Supporting information

Experimental procedures in Supporting information

Strain construction and confirmation.

Construction of pIJ12141: Two 2 kb fragments PCR-amplified with primer pairs act71/act73 and act90/act92 were cloned into pKC1132 using *Hin*dIII/*Bam*HI and *Bam*HI/*Eco*RI restriction sites contained in the vector or designed into the oligonucleotide primers.

Construction of pIJ12142: Two 2 kb fragments PCR-amplified with primer pairs red77/red78 and red96/red98 were cloned into pKC1132 using *Hin*dIII/*Xba*I and *Xba*I/*Eco*RI restriction sites contained in the vector or designed into the oligonucleotide primers.

Construction of pIJ12143: Two 2 kb fragments PCR-amplified with primer pairs cpK69/cpk70 and cpk87/cpk88 were cloned into pKC1132 using *Hin*dIII/*Xba*I and *Xba*I/*Eco*RI restriction sites contained in the vector or designed into the oligonucleotide primers.

Construction of pIJ12144: The kanamycin resistance gene *neo* was excised from pTC192-Km with *Xba*I and cloned into the *Xba*I site of pIJ12143, with a 5'-3' orientation from cpk70 to cpk87.

Construction of pIJ12145: Cosmid StE63 was PCR-targeted with the *aac(3)IV-oriT* cassette from pIJ773 amplified with primers cdaRDT1 and cdaRDT2 as previously described (Gust *et al*., 2003; Gust *et al*., 2004) resulting in the deletion of *cdaPSI* and most of the *cdaPSII* open reading frame. Desired clones were confirmed by PCR with primers cdaTST1 and cdaTST2.

Construction of pIJ12146: Two 2 kb fragments PCR-amplified with primer cda10/cda11 and cda47/cda49 were cloned into pKC1132 using *Hin*dIII/*Xba*I and *Xba*I/*Eco*RI restriction sites contained in the vector or designed into the oligonucleotide primers.

Construction of pIJ12149: A 759 bp *Pst*I/*Bam*HI DNA fragment containing *rpsL* was subcloned from cosmid StD40A in pBluescript II KS(+) digested with the same enzymes, and used for *in vitro* directed mutagenesis using QuikChange® Kit (Stratagene) and the oligonucleotides a43g_c52t/a43g_c52t_antisense, designed with the web-based tool "QuikChange® Primer Design Program" provided by Stratagene. After identifying a mutant clone by sequencing, a 3978 bp *Bam*HI/*Not*I fragment containing the region downstream of *rpsL*, and a 6090 bp *Not*I/*Pst*I fragment containing the region upstream of *rpsL*, were cloned from cosmid StD40A into the mutant clone to provide sufficient flanking homology for efficient recombination. Finally, the *aac(3)IV-oriT* cassette from pIJ773 was inserted into the construct with *Eco*RI/*Hin*dIII, yielding pIJ12149.

Construction of pIJ12150: A 13166 bp *Pst*I fragment containing *rpoB* was subcloned from cosmid StD40A in pBluescript II KS(+). The *aac(3)IV-oriT* cassette from pIJ773 was introduced upstream of the putative promoter of *rpoB* by PCR-targeting with primers rpoB_RDT1 and rpoB_RDT2 (Gust *et al*., 2003; Gust *et al*., 2004). Desired clones were confirmed by PCR with primers rpoB_TST1 and rpoB_TST2.

Construction of pIJ12151: A *Bam*HI/*Pst*I 4825 bp DNA fragment containing *rpoB* was subcloned from cosmid StD40A in pBluescript II KS(+) and used for *in vitro* directed mutagenesis using QuikChange® kit (Stratagene) and the oligonucleotides c1633t/c1633t_antisense, designed with the web-based tool "QuikChange® Primer Design Program" provided by Stratagene. After identifying a mutant clone by sequencing with oligonucleotides rpoBmut_tst1 and rpoBmut_tst2, a 3496 bp *Not*I/*Mlu*I fragment containing the region upstream of *rpoB* was cloned from cosmid StD40A into the same sites of the mutant clone, providing sufficient homology for efficient recombination. Finally, the *neo-oriT* cassette from pIJ776 was inserted into the construct with *Eco*RI/*Cla*I, yielding pIJ12151

