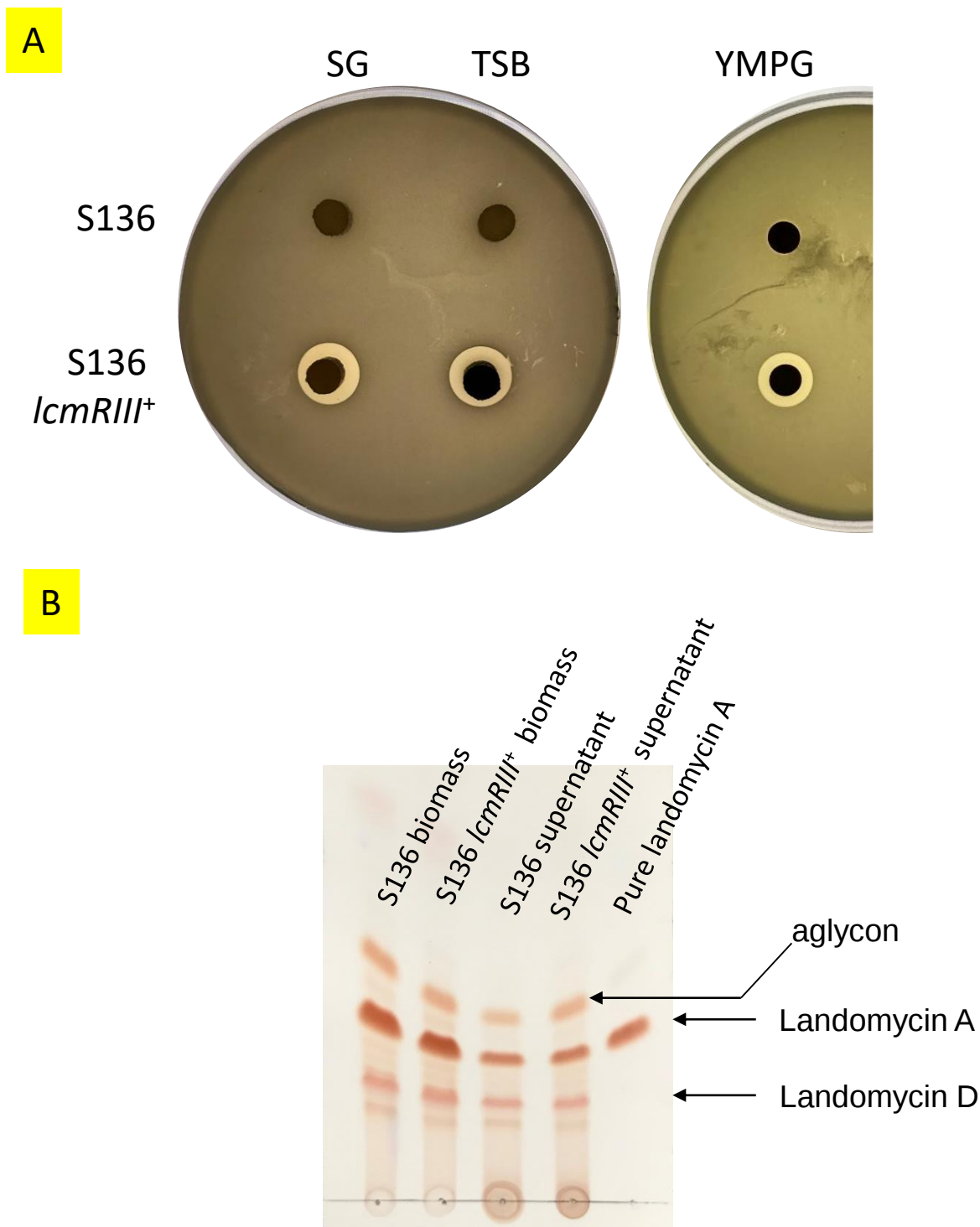


Fig. S8. Pathway-specific regulatory gene *lcmRIII* under control of constitutive promoter *ermEp* induces Lcm production in AdpA- and medium-independent manner while leaving landomycin production unperturbed. **(A)** Strains and media are labeled around the photo. Agar plugs for the bioassay were taken from lawns grown for 120 h. **(B).** TLC of landomycins extracted separately from biomass and spent medium (5-mL samples) of the strains labeled on the photo above. Differences in the biomass (dry weight) did not exceed 10% of the mean value. The TLC image represents typical result of six biological replicates.

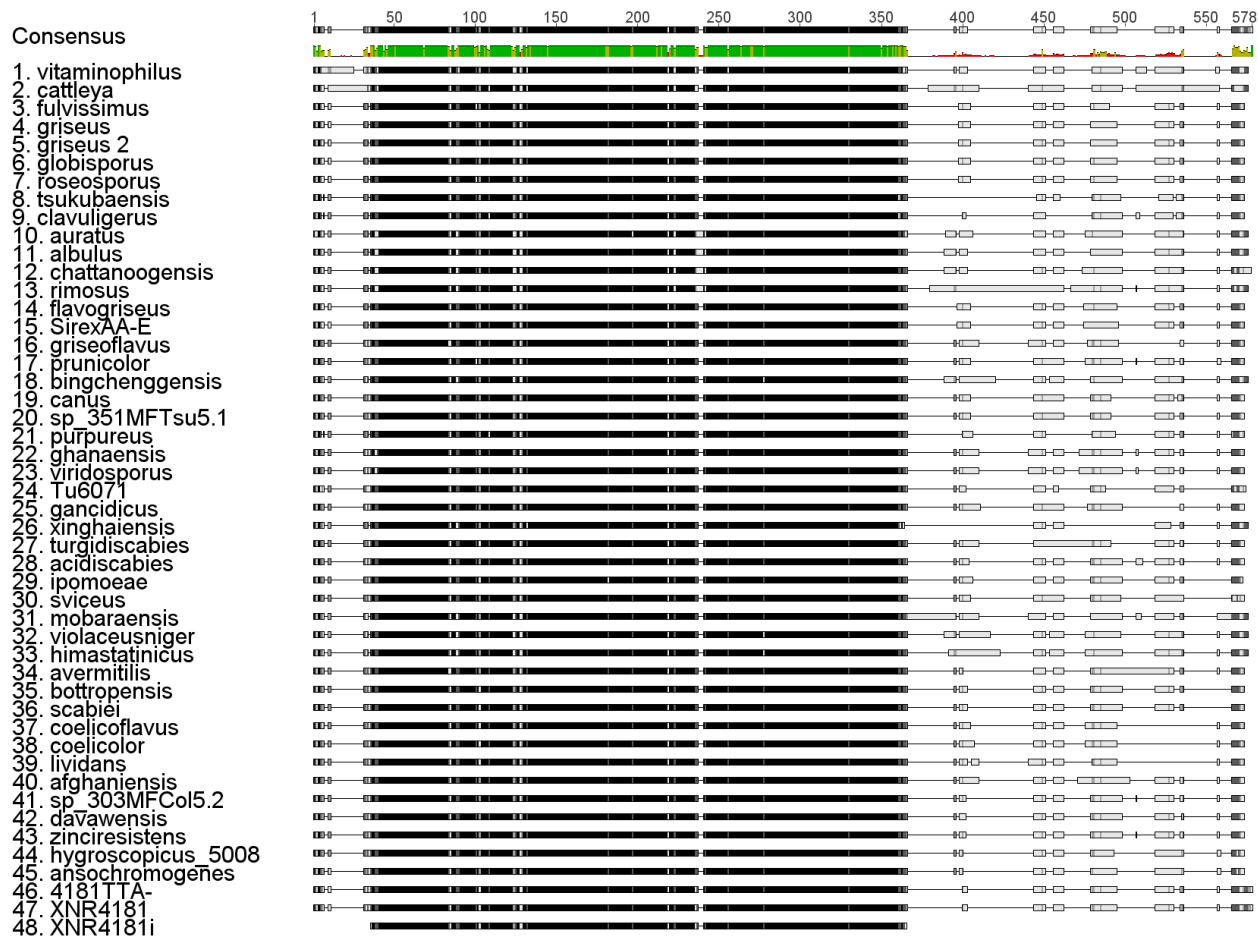


Fig. S9. Multiple amino acid sequence alignment constructed to discover the most conserved region of AdpA proteins (Geneious alignment). Novel AdpA variants constructed in this work – 4181_{TTA-} (XNR_4181_{TTA-}), XNR4181i (XNR_4181i) – together with *S. albus* adpA (XNR_4181) – are shown at the bottom of the alignment.

