

Supporting Information

Food-Poisoning Bacteria Employ a Citrate Synthase and a Type II NRPS To Synthesize Bolaamphiphilic Lipopeptide Antibiotics**

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Experimental procedures

Bacterial strains and media

Bacterial and fungal strains used in this study are listed in table S1. *Burkholderia gladioli* pv. *cocovenenans* HKI0521 was continuously cultured in MGY+M9, PDB or CYMG medium (Table S2). For long-term storage, the bacteria were frozen with 1:1 volume of 50% glycerol at -80 °C. *Burkholderia* gladioli pv. *cocovenenans* HKI0521 mutant strains were either cultured in MGY+M9 or PDB medium/agar with addition of kanamycin (50 μ g mL⁻¹). *E. coli* strains were cultured in LB medium or on LB agar plates at 37 °C with appropriate antibiotic concentrations (kanamycin 50 μ g mL⁻¹).

Table S1. Bacterial and fungal strains used in this study.

Bacterial strain	Additional information	Reference
B. gladioli pv. cocovenenans	HKI0521	-
B. gladioli DSM4285	ATCC 10248 ^[1]	
Escherichia coli	One Shot™ TOP10 Electrocomp™ <i>E. coli</i>	ThermoFisher Scientific

 Table S2. Composition of media used in this study.

Medium or medium additive	Composition (L ⁻¹)
MGY+M9 medium	10 g Glycerol, 1.25 g yeast extract (technical yeast extract, BD, Bacto®), 960 mL distilled water, sterilization at 120 °C for 20 min, then add: 20 mL M9 salt A, 20 mL M9 salt B
CYMG	10 g Glycerol, 8 g casein peptone, 4 g yeast extract (technical yeast extract, BD, Bacto®), 6.29 g MgCl ₂ *6 H ₂ O, sterilization at 120 °C for 20 min
PDB	Potato dextrose broth (BD, Bacto®)
PDA	PDB with addition of 1.5% agar (BD, Bacto®)
LB medium/agar	10 g Tryptone (BD, Bacto®), 5 g yeast extract (BD, Bacto®), 10 g NaCl, sterilization at 120 °C for 20 min; for agar: addition of 1.5% agar
NAG agar	34 g Standard I nutrient agar (Merk KGaA), 10 g glycerol, sterilization at 120 °C for 20 min
NA	Standard I nutrient agar (Merk KGaA)
M9 salt solution A M9 salt solution B	350 g K ₂ HPO ₄ , 100 g KH ₂ PO ₄ , sterilization at 120 °C for 20 min 29.4 g Sodium citrate, 50 g (NH ₄) ₂ SO ₄ , 5 g MgSO ₄ , sterilization at 120 °C for 20 min

General analytical methods

LC-MS analysis was performed using an Exactive Orbitrap High Performance Benchtop LC-MS (Thermo Fisher Scientific) with an electron spray ion source and an Accela HPLC System, C18 column (Betasil C18, 150 x 2.1 mm, Thermo Fisher Scientific, Germany), solvents: acetonitrile and water (both supplemented with 0.1% formic acid), flow rate: 0.2 mL min⁻¹; program: hold 1 min at 5% acetonitrile, 1–16 min 5–99% acetonitrile, hold 16–31 min 99% acetonitrile, 31-32 min 99–5%, 32–43 min to 5% acetonitrile. For MS-MS measurements a QExactive Orbitrap High Performance Benchtop LC-MS (Thermo Fisher Scientific) with an electron spray ion source and an Accela HPLC System was used (column: Accucore C18 2.6 μ m, 100 x 2.1 mm, Thermo Fisher Scientific solvents: acetonitrile and water (both supplemented with 0.1% formic acid), flow rate: 0.2 mL min⁻¹; program: hold 1 min at 5% acetonitrile, hold 16-31 min 99% acetonitrile, 31–32 min 99–5%, 32–43 min to 5% acetonitrile and water (both supplemented with 0.1% formic acid), flow rate: 0.2 mL min⁻¹; program: hold 1 min at 5% acetonitrile, 1–16 min 5–99% acetonitrile, solvents: acetonitrile and water (both supplemented with 0.1% formic acid), flow rate: 0.2 mL min⁻¹; program: hold 1 min at 5% acetonitrile, 1–16 min 5–99% acetonitrile, hold 16-31 min 99% acetonitrile, 31–32 min 99–5 %, 32–43 min to 5% acetonitrile. NMR spectra were recorded with a Bruker 500 or 600 MHz Avance III Ultra Shield (Bruker BioSpin GmbH) in DMSO-d₆ ¹H 600 MHz; ¹³C 150 MHz.

