Supporting Information

**A Specialized Polythioamide-Binding Protein Confers Antibiotic Self-Resistance in Anaerobic Bacteria**

_F. Gude, E. M. Molloy, T. Horch, M. Dell, K. L. Dunbar, J. Krabbe, M. Groll*, C. Hertweck*}_
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Experimental Procedures

General materials and methods

Sequencing, oligonucleotide primer synthesis and gene synthesis were performed by Eurofins Genomics (Germany) or GENEWIZ (Germany). All chemicals and media components were purchased from Sigma-Aldrich (USA) and Roth (Germany). Enzymes for molecular cloning were purchased from Thermo Fisher Scientific (USA) and New England Biolabs (NEB, USA).

Bacterial strains and culturing conditions

*Escherichia coli* strains were grown in lysogeny broth (LB) with agitation or on LB agar plates at 37 °C containing the appropriate antibiotic (25 µg mL⁻¹ chloramphenicol, 50 µg mL⁻¹ kanamycin). Plasmid construction and storage was performed with *E. coli* TOP10, while *E. coli* Rosetta (DE3) was used for heterologous protein overproduction. *Ruminoclostridium cellulolyticum* DSM 5812 was obtained from the Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH culture collection and maintained under an anaerobic atmosphere (N₂:CO₂: 85:5:10 v/v/v) in a Whitley A35 anaerobic work station (Don Whitley Scientific) operating at 37 °C. Routine cultivation of the wild-type and mutant *R. cellulolyticum* strains was performed in modified CM3 medium with cellobiose (6 g L⁻¹) as previously described. CTA induction conditions involved cultivation in DSMZ medium 165 (M165) as previously described. To allow for gas exchange, all cultures were grown in unsealed test-tubes or glass bottles with a loosened lid.

Phylogenetic analyses

Protein sequence similarity searches were performed with BLASTp (default parameters), and sequences sharing a minimum identity of 46 % were selected for the alignment. The sequences were aligned with ClustalW and trimmed to an overlapping region present in all sequences using MEGA7 (v. 7.0.26). Positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The phylogenetic tree was reconstructed using the neighbor-joining method with 1,000 bootstrap replicates in MEGA7. See Figure S2 for the final alignment.

Plasmid construction for the generation of *R. cellulolyticum ΔctaA* and *R. cellulolyticum ΔctaA ΔctaZ*

For the creation of the in-frame nonsense mutation of ctaZ in the genome of *R. cellulolyticum* and *R. cellulolyticum ΔctaA* (Figure S3), we generated a CRISPR-based knockout plasmid as previously described, with minor changes. In short, we used the webtool CRISPy-web to determine a protospacer flanked by a PAM sequence (NGG) suited for CRISPR-nCas9-based genome editing. A fragment containing the sgRNA under the regulation of the mini-P4 promoter as well as the template for homology-directed repair was obtained by gene synthesis (GENEWIZ) (Table S4). The obtained vector was BsaI-digested, the desired fragment extracted and ligated into BsaI-linearized pCasC using T4 Ligase (Thermo Fisher Scientific) to result in pCasC-ctaZ. For final verification, the plasmid was sequenced by GENEWIZ using primer Cc3263-KO Seq (Table S3). For transformation of *R. cellulolyticum* and *R. cellulolyticum ΔctaA* by electroporation, the plasmid was methylated using the MspI methyltransferase.

Plasmid construction for genetic complementation of *R. cellulolyticum ΔctaZ*, and CtaZ heterologous production in *E. coli*

The gene encoding CtaZ was amplified by PCR from genomic DNA of *R. cellulolyticum* DSM 5812 using the primers CtaZ-F and CtaZ-R for pET28a-ctaZ and gyrP-4 fw and gyrP-CTA rv for pMTL-ctaZ (Table S3). PCR amplifications were performed using the Phusion High-Fidelity DNA Polymerase (NEB) and amplicons were purified using a Monarch PCR & DNA Cleanup Kit (NEB). For genetic complementation, the purified PCR product was assembled with Scal- and FspI-digested pMTL0 vector using NEBuilder HiFi DNA Assembly Master Mix (NEB) according to the manufacturer’s protocol. This yielded pMTL-ctaZ. For heterologous production, the purified PCR product was digested with restriction enzymes NheI and XhoI and ligated with an appropriately digested pET-28a vector using T4 DNA ligase (NEB) according to the manufacturer’s protocol. This yielded pET28a-ctaZ. Assembled or ligated expression vectors were introduced into *E. coli* TOP10 through electroporation and transformants were selected on LB agar plates supplemented with the relevant antibiotic (Table S2). Plasmids were isolated from the transformants using a Monarch Plasmid Miniprep Kit (NEB) and verified by sequencing using the primers T7 Seq F and T7Seq R for pET28a-ctaZ and primers gyrP-4 fw and gyrP-CTA rv for pMTL-ctaZ (Table S3).
The single knock-out mutant R. cellulolyticum ΔctaZ and double knock-out mutant R. cellulolyticum ΔctaA ΔctaZ were created as previously described,[2, 9] with minor changes. Briefly, R. cellulolyticum and R. cellulolyticum ΔctaA[9] were transformed with the plasmid pCasC-Coei_3263. Successful editing of the target gene resulted in the introduction of an in-frame STOP codon and an EcoRV restriction site (5′-TAAGATATC-3′). Colonies that appeared after three days were streaked on selective GS2 agar plates containing 15 µg mL⁻¹ erythromycin. A number of single transformant colonies were screened for incorporation of the desired mutation by colony PCR and restriction analysis as follows. Colony PCR was performed using OneTaq® 2x Master Mix with Standard Buffer (NEB) with primers Cc3263-KO F and Cc3263-KO R (Table S3), resulting in PCR products of the relevant region of ctaZ (565 bp). Colonies that carried the restriction site in ctaZ (corresponding to edited ctaZ) were distinguished from those maintaining the wild-type locus by EcoRV digest of the PCR amplicons for 1 h at 37 °C. The obtained DNA fragments were analyzed on a 2% agarose gel stained with ethidium bromide and visualized with UV light. The PCR amplicons from ΔctaZ strains were digested into two fragments (279 and 289 bp), whereas the PCR amplicons from the unedited strains were not digested. Candidate mutants that resulted in complete digestion in the restriction analysis were verified by DNA sequencing of the relevant region of ctaZ using the primer Cc3263-KO Seq (Table S3).

Once verified, R. cellulolyticum ΔctaZ pCasC-ctaZ and R. cellulolyticum ΔctaA ΔctaZ pCasC-ctaZ were subcultured without antibiotic selection until erythromycin resistance was lost (7 and 5 passages, respectively), then the absence of pCasC-ctaZ was confirmed in each case by colony PCR using primers pUCori-seq-rev and HB1-seq-rev (Table S3).

The cured mutant strains, R. cellulolyticum ΔctaZ and R. cellulolyticum ΔctaA ΔctaZ, were transformed with the relevant plasmids for genetic complementation experiments (Table S2). Transformants were selected on erythromycin-containing plates and verified by colony PCR using primers M13R and pMTLseq-rev (Table S3).