SCO2792	ATGAGCCACGACTCCACGCCGCGCGGAGCGCGGCCGGAAGCTGTCTGGGCGACGC	60	SCO2792	GCCTTGGAGACCTTCCACGAGCAGTTGACGTGGAGACGTGGCCGCGCGCTACATG	780
XNR4181ii	-----ATGAAACTCTCTGGGCGGCGC	21	XNR4181ii	GCCTTGGAGACCTTCCACGAGCAGTTGACGTGGAGACGTGGCCGCGCGCTACATG	741
XNR4181TTA-	ATGAGCCACGACTCCACGCGGACCTCGGGGCGGAAACTCTCTGGGCGGCGC	60	XNR4181TTA-	GCCTTGGAGACCTTCCACGAGCAGTTGACGTGGAGACGTGGCCGCGCGCTACATG	780
XNR4181	ATGAGCCACGACTCCACGCGGACCTCGGGGCGGAAACTCTCTGGGCGGCGC	60	XNR4181	GCCTTGGAGACCTTCCACGAGCAGTTGACGTGGAGACGTGGCCGCGCGCTACATG	780
	*****			*****	
SCO2792	CGCAAGGAGATCGTCGCGGTGCTGCTTCTCAGCGGCGGCCCATCTTCGAGAGTTCCATA	120	SCO2792	AGCCGCCGACCTTCGACCGCGGTTCCGCTCGCTGACGGGAGCGCCCGCTCAGTG	840
XNR4181ii	CGCAAGGAGATCGTCGCGGTCTCTCTTCTCAGCGGCGGCCCATCTTCGAGAGTTCCATC	81	XNR4181ii	AGCCGCCGACCTTCGACCGCGGCTTCCTGTTCTGCTGACGGGAGCGACCGCTCAGTG	801
XNR4181TTA-	CGCAAGGAGATCGTCGCGGTCTCTCTTCTCAGCGGCGGCCCATCTTCGAGAGTTCCATC	120	XNR4181TTA-	AGCCGCCGACCTTCGACCGCGGCTTCCTGTTCTGCTGACGGGAGCGACCGCTCAGTG	840
XNR4181	CGCAAGGAGATCGTCGCGGTCTCTCTTCTCAGCGGCGGCCCATCTTCGAGAGTTCCATC	120	XNR4181	AGCCGCCGACCTTCGACCGCGGCTTCCTGTTCTGCTGACGGGAGCGACCGCTCAGTG	840
	*****			*****	
	AS				
SCO2792	CCGCTGTCGGTGTTCGGGATGACCGCGAGGACGCGGCGTCCGCGCTACCGGCTGCTG	180	SCO2792	CTGATCACCCAGCGGGTGTCTCAGGCGAGCGCTGCTGGAGACGTGGAGTACTCGTG	900
XNR4181ii	CCGCTCTCCGCTTCGCGATCGACCGCGAGGACGCGGCGTCCGCGCTACCGGCTGCTG	141	XNR4181ii	CTCATACCCAGCGCGTGTGACGCGCGAGCGGCTCTGGAGACCTCCGACTACTCGTG	861
XNR4181TTA-	CCGCTCTCCGCTTCGCGATCGACCGCGAGGACGCGGCGTCCGCGCTACCGGCTGCTG	180	XNR4181TTA-	CTCATACCCAGCGCGTGTGACGCGCGAGCGGCTCTGGAGACCTCCGACTACTCGTG	900
XNR4181	CCGCTCTCCGCTTCGCGATCGACCGCGAGGACGCGGCGTCCGCGCTACCGGCTGCTG	180	XNR4181	CTCATACCCAGCGCGTGTGACGCGCGAGCGGCTCTGGAGACCTCCGACTACTCGTG	900
	*****			*****	
SCO2792	GTGTGCGCCGGCGAGGACGGCCGCTGCGACACCGAGGGGCTGGAACCTACCGCGCCG	240	SCO2792	GACGAGGTGCGCGGGGCTGCGGGTTCGCGTCCCGGTGGCGCTGCGCGGGCACCTTCGCG	960
XNR4181ii	GTCTGCGCCGGCGAGGACGGACCGCTACGAGCACCGCGGACTGGAACCTACCGCGCCG	201	XNR4181ii	GACGAGGTGCGCGGGGCTGCGGGTTCGCGTCCCGGTGGCGCTGCGCGGGCACCTTCGCG	921
XNR4181TTA-	GTCTGCGCCGGCGAGGACGGACCGCTACGAGCACCGCGGACTGGAACCTACCGCGCCG	240	XNR4181TTA-	GACGAGGTGCGCGGGGCTGCGGGTTCGCGTCCCGGTGGCGCTGCGCGGGCACCTTCGCG	960
XNR4181	GTCTGCGCCGGCGAGGACGGACCGCTACGAGCACCGCGGACTGGAACCTACCGCGCCG	240	XNR4181	GACGAGGTGCGCGGGGCTGCGGGTTCGCGTCCCGGTGGCGCTGCGCGGGCACCTTCGCG	960
	*****			*****	
SCO2792	CAGGGACTGGAGGCGATCTCGCGCGGGCAGCGTGTGCTGCGCGGCTGGCGGTGATC	300	SCO2792	CGCCAGTGTGGGCTGTCTCCCGCGGCTACCGGGCGGCTACCGGGCGCGCTCCCGAG	1020
XNR4181ii	CAGGGCTTGGAGGCGCTCGCCGGGAGGAGCGGTGGTGGTCCCGCTGGCGGTCCATC	261	XNR4181ii	CGCCAGTGTGGGCTGTCTCCCGCGGCTACCGGGCGGCTACCGGGCGCGCTCCCGAG	981
XNR4181TTA-	CAGGGCTTGGAGGCGCTCGCCGGGAGGAGCGGTGGTGGTCCCGCTGGCGGTCCATC	300	XNR4181TTA-	CGCCAGTGTGGGCTGTCTCCCGCGGCTACCGGGCGGCTACCGGGCGCGCTCCCGAG	1020
XNR4181	CAGGGCTTGGAGGCGCTCGCCGGGAGGAGCGGTGGTGGTCCCGCTGGCGGTCCATC	300	XNR4181	CGCCAGTGTGGGCTGTCTCCCGCGGCTACCGGGCGGCTACCGGGCGCGCTCCCGAG	1020
	*****			*****	
SCO2792	ACCTCGCGCCGCGCGAGGAAGCACTCGACGCACTGCGAGGGCGCACGAGGAGGGGCG	360	SCO2792	GGCGAACGCGACGCGGACCGGACCGACGCGCGGGCGGCGCCACCGCGCGCTGCCCGG	1080
XNR4181ii	ACCTCACCCCGCGCGCGCGCGCTGGAGCGGCTGCGCGCGGCGCACGAGGAGGGGCG	321	XNR4181ii	GGCGAACGCGACGCGGACCGGACCGGACCGCGGGCGGCGCCACCGCGCGCTGCCCGG	981
XNR4181TTA-	ACCTCACCCCGCGCGCGCGCGCTGGAGCGGCTGCGCGCGGCGCACGAGGAGGGGCG	360	XNR4181TTA-	CAGGAGCGACGACGGCGCGAGCGGCTGCGGCGGCGGCGGCGGCGGCGGCGGCGGCG	1071
XNR4181	ACCTCACCCCGCGCGCGCGCGCTGGAGCGGCTGCGCGCGGCGCACGAGGAGGGGCG	360	XNR4181	CAGGAGCGACGACGGCGCGAGCGGCTGCGGCGGCGGCGGCGGCGGCGGCGGCGGCG	1071
	*****			*****	
SCO2792	CGCATGTGGGCTGTGACGCGGCGCTTCTGCTCTCGCGGGCGCGGCGTGTGGAGCGG	420	SCO2792	TTCGACCGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1128
XNR4181ii	CGCATGTGGGCTGTGACGCGGCGCTTCTGCTCTCGCGGGCGCGGCGTGTGGAGCGG	381	XNR4181ii	-----CTCCCTCGCCCGGAGA-----ACGCGGTCCCGTTCAG---	981
XNR4181TTA-	CGCATGTGGGCTGTGACGCGGCGCTTCTGCTCTCGCGGGCGCGGCGTGTGGAGCGG	420	XNR4181TTA-	TTCGACCGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1131
XNR4181	CGCATGTGGGCTGTGACGCGGCGCTTCTGCTCTCGCGGGCGCGGCGTGTGGAGCGG	420	XNR4181	TTCGACCGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG	1131
	*****			*****	
SCO2792	CGCCCCGACACGACGACTGGATGTACGCGCCGACGCTGGCGAAGCGTATCCGTCGGTG	480	SCO2792	----ACCGCGCGACCGCACCCGGA----TGCGCGGGGCGGCGGCGGCGGCGGCGGCGG	1179
XNR4181ii	AGGCGCGGCGACACGACGACTGGATGTACGCGCCGACGCTGGCGAAGCGTATCCCTCGTG	441	XNR4181ii	GGCGACCCGGGCGGGGAGCACCGGAGGCTACTCCAGGGGCGCGGGCACTCCGGGCG	981
XNR4181TTA-	AGGCGCGGCGACACGACGACTGGATGTACGCGCCGACGCTGGCGAAGCGTATCCCTCGTG	480	XNR4181TTA-	GGCGACCCGGGCGGGGAGCACCGGAGGCTACTCCAGGGGCGCGGGCACTCCGGGCG	1191
XNR4181	AGGCGCGGCGACACGACGACTGGATGTACGCGCCGACGCTGGCGAAGCGTATCCCTCGTG	480	XNR4181	GGCGACCCGGGCGGGGAGCACCGGAGGCTACTCCAGGGGCGCGGGCACTCCGGGCG	1191
	*****			*****	
SCO2792	CACGTGACCCGCGGAACTCTTGCGGACGACGGGACGTGCTGACGTCCGCGGGACCC	540	SCO2792	CAGCGCAGCGCGCC-GTGA----- 1197	
XNR4181ii	CACGTGACCCGCGGGAGCTCTTGCTGACGACGGGAGGTGCTCACTCTGCGCGGGACCC	501	XNR4181ii	----- 981	
XNR4181TTA-	CACGTGACCCGCGGGAGCTCTTGCTGACGACGGGAGGTGCTCACTCTGCGCGGGACCC	540	XNR4181TTA-	CAGCGGGAACGTCCGCTGGGCGGCTCTGA 1221	
XNR4181	CACGTGACCCGCGGGAGCTCTTGCTGACGACGGGAGGTGCTCACTCTGCGCGGGACCC	540	XNR4181	CAGCGGGAACGTCCGCTGGGCGGCTCTGA 1221	
	*****			*****	
SCO2792	GCGGCCGGGATCGACCTGTGCTGCACATCTGCGGACGAGCACGGCAACGAGGCGGCC	600			
XNR4181ii	GCGGCCGGGATCGATCTTGCTGCACATCTGCTCGGACCGATCACGGCAGCGAGGCGCG	561			
XNR4181TTA-	GCGGCCGGGATCGATCTTGCTGCACATCTGCTCGGACCGATCACGGCAGCGAGGCGCG	600			
XNR4181	GCGGCCGGGATCGATCTTGCTGCACATCTGCTCGGACCGATCACGGCAGCGAGGCGCG	600			
	*****			*****	
SCO2792	GGTGGCTGGCGCGCGGCTGGTGGTCCCCCGCGCGGCGGCGGCGGCGGAGGCGCTAC	660			
XNR4181ii	GGCGCCCTGGCGCGCGCTGGTGGTGGTCCCCCGCGCGGCGGCGGCGGCGGAGGCGCTAC	621			
XNR4181TTA-	GGCGCCCTGGCGCGCGCTGGTGGTGGTCCCCCGCGCGGCGGCGGCGGCGGAGGCGCTAC	660			
XNR4181	GGCGCCCTGGCGCGCGCTGGTGGTGGTCCCCCGCGCGGCGGCGGCGGCGGAGGCGCTAC	660			
	*****			*****	
SCO2792	CTCGACAGGTCTTACCGGAGGAGATCGGCGCGGACCGCTCGCGAGGTGCTCGCTGG	720			
XNR4181ii	CTCGACAGGTCTTACCGGAGGAGATCGGCGCGGACCGCTGGCGAGGTGCTCGCTGG	681			
XNR4181TTA-	CTCGACAGGTCTTACCGGAGGAGATCGGCGCGGACCGCTGGCGAGGTGCTCGCTGG	720			
XNR4181	CTCGACAGGTCTTACCGGAGGAGATCGGCGCGGACCGCTGGCGAGGTGCTCGCTGG	720			
	*****			*****	