Genomotyping with Affymetrix arrays. Genomic DNA was isolated from M145 and M1146 using a phenol-based method (Weaver *et al*., 2004) and from M145 and M1154 using a salting-out procedure (Kieser *et al*., 2000). Both methods gave equivalent labeling efficiency. Labeling was performed with BioPrime DNA Labelling System Kit (Invitrogen) using a modified protocol: 60 μl of 2.5X random primers were added to 300 ng of DNA dissolved in 72 μl water, the DNA denatured at 98 ºC for 10 min, followed by cooling to 4 ºC for 10 min; 15 μl 10X dNTP mix and 3 μl Klenow (40 U/μl) were added and the mixture incubated at 25 ºC for 18 h (all previous incubation steps were performed in a thermocycler); DNA was precipitated by adding 15 μl sodium acetate 3 M pH 5.2 and 400 μl ethanol -20 ºC, followed by incubation at -80 ºC for 1 h, and centrifugation at 13000 rpm at 4 ºC for 1 h; the pellet was washed with 70% ethanol in water, air dried for 10 min, and re-suspended in 110 μl of water. Labeling quality control was performed using a gel-shift assay: For a positive control, 5 μl of labeled product were mixed with 2 μl of 2 mg/ml NeutrAvidin; for a negative control, 5 μl of labeled product were mixed with 2 μl water; both were loaded immediately onto a 2% agarose gel in TBE buffer (Sambrook *et al*., 1989) and run at 99 V for 15 min. Affymetrix chip hybridization, washing and scanning was performed as described (Hesketh *et al*., 2007). Raw data files (CEL files) were loaded into GeneSpring 7, processed with the RMA algorithm and "per chip" normalized to the 50th percentile. M145 versus M1146 genomotyping was performed once, while M145 versus M1154 genomotyping was performed three times. Normalized intensities were displayed on a scatter-plot, with M145 and derived mutants along the X and Y axis, respectively (Fig. S1). We chose a two-fold threshold to identify differentially represented genes. Deleted genes were used as negative controls and the fragments used for homologous recombination as positive controls. All of the positive controls were within the +/- two-fold threshold, while most of the negative controls were well below the two-fold threshold. The higher than expected ratio of some of the negative controls is thought to reflect cross-hybridization with other regions of the genome. For example, while several deleted genes from the *cda* cluster gave higher than expected intensities, Southern blotting and hybridization with a probe comprising the deleted genes confirmed their absence, but also identified cross-hybridising DNA fragments from elsewhere in the genome (Fig. S1C). All of the probes representing non-deleted genes grouped along the diagonal, within the +/- two-fold threshold, indicating that no gene deletions other than those expected or extraneous gene amplifications had occurred during strain construction (Fig. S1A,B).

Table S1: Oligonucleotides used in this study.

