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Carbon catabolite repression regulates the production of the unique volatile sodorifen of *Serratia plymuthica* 4Rx13

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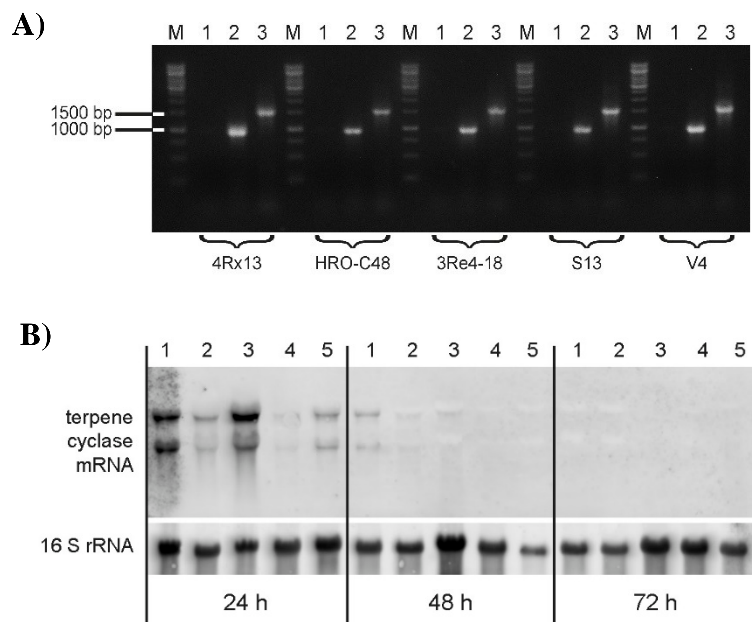

Figure S1: Expression of sodorifen cluster in different producer strains. Expression status of the sodorifen cluster genes was assessed in the different producer strains *Serratia plymuthica* 4Rx13, HRO-C48, 3Re4-18, S13 and V4 after 24 h cultivation in minimal medium + 55mM succinate. **A)** RT-PCR was performed with primers specific for the terpene cyclase gene of the sodorifen cluster to prove expression in all producing strains (lane 2). Lane 1: negative control (without reverse transcriptase), lane 3: positive control (16 S rRNA). For each bacterial isolate 1 µg of RNA was used as a template. M = marker (GeneRuler 1 kb). **B)** Northern blot was performed to quantify expression levels of the terpene cyclase gene in the sodorifen producers after 24 h, 48 h and 72 h cultivation (1: *S.p.* 4Rx13, 2: *S.p.* HRO C48, 3: *S.p.* 3Re4-18, *S.p.* S13, 5: *S.p.* V4). Blotting was done with 5 µg RNA each and DIG-dUTP-labelled probes specific for the terpene cyclase (upper panel) and the 16 S rRNA (lower panel) as a positive control. Finally, labelled RNA molecules were detected by fluorescence measurement for 1 min.

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4Rx13       GGTATTTCCTCTTACTGGCTCGCTATAGATCCATCCGATTTGACACCATGACGCAGGCGG   60
HRO-C48     GGTATTTCCTCTTGCTGGCTCGCTATAGATCCATCCGATTTGACACCATGACGCAGGCGG   60
3Re4-18     GGTATTTCCTCTTGCTGGCTCGCTATAGATCCATCCGATTTGACACCATGACGCAGGCGG   60
S13         GGTATTTCCTCTTGCTGGCTCGCTATAGATCCATCCGATTTGACACCATGACGCAGGCGG   60
V4          GGTATTTCCTCTTGCTGGCTCGCTATAGACCCATCCGATTTGACACCATGACGCAGGCGG   60
            ************* *************** ******************************

4Rx13       GAACAAGAAGTAAATGTTATGTTAACTGCAGCGACGCACAATAACCCCACTCTGCAATCA   120
HRO-C48     GAACAAGAAGTAAATGTTATGTTAACTGCAGCGACGCACAATAACCCCACTCTGCAATCA   120
3Re4-18     GAACAAGAAGTAAATGTTATGTTAACTGCAGCGACGCACAATAACCCCACTCTGCAATCA   120
S13         GAACAAGAAGTAAATGTTATGTTAACTGCAGCGACGCACAATAACCCCACTCTGCAATCA   120
V4          GAACAAGAAGTAAATGTTATGTTAACTGCAGCGACGCACAATAACCCCACACTGCAATCA   120
            ************************************************** *********