Fig. S10. Multiple nucleotide sequence alignment of *adpA_{SCO}*, *XNR_4181* and the new alleles of the latter. Positions of *adpA_{SCO}* antisense RNA transcription start point (AS) and rare TTA codon are highlighted.

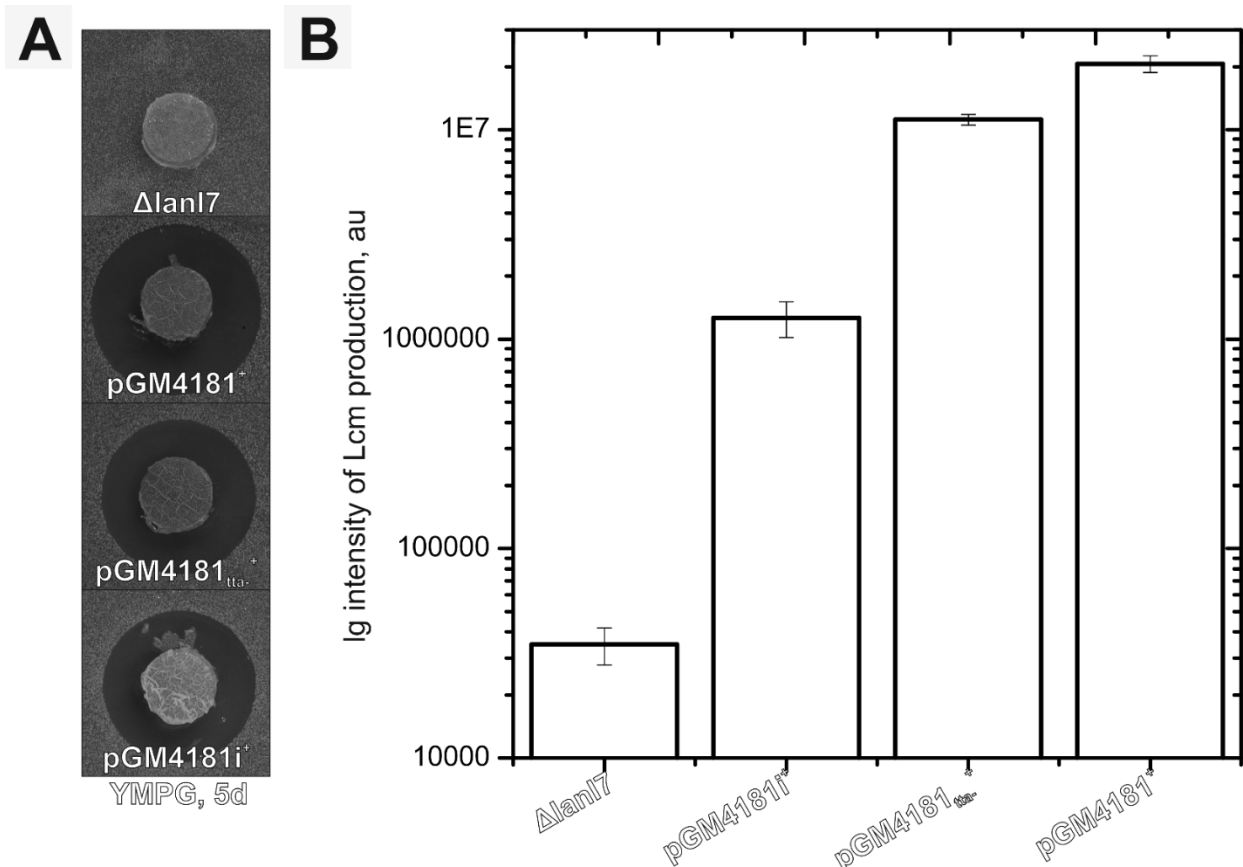
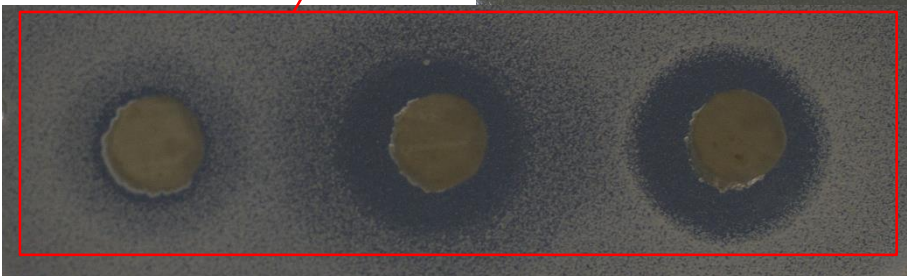
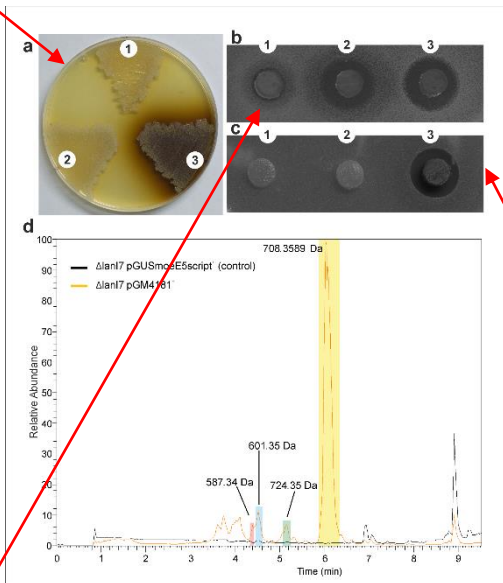
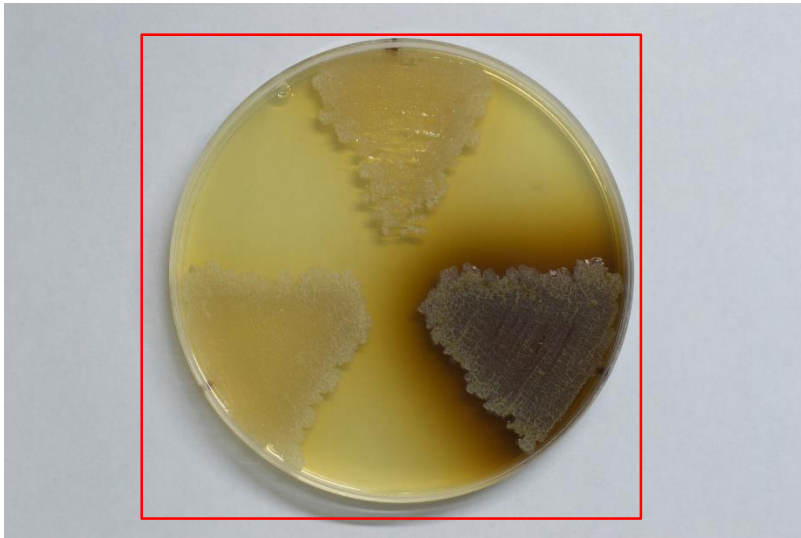


Fig. S11. Novel *adpA* alleles activate Lcm production by *S. cyanogenus* $\Delta lanI7$, as judged from agar plug assay (**A**) and LC-MS results (**B**). Areas of Lcm mass peaks (708.35 Da (M+H)⁺) were integrated and represented as arbitrary units (au); please note logarithmic scale of the y-axis. Data represent mean values of three independent experiments $\pm$ 2SD.