Determination of minimum inhibitory concentration values

To quantify the contribution of CtaZ to CTA immunity, MIC determinations were performed by agar diffusion assay.[10] Inocula (150 µL) from actively growing cultures (optical density at 600 nm (OD₆₀₀) of ~0.6) in VM broth[11] were spread on VM agar plates of uniform thickness (25 mm).[12] Standard 9 mm paper discs[13] were placed on the surface of the agar and 40 µL volumes of the CTA solutions were added. For each biological replicate, CTA was freshly weighed and a 1 mM stock solution in DMSO was prepared, before diluting to the relevant concentrations in DMSO. Two technical replicates were performed for each strain, each consisted of a single plate with a DMSO vehicle control and a range of CTA concentrations depending on the susceptibility of the test strain. In this way, the inhibition zones within each experiment were obtained under the same experimental conditions. After three days incubation, the diameters of the inhibition zones were measured. For each of the three biological replicates performed per strain, the diffusion distances were determined as half of the inhibition zone diameter (mm) taken from the average of the two technical replicates less the disc diameter. The experimental results were analyzed by both the standard free diffusion model (size of inhibition zones increases quadratically with the logarithm of antibiotic concentration) and the dissipative diffusion model (size of inhibition zones increases linearly with the logarithm of antibiotic concentration) (Figure S4).[14] Initial data analysis was performed using a Microsoft Excel spreadsheet and a web tool at http://www.agardiffusion.com. Each biological replicate was analyzed individually to determine the MIC, which was derived from the zero intercept of a linear regression of the zone diameters (mm), or their squared values $x^2$, plotted against the natural logarithm of the tested CTA concentrations $c$ (µM). The resulting MIC values, together with the corresponding regression coefficients ($R^2$) from the regression analysis, are summarized in Table S6. In all cases, use of the dissipative diffusion model resulted in $R^2$ values that were closer to 1, indicating a better linear fit. Therefore, the average values from three biological replicates analyzed by this model were taken in the determination of the final MICs (Figure 2B).

Bioinformatics

The multiple sequence alignment of CtaZ with YtkR7 and C10R6 was performed with the Clustal Omega webtool using the default parameters.[15] The sequences aligned in Figure 3A are marked in Table S5.

The Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST)[16] was used to generate a sequence similarity network (SSN) for the Gyrl-like protein superfamily (PF06445, comprising of 39,345 sequences in September 2021) and CtaZ-like proteins. The sequences were retrieved using the “sequence BLAST” option with default parameters, using CtaZ as a query and the Gyrl-like protein superfamily (PF06445) as additional input. Sequences with ≥ 50 % identity over 80 % of the sequence length (UniRef50) were visualized as a single node resulted in a network composed of 4,318 nodes. The network was constructed at an alignment score of 40 and visualized using Cytoscape (v. 3.8.0).[17] Figure 5A shows selected clusters with nodes containing sequences with characterized cellular functions. Additionally, Figure S9 shows the complete SSN with nodes highlighted containing sequences characterized by protein structure, in vitro or in vivo experiments.

Protein sequences for the genomic analysis of CtaZ homologs (Figure 5B) were retrieved using BLASTp against the non-redundant GenBank database and CtaZ (Coei_3263) as a query. This search returned 200 homologs sharing at least 44 % sequence identity with CtaZ over 82 % of the sequence (Table S8). The local genomic region (15 open reading frames upstream and downstream) surrounding the ctaZ homolog was checked for the presence of genes encoding a peptidyl carrier protein, a CtaC-like thioamide synthetase, transcriptional regulators, transporter proteins and common secondary metabolite biosynthetic enzymes for nonribosomal peptides,
polyketides and ribosomally synthesized and post-translationally modified peptides. Due to the poor annotation status of the sequences, HHpred,[18] BLASTp,[19] and antiSMASH[19] were used to manually check the genomic regions for open reading frames potentially encoding secondary metabolite biosynthetic proteins.

Heterologous production and purification of CtaZ for in vitro analysis

E. coli Rosetta (DE3) cells were transformed with pET28a-ctaZ and transformants were selected on LB agar plates supplemented with the relevant antibiotics. A 500 mL volume of LB medium supplemented with the relevant antibiotics was inoculated with 5 mL of an overnight pre-culture of E. coli Rosetta (DE3) harboring recombinant plasmid, and grown at 37 °C with agitation, until an OD$_{600}$ of 0.5–0.75 was reached. Cultures were then incubated for 10 min before protein overproduction was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.4 mM. Following induction, cells were grown at 18 °C with agitation overnight and afterwards harvested by centrifugation at 4,000 × g and 4 °C for 10 min. The resulting pellet was washed with Tris buffered saline (50 mM Tris pH 7.5, 150 mM NaCl) and stored at −20 °C.

Frozen cell pellets were resuspended in 40 mL of lysis-wash buffer (50 mM Tris pH 8.2, 300 mM NaCl, 25 mM imidazole, 1 mM DTT, 5 % glycerol v/v). Cells were sonicated at 4 °C using a SONOPLUS ultrasonic homogenizer with a MST73 microtip (Bandelin) and the following parameters: 30 % power, six 60 s cycles with 5 min breaks between cycles. The lysate was then centrifuged at 4 °C for 30 min at 12,000 × g to pellet insoluble debris. The supernatant was loaded onto a 5 mL HisTrap HP column (Cytiva), which was equilibrated with lysis-wash buffer using an AKTA Pure system (GE Healthcare). Unbound proteins were removed by washing with 150 mL lysis-wash buffer. Bound proteins were eluted with 15 mL elution buffer (50 mM Tris pH 8.2, 300 mM NaCl, 250 mM imidazole, 1 mM DTT, 5 % glycerol v/v). Protein-containing fractions were pooled and diluted 1:1 (15 mL) with gel filtration buffer (GF buffer; 30 mM HEPES pH 8.2, 250 mM NaCl, 0.5 mM TCEP) and 30 U thrombin (bovine, Sigma) was added to remove the His-tag. The solution was dialyzed overnight at 4 °C against 1.5 L GF buffer. To remove thrombin, the dialyzed sample was added to 3 mL of pre-equilibrated benzamidine resin (GE Healthcare) and incubated under rotation for 2 h at room temperature (RT). The resin was removed using a syringe column (MoBiTec) and the eluate was concentrated to 1.5 mL using Amicon Ultra-3kDa MWCO centrifugal filters (Merck Millipore). The concentrated sample was applied to a HiLoad 16/600 Superdex 200 pg column (GE Healthcare) and eluted using GF buffer. Single-peak fractions were concentrated to a volume of 1 mL and dialyzed overnight at 4 °C against 1 L storage buffer (30 mM HEPES pH 8.2, 250 mM NaCl, 0.5 mM TCEP, 20 % glycerol v/v). Protein was stored at −80 °C, and protein concentration was determined by absorbance at 280 nm.

CTA modification assays with CtaZ

Reactions were performed with 15 µM purified CtaZ and 100 µM CTA (1 mM stock in DMSO) in phosphate reaction buffer (50 mM K$_2$HPO$_4$ pH 7.5, 125 mM NaCl). In control reactions, CtaZ was either absent or replaced by heat-inactivated protein. Assays were incubated at 30 °C and were allowed to proceed for 30 min, 24 h or 64 h, and quenched by the addition of one volume of methanol. For HPLC measurements, the samples were dried under vacuum and dissolved in 90 µL methanol, centrifuged for 10 min at RT and 18,000 × g, and filtered (3 mm CHROMAFIL O-20/3 syringe filters, 0.2 µm, PTFE membrane, Macherey-Nagel) prior to analysis. HPLC measurements for the detection of CTA and CTA degradation products were performed with an Accela HPLC system (Thermo Fisher Scientific). Separation was performed with a Betasil C18 column (2.1 × 150 mm, 3 µm, Thermo Fisher Scientific) operating at a flow rate of 200 µL min$^{-1}$, with 0.1 % formic acid (solvent A) and acetonitrile + 0.1 % formic acid (solvent B) and the following gradient: 5 % solvent B for 1 min, 5 % to 98 % solvent B over 15 min, hold 98 % solvent B for 3 min, 98 % to 5 % solvent B over 1 min, hold 5 % solvent B for 13 min. UV-Vis spectra were recorded in a wavelength range from 200 to 600 nm with a bandwidth of 1 nm and are depicted as total scan overlays.