Name	Sequence	Genome coordinates	Notes
act71	GGCGGaAGCTtAGCAGCAACTC	5513841..5513862	Deletion of actinorhodin gene cluster*
act73	GACCGgaTCCCTGCTCCAGGAC	c(5515912..5515933)	
act90	ACGCTGGATCCCGACACCCTGA	5532919..5532940	
act92	GCAGGaAttCGCCGCCGAGCAC	c(5535028..5535049)	
red77	CGGCAAGcTtCGTACGCTGGTG	6432862..6432883	Deletion of prodiginines gene cluster*
red78	CCGtCtAGaCCGGCGTTGGCGAA	c(6434856..6434878)	
red96	GTCGTCtAGAGCGCGGACACC	6462141..6462161	
red98	CGTCGAAttCCTCGCCGTATCGC	c(6464140..6464162)	
cpk69	TCCCCAagcTTGAACAACGTG	6894164..6894184	Deletion of *cpk* gene cluster*
cpk70	ACCTTCtAGaCGGAGGACGAGA	c(6896173..6896194)	
cpk87	CGTGGTCtaGATCGTCGAGCC	6946389..6946409	
cpk88	GCGAGGAatTCGTCGGAGCCGA	c(6948344..6948365)	
cdaRDT1	CACACATCTTGTTCGTTTTGCATTGTGAGGAG ACAGGTGATTCCGGGGATCCGTCGACC	3543298..3543336	Substitution of *cdaPSI* and *cdaPSII* with *aac(3)IV* by PCR-targeting†
cdaRDT2	CCGGTCCTCGCGCACCACGGCCACCGCCTCC GGCACCCCTGTAGGCTGGAGCTGCTTC	c(3574692..3574730)	
cdaTST1	GCTCCTACCTGCGGTTATCTTCC	3543187..3543209	
cdaTST2	AACGCCGACGGCACCATGTAGTC	c(3574833..3574855)	
cda10	CCGCGaAGCTtACGCAGCAACC	3519458..3519479	Deletion of CDA gene cluster*
cda11	CATCGtCtAGATGAAGCCCCGCA	c(3521498..3521520)	
cda47	CGACCTCtAGaCGGGCCAGCAC	3600279..3600300	
cda49	GCACGGaATTCTGGCCGAGGATC	c(3602271..3602293)	
rpsL_RDT1	AGCGGGCGGCGATGCCGGTGTCGCCCATTTG TTTTGACCATTCCGGGGATCCGTCGACC	5088690..5088728	Introduction of *rpsL* K88E and P91S mutations†
rpsL_RDT2	CCAGGCACAAGGTATGAGCGTACCTAGCGCA TTCGCTGCTGTAGGCTGGAGCTGCTTC	c(5088729..5088767)	
rpsL_TST1	CTGCTGGTCTACACGCTGAC	5088417..5088436	
rpsL_TST2	TTCAGCAGCACCTTGTTGAT	c(5089397..5089416)	
a43g_c52t	CGGCCGTGTGgAGGACCTGtCGGGTGTTCG	5089196..5089225	
a43g_c52t_antisense	CGAACACCCGaCAGGTCCTcCACACGGCCG	c(5089196..5089225)	

Table S1 continues on the next page.

Table S1: Oligonucleotides used in this study (continued).

Name	Sequence	Genome coordinates	Notes
rpoB_RDT1	CGAAGAGCGATCATCCATCCGGGTGGTCGCT CTTCGGCGATTCCGGGGATCCGTCGACC	5077519..5077557	Introduction of *aac(3)IV* upstream of *rpoB* by PCR-targeting†
rpoB_RDT2	CGTCACTCGTGCAAGGCGACCGCCGCCGGAC CCCGGATATGTAGGCTGGAGCTGCTTC	c(5077558..5077596)	
rpoB_TST1	TGCCGAATTAGTGGAAGGAC	5077060..5077079	
rpoB_TST2	TCAGACATGCTCTGGTCGTC	c(5077943..5077962)	
rpoBmut_tst1	GATCCAGAACCAGGTCCGTA	5079169..5079188	Introduction of *rpoB* S433L mutation‡
rpoBmut_tst2	GATGACGAAGCGGTCCTC	c(5079623..5079640)	
c1633t	CCAGAACAACCCGCTGTtGGGGCTGAC	5079340..5079366	
c1633t_antisense	GTCAGCCCCaACAGCGGGTTGTTCTGG	c(5079340..5079366)	
rpoBsq1	GGTACGGCGTCTCGATGAAG	c(5079547..5079566)	
Cm84F	GACATCCAGCGGTCCAAC	Not applicable	Analysis of expression of chloram-phenicol genes by qRT-PCR
Cm84R	CGGAGTTCCTCGAAGACG	Not applicable	
Cm86F	ACGAGATCTGCCTGACCAAC	Not applicable	
Cm86R	CCTTGATGGGCTTGGACTC	Not applicable	
Cm88F	GGACTTCCTGCCCCTGTC	Not applicable	
Cm88R	AACCAGTCGTCCTTGAGGTG	Not applicable	
Sco4742F	CAGCCGTGCCATCAGTTC	5157225..5157242	qRT-PCR controls (Hesketh *et al.*, 2009)
Sco4742R	ACTGCGTACAGCGTGGAAAC	c(5157262..5157281)	
hrdBF	TCGACTACACCAAGGGCTAC	6368617..6368636	
hrdBR	GTTGATGACCTCGACCATGT	c(6368725..6368744)	

*Sites for restriction endonucleases are underlined. Lower case letters indicate differences from the original sequence.

†Universal sequences for cassette amplification (Gust *et al.*, 2003; 2004) are underlined. Lower case letters indicate differences from the original sequence.

‡Lower letters indicate differences from the original sequence.