4Rx13       ACATGACCGAGACGCTTCATTACGGTCGG-GACAGAGGGTATCGGTGAGCACGTAATATC   179
HRO-C48     ACATGACCGAGACGCTTCATTACGGTCGG-GACAGAGGGTATCGGTGAGCACGTAACATC   179
3Re4-18     ACATGACCGAGACGCTTCATTACGGTCGG-GACAGAGGGTATCGGTGAGCACGTAACATC   179
S13         ACATGACCGAGACGCTTCATTACGGTCGG-GACAGAGGGTATCGGTGAGCACGTAACATC   179
V4          ACATGACCGAGACGCTTCATTACGGTCGGGGACAGAGGGTATCGGTGAGCACGTAACATC   180
            ***************************** ************************** ***

4Rx13       ATTGGGCTAGCCGACCCCTTTGTTTCTGTAGCCGAAAACATCCATCGCCTGGCTTTAACA   239
HRO-C48     ATTGGGCTAGCCGACCCTTTTGTTTCTGTAGCCGAAAACATCCATCGCCTGGCTTTAACA   239
3Re4-18     ATTGGGCTAGCCGACCCTTTTGTTTCTGTAGCCGAAAACATCCATCGCCTGGCTTTAACA   239
S13         ATTGGGCTAGCCGACCCTTTTGTTTCTGTAGCCGAAAACATCCATCGCCTGGCTTTAACA   239
V4          ATTGGACTAGCCGACCCTTTTGTTTCTGTAGCCAAAAATATTCATCACCTGGCTTTGACA   240
            ***** *********** *************** **** ** **** ********* ***

4Rx13       AAAAATAGTTGCACCCTAGCAAAACTCCCACATCGTTGAATTAACATTAAGTTAAACCAT   299
HRO-C48     AAAAATAGTTGCACCCTAGCAAAACTCCCACATCGTTGAATTAACATTAAGTTAAACCAT   299
3Re4-18     AAAAATAGTTGCACCCTAGCAAAACTCCCACATCGTTGAATTAACATTAAGTTAAACCAT   299
S13         AAAAATAGTTGCACCCTAGCAAAACTCCCACATCGTTGAATTAACATTAAGTTAAACCAT   299
V4          TAAAATAGTTTCGCCATAGCAAAACTCCCACATCGTTGAATTAACATTAAGTTAAACCAT   300
             ********* * ** ********************************************

4Rx13       AAGTTAAAAATATATCACTCGCTGATACTTCATCATAAAATAGTCATCATCATGATTAAT   359
HRO-C48     AAGTTAAAAATATATCACCAGCTGATACTTCATCATAAAATAGTCATCATCATGATTAAT   359
3Re4-18     AAGTTAAAAATATATCACCAGCTGATACTTCATCATAAAATAGTCATCATCATGATTAAT   359
S13         AAGTTAAAAATATATCACCAGCTGATACTTCATCATAAAATAGTCATCATCATGATTAAT   359
V4          AAGTTAAAAATATATCACCAGCTGATACTTCATCATAAAATAGTCATCATCATGATTAAT   360
            ******************  ****************************************

4Rx13       TTATTTTTCTTAAAAACCGTCATTAAATAAAATTTGACAATGAAAGAAATTCGTCTTTAA   419
HRO-C48     TTATTTTTATTAAAAGCCATCATTAAATAAAATTTGACAATGAAAGAAATGCGTCTTTAA   419
3Re4-18     TTATTTTTATTAAAAGCCATCATTAAATAAAATTTGACAATGAAAGAAATGCGTCTTTAA   419
S13         TTATTTTTATTAAAAGCCATCATTAAATAAAATTTGACAATGAAAGAAATGCGTCTTTAA   419
V4          TTATTTTTCTTAAAAACCATCATTAAATAAAGTTTGACAATGAAAAAAATGCGTCTTTAA   420
            ******** ****** ** ************ ************* **** *********