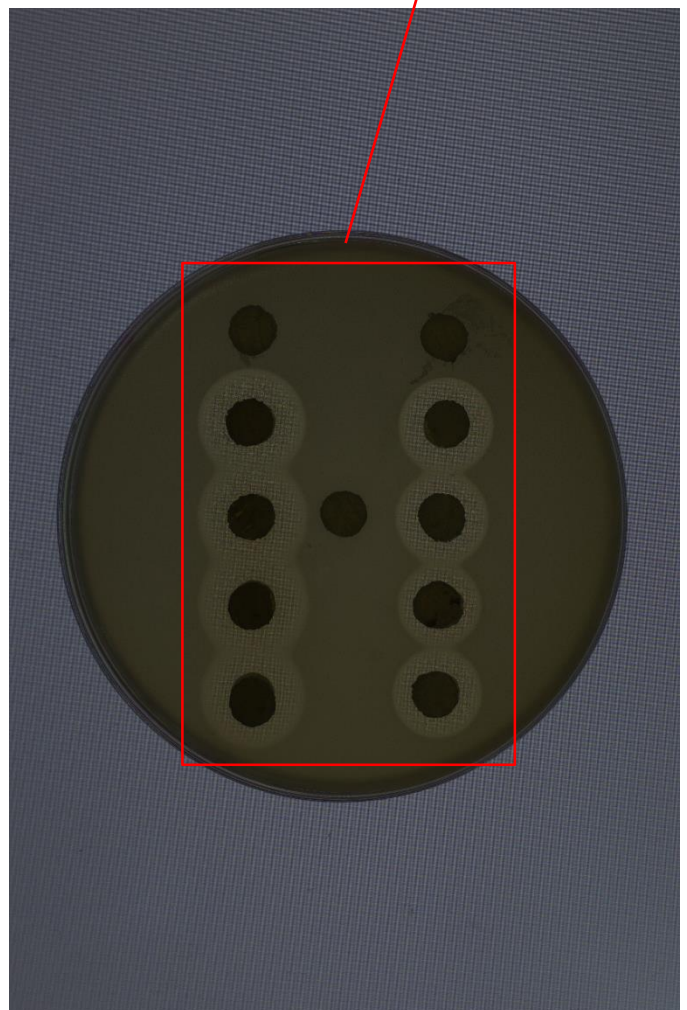
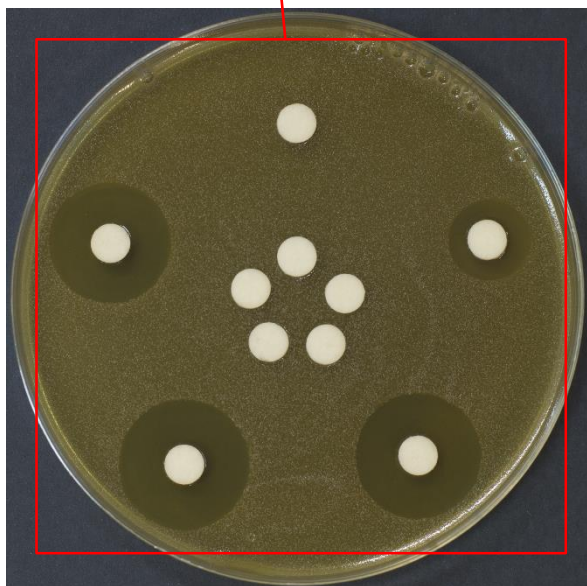
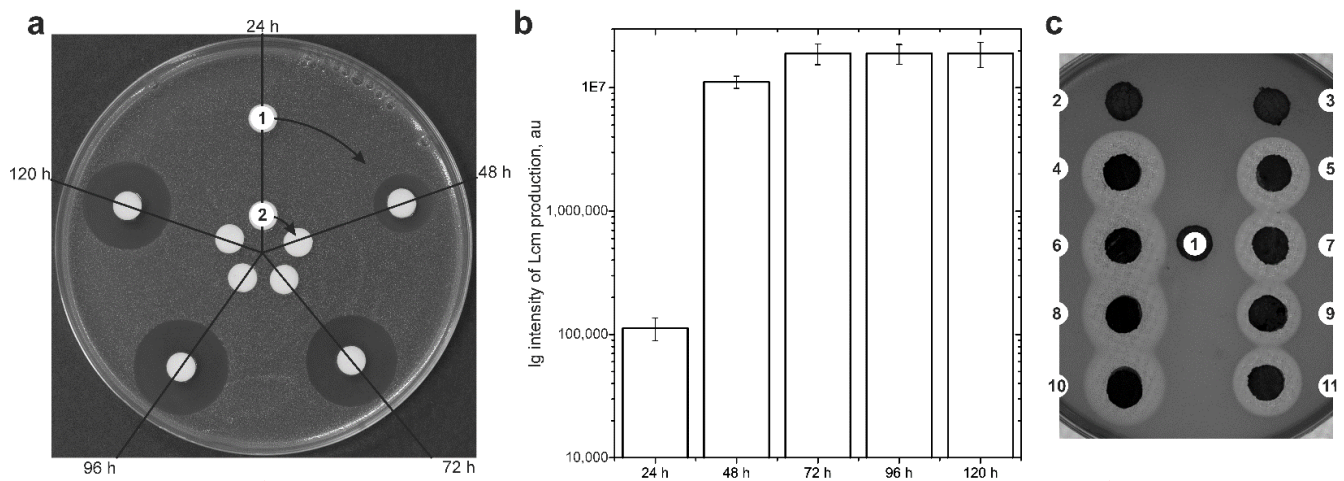
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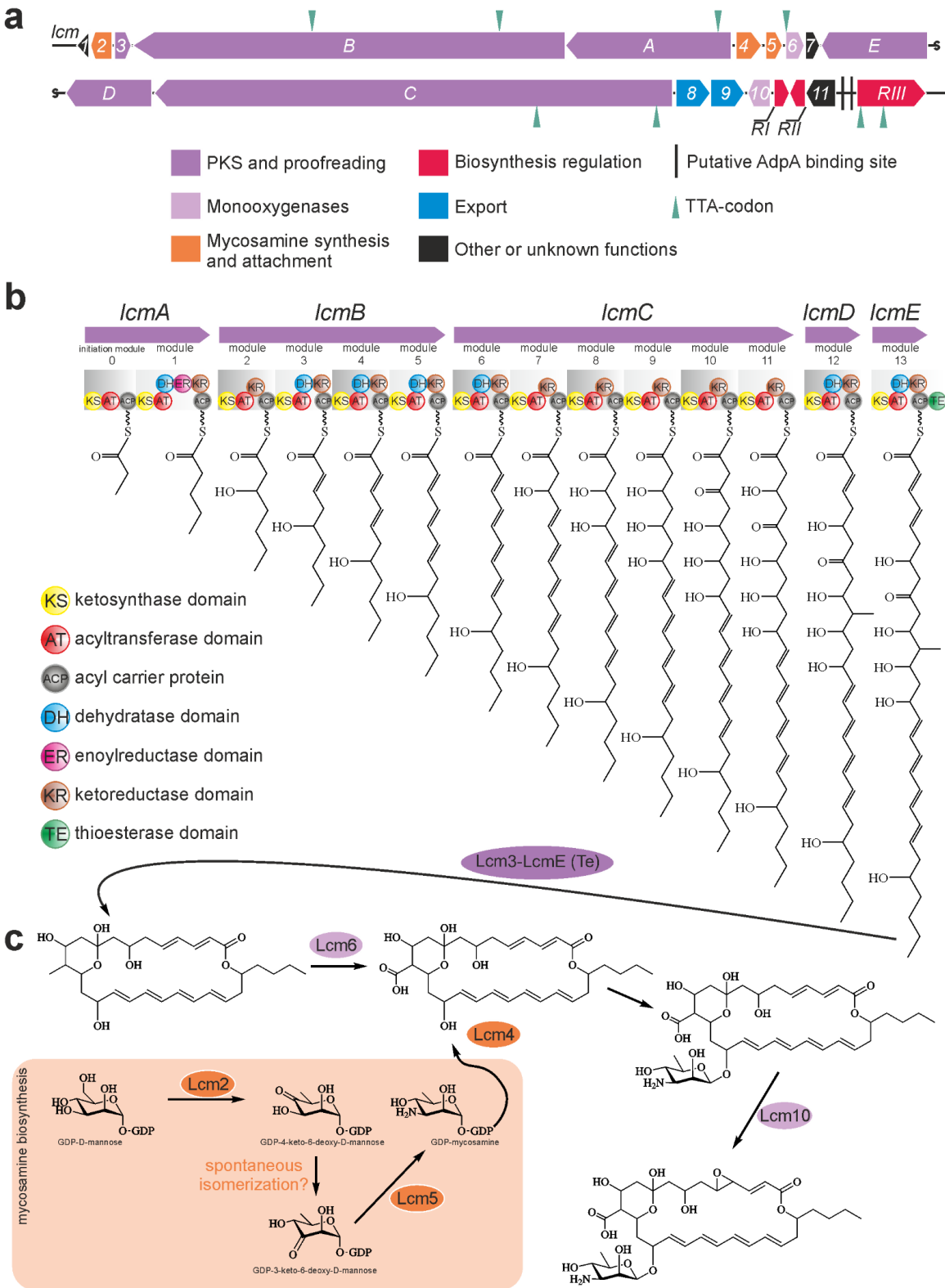
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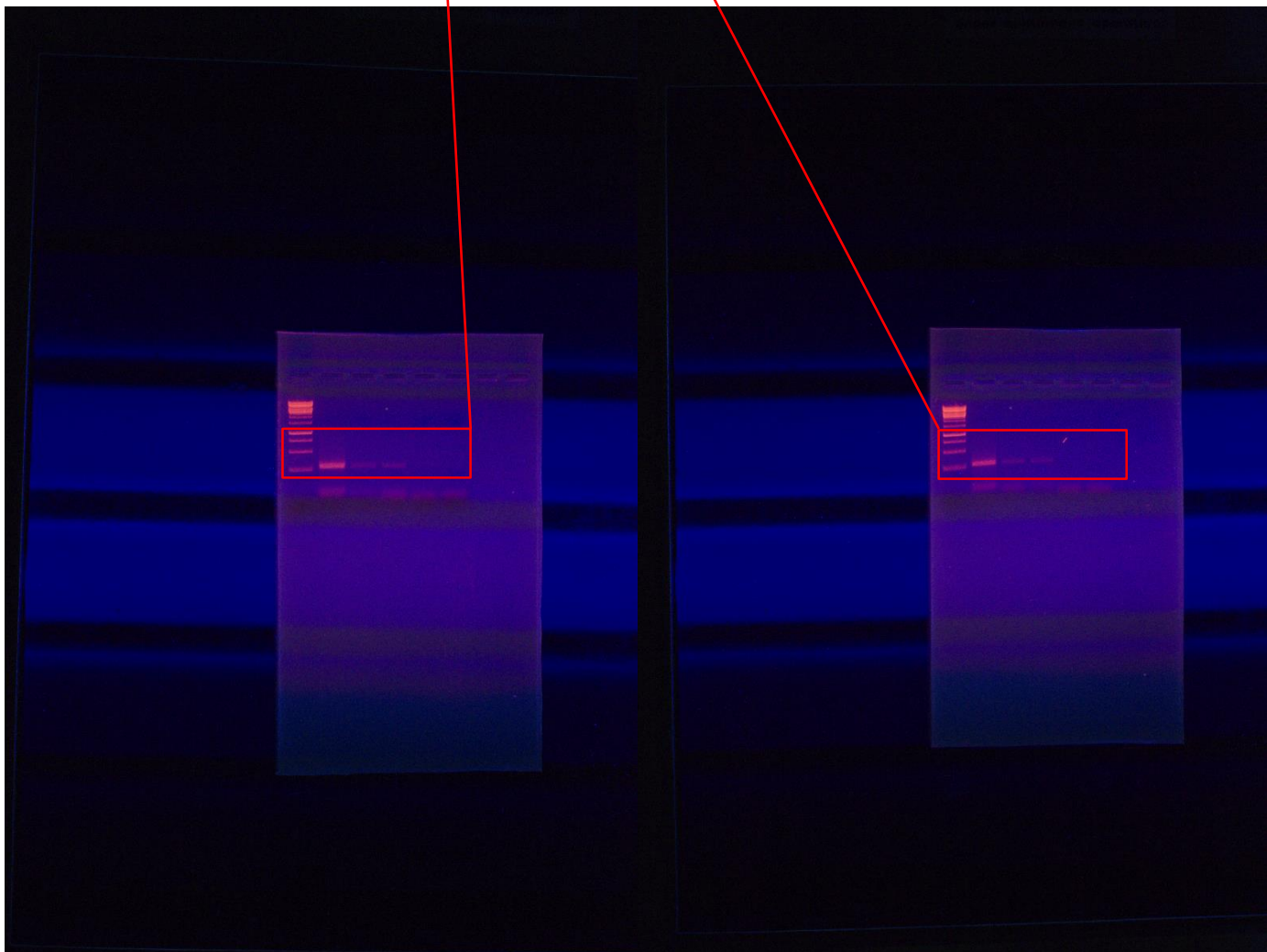
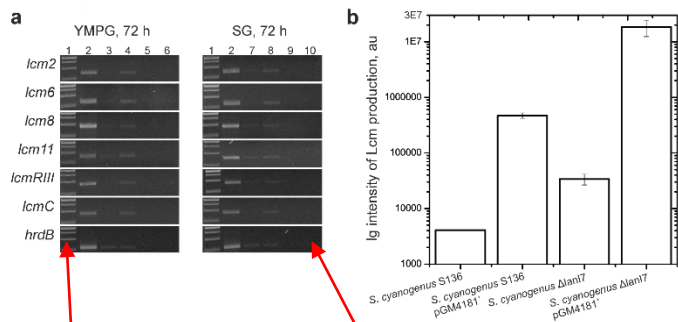
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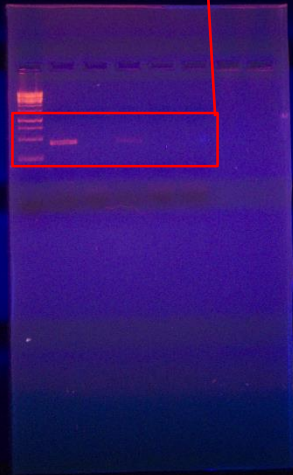
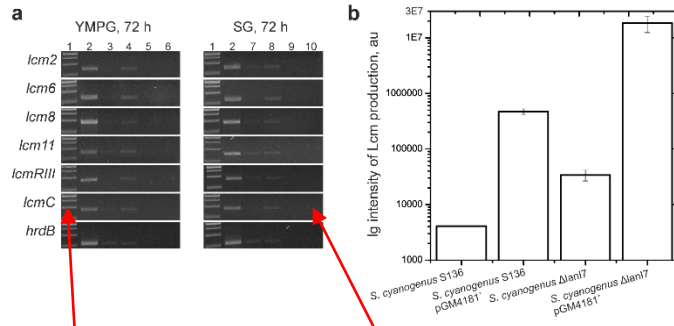