CTA-CtaZ in vitro binding assays

Binding assays were performed with 40 µM purified CtaZ and 40 µM CTA (5 mM stock solution in DMSO) in HEPES reaction buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 20 mM MgCl$_2$, 1 mM TCEP) at RT. In control assays, CtaZ was replaced by buffer or CTA was replaced by DMSO. After 2 h incubation, the assays were centrifuged for 10 min at RT and 18,000 × g. The supernatant was filtered (13 mm Acrodisc syringe filters, 0.45 µm, GHP membrane, Pall) prior to measurement by LC-HR-MS (Figure 3C).

In a control experiment, the influence of precipitate removal by filtration during sample preparation was tested. Therefore, the binding assays described above were repeated with and without filtering samples prior to LC-HR-MS analysis (Figure S6). LC-HR-MS measurements for the detection of CtaZ and CTA were performed with a Thermo Ultimate3000 UHPLC-system coupled to a QExactive HF-X Hybrid-Quadrupole-Orbitrap (Thermo Fisher Scientific) mass spectrometer equipped with an electrospray ion source. Separation was performed with a BioResolve RP mAb Polyphenyl column (2.1 × 100 mm, 45 Å, 2.7 µm, Waters) operating at a flow rate of 300 µL min$^{-1}$, with 0.1 % formic acid (solvent A) and acetonitrile + 0.1 % formic acid (solvent B) and the following gradient: 15 % to 40 % solvent B over 1 min, hold 40 % solvent B for 0.5 min, 40 % to 45 % solvent B over 0.5 min, hold 45 % solvent B for 0.5 min, 45 % to 50 % solvent B over 0.5 min, hold 50 % solvent B for 0.5 min, 50 % to 55 % solvent B over 0.5 min, hold 55 % solvent B for 0.5 min, 55 % to 80 % solvent B over 0.5 min, hold 80 % solvent B for 1.5 min, 80 % to 15 % solvent B over 0.5 min, hold
Heterologous protein production and purification of CtaZ for crystallization

_E. coli_ Rosetta (DE3) pET28a-ctaZ was grown in Fernbach shaking flasks at 37 °C containing 3 L of LB medium supplemented with the relevant antibiotic. At an OD600 of 0.6, IPTG was added to a final concentration of 1 mM and incubation was continued overnight at 20 °C. Cells were harvested by centrifugation, washed with 0.9% (w/v) NaCl and stored at −20 °C. Frozen bacterial cell mass (25 g) was thawed in 50 mL of 100 mM Tris/HCl, pH 7.5, containing 500 mM NaCl, and 20 mM imidazole/HCl (buffer A). The cells were disrupted by sonification (Branson Digital Sonifier 250). The resulting suspension was centrifuged at 40,000 × _g_ for 20 min at 4 °C. The supernatant was applied to a 5 mL HisTrap HP column (GE Healthcare), which had been equilibrated with buffer A (flow rate 5 mL min⁻¹) using an ÄKTA Pure system (GE Healthcare). Unbound or loosely associated proteins were removed by washing with buffer A. CtaZ protein was eluted by applying a 50 mL linear gradient from buffer A to buffer B (100 mM Tris/HCl pH 7.5, 500 mM NaCl, 500 mM imidazole). Fractions were combined and 200 U of thrombin from bovine plasma (Serva) was added. The solution was dialyzed overnight at 4 °C against 20 mM Tris/HCl, pH 7.5, containing 100 mM NaCl and again applied to the HisTrap HP column that was equilibrated with buffer A. The percolate was concentrated to 1 mL and the solution was applied to a HiLoad 16/60 Superdex 75 pg column (GE Healthcare, flow rate 1.5 mL min⁻¹). Single-peak fractions were concentrated to 40 mg mL⁻¹ using Amicon Ultra-10kDa MWCO centrifugal filters (Merck Millipore), and stored at 4 °C.

Crystallization of CtaZ(apo) and CtaZ:CTA

Crystallization experiments of CtaZ (20 mg mL⁻¹) were performed by the sitting drop vapor diffusion method at 20 °C. For co-crystallizing experiments, the natural ligand CTA (100 mM stock solution in DMSO) was added to CtaZ (24 mg mL⁻¹) to a final concentration of 2 mM. Crystallization drops had a maximum volume of 0.4 μL with either a 1:1, 2:1, or 3:1 ratio of protein and reservoir solution. CtaZ(apo) crystals preferentially grew in 2.8 M sodium acetate, pH 7.0, whereas CtaZ:CTA crystals were obtained with 0.2 M zinc acetate, 0.1 M sodium cacodylate, pH 6.5 and 18 % polyethylene glycol 8000. Crystals were cryoprotected by a 7:3 mixture of mother liquor and 100 % v/v glycerol and subsequently vitrified in liquid nitrogen.

Structure determination of CtaZ(apo) and CtaZ:CTA

High resolution datasets of CtaZ(apo) and CtaZ:CTA crystals were recorded using synchrotron radiation of _λ_ = 1.0 Å at the beamline X06SA, Swiss Light Source (SLS), Paul Scherrer Institute, Villigen, Switzerland. Reflection intensities were evaluated with the program package XDS and data reductions were carried out with XSCALE[20] (Table S7). Due to the presence of zinc in the crystallization buffer of CtaZ:CTA crystals, the fluorescence spectrum depicted strong X-ray absorption for this heavy metal. Therefore, we performed a scan at the zinc edge and collected anomalous diffraction data at the peak wavelength ( _λ_ = 1.281 Å). A total of two zinc sites were located with SHELXD.[21] Phasing, density modification and initial model building of the CtaZ:CTA complex were carried out with the program CRANK2.[22] With these improved phases, we could unambiguously assign the entire CtaZ sequence, the last missing reductions were carried out with XSCALE.[20] Since the CtaZ:CTA crystal was highly resistant to radiation damage, we also collected a dataset at 2.0 Å to yield anomalous signals for sulfur (Table S7). With the refined CtaZ:CTA model in hands, we were able to depict the anomalous signal for each of the sulfur atoms in the natural product (Figure 4A). Next, we solved the structure of CtaZ(apo) by Patterson search calculations with PHASER[24] using the coordinates of CtaZ:CTA. After model building of CTA(apo) and CtaZ:CTA was completed, water molecules were automatically placed with ARP/wARP solvent,[25] Restrained and TLS (Translation/Libration/Screw) refinements with REFMAC[26] yielded superb _R_<sub>free</sub> and _R_<sub>free</sub> as well as root-mean-square deviation (RMSD) bond and angle values for both datasets (Table S7). Crystal structures have been deposited in the RCSB Protein Data Bank under the accession codes 7ZHE for CtaZ(apo) and 7ZHD for the CtaZ:CTA complex.
Results and Discussion

Figure S1. Detailed phylogenetic tree of GyrI-like proteins. Neighbor-joining phylogenetic tree of diverse GyrI-like domains from multidrug resistance and self-resistance proteins with 1,000 bootstrap replicates. The color-code refers to the cellular function assigned to at least one member of each group (highlighted in bold) except in the case of the CtaZ-like group, which has no characterized members. Protein accession numbers are indicated in brackets. See Figure S2 for the sequence alignment used to generate this tree.
SUPPORTING INFORMATION

Figure S2. Multiple sequence alignment of GyrI-like proteins. The multiple sequence alignment was used for the construction of the phylogenetic tree in Figure 1C and Figure S1. See Table S5 for protein accession numbers. Alignment continues on the next page.
Figure S2. Multiple sequence alignment of Gyr-like proteins (continued).