4Rx13       TTAAATTTCATTGATTAAAAAACACCCAATGCTCAATCGAATTTATTTCGGGGGTCACTC   479
HRO-C48     TTAAATTTCATTGATCAAAAAACACCCAATGCTCAATCGAATTTATTTCGGGGGTCACTC   479
3Re4-18     TTAAATTTCATTGATCAAAAAACACCCAATGCTCAATCGAATTTATTTCGGGGGTCACTC   479
S13         TTAAATTTCATTGATCAAAAAACACCCAATGCTCAATCGAATTTATTTCGGGGGTCACTC   479
V4          TTAAATTTCATTGATCAAAAAACACCCTATCTGCAATCGAATTTATTTCGGGGGTCACTC   480
            *************** *********** **   ***************************

4Rx13       G--------       480
HRO-C48     GTGGAAGAA       488
3Re4-18     ---------       479
S13         GTGGAAGAA       488
V4          ---------       480
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Figure S2: Analysis of the 5'-UTR in the sodorifen producer strains *Serratia plymuthica.* 4Rx13, HRO-C48, 3Re4-18, S13 and V4. Alignment was performed with the Clustal Omega online software (Sievers et. al. 2011). Red letters indicate differences in the sodorifen producing strains in comparison to *S. p.* 4Rx13. Asterisks represent matches between all sequences and dashes deletions.

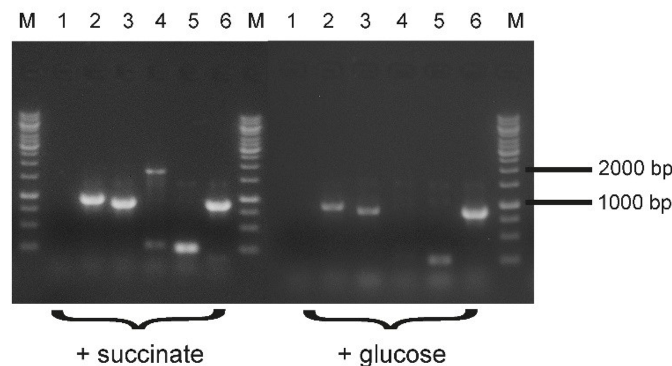
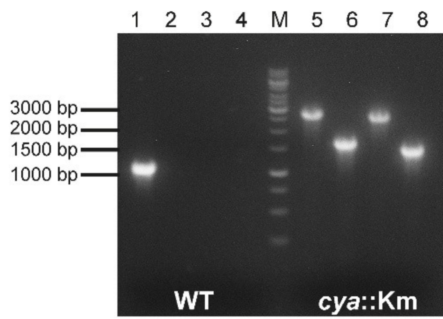


Figure S3: Expression of the sodorifen cluster depending on the carbon source. Expression status of the sodorifen cluster genes was assessed in *Serratia plymuthica* 4Rx13 after 24 h cultivation in minimal medium supplemented with either 55 mM succinate or glucose. RT-PCR was performed with primers specific for the terpene cyclase (lane 2), methyltransferase (lane 3), DOXP synthase (lane 4) and IPP isomerase (lane 5) of the sodorifen cluster. Lane 1: negative control (without reverse transcriptase), lane 6: positive control (GAPDH). For each cultivation condition, 1 µg of RNA was used as a template. M = marker (GeneRuler® 1kb).

A)



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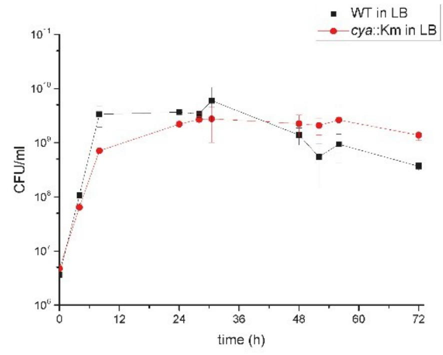
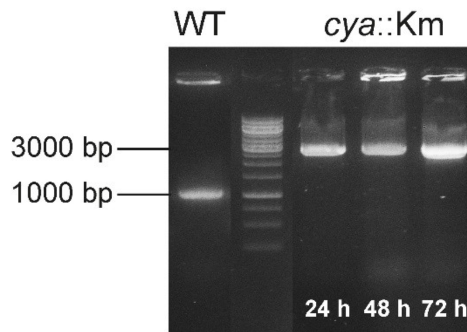
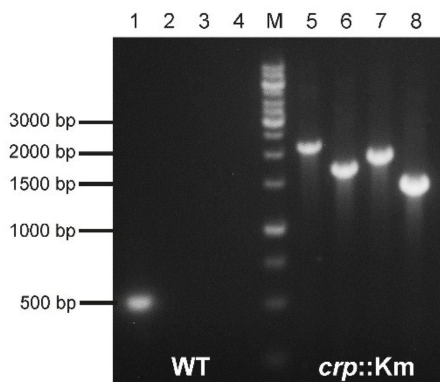