Table S2: Vectors and constructs used in this study.

Vector/Construct	Description	Reference
pAH91	Conjugative and ϕC31-integrative cosmid containing chloramphenicol gene cluster from *S. venezuelae*	Andy Hesketh, unpublished
pAH88	Conjugative and ϕBT1-integrative SCBAC28G1	Andy Hesketh, unpublished
pBluescript II KS(+)	General cloning vector	Alting-Mees and Short, 1989
pCGC002	Conjugative and ϕC31-integrative cosmid containing congocidine gene cluster from *S. ambofaciens*	Juguet *et al.*, 2009
pGEM-T-easy	T-cloning vector for Taq-PCR products	Promega Corp.
pIJ773	pBluescript II KS(+) containing *aac(3)IV-oriT* cassette	Gust *et al.*, 2003; 2004
pIJ776	pBluescript II KS(+) containing *neo-oriT* cassette	Gust *et al.*, 2003; 2004
pKC1132	Cloning vector, RP2-conjugative	Bierman *et al.*, 1992
pTC192-Km	Source of *neo* (kanamycin resistance gene)	Rodríguez-García *et al.*, 2006
StE63	Cosmid from *S. coelicolor* genomic library	Redenbach *et al.*, 1996
pIJ12141	Deletion of actinorhodin gene cluster	This work
pIJ12142	Deletion of prodiginines gene cluster	This work
pIJ12143	Deletion of PKS-I *cpk* gene cluster	This work
pIJ12144	Replacement of PKS-I *cpk* gene cluster with *neo*	This work
pIJ12145	Replacement of *cdaPSI* and *cdaPSII* with *aac(3)IV*	This work
pIJ12146	Deletion of remaining CDA gene cluster	This work
pIJ12149	Replacement of wild-type *rpsL* with mutated *rpsL*	This work
pIJ12150	Introduction of *aac(3)IV* upstream of *rpoB*	This work
pIJ12151	Replacement of wild-type *rpoB* with mutated *rpoB*	This work

Table S3: Strains used in this study.

Strain	Genotype or Comments	Reference
Escherichia coli DH5alpha	F$^-$ ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-	Hanahan, 1983
E. coli BW25113[pIJ790]	K-12 derivative (Δ*araBAD*, Δ*rhaBAD*) carrying plasmid pIJ790	Gust *et al.*, 2003; 2004
E. coli ET12567[pUZ8002]	*dam dcm hsdM hsdS hsdR cat tet*, carrying plasmid pUZ8002	Gust *et al.*, 2003; 2004
Micrococcus luteus ATCC 4698	Bioassay indicator microorganism	ATCC
S. coelicolor M145	SCP1$^-$ SCP2$^-$ derivative from A3(2) [wild type]	Kieser *et al.*, 2000
S. coelicolor M1141	Δ*act*	This work
S. coelicolor M1142	Δ*act* Δ*red*	This work
S. coelicolor M1143	Δ*act* Δ*red* Δ*cpk::neo*	This work
S. coelicolor M1144	Δ*act* Δ*red* Δ*cpk*	This work
S. coelicolor M1145	Δ*act* Δ*red* Δ*cpk* Δ*cdaPSI&II::aac(3)IV*	This work
S. coelicolor M1146	Δ*act* Δ*red* Δ*cpk* Δ*cda*	This work
S. coelicolor M1151	Δ*act* Δ*red* Δ*cpk* Δ*cda* with *aac(3)IV* upstream *rpoB*	This work
S. coelicolor M1152	Δ*act* Δ*red* Δ*cpk* Δ*cda* *rpoB*(C1298T)[†]	This work
S. coelicolor M1153	Δ*act* Δ*red* Δ*cpk* Δ*cda* *rpoB*(C1298T)[†] *rpsL*(A262G C271T)[‡]	This work
S. coelicolor M1154	Δ*act* Δ*red* Δ*cpk* Δ*cda* *rpoB*(C1298T)[†] *rpsL*(A262G)[§]	This work*
S. coelicolor M1155	Δ*act* Δ*red* Δ*cpk* Δ*cda* *rpsL*(A262G C271T)[‡]	This work
S. coelicolor M1156	Δ*act* Δ*red* Δ*cpk* Δ*cda* *rpsL*(A262G)[§]	This work*

*Selected as spontaneously resistant mutant at 5 μg/ml of streptomycin.