| 110 | 120 | 130 |
|-----|-----|-----|
| R. cellulolyticum CtaZ | AIRSYAREKNMLILQPPPPREVYKSPGKGNPNKYITFL | ...|
| C. difficile CtaZ | NIKR1IEKNIDTVGIFWIFIEKSPKGNPKNYITEIVF | ...|
| C. botulinum CtaZ | AIAVAYAKELTVQPPWREVFKSPKGKPNKYTEILP | ...|
| F. necrophorum CtaZ | KIRNY1IEKIEVQVFVFKSPKGKPNKYTEIFV | ...|
| E. coli GyrI | QKFSLLQDSAYELPKFCFVYNLNGARDYWDIEYVAV | ...|
| E. sp. 638 GyrI | SFFSLQDNHYAPKPCFERYLNDGADGWIDEMFV | ...|
| C. sakazakii GyrI | AFFRLQQVRQAAARCPFEIYLRDKGDYWDIEMIVF | ...|
| E. coli Rob | TVYTCMFLNLTRKLDIERFYPEDEQAFPILRCYI | ...|
| K. pneumoniae Rob | TVYTCMFLNLTRKQQDERFPHKHEQPPIQLREYLI | ...|
| S. sp. TP-A2060 YtkR7 | GLHAFIESGQAASQTHHEEYLMSPRTAPRLRTI | ...|
| S. ventii YtkR7 | ALHAFLDNGQRASSQTEHHEYLMSPRTAPRLRTI | ...|
| S. bohaisens YtkR7 | ALHAFLDNGQRASSQTEHHEYLMSPRTAPRLRTI | ...|
| L. innocua Lin2189 | EMHQFMETQGKRISKHEIYLYSDPRKANPKMKTLR | ...|
| L. monocytogenes Lin2189 | EMHQFMETQGKRISKHEIYLYSDPRKANPKMKTLR | ...|
| S. zelensis C10R6 | RMHE1MPFDKFRTHHEIYLYSDARTRPLRNTL | ...|
| S. carminius C10R6 | RMHE1MPFDKFRTHHEIYLYSDARTRPLRNTL | ...|
| S. yokosukanensis C10R6 | RMHE1MPFDKFRTHHEIYLYSDARTRPLRNTL | ...|
| B. subtilis BmrR | KLIYIDTVSDYELIIPIHYSFKQEEYR-VEMKIRI | ...|
| B. vallismortis BmrR | KLIYIDTVSDYELIIPIHYSFKQEEYR-LBEMKIRI | ...|
| B. halotolerans BmrR | KLIYIDTVSDYELIIPIHYSFKQEEYR-VEMKIRI | ...|
Figure S3. Generation of \textit{R. cellulolyticum} Δ\textit{ctaZ} and \textit{R. cellulolyticum} Δ\textit{ctaZ} Δ\textit{ctaA} by CRISPR-nCas9 genome editing. (A) The indicated region of the ctaZ (Ccel_3263) gene sequence from wild-type \textit{R. cellulolyticum} is displayed above the corresponding mutated sequence of Δ\textit{ctaZ} (marked in red). The incorporated STOP codon is denoted by a bold “TAA” and an asterisk in the resulting amino acid sequence. The incorporated EcoRV recognition site is italicized. (B) Agarose gel (2\%) showing EcoRV-digested ctaZ-specific PCR products amplified from single colonies of the indicated \textit{R. cellulolyticum} strains. The PCR amplicon from the Δ\textit{ctaZ} strain (565 bp) contains one EcoRV recognition site so is digested to the expected 279 bp and 289 bp fragments by EcoRV, which converge as one band on the agarose gel. The PCR amplicon from the wild type (WT) control (565 bp) does not contain an EcoRV recognition site so is not digested by EcoRV. M, GeneRuler DNA Ladder Mix (Thermo Fisher Scientific); neg., negative control (PCR without template). Restriction analysis for \textit{R. cellulolyticum} Δ\textit{ctaA} Δ\textit{ctaZ} is shown as a representative; data for \textit{R. cellulolyticum} Δ\textit{ctaA} Δ\textit{ctaZ} is analogous. (C) DNA sequence chromatogram confirming the correct insertion of the desired mutated sequence (marked in grey). Sequencing data for \textit{R. cellulolyticum} Δ\textit{ctaA} Δ\textit{ctaZ} is shown as a representative; data for \textit{R. cellulolyticum} Δ\textit{ctaZ} is analogous.
Figure S4. Results of the closthoamide agar diffusion assay analyzed by the free and dissipative diffusion models. Results from the agar diffusion assay showing the susceptibility of the listed strains to closthoamide (CTA). Three biological replicates, each consisting of two technical replicates, were performed. The graphs depict linear regression analyses using quadratic (free diffusion model) or linear (dissipative diffusion model) dependence of zone diameter $x$ (mm) on the natural logarithm (ln) of the tested CTA concentrations $c$ (µM). The minimum inhibitory concentration (MIC) values (derived from the zero intercepts, µM) and corresponding $R^2$ (regression coefficient) values are summarized in Table S6. Better linear fits ($R^2$ values closer to 1) are obtained using the dissipative diffusion model; therefore, the average values from the three biological replicates analyzed by this model were used for the determination of the final MICs (Figure 2B).
Figure S5. SDS-PAGE analysis of purified CtaZ. A Coomassie-stained SDS-PAGE protein gel of CtaZ is depicted. The expected size of the protein is indicated.
Figure S6. Influence of sample preparation on CtaZ-CTA binding assays. A control experiment for the CtaZ-CTA binding assay (shown in Figure 3C) is depicted to illustrate the influence of precipitate removal by filtration during sample preparation. HPLC profiles (left) and HPLC-HR-MS profiles (right) are shown; traces correspond to the extracted ion chromatogram of the [M+H]⁺ ionic species for CTA (m/z 695.1453) and are displayed with m/z values ± 5 ppm from the calculated exact mass. (A) Samples were filtered through a GHP membrane (PALL) prior to LC-HR-MS analysis. In an aqueous solution, CTA can pass the filter membrane only when CtaZ is present, presumably due to CTA solubilization caused by binding to CtaZ. (B) Samples were not filtered prior to LC-HR-MS analysis. Unlike in Figure S6A, CTA is detectable in the absence of CtaZ albeit at a lower intensity than in the sample containing both CtaZ and CTA, indicating that CtaZ enhances the solubility of CTA in aqueous solutions. It seems that unbound CTA in aqueous solution is removed by filtration, rendering the compound undetectable in LC-HR-MS analysis in Figure 3C and Figure S6A.
Figure S7. Comparison of the protein structures of CtaZ and EcmrR. (A) Ribbon diagram of CtaZ (PDB ID: 7ZHE). Residues 132–139 (shown as dots) are not resolved in the electron density map. (B) A DALI search\(^{(27)}\) revealed highest similarity of CtaZ (black) to transcriptional regulator EcmrR (residues 112–269) (grey; PDB ID: 6WL5, 1.3 Å root-mean-square deviation (RMSD) for 110 Cα atoms, Z-score 17.4).\(^{(28)}\) Both proteins are depicted adopting the characteristic GyrI-like protein structure.
Figure S8. Structural comparison of the ligand binding sites of CtaZ and other Gyrl-like proteins. Structural superposition of CtaZ:CTA (PDB ID: 7ZHD) with (A) EcmrR (residues 112–269) bound to cetyltrimethylammonium (CMA, cyan; PDB ID: 6WL5, 1.3 Å RMSD for 122 Cα atoms), (B) Lin2189 (residues 20–208) bound to yatakemycin (YTM, gold; PDB ID: 5X5M, 1.5 Å RMSD for 106 Cα atoms), and (C) BmrR (residues 112–207) bound to kanamycin (KAN, tan; PDB ID: 3Q5R, 1.8 Å RMSD for 88 Cα atoms).