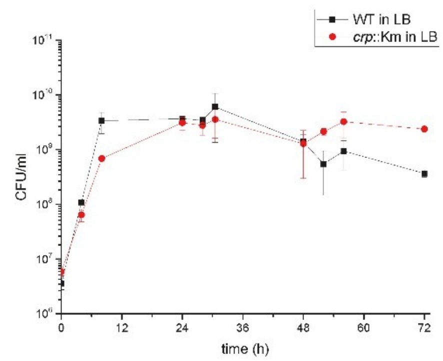
C)



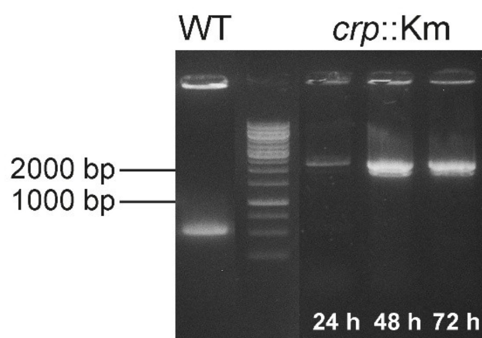
D)



E)



F)



G)

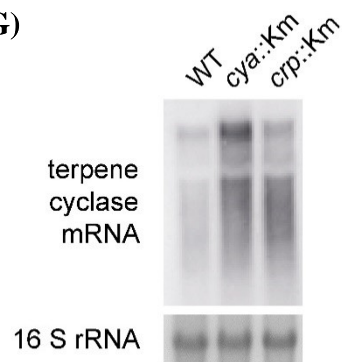


Figure S4: Characterization of the *cya*::Km and *crp*::Km insertion mutants of *Serratia plymuthica* 4Rx13. A)-C) Characterization of *cya*::Km. **A)** Verification of correct insertion of the functional cassette by PCR. In lanes 1-4 results for the *S. p.* 4Rx13 wildtype are shown and in lanes 5-8 for the *cya* mutant. Lanes 1 & 5 represent amplification products with gene-specific primers for the *cya* gene (1.1 kb for the WT; 2.7 kb for *cya*::Km). Lanes 4 & 8 depict amplification products of primers specific for the functional cassette (1.4 kb). Lanes 2 & 6 show PCR products after amplification with gene- and cassette-specific primers (1.6 kb; left border), as well as lanes 3 & 7 (right border; 2.5 kb). M = marker (GeneRuler® 1 kb). **B)** Growth comparison of *S. p.* 4Rx13 WT and *cya*::Km in complex medium (NB). The living cell number (CFU/ml) was determined periodically for up to 72 h. Error bars represent standard deviation (n=3). **C)** Stability of the mutation of *cya* in *S. p.* 4Rx13 was determined in 24 h intervals for up to 72 h of cultivation in complex medium (LB) using PCR. **D)-F)** Characterization of *crp*::Km. **D)** Verification of correct insertion of the functional cassette by PCR. In lanes 1-4 results for the *S. p.* 4Rx13 wildtype are shown and in lanes 5-8 for the *crp* mutant. Lanes 1 & 5 represent amplification products with gene-specific primers for the *crp* gene (537 bp for the WT; 2.15 kb for *crp*::Km). Lanes 4 & 8 depict amplification products of primers specific for the functional cassette (1.4 kb). Lanes 2 & 6 show PCR products after amplification with gene- and cassette-specific primers (1.68 kb; left border), as well as lanes 3 & 7 (right border; 1.89 kb). M = marker (GeneRuler® 1 kb). **E)** Growth comparison of *S. p.* 4Rx13 WT and *crp*::Km in complex medium (NB). The living cell number (CFU/ml) was determined periodically for up to 72 h. Error bars represent standard deviation (n=3). **F)** Stability of the mutation of *crp* in *S. ply.* 4Rx13 was determined in 24 h intervals for up to 72 h of cultivation in complex medium (NB) using PCR. **G)** Expression of the terpene cyclase in *S. p.* 4Rx13 *cya*::Km and *crp*::Km in comparison to the wild type. Northern blot of *S. p.* 4Rx13 wild type, *cya*::Km and *crp*::Km was performed after 24 h cultivation in complex medium (NB). Detection of the terpene cyclase mRNA (upper panel) was conducted using hybridization with a DIG-labelled probe followed by fluorescence measurement for 1 min. 16 S rRNA detection (lower panel) was performed on the same blot as a positive control.