[†]*rpoB*(S433L) in amino acid notation

[‡]*rpsL*(K88E P91S) in amino acid notation

[§]*rpsL*(K88E) in amino acid notation

Results in Supporting information

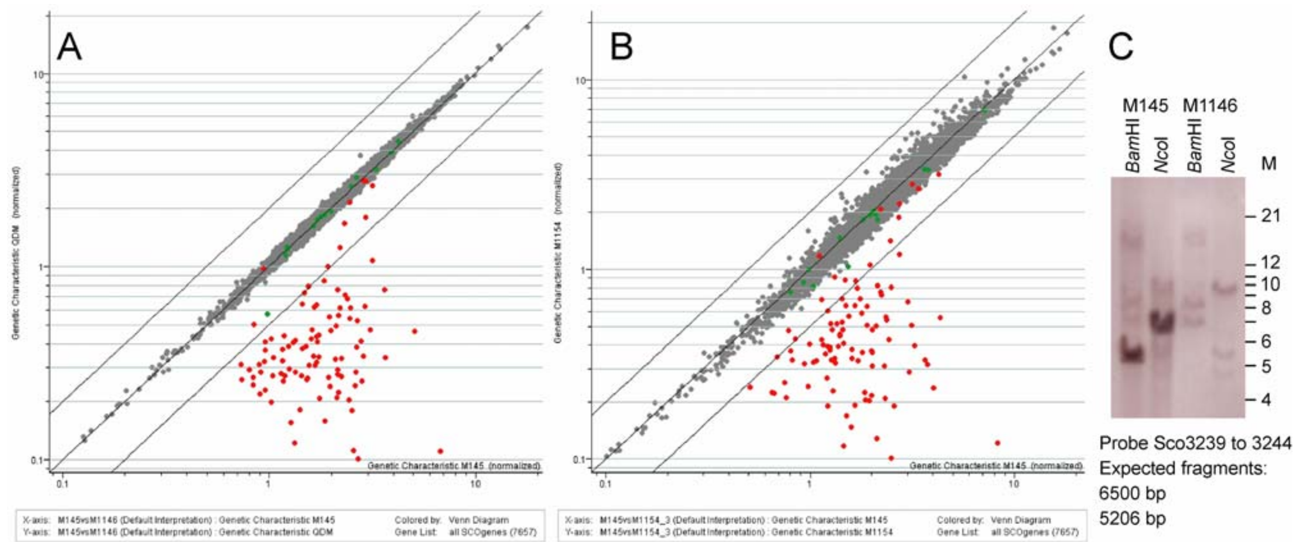


Fig. S1. Genomotyping analysis. **A** Scatter plot of normalized data from the quadruple deletion mutant (QDM) M1146 (Y-axis) versus M145 (X-axis). **B** Scatter plot of normalized data from M1154 (Y-axis) versus M145 (X-axis). In green, positive controls (fragments used for homologous hybridization); in red, negative controls (deleted genes); in grey, rest of the genes; diagonal lines from top to bottom, two-fold increase in intensity, 1:1 ratio and two-fold decrease in intensity. **C** Southern blot of digested DNA from M145 and M1146 hybridized with a probe derived from some of the genes deleted from the *cda* gene cluster that gave higher than expected intensities upon genomotyping. The expected fragments are detected only in M145 and not in M1146, while several cross-hybridizing fragments are observed in both strains.