Identification, isolation and purification of bolagladin

For isolation of bolagladins, bacteria were cultured in PDB media. 50 mL of PDB medium was inoculated ($OD_{600} \sim 0.4$) and incubated at 30 °C overnight, at 120 rpm. Fourteen 1 L flasks with 250 mL of PDB each were inoculated with 4 mL of this bacterial preculture and incubated without shaking at 30 °C for 4 d. The cultures were thoroughly extracted twice with 1:1 volume of ethyl acetate. The organic extract was dried with sodium sulfate and concentrated under reduced pressure. The residue was dissolved in methanol and separated by size exclusion chromatography using Sephadex LH-20 (Pharmacia Fine Chemicals) with MeOH as eluent. Bolagladin-containing fractions were concentrated (minimum: 5 mL methanol). Final purification was achieved by preparative reversed-phase HPLC (Shimadzu LC-8A HPLC system) using either a Phenomenex Synergi 4 µm Fusion-RP 80 Å, 250 × 21.2 mm column with gradient MeCN/H₂O supplemented with 0.01% trifluoroacetic acid (*v/v*) (58/42 in 20 min to 80/20, 80/20 in 2 min to 100/0. MeCN 100% for 10 min, flow rate 12 mL min⁻¹).

Absolute configuration of amino acids

Bolagladins were hydrolyzed with 6 M HCl for 12 h at 105 °C and subsequently dried under reduced pressure. After the addition of 100 μ L 1 M NaHCO₃ and 50 μ L 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide solution (L-FDAA, Marfey reagent, 10 mg mL⁻¹ acetone) the samples were heated for 1 h at 50 °C. 50 μ l 2 M HCl was added as well as 200 μ L 50% (*v/v*) MeCN. The samples were analyzed with an analytical HPLC and a Kinetex 5 μ m XB-C18 100 Å 250 × 4.6 μ m (Phenomenex) column and a flow rate of 0.5 mL min⁻¹. MeCN and H₂O both supplemented with 0.1% trifluoroacetic acid were used. The gradient can be found in Table S3.

Table S3. Gradient used for Marfey method.

Zeit (min)	dH ₂ O (%)	ACN (%)
5	70	30
30	50	50
35	0	100
37	70	30
42	70	30

The retention times of derivatized amino acids can be found in Table S4 and Table S5. To distinguish isoleucine and *allo*-isoleucine the samples were treated with L-FDAA 50 μ l 1-fluoro-2,4-dinitrobenzol (Sanger reagent) (10 mg mL⁻¹ acetone). A Eurospher C18 5 μ m 100 Å 250 × 4.6 μ m column and an isocratic flow of 40% MeCN in H₂O supplemented with 0.1% trifluoroacetic acid was used.

Table S4. Analysis of the absolute configuration of amino acids bolagladin A (1) and B (2) using Marfey method.

Amino acid	Configuration	Retention time of derivatized standard amino acid [min]	Retention time of derivatized amino acid in bolagladin A (1) [min]	Retention time of derivatized amino acid in bolagladin B (2) [min]
Serine	D	11.6	11.4 (L)	11.4 (L)
	L	11.4		
Valine	D	30.4	30.4 (d)	-
	L	25.3		
Homoserine	DL	12.0 and 12.4	12.0 (L)	12.0 (L)
	L	12.0		
Isoleucine	D	35.1	-	35.1 (D)
	L	29.8		

Table S5. Analy	sis of the configuratio	n of Isoleucine in bolagladin E	3 (2) usino	o Sanger reagent.
	fold of allo bornigarado			g ounger rougene.

Amino acid	Configuration	Retention time of standard	Retention time of amino acid in
		amino acid [min]	bolagladin B (2)[min]
Isoleucine	D	25.5	25.5 (D)
	D- allo	26.5	

Comparison of the retention times of the standard amino acid and the samples allowed for identification of the amino acid building blocks of bolagladin A (L-serine, D-valine, L-

homoserine and L-serine) and bolagladin B (L-serine, D-*allo*-isoleucine, L-homoserine and L-serine). This is in accordance with the predictions based on the Stachelhaus code.

Sequencing and annotation of bolagladin gene cluster

The bolagladin gene cluster (*bol*) was uploaded to the NCBI database, accession number: (MT844061). The webtool antiSMASH 5.0^[2] was used to for the identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in the genome of *Burkholderia gladioli* pv. *cocovenenans* HKI0521. The bolagladin gene cluster consists of a 13.6 kb NRPS gene that contains four modules (Table S6). The predicted amino acid and the Stachelhaus code were taken from the antiSMASH analysis.

Table S6. Prediction of the NRPS modules of BolH according to antiSMASH 5.0; M - Module; C -
condensation, A – adenylation, T – peptidylcarrier domain, TE – thioesterase, X – no prediction
available. Ser – serine. Hse – homoserine. Val - valine.