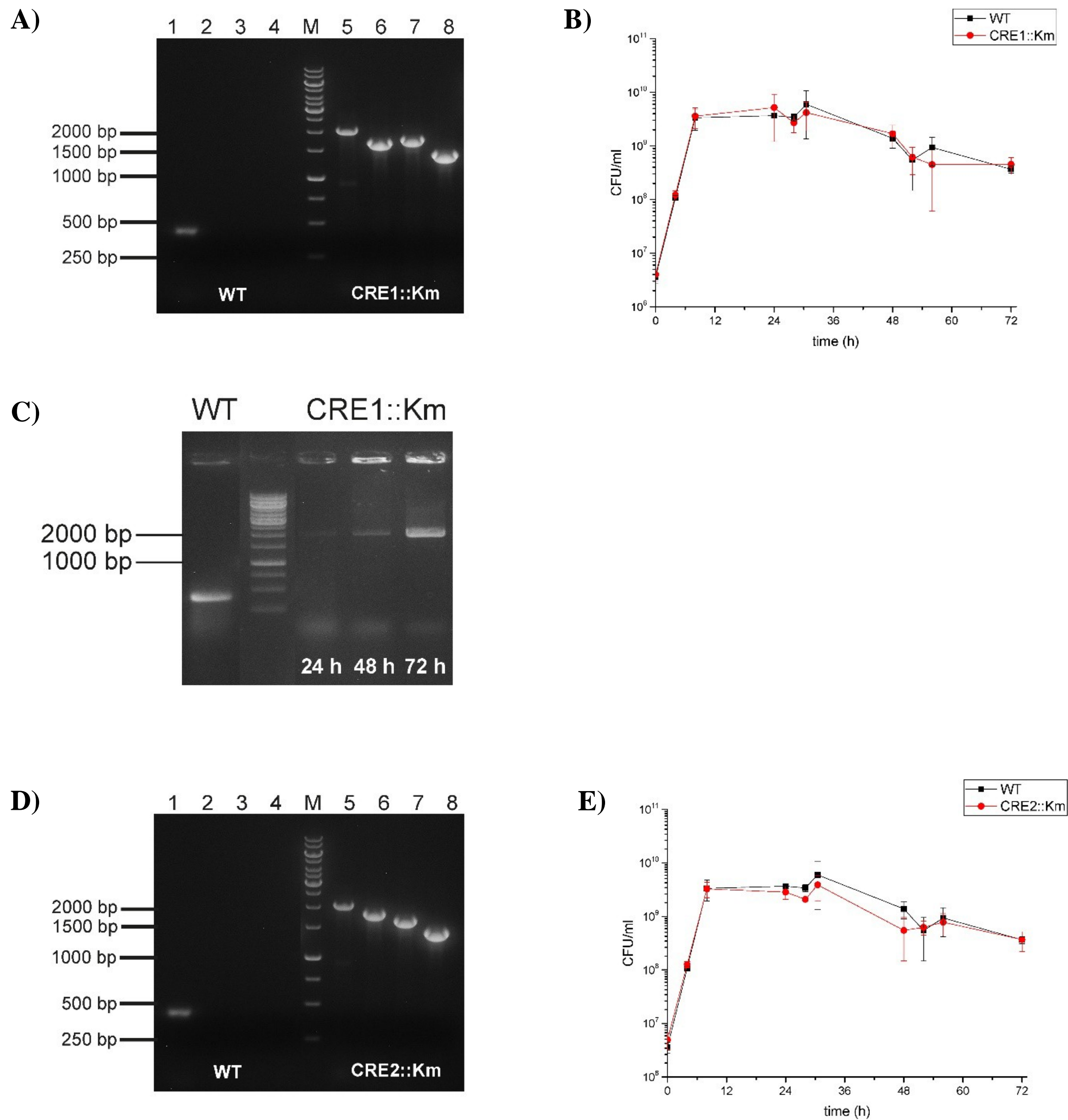


Figure S5: Characterization of the CRE1::Km and CRE2::Km deletion mutants of *Serratia plymuthica* 4Rx13. A)-C) Characterization of CRE1::Km. **A)** Verification of correct insertion of the functional cassette by PCR. In lanes 1-4 results for the *S. p.* 4Rx13 wildtype are shown and in lanes 5-8 for the CRE1 mutant. Lanes 1 & 5 represent amplification products with specific primers for the 5'-UTR of the sodorifen cluster (434 bp for the WT; 2.1 kb for CRE1::Km). Lanes 4 & 8 depict amplification products of primers specific for the functional cassette (1.4 kb). Lanes 2 & 6 show PCR products after amplification with 5'-UTR- and cassette-specific primers (1.68 kb; left border), as well as lanes 3 & 7 (right border; 1.8 kb). M = marker (GeneRuler® 1 kb). **B)** Growth comparison of *S. p.* 4Rx13 WT and CRE1::Km in complex medium (NB). The living cell number (CFU/ml) was determined periodically for up to 72 h. Error bars represent standard deviation (n=3). **C)** Stability of the deletion of CRE1 in *S. p.* 4Rx13 was determined in 24 h intervals for up to 72 h of cultivation in complex medium (NB) using PCR. **D)-E)** Characterization of CRE2::Km. **D)** Verification of correct insertion of the functional cassette by PCR. In lanes 1-4 results for the *S. p.