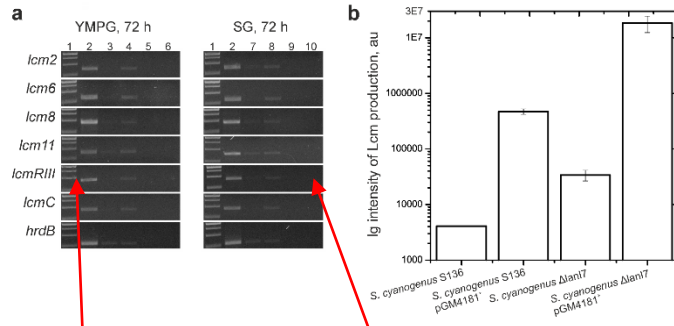
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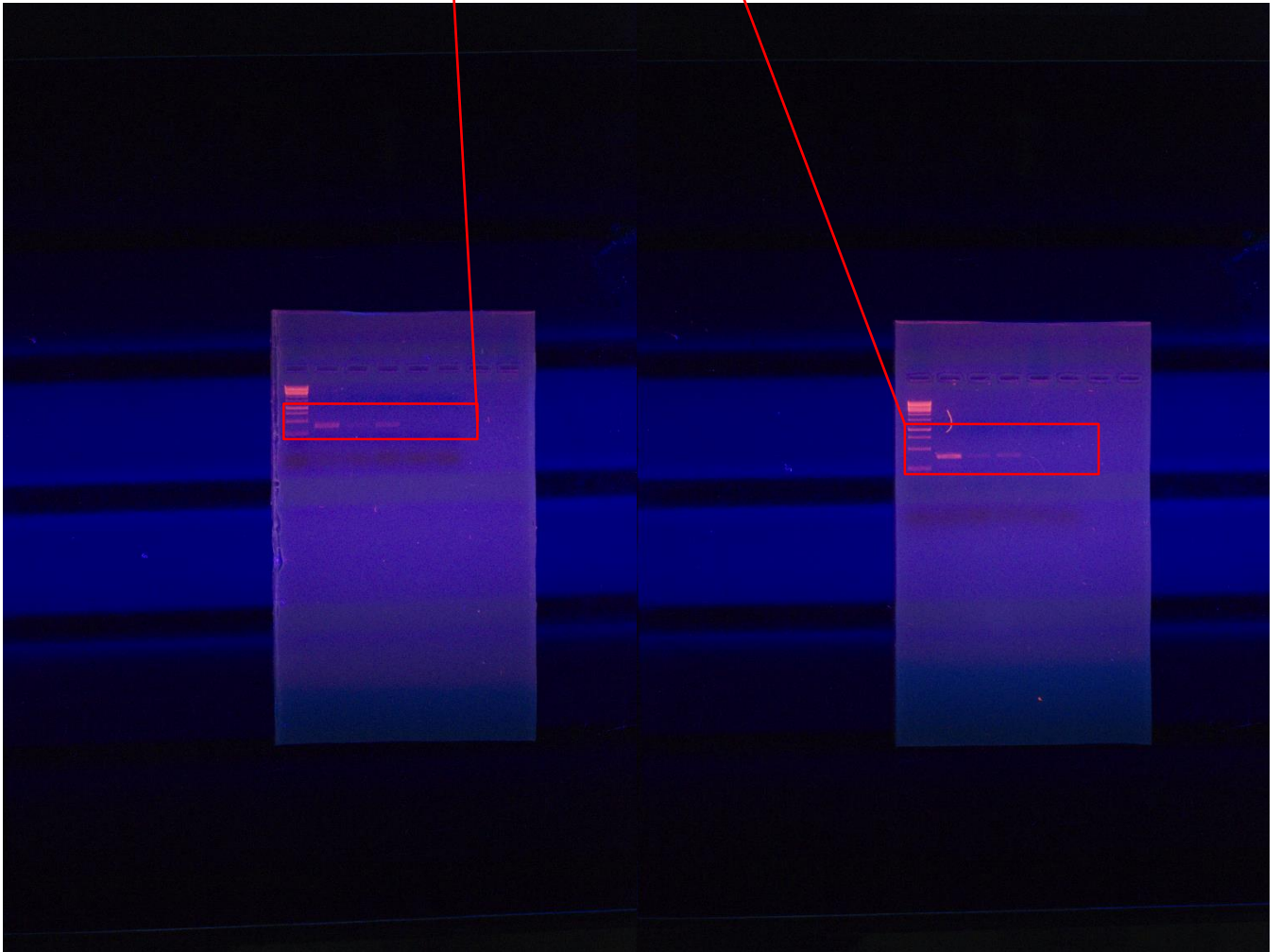
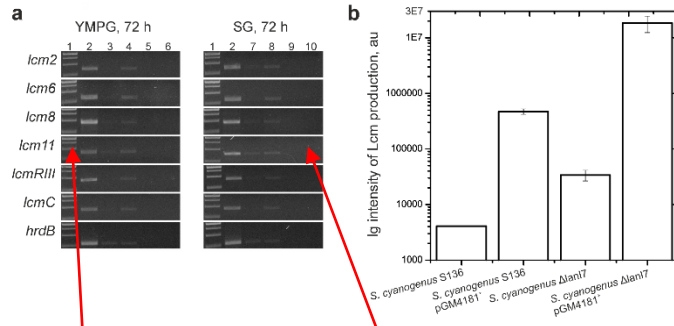