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**A**

CtaZ:CTA  
EcmrR:CMA

**B**

CtaZ:CTA  
Lin2189:YTM

**C**

CtaZ:CTA  
BmrR:KAN
Figure S9. Complete sequence similarity network for CtaZ homologs and the GyrI-like protein superfamily. GyrI-like protein superfamily: PF06445. Proteins that share ≥ 50% sequence identity over 80% of the sequence length are conflated resulting in a network composed of 39,384 sequences and represented with 4,318 nodes; alignment score 40. Nodes are colored based on a known resistance or self-resistance function. Node outline color indicates the protein domain architecture, while the node shape corresponds to protein activity. Node labels correspond to a representative protein and are colored based on the phylum of the respective organism. Nodes containing sequences common to the genome mining approach shown in Figure 5B are highlighted by an asterisk * (Anaeroviringula multivorans DSM 17722 and Blautia producta DSM 2950) or a cross † (Clostridium botulinum Prevot_594 and Robinsonella peoriensis NRRL B-23885).
Table S1. Strains used in this study.

| Strain                          | Relevant genotype and descriptions                        | Source                  |
|---------------------------------|----------------------------------------------------------|-------------------------|
| *Escherichia coli* TOP10        | General cloning strain                                    | Laboratory strain       |
| *E. coli* Rosetta (DE3)         | Protein production strain                                 | New England Biolabs     |
| *E. coli* Rosetta (DE3) pET28a-ctaZ | Strain contains pET28a-ctaZ                              | This study              |
| *Ruminiclostridium cellulolyticum* | Wild type (DSM 5812)                                    | DSMZ                    |
| *R. cellulolyticum* ΔctaA       | CRISPR-nCas9-inactivated Ccel_3260                       | [2]                     |
| *R. cellulolyticum* ΔctaA pMTL₀ | CRISPR-nCas9-inactivated Ccel_3260, strain contains pMTL₀ | This study              |
| *R. cellulolyticum* ΔctaZ       | CRISPR-nCas9-inactivated Ccel_3263                       | This study              |
| *R. cellulolyticum* ΔctaA ΔctaZ | CRISPR-nCas9-inactivated Ccel_3260 and Ccel_3263         | This study              |
| *R. cellulolyticum* ΔctaA ΔctaZ pMTL₀ | CRISPR-nCas9-inactivated Ccel_3260 and Ccel_3263, strain contains pMTL-ctaZ | This study              |
| *R. cellulolyticum* ΔctaA ΔctaZ pMTL-ctaZ | CRISPR-nCas9-inactivated Ccel_3260 and Ccel_3263, strain contains pMTL-ctaZ | This study              |
### Table S2. Plasmids used in this study.

| Plasmid                | Description                                                                 | Source      |
|------------------------|-----------------------------------------------------------------------------|-------------|
| pET28a                 | Expression vector for the production of N-terminally His-tagged proteins, Kan\(^R\) | Novagen     |
| pET28a-ctaZ            | N-terminal His-tag on protein product of Ccel_3263 (ctaZ)                   | This study  |
| pMTL∅                  | Expression plasmid (pTargetC) for Clostridia carrying P4 promoter, Thia\(^R\) | [2]         |
| pMTL-ctaZ              | pMTL∅ with ctaZ                                                              | This study  |
| pCasC                  | pSOS-Cas-Gm with D10A Cas9 mutation; Clostridia-adapted vector for CRISPR/Cas editing in *R. cellulolyticum*, Thia\(^R\) | [2, 9]      |
| pCasC-ctaZ             | pCasC-based CRISPR-nCas9 vector for Ccel_3263 (ctaZ) inactivation          | This study  |

Kan\(^R\), kanamycin resistance; Thia\(^R\), thiamphenicol resistance
Table S3. Oligonucleotide primers used in this study.

| Primer Name | Sequence (5' → 3') | Use                      |
|-------------|---------------------|--------------------------|
| T7 Seq F    | TAATACGACTCTATAGGG  | pET28a sequencing        |
| T7 Seq R    | CTAGTTATGCTACGCGT   | pET28a sequencing        |
| CtaZ-F      | AAAGCTAGCATGAATTGAAATGTTAAGGAC | pET28a-c3aZ cloning      |
| CtaZ-R      | TTTCTCGAGCTACTCTCTTAATGGGAAAC | pET28a-c3aZ cloning      |
| Cc3263-KO F | CACCGAGAAAAAGATAGAGG | Δc3aZ colony PCR         |
| Cc3263-KO R | CCTCTATTGCAAGCATCGAACACTAC | Δc3aZ colony PCR         |
| Cc3263-KO Seq | GGGCATCTCTGAGGTCTTG | Δc3aZ sequencing         |
| pUC0ri-seq-rev | AACAAGCCATGAAAACCG | pCasC-c3aZ sequencing   |
| HB1-seq-rev | AACAAGCCATGAAAACCG | pCasC-c3aZ sequencing   |
| gyrI-P4-fw  | TTTTTAAAGTTAAATAAGGTTATAAGGAGGAAAATC | pMTL-c3aZ cloning and sequencing |
| gyrI-CTA_rv | CCATTCCGCATCATCAGGCTAAGCCTCTCTCGTTAATGC | pMTL-c3aZ cloning and sequencing |
| pTargetC-Seq-F | CTTGCGACAGCTGATATG | pMTL-c3aZ sequencing   |
| M13R        | CAGGAAACAGCTATGACC | pMTL-c3aZ colony PCR    |
| pMTLseq-rev | CATCTCGTCATAGTACC | pMTL-c3aZ colony PCR    |
Table S4. Sequence of the sgRNA cassette for CRISPR-nCas9-based knockout of ctaZ. Sequence of the cassette includes the sgRNA cassette (P4 promoter, sgRNA, spy terminator), N20 sequence and mutated homology arms for Ccel_3263. Homology arms are marked in grey, the STOP codon is underlined and the EcoRV recognition site is italicized.

| Target     | Sequence of gene knockout cassette (5' → 3')                                                                 |
|------------|-------------------------------------------------------------------------------------------------------------|
| Ccel_3263  | ACCCGAGACCATATGGATGCTTGACTTGGACAAATTTTTTTAAAAAGTTAAAGTTGCTATGTTGTCTCTACAGGTTTTAGGC                     |
|            | TAGAAATAGCAAGGTTAAATAAGGGCTAGTCCCTTATACACTGGAATTCTCTAGAGTCG                                      |
|            | TGGAGCTGTGATTCGGTTAAACACGCAAAAAATAATTATTTTAAAGGGAAGAATCTAATGAATTATGAATTAAATAGGACGTG                  |
|            | ACACCCATTAGAGTGTCTATGACTTTATGACTATAGGAGGCTGGCTGGCCAGGACATGAAAGGTGGGCACAGGCTAAGGTCAGTACAGGG              |
|            | AAAAGCCAAATGGAGACCTTTATATGCTATTATGAGTTAGCTACAGGGAATATGCTATAGGACGTG                                     |
|            | CAGGACCGCGGTTGAGAAAATGATGCGCAAGAATAAAAGCAGATGAGCTACGTATGGAGAAGCTATGGAATAAAGTCCTTTCGGGGAAGTTTTATAAGG     |
|            | CCAGTATACAGGGCATTTGAGAATTATGACGTAAAGAATATAGTCTACGTATGGAGAAGCTATGGAATAAAGTCCTTTCGGGGAAGTTTTATAAGG       |
|            | CCGGGAATGATGACTAAAAAGTTAACACGCTAATAAGTATATAACTGAAGTTCTGTTTCCATTAAAGGAGGCCTGCAGACATGCAAGCTG            |
Table S5. List of GyrI-like and CtaZ-like proteins used to construct the phylogenetic tree. Sequences used for the multiple sequence alignment in Figure 3A are marked with an asterisk (*).