* 4Rx13 wildtype are shown and in lanes 5- 8 for the CRE2 mutant. Lanes 1 & 5 represent amplification products with specific primers for the 5'- UTR of the sodorifen cluster (434 bp for the WT; 2.1 kb for CRE2::Km). Lanes 4 & 8 depict amplification products of primers specific for the functional cassette (1.4 kb). Lanes 2 & 6 show PCR products after amplification with 5'-UTR- and cassette-specific primers (1.8 kb; left border), as well as lanes 3 & 7 (right border; 1.66 kb). M = marker (GeneRuler® 1 kb). **E)** Growth comparison of *S. p.* 4Rx13 WT and CRE2::Km in complex medium (NB). The living cell number (CFU/ml) was determined periodically for up to 72 h. Error bars depict standard deviation (n=3).

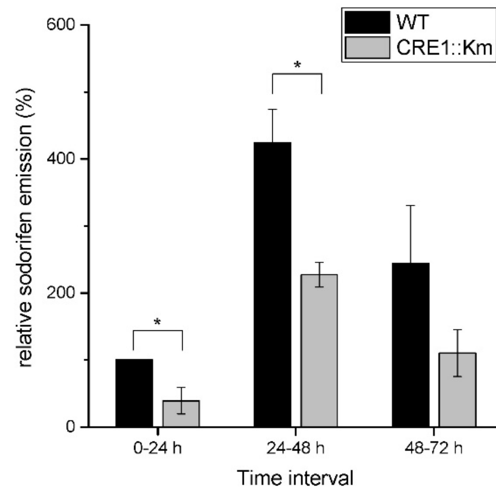


Figure S6: Relative sodorifen emission of *S. ply.* CRE1::Km in complex medium. Sodorifen emission of *S.ply.* 4Rx13 wild type and CRE1::Km during cultivation in complex medium (NBII) was determined using the closed VOC collection system (modified after Kai et. al. 2010) with subsequent GC/MS analysis. Relative sodorifen emission was calculated by correlating the living cell number (CFU/ml) to the sodorifen amount measured. As a reference, wild type emission until 24 h cultivation was used (100 %). Error bars indicate standard deviation (n=3). $^{*}p < 0.05$

Table S1: Primer sequences and annealing temperatures. Underlined parts of primers represent the part binding to the functional cassette.