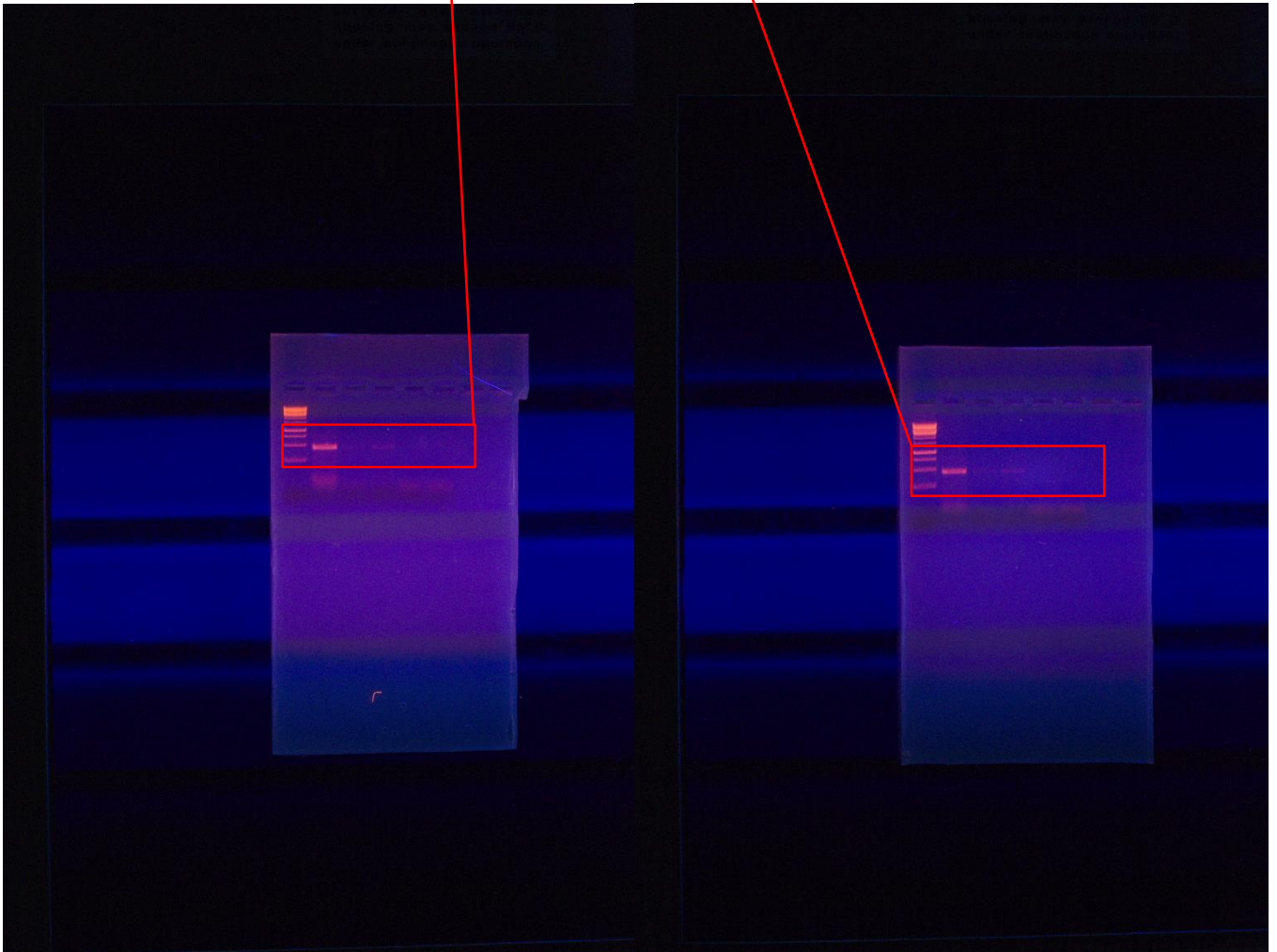
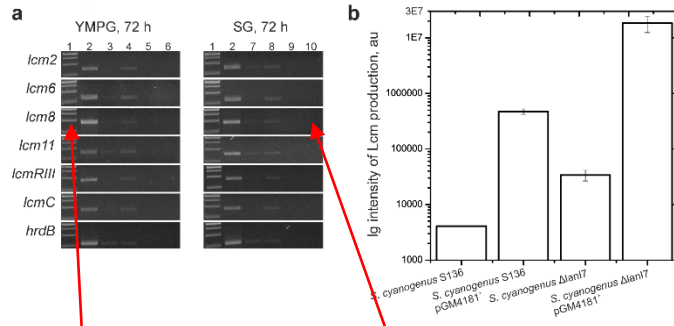
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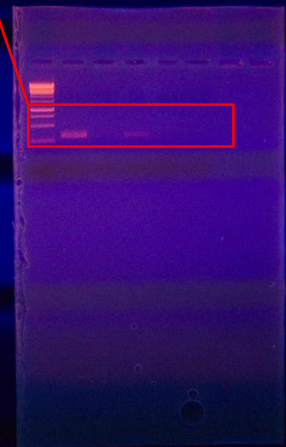
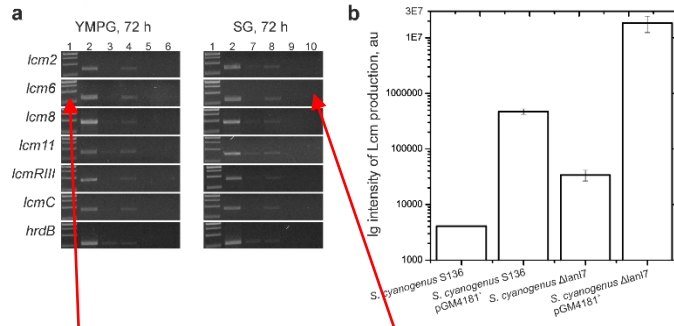


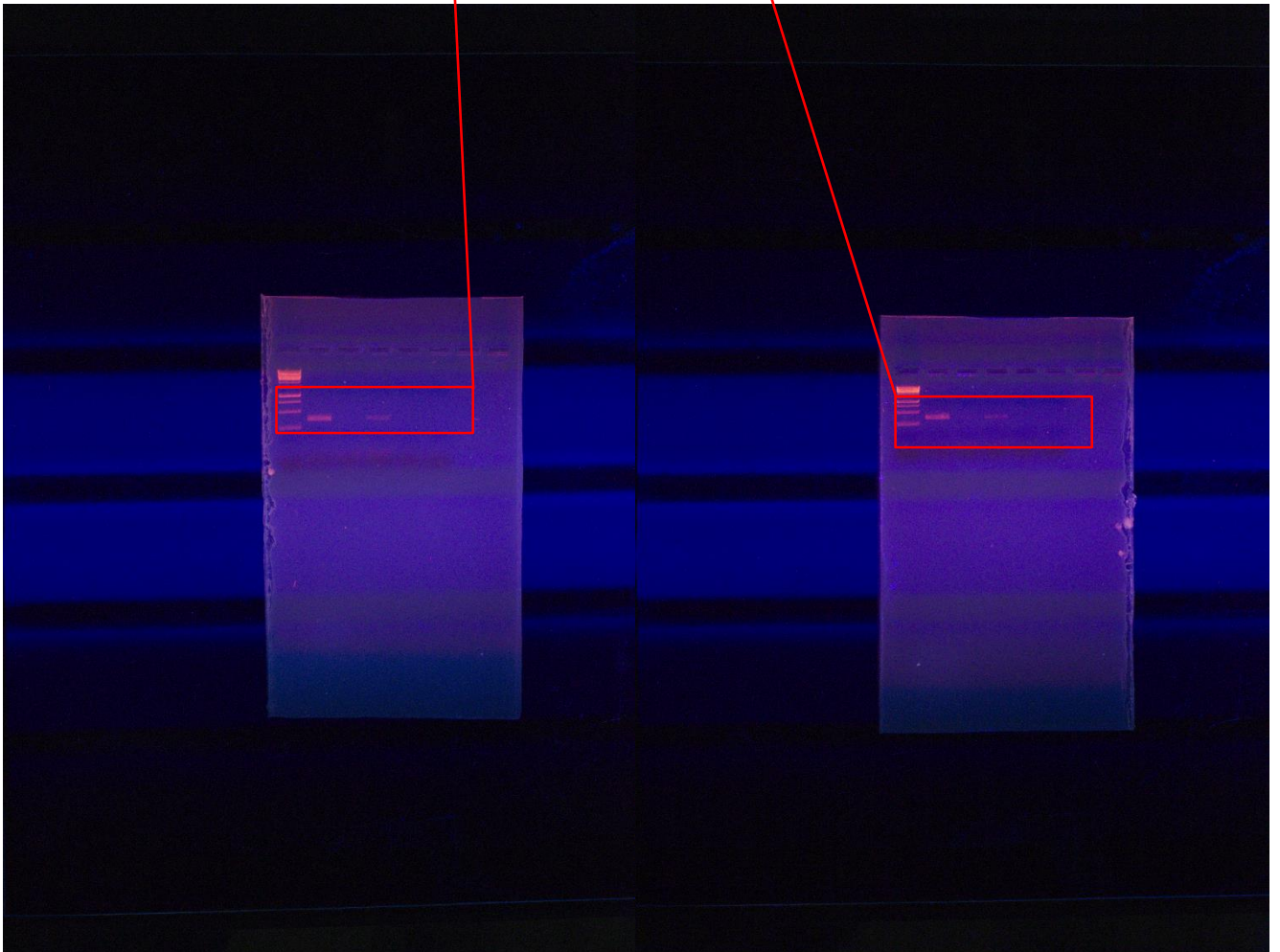
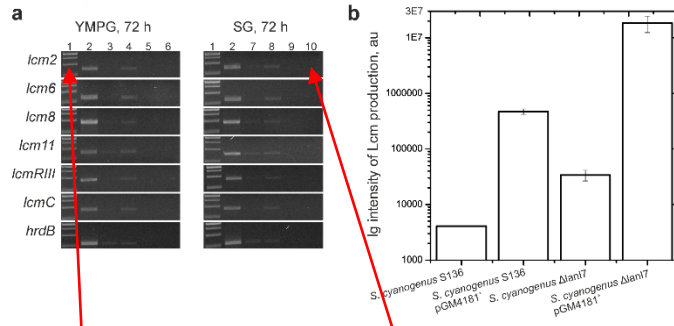