Modules of BolH	Specificity code (antiSMASH)	Predicted amino acid (Stachelhaus code match)	Amino acid sequence of bolagladin A (1)
M1 (C-A-T)	DVWHMSLVDK	Ser (100%)	Ser
M2 (C-A-T)	DALWMGGVFK	Val (80%)	Val
M3 (C-A-T)	DLKNVGSDVK	Х	Hse
M4 (C-A-TE)	DVWHVSLIDK	Ser (100%)	Ser

NCBI Blast and HHpred^[3] were used for sequence database searching to find homologous genes or proteins that allow for speculations on their functionalities. A promotor search was conducted using PromotorHunter (<u>http://www.phisite.org/promoterhunter</u>). Based on the calculated Gibbs energy and a position-specific weight matrix, a scoring system was used to predict putative promotor sites.

Matrix (-10 region):
A: 5 76 15 61 56 6
C: 10 6 11 13 20 7
G: 8 6 14 14 8 5
T: 77 12 60 12 15



Figure S1: Bolagladin biosynthetic gene cluster (bol).

Further information on the genes respectively encoded proteins can be found in Table S7.

Gene	Lengt	Putatively encoded	Closest characterized	Accession	Identity/
	h [AA]	protein	orthologous protein and (organism)	number	similarity
<i>bolU</i> (+13)	804	TonB-dependent Rezeptor	Enterobactin-iron-transporter (<i>Pseudomonas aeruginosa</i>)	Q05098.1	22%/37%
bolV (+12)	333	Ribokinase	Ribokinase (<i>Homo sapiens</i>)	Q9H477.1	38%/54%
<i>bolW</i> (+11)	296	Transcriptional regulator	Deoxyribose operon repressor (<i>Escherichia coli</i> O157)	P0ACK7.1	43%/57%
<i>bolX</i> (+10)	226	Ι	Deoxyribose-phosphate aldolase (<i>Burkholderia lata</i>)	Q39NL8.1	85%/90%
bolY (+9)	140	Putative plasmid stability protein	VapC ribonuclease (Sinorhizobium fredii)	P55511.1	32%/48%
<i>bolZ</i> (+8)	54	1	1	1	1
bolA (+7)	54	HTH-type transcriptional regulator	Transcriptional activator (<i>Pseudomonas aeruginosa</i> PAO1)	P24734.3	36%/49%
<i>bolB</i> (+6)	583	Long-chain-fatty- acid-CoA ligase	Long-chain-fatty-acid ligase (<i>Mycobacterium tuberculosis</i>)	P0A4X9.1/	37%/48%
bolC (+5)	82	ACP	Acyl carrier protein (<i>Nitrosomonas europaea</i>)	2LKI_A	15%/18%
bolD (+4)	352	Oxygenase/ reductase	p-Aminobenzoate N-oxygenase (<i>Streptomyces thioluteus</i>)	3CHH_B	15%/4%
bolE (+3)	480	Dehydrogenase, putative oxidoreductase	D-2-hydroxyglutarate dehydrogenase (<i>Oryza sativa</i>)	Q7XI14.1	33%/50%
bolF (+2)	343	Desaturase	Sphingolipid ∆(4)- desaturase/C4-monooxygenase DES2 (<i>Homo sapiens</i>)	Q6QHC5. 2	28%/41%
<i>bolG</i> (+1)	441	Major facilitator superfamily (MFS)	MFS-type transporter MT0042 (<i>Mycobacterium tuberculosis</i>)	P9WJY0.1	28%/28%
<i>bol</i> H (0)	4,547	NRPS	Tyrocidine synthase 3 (<i>Brevibacillus parabrevis</i>)	O30409.1	35%/51%

Table S7. Proteins encoded up- and downstream of *bolH*.

Gene	Length [AA]	Putatively encoded protein	Closest characterized orthologous protein and (Organism)	Accession number	ldentity/ similarity
<i>boll</i> (-1)	69	Domain binding protein	MbtH-Homolog (<i>Geobacillus</i> sp. Y4.1MC1)	5U89_B	40%/90%
bolJ (–2)	140	/	1	/	1
bolK (-3)	162	1	1	/	/
bolL (–4)	281	Reductase/ desaturase	Possible acyl desaturase (<i>Mycobacterium tuberculosis</i>)	1ZA0_A	19%/20%
bolM (–5)	364	Transferase	Alkyldiketide–CoA synthase; polyketidesynthase (<i>Tetradium ruticarpum</i>)	5WX6_A	
<i>bolN</i> (–6)	392	Decarboxylase	Diaminopimelate decarboxylase (<i>Actinosynnema pretiosum</i> subsp. <i>auranticum</i>)	O69203.1	28%/36%
bolO (-7)	527	Ligase/A-domain	Long chain fatty acid-CoA ligase (<i>Bacillus subtilis</i>)	O07610.2	29%/44%
bolP (–8)	260	Fatty acid biosynthesis	3-Oxoacyl-acyl-carrier-protein synthase III (<i>Haemophilus</i> <i>influenzae</i>)	3IL3_A	17%/11%
bolQ (–9