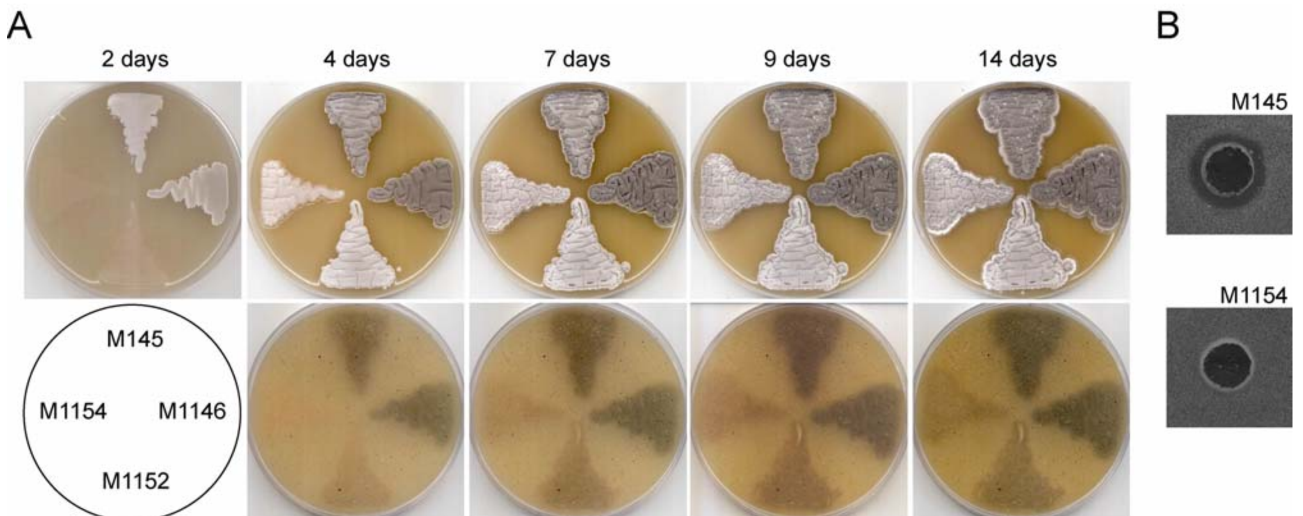


Fig. S2. Growth and differentiation. **A** Growth and sporulation on SFM agar medium. Plates were scanned from above (top row) and below (bottom row; the image was inverted horizontally for easier interpretation) at the indicated times. Note the delayed growth and reduction in spore pigmentation in M1152 and M1154. **B** Antimicrobial activity detected in M145 but not in M1154 GYM culture supernatants.

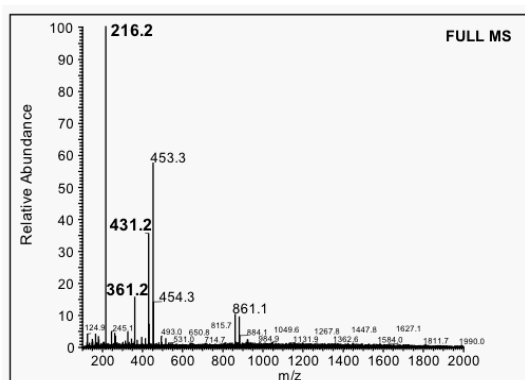
Table S4: Data for Figure 3 for chloramphenicol (CP) (Fig. 3A) and congocidine (CGC) (Fig. 3B) production. Strains were grown in liquid GYM medium inoculated with pre-germinated spores (chloramphenicol gene cluster) or with mycelium from a seed culture (congocidine gene cluster).