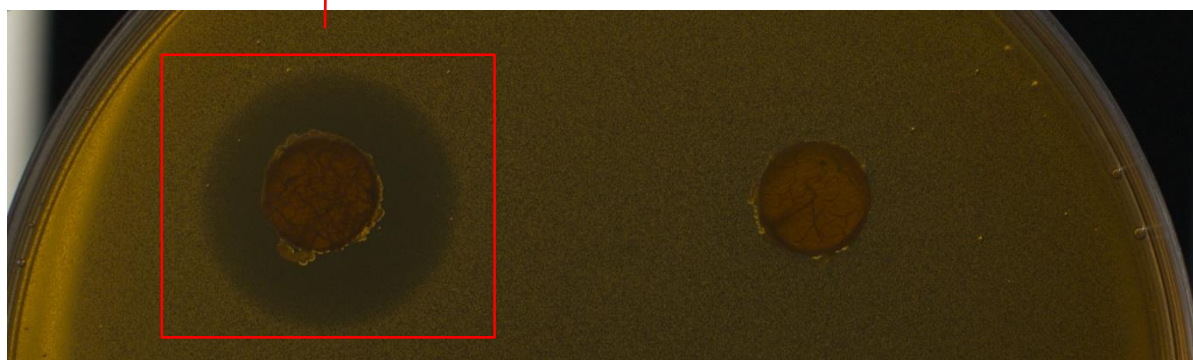
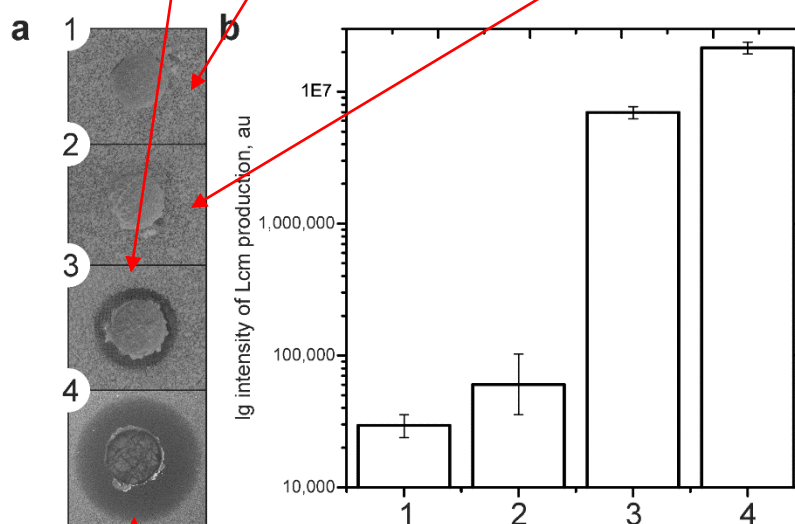
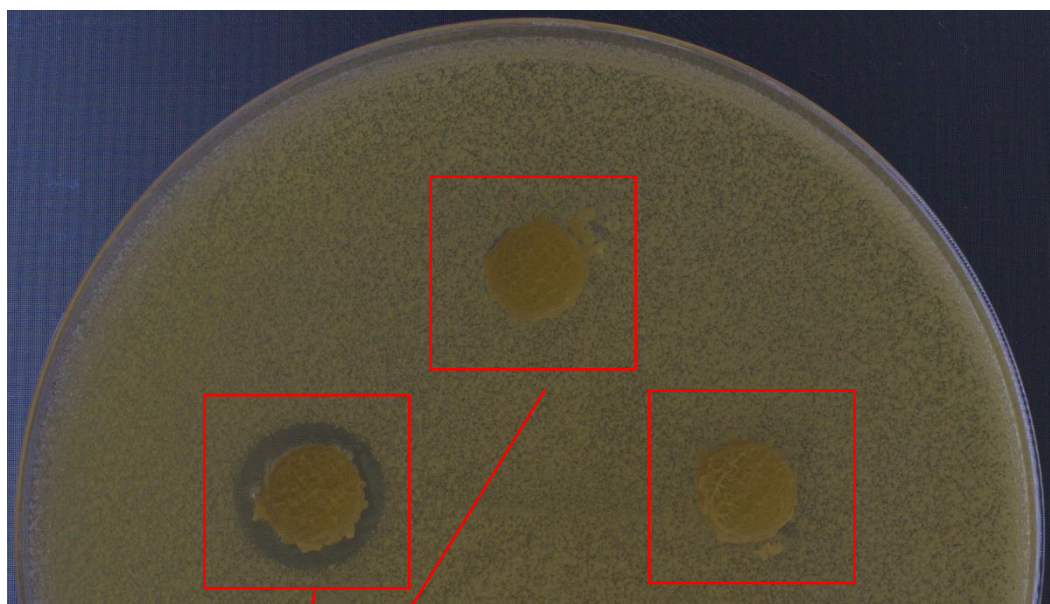




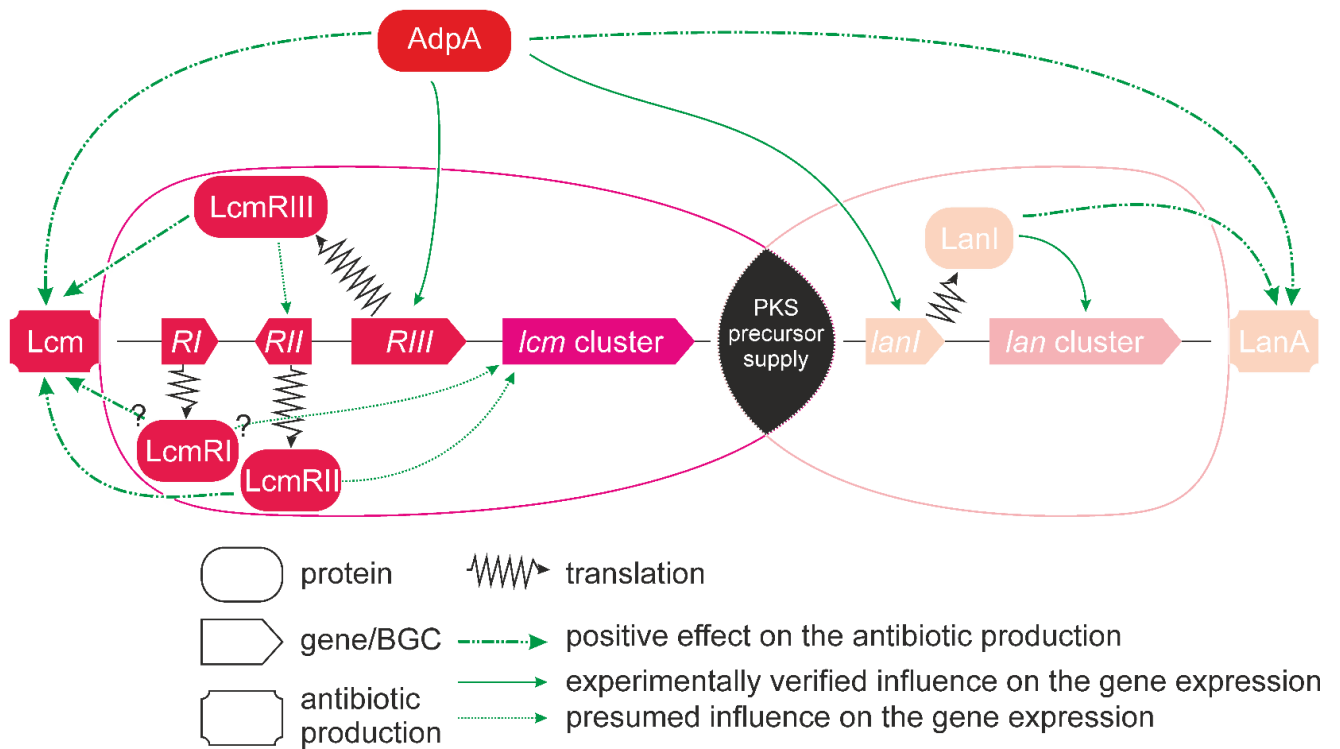




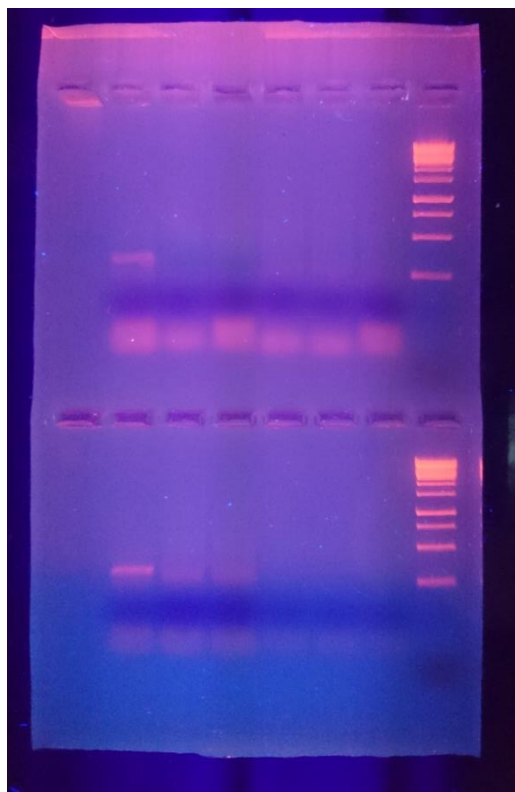
Original versions of photos from main text Fig. 5.