Gene	Direction	Tm (°C)	Sequence (5’ ⟶ 3’)	function
SOD_c01380 (*cya*)	sense	65	GAGACTGGATGCGATCAACC	gene specific primers
	antisense	67	CCCGTTCGATCTTCCAGTTG	
	sense	62	CCCGCGTTTCAACGGGTATACAGTCTGCTGCCTACCTTACTGCACTACCAAATTAACCCTCACTAAAGGGCGG	primers for mutagenesis
	antisense	62	CAGGCGTGTAAAGGCAAACGCCGTGGGGAACGTTACCGTTCAGATAGCCCTAATACGACTCACTATAGGGCTC	
SOD_c43810 (*crp*)	sense	71	TCTCGGCAAACCGCAAACAG	gene specific primers
	antisense	67	CGTGAACAACCGACGATCTG	
	sense	62	GCTCCGTCGCGGTGCTGATTAAAGATGAAGAGGGTAAAGAGATGATCCTGAATTAACCCTCACTAAAGGGCGG	primers for mutagenesis
	antisense	62	CTGCGCTCCTGGCCTTCTTCAAACAATCCAAGCTCGCCGATGAAATCCCCTAATACGACTCACTATAGGGCTC	
5’UTR	sense	64.5	GATCCATCCGATTTGACACC	5’-UTR specific primers
	antisense	65	ATTCGATTGAGCATTGGGTG	
CRE1::Km	sense	62	GGGTATCGGTGAGCACGTAATATCATTGGGCTAGCCGACCCCTTTGTTTCAATTAACCCTCACTAAAGGGCGG	primers for mutagenesis
	antisense	62	TGGGAGTTTTGCTAGGGTGCAACTATTTTTTGTTAAAGCCAGGCGATGGATAATACGACTCACTATAGGGCTC	
CRE2::Km	sense	62	ATTAAGTTAAACCATAAGTTAAAAATATATCACTCGCTGATACTTCATCAAATTAACCCTCACTAAAGGGCGG	primers for mutagenesis
	antisense	62	TGTCAAATTTTATTTAATGACGGTTTTTAAGAAAAATAAATTAATCATGATAATACGACTCACTATAGGGCTC	
SOD_c20750 (terpene cyclase)	sense	65.5	ATCCCTTCAACCGGCAGTAC	gene specific primers
	antisense	64	CTGAAGCGGTATAAGCCAGC	
16 S rRNA	sense	62	AGAGTTTGATCATGGCTCAGA	gene specific primers
	antisense	70	AGGTGATCCAACCGCAGGTTC	
SOD_c38830 (GAPDH)	sense	62	ACCTGTTGGACGCAGAGTAC	gene specific primers
	antisense	64	GAGCGATCAGATCCAGAACC	
Primer name				
Q_O	sense	61.5	CCAGTGAGCAGAGTGACG	primers for 5‘-RACE-PCR
Q_I	sense	61	GAGGACTCGAGCTCAAGC	
Q_T	sense	48	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTTTT	
GSP1	antisense	69	CTTCCTGTAAACGCCGCTGG	
GSP2	antisense	73	ATGGCCGCAGCAACTGTTGG	
pJet1-FP	sense	59	ACTACTCGATGAGTTTTCGG	Primers for sequencing (provided by GATC)
pJet1-RP	antisense	57.5	TGAGGTGGTTAGCATAGTTC	

Table S2: Protocol for Reverse Transcriptase PCR.

Component	Volume
RNA (DNaseI-digested)	10 µl
antisense Primer (10 pmol/µl)	2 µl
dNTPs (10 mM each)	1 µl
DEPC-H_2O	1.5 µl
5 min, 65 °C, 1 min on ice	
5x RT-buffer (Thermo Fisher Scientific, Waltham, USA)	4 µl
Ribolock (Thermo Fisher Scientific, Waltham, USA)	0.5 µl
Maxima Reverse Transcriptase (Thermo Fisher Scientific, Waltham, USA) or DEPC-H_2O (negative control)	0.4 µl
DEPC-H_2O	0.6 µl

Amplification conditions

30 min, 50 °C
5 min 85 °C
1 min on ice

Table S3: Protocol for DIG-labelling of Northern blot probes. X = annealing temperature specific for the primers used.

Component	Volume
10x Taq buffer	5 µl
sense Primer	2 µl
antisense Primer	2 µl
dATP, dCTP, dGTP (10 mM each)	each 1 µl
dTTP (1 mM)	6.5 µl
DIG-dUTP (Roche Diagnostics Deutschland GmbH, Mannheim, Germany)	1 µl
Taq polymerase	1 µl
DNA template	1 µl

Amplification conditions

Step	
2 min 95 °C	
30 s 95 °C	40 cycles
30 s x °C	40 cycles
1 min 72 °C	40 cycles
7 min 72 °C	40 cycles
∞ 10 °C	