| Accession number | Organism                  | Class |
|------------------|---------------------------|-------|
| CtaZ (Ccel_3263)* | Ruminiclostridium cellulolyticum | CtaZ  |
| WP_022620692.1   | Clostridoides difficile    | CtaZ  |
| WP_053337826.1   | Clostridium botulinum      | CtaZ  |
| WP_035914910.1   | Fusobacterium necrophorum  | CtaZ  |
| P33012           | Escherichia coli           | GyrI  |
| A4WC16           | Enterobacter sp. 638       | GyrI  |
| A7MJM9           | Cronobacter sakazakii      | GyrI  |
| P39075           | Bacillus subtilis          | BmrR  |
| WP_010328316.1   | Bacillus vallismortis      | BmrR  |
| WP_202853084.1   | Bacillus halotolerans      | BmrR  |
| P0AC10           | Escherichia coli           | Rob   |
| A0A378E9L4       | Klebsiella pneumoniae      | Rob   |
| E3GAQ7           | Enterobacter lignolyticus  | Rob   |
| Q929T5           | Listeria innocua           | Lin2189 |
| WP_031541452.1   | Listeria monocytogenes     | Lin2189 |
| WP_194349349.1   | Listeria welshimeri        | Lin2189 |
| I3NN73*          | Streptomyces sp. TP-A2060  | YtkR7 |
| WP_167933938.1   | Streptomyces ventii        | YtkR7 |
| WP_168088698.1   | Streptomyces bohaisiens    | YtkR7 |
| A0A1W6EUW3*      | Streptomyces zelensis      | C10R6 |
| A0A2MBM633       | Streptomyces carminius     | C10R6 |
| A0A101P4J0       | Streptomyces yokosukanensis| C10R6 |
Table S6. Comparison of the diffusion of CTA by the free and dissipative diffusion models. The MIC values (derived from the zero intercepts, µM) and corresponding $R^2$ (regression coefficient) values presented are the averages determined from the analysis of three biological replicates (Figure S4). Diffusion of CTA is better described by the dissipative diffusion model ($R^2$ values closer to 1), which was therefore used in the determination of the final MIC values (underlined) displayed in Figure 2B.

| Model            | Value | R. cellulolyticum ΔctaA pMTL∅ | R. cellulolyticum ΔctaA ΔctaZ pMTL∅ | R. cellulolyticum ΔctaA ΔctaZ pMTL-ctaZ |
|------------------|-------|-------------------------------|-------------------------------------|------------------------------------------|
| dissipative diffusion: $x / \ln(c)$ | $R^2$ | 0.993                         | 0.992                               | 0.973                                    |
|                   | MIC (µM) | 57                            | 1.6                                 | 18                                       |
| free diffusion: $x^2 / \ln(c)$      | $R^2$ | 0.957                         | 0.969                               | 0.855                                    |
|                   | MIC (µM) | 93                            | 2.7                                 | 29                                       |
### Table S7. Crystallographic data collection and refinement statistics.

| Crystal parameters | CtaZ | CtaZ:CTA | CtaZ:CTA (ano zinc) | CtaZ:CTA (ano sulfure) |
|--------------------|------|----------|---------------------|------------------------|
| **Space group**    | R32  | C2       | C2                  | C2                     |
| **Cell constants** | a = b = 121.3 Å, c = 108.4 Å | a = 104.6 Å, b = 40.9 Å, c = 44.8 Å, β = 91.6° | a = 104.6 Å, b = 40.9 Å, c = 44.8 Å, β = 91.6° | a = 104.6 Å, b = 40.9 Å, c = 44.8 Å, β = 91.6° |
| **Subunits / AU**  | 1    | 1        | 1                   | 1                      |
| **Data collection**|      |          |                     |                        |
| **Beam line**      | X06SA, SLS | X06SA, SLS | X06SA, SLS | X06SA, SLS |
| **Wavelength (Å)** | 1.0  | 1.0      | 1.281              | 2.0                    |
| **Resolution range (Å)** | 30–2.0, (2.1–2.0) | 30–1.65, (1.75–1.65) | 30–1.85, (1.95–1.85) | 30–2.4, (2.5–2.4) |
| **No. observations** | 87,441 | 68,561 | 105,190 | 78,496 |
| **No. unique reflections** | 20,682 | 22,520 | 30,691 | 14,120 |
| **Completeness (%)** | 99.0 (99.9) | 97.9 (99.3) | 99.6 (99.7) | 97.3 (92.3) |
| **R_merge (%)** | 4.9 (56.2) | 3.3 (50.7) | 4.5 (28.1) | 6.3 (37.8) |
| **I/σ (I)** | 16.4 (2.2) | 15.9 (2.2) | 14.5 (3.8) | 18.8 (4.8) |
| **Refinement (REFMAC5)** |      |          |                     |                        |
| **Resolution range (Å)** | 30–2.0 | 30–1.65 |
| **No. refl. working set** | 19,640 | 21,391 |
| **No. refl. test set** | 1,034 | 1,125 |
| **No. non hydrogen** | 1,281 | 1,282 |
| **No. of ligand atoms** | - | 43 |
| **Solvent** | 106 | 76 |
| **R_work/R_free (%)** | 18.1 / 20.7 | 17.4 / 19.9 |
| **r.m.s.d. bond (Å) / angle (°)** | 0.003 / 1.2 | 0.003 / 1.2 |
| **Average B-factor (Å²)** | 45.5 | 35.7 |
| **Ramachandran Plot (%)** | 98.0 / 2.0 / 0 | 100 / 0 / 0 |

**Notes:**
- Asymmetric unit
- The values in parentheses for resolution range, completeness, R_merge and I/σ (I) correspond to the highest resolution shell
- Data reduction was carried out with XDS and from a single crystal. Friedel pairs were treated as identical reflections.
- Data reduction was carried out with XDS and from the same CtaZ:CTA crystal. Friedel pairs were treated as different reflections to record anomalous scattering effects.
- Rmerge(l) = Σhkl |I(lhk)| - <l(lhk)> / Σhkl |I(lhk)|, where l(lhk) is the measurement of the intensity of reflection hkl and <l(lhk)> is the average intensity
- R = Σhkl |F_obs| - |F_calc| / Σhkl |F_obs|, where Rwork is calculated without a sigma cut off for a randomly chosen 5% of reflections, which were not used for structure refinement, and Rfree is calculated for the remaining reflections.
- Deviations from ideal bond lengths / angles
- Percentage of residues in favored region / allowed region / outlier region
Table S8. List of sequences used for genomic survey of CtaZ homologs. PCP = peptidyl carrier protein; CtaC = thioamide synthetase homolog; NRPS = nonribosomal peptide synthetase; PKS = polyketide synthase; RiPP = ribosomally synthesized and post-translationally modified peptide.

| Organism name                      | Accession ID          | Phylum          | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RiPP |
|------------------------------------|-----------------------|-----------------|-----|------|----------------------|-----------------------------|--------------|------|
| Anaerovigula multivorans           | WP_089285069.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. CSS11              | WP_224035564.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sporogenes             | WP_163257497.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum              | WP_207130621.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. CM028              | WP_220320532.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sporogenes             | WP_072584225.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sporogenes             | WP_163224385.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum              | WP_030035115.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium estertheticum          | WP_226137092.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. CM027              | WP_220287615.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium estertheticum          | WP_226130650.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum              | WP_191598774.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sporogenes             | WP_096043889.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum strain Mfbjulcb3 | WP_061327743.1      | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum              | WP_100489189.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium estertheticum          | WP_216103447.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium estertheticum          | WP_152753967.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | Y    |
| Clostridium botulinum              | WP_159035817.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium estertheticum          | WP_220713777.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sporogenes             | WP_003493867.1        | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
| Thermoanaerobacterium saccharolyticum | WP_048411500.1      | Firmicutes      | N   | N    | Y                    | Y                           | N            | N    |
# Supporting Information