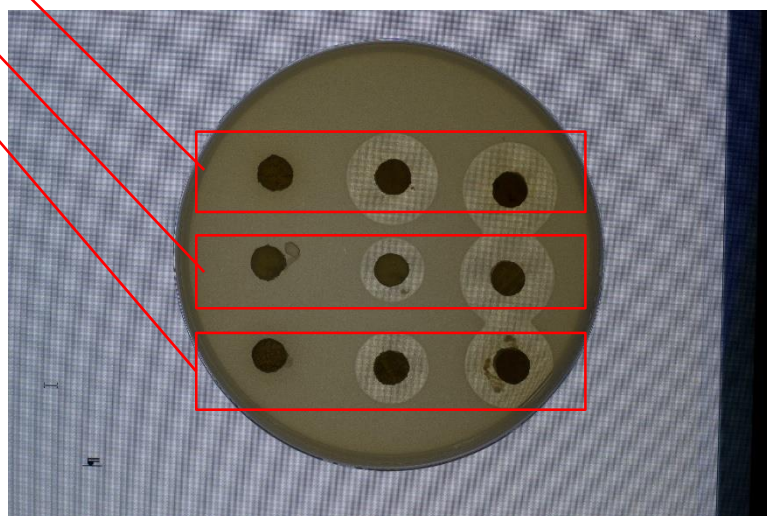
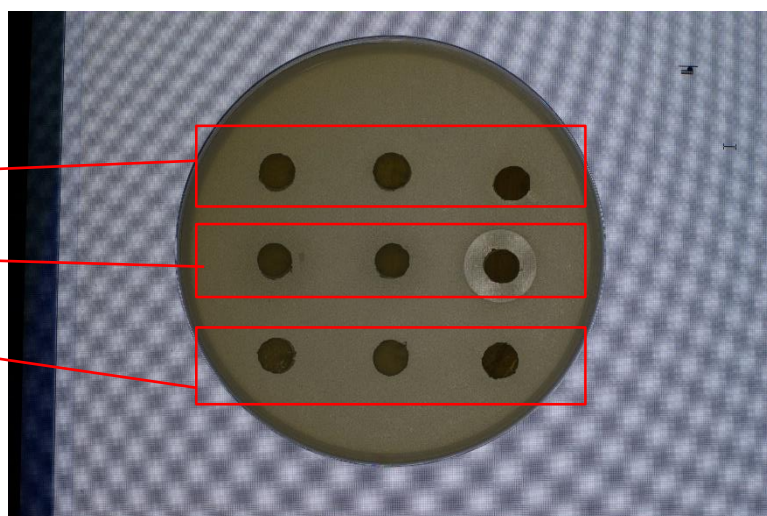
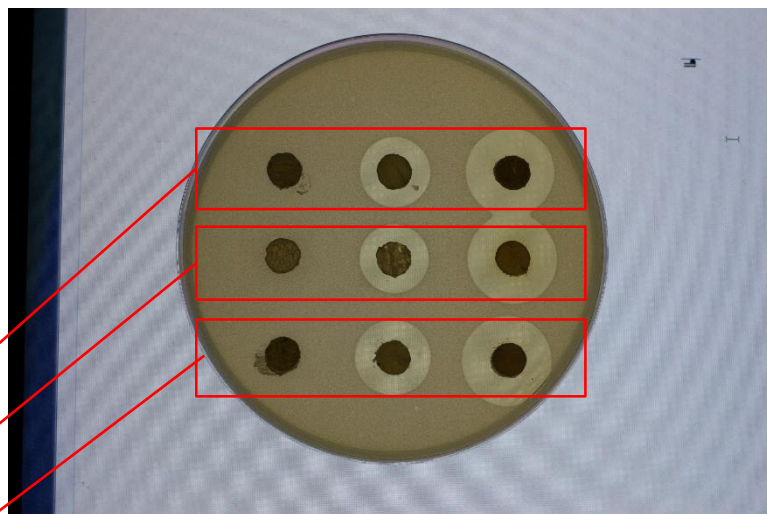
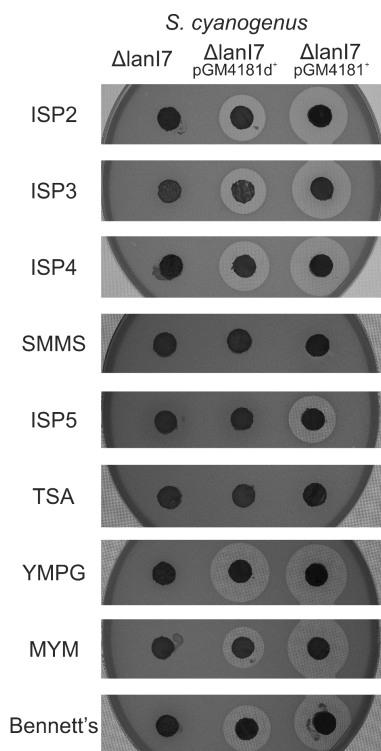
Original version of Fig. 6.



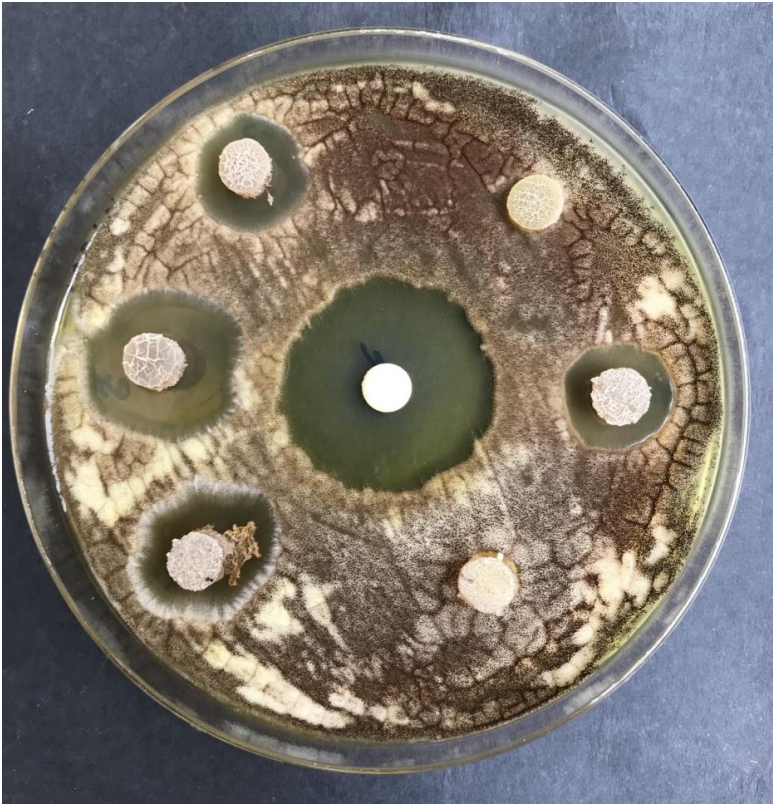
Original version of the agarose gel shown in Fig. S2



Original versions of the photos from ESM Fig. S3



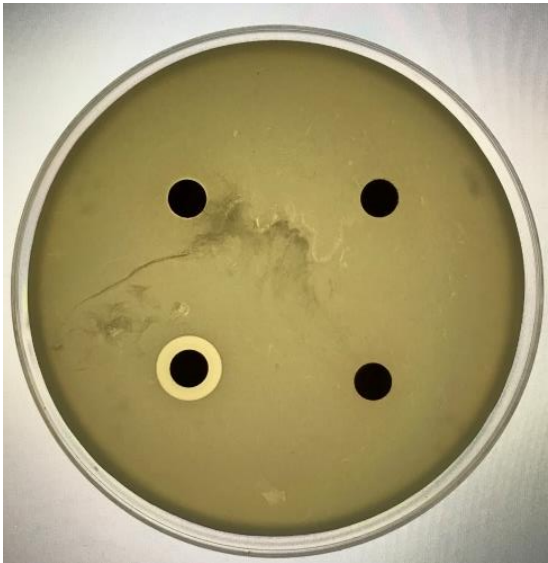
Original versions photos of Petri dishes shown in Fig. S5



Original versions photos of Petri dishes shown in Fig. S7a



Original versions photos of Petri dishes shown in Fig. S8a



Original versions of the photo from ESM Fig. S11