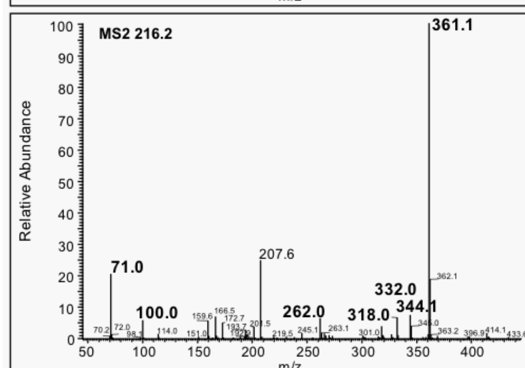
Fig.3A		Exponential growth phase		Stationary growth phase	
		Average	**Std. Dev.**	**Average**	**Std. Dev.**
Growth	**M145**	1.0767	0.0666	2.9500	0.0707
	M1146	0.9933	0.0513	2.2767	0.4637
	M1152	0.8767	0.1172	1.5200	0.0458
	M1154	1.0433	0.0306	1.2600	0.2007
µg CP/ml	**M145**	0.0387	0.0085	1.6068	0.5586
	M1146	0.0365	0.0030	4.6783	1.8549
	M1152	1.4349	0.3466	41.2412	5.7272
	M1154	5.0205	1.4593	26.2375	4.5116
µg CP/mg DCW	**M145**	0.0359	0.0075	0.5471	0.2025
	M1146	0.0367	0.0020	2.1439	1.0503
	M1152	1.6487	0.4385	27.2231	4.5859
	M1154	4.8014	1.3125	21.1335	4.8376
***hrdB* qPCR**	**M145**	117325.8333	68642.2372	347097.8333	32145.3100
	M1146	186474.2556	121387.0212	541392.7778	374769.5212
	M1152	117831.2444	17886.4626	912278.6667	279271.6165
	M1154	236464.0000	52542.6905	222504.2222	125190.6784
***sco4742* qPCR**	**M145**	8537.9511	20930.4167	4357.5732	5296.4419
	M1146	11419.1344	18153.6444	4222.9909	1950.2305
	M1152	12918.5111	43531.3111	285.0669	34175.8477
	M1154	4387.8222	13101.7189	2213.0560	3519.5415
***orf2* qPCR**	**M145**	5997.6789	2708.6750	12902.7733	4417.0981
	M1146	9368.1344	6863.8200	37073.1889	8630.9096
	M1152	37480.0333	25012.4300	82189.9333	60593.1332
	M1154	150824.9333	86713.3717	53790.1944	13202.7759
***orf3* qPCR**	**M145**	5515.3667	2638.7566	12976.3833	2234.6696
	M1146	7523.9933	4836.7022	40799.3444	8702.7966
	M1152	37306.2778	19727.5478	88491.8444	71472.0926
	M1154	165817.4000	103186.7773	47353.8889	12366.3220
***orf8* qPCR**	**M145**	7893.9733	4254.7533	20145.3333	4968.6979
	M1146	14153.6789	8041.3617	60031.9000	15219.8171
	M1152	55394.2556	23811.3928	99400.3778	76083.8014
	M1154	209497.4667	107438.6929	69342.2667	17018.3550

Table S4 continues on the next page.

Table S4: Data for Figure 3 (continued).

Fig.3B		8 hours		3.3 days		6 days	
		Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
Growth	M145	1.8633	0.1007	2.4350	0.4031	1.9367	0.2053
	M1146	2.4367	0.3800	2.3733	0.3592	1.8700	0.1905
	M1152	2.6200	0.3161	2.1900	0.0854	1.9300	0.3291
	M1154	3.1667	0.6901	2.7200	0.3372	1.9133	0.2250
CGC(Area Units/ml)	M145	20.0000	7.2111	1744.6667	673.0002	1609.3333	338.5444
	M1146	1465.0000	1062.0909	10420.3333	8567.3020	9765.0000	8459.9490
	M1152	205.3333	38.5270	39214.0000	523.3670	38110.3333	1105.3417
	M1154	264.6667	38.5270	52856.6667	21188.5856	52527.0000	21436.3807
CGC (Area Units/mg DCW)	M145	10.6501	3.3488	850.0667	55.0184	834.0908	168.0351
	M1146	604.3239	429.0248	4171.7267	2877.3079	5167.5758	4175.6145
	M1152	80.2932	24.4773	17927.9372	861.6937	20100.9375	3225.0864
	M1154	85.2809	16.2938	20010.9101	9014.9016	27264.0233	9473.3562





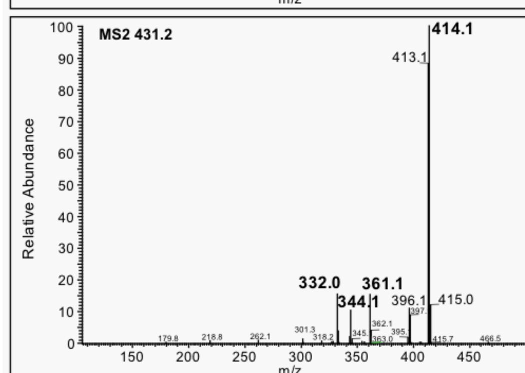


Fig. S3. Identification of congocidine. MS/MS analysis of a fraction of the large peak eluting at 10.5 min in the chromatogram from M1154 containing the congocidine gene cluster (Fig. 4). 431 m/z corresponds to congocidine + 1 hydrogen ion ; 453 m/z corresponds to the sodium adduct; 216 m/z corresponds to doubly charged congocidine + 2 hydrogen ions; See Juguet *et al.* (2009) for further explanation of the MS/MS data and fractionation spectra.

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