| Organism name                  | Accession ID          | Phylum   | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPI |
|--------------------------------|-----------------------|----------|-----|------|----------------------|----------------------------|-------------|------|
| Clostridium estertheticum      | WP_216108708.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium sp.                | WP_003485938.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium botulinum          | AVP62490.1            | Firmicutes | N   | Y    | Y                    | Y                          | N           | N    |
| Clostridales bacterium         | NLH02048.1            | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium jeddahense         | WP_198465122.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Anaerostilbacter massiliensis  | WP_042682962.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Abyssisolbacter fermentans    | WP_066497819.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium pasteurianum strain GL11 | WP_066023457.1  | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium minihomine         | WP_101696201.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium algidicarnis       | WP_029452272.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium tetani             | WP_023438841.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium lundense           | WP_027626370.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Hathewayana proteolytica       | WP_072904169.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Lachnoclostridium phytofermentans | WP_029503460.1     | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium tetanomorphum      | WP_035146273.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium tetani             | WP_129029858.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium oryzae             | WP_079422320.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium tetani             | WP_039261565.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Ruminococcaceae bacterium BL-4 | CAB1245269.1          | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridaceae bacterium        | MW4829480.1           | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Clostridium sp. DMHC 10        | WP_053242394.1        | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Oscillospiraceae bacterium     | MBE631230.1           | Firmicutes | N   | N    | Y                    | Y                          | N           | N    |
| Organism name                          | Accession ID       | Phylum        | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RfPP |
|---------------------------------------|--------------------|---------------|-----|------|----------------------|-----------------------------|-------------|------|
| Clostridium amylolyticum              | WP_073003908.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Clostridium sp. MSJ-11                | WP_216438309.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Clostridium tetani                    | WP_115605970.1     | Firmicutes    | Y   | N    | Y                    | Y                           | N           | N    |
| Anaerocolumna sedimenticola           | WP_161840029.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Clostridicaceae bacterium             | HCJ58251.1         | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Emergencia timonensis                 | WP_067542616.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Robinsoniella sp. KNHs210             | WP_027292864.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Ruminococcus sp. OM05-7               | WP_118633935.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Lachnospiraceae bacterium             | GFI46271.1         | Firmicutes    | N   | N    | N                    | Y                           | N           | N    |
| Clostridium botulinum                 | WP_053337826.1     | Firmicutes    | Y   | Y    | Y                    | Y                           | N           | N    |
| Clostridium tagluense                 | WP_220322709.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Robinsoniella peoriensis              | WP_138603146.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Clostridicaceae bacterium strain 1XD21-29 | NBH36554.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Clostridium sp. FP2                   | WP_164952462.1     | Firmicutes    | N   | Y    | Y                    | Y                           | N           | N    |
| Clostridiales bacterium LR776134.1    | CAB1245269.1       | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Robinsoniella peoriensis              | WP_070049092.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Robinsoniella peoriensis              | WP_044294841.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Lachnospiraceae bacterium             | WP_117980771.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Alkalibaculum sporogenes              | WP_152802938.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Anaerosphaera multitolerans           | WP_127723140.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Lachnospiraceae bacterium             | GFI04794.1         | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Tissierella sp. P1                    | WP_094903040.1     | Firmicutes    | N   | N    | Y                    | Y                           | N           | N    |
| Organism name                      | Accession ID    | Phylum       | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|-----------------------------------|----------------|--------------|-----|------|----------------------|-----------------------------|--------------|------|
| Tissierella carlieri             | WP_216561500.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Coprobacillus sp. BIOML-A1       | MZK55450.1     | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiales bacterium          | MBSS080537.1   | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Blautia sp. RD014234             | MCA5961946.1   | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Bacillus sp. WMMC1349            | NPC91583.1     | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Flavonifractor sp. An92         | WP_087257743.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Faecalibacterium faecalis       | WP_216241779.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Lachnospiraceae bacterium AM48-27BH | RHQ12924.1    | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Firmicutes bacterium             | WP_023043102.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Bacillus sp. WMMC1349            | WP_216664710.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Blautia marasmi                  | WP_095170587.1 | Firmicutes   | N   | N    | Y                    | Y                           | Y            | N    |
| Clostridium beijerinckii         | WP_185670391.1 | Firmicutes   | N   | N    | N                    | N                           | N            | N    |
| Blautia pseudococoides           | WP_065542609.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Streptococcus uberis             | WP_203261662.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Coprobacillus cateniformis       | MBM6798211.1   | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium cavendishii          | WP_072985642.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Haloimpatiens massiliensis       | WP_102399849.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. KNHs214          | WP_035291884.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Eubacteriales sp.                | WP_117596986.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Tepidanaerobacter acetatoxydans  | WP_013777987.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Sporanaerobacter acetigenes      | WP_072744831.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Enterocloster bolteae            | WP_118036403.1 | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Organism name                    | Accession ID          | Phylum       | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|---------------------------------|-----------------------|--------------|-----|------|----------------------|-----------------------------|--------------|------|
| Marinisporobacter balticus      | WP_132247045.1        | Firmicutes   | Y   | N    | Y                    | Y                           | Y            | Y    |
| Treponema pedis                 | WP_024469039.1        | Spirochaetes | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile         | WP_054276562.1        | Firmicutes   | Y   | N    | Y                    | Y                           | N            | N    |
| Coprobacillus cateniformis      | WP_008786319.1        | Firmicutes   | Y   | N    | Y                    | Y                           | N            | N    |
| Blautia cocoides strain NCTC11035 | WP_115622679.1    | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. BL-8            | WP_077861566.1        | Firmicutes   | Y   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile         | WP_167640986.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium saccharoperbutylfaceticum | WP_015392150.1    | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium sp. FP1             | WP_164946488.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridium botulinum           | WP_045905952.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile         | HBH180654.1           | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Blautia producta                | WP_130183090.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae bacterium         | WP_054270090.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Vallitalea guaymasensis         | WP_212690078.1        | Firmicutes   | Y   | N    | Y                    | Y                           | Y            | N    |
| Clostridiodae difficile         | WP_021384377.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae sp. ZZV15-6598    | WP_227849129.1        | Firmicutes   | N   | N    | N                    | Y                           | N            | N    |
| Clostridiodae difficile         | WP_107595359.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile         | WP_223195515.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile         | HBG4224319.1          | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Clostridiodae difficile strain RA09-70 | WP_054273608.1    | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Vallitalea okinawensis          | WP_105614688.1        | Firmicutes   | N   | N    | Y                    | Y                           | N            | N    |
| Blautia producta                | WP_173724357.1        | Firmicutes   | Y   | N    | Y                    | Y                           | N            | N    |
| Organism name                              | Accession ID       | Phylum     | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RRIP |
|--------------------------------------------|--------------------|------------|-----|------|----------------------|-----------------------------|-------------|------|
| Clostridioides sp. ZZV15-6388              | WP_227827463.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Streptococcus pneumoniae                   | WP_044727499.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Clostridioides sp. ZZV15-6597              | WP_227840904.1     | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Blautia marasmi strain SL 2.02             | WP_033140923.1     | Firmicutes | N   | N    | Y                    | Y                           | Y           | N    |
| Clostridioides sp. ZZV15-6383              | WP_227825934.1     | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Clostridioides difficile strain 2021EL-00908| MBY2476754.1       | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Clostridioides sp. ES-S-0001-03            | WP_227482395.1     | Firmicutes | N   | N    | Y                    | Y                           | Y           | N    |
| Clostridioides sp. ZZV14-5902              | WP_227850812.1     | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Vallitalea guaymasensis                    | WP_113673400.1     | Firmicutes | Y   | N    | Y                    | Y                           | Y           | N    |
| Clostridioides difficile                    | WP_169468134.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Blautia sp. RD014234                       | MCA5964481.1       | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Clostridioides bacterium                   | WP_227452510.1     | Firmicutes | N   | N    | Y                    | Y                           | Y           | N    |
| Clostridium sp. D2Q-14                     | WP_203373103.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Clostridioides difficile                    | HBG5346294.1       | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Clostridioides difficile                    | MBH6948933.1       | Firmicutes | N   | N    | N                    | Y                           | N           | N    |
| Clostridioides sp. ES-S-0006-03            | WP_227433052.1     | Firmicutes | N   | N    | N                    | Y                           | N           | N    |
| Clostridioides difficile                    | WP_003430201.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Clostridioides difficile                    | WP_021363516.1     | Firmicutes | N   | N    | Y                    | Y                           | N           | N    |
| Clostridioides difficile                    | WP_038812121.1     | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Clostridioides difficile                    | WP_107598297.1     | Firmicutes | N   | N    | Y                    | Y                           | Y           | N    |
| Clostridioides difficile                    | WP_077716979.1     | Firmicutes | N   | N    | N                    | Y                           | Y           | N    |
| Clostridioides difficile                    | HBF5456700.1       | Firmicutes | N   | N    | N                    | Y                           | N           | N    |
| Organism name          | Accession ID   | Phylum       | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|------------------------|---------------|--------------|-----|------|----------------------|----------------------------|-------------|------|
| Clostridioides difficile| HBF6357449.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Blautia wexlerae        | WP_207739572.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| MBY1948980.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| HBE9729668.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_045145608.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| MBY2849528.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| EGT3916955.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_021360546.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_009889503.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_021406380.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_016728863.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_095905945.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| HBG3483912.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_021359212.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_003436480.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| MBZ1209411.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| HBF7860643.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| HBG0099204.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_077709975.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| HBF2804837.1  | Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_021368671.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile| WP_107613022.1| Firmicutes   | N   | N    | Y                    | Y                          | N           | N    |
| Organism name                     | Accession ID       | Phylum          | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|----------------------------------|-------------------|-----------------|-----|------|----------------------|-----------------------------|-------------|------|
| *Clostridoides difficile*        | HBG7672763.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | HBG2772691.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | HBF8490363.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | HBBH3928631.1     | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | MBH6866322.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | WP_0139625554.1   | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | WP_021375263.1    | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | HBF0377810.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | VIF98551.1        | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | MBY2486030.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | WP_107615446.1    | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridoides difficile*        | WP_003420216.1    | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridium innocuum*           | EHU7845026.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Hydrogenispora ethanolica*      | WP_132014954.1    | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Firmicutes bacterium*           | WP_008818942.1    | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Erysipelotrichaceae bacterium 2_2_44A* | EGX77340.1        | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Erysipelotrichaceae bacterium 146* | ANU69031.1        | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Erysipelotrichaceae bacterium*  | MBSS26719.1       | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridium innocuum*           | MCC2846297.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridium innocuum*           | MBV4067596.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Clostridium innocuum*           | KGJ53145.1        | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
| *Erysipelotrichaceae bacterium*  | MBG6180612.1      | Firmicutes      | N   | N    | Y                    | Y                           | N           | N    |
### Supporting Information

| Organism name                 | Accession ID        | Phylum            | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|------------------------------|---------------------|-------------------|-----|------|----------------------|----------------------------|-------------|------|
| Clostridioides difficile DA00065 | EQG21184.1          | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Treponema pedis              | WP_194075959.1      | Spirochaetes      | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile CD9  | EQE05482.1          | Firmicutes        | N   | N    | Y                    | N                          | N           | N    |
| Clostridioides difficile     | WP_012816159.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile     | WP_021368997.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Enterococcus sp.             | WP_086349861.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Enterococcus sp.             | OTP14431.1          | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile Y343| EQI65987.1          | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile DA00215 | EQH29665.1          | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile     | WP_021367798.1      | Firmicutes        | N   | N    | N                    | N                          | N           | N    |
| Clostridioides difficile     | HBF2339718.1        | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Clostridioides difficile     | WP_059027001.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Enterococcus hulanensis      | WP_137665830.1      | Firmicutes        | N   | N    | Y                    | N                          | N           | N    |
| Enterococcus hulanensis      | WP_206921006.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Sebaldella termidids         | WP_012863261.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Fusobacterium sp.            | WP_032840620.1      | Fusobacteria      | Y   | Y    | Y                    | Y                          | N           | N    |
| Fusobacterium necrophorum    | WP_035914910.1      | Fusobacteria      | Y   | Y    | Y                    | Y                          | N           | N    |
| Clostridioides difficile     | WP_022606962.1      | Firmicutes        | Y   | Y    | Y                    | Y                          | N           | N    |
| Oceanotoga teriensis         | WP_109604299.1      | Thermotogae       | N   | N    | Y                    | Y                          | N           | N    |
| Desulfosporosinus sp. Td-M   | KGP75389.1          | Firmicutes        | N   | N    | N                    | N                          | N           | N    |
| Vagococcus sp. BWB3-3         | WP_209529320.1      | Firmicutes        | N   | N    | Y                    | Y                          | N           | N    |
| Bacterium D16-54             | RJK01155.1          | unclassified      | N   | N    | Y                    | Y                          | N           | N    |
### Organism Details

| Organism name | Accession ID | Phylum | PCP | CtaC | Upstream DNA-binding | Two downstream transporters | NRPS or PKS | RIPP |
|---------------|--------------|--------|-----|------|----------------------|-----------------------------|-------------|------|
| Firmicutes bacterium | MTI67946.1 | Firmicutes | N   | N    | Y                   | Y                           | N           | N    |
| Eubacterium sp.    | SCJ76062.1  | Firmicutes | N   | N    | Y                   | Y                           | N           | N    |
| Lachnospiraceae bacterium TB5 | BCN31760.1 | Firmicutes | N   | N    | N                   | N                           | N           | N    |
Author Contributions

F.G., E.M.M., K.L.D., M.G., and C.H. designed research; F.G., E.M.M., T.H., M.D., K.L.D., J.K., and M.G. performed research; F.G., E.M.M., T.H., M.D., K.L.D., J.K., and M.G. analyzed data; and F.G., E.M.M., M.G. and C.H. wrote the manuscript.

References

[1] T. Lincke, S. Behnkens, K. Ishida, M. Roth, C. Hertweck, Angew. Chem. Int. Ed. 2010, 49, 2011–2013.
[2] K. L. Dunbar, H. Büttner, E. M. Molloy, M. Dell, J. Kumpfmüller, C. Hertweck, Angew. Chem. Int. Ed. 2018, 57, 14080–14084.
[3] G. M. Boratyn, A. A. Schäffer, R. Nagarwala, S. F. Altschul, D. J. Lipman, T. L. Madden, Biol. Direct 2012, 7, 12.
[4] J. D. Thompson, D. G. Higgins, T. J. Gibson, Nucleic Acids Res. 1994, 22, 4673-4680.
[5] S. Kumar, G. Stecher, K. Tamura, Mol. Biol. Evol. 2016, 33, 1870-1874.
[6] N. Saitou, M. Nei, Mol. Biol. Evol. 1987, 4, 406-425.
[7] K. Blin, E. P. Pedersen, T. Weber, S. Y. Lee, Synth. Syst. Biotechnol. 2016, 1, 118-121.
[8] I. Fedorova, A. Arseniev, P. Selkova, G. Pobegalov, I. Goryanin, A. Vasileva, O. Musharova, M. Abramova, M. Kazakov, T. Zyubko, T. Artamonova, D. Artamonov, S. Shmakov, M. Khodorkovskii, K. Severinov, I. Fedorova, A. Arseniev, P. S. Artamonova, S. Bachas, C. Eginton, D. Gunio, H. Wade, Proc. Natl. Acad. Sci. USA 2011, 108, 11046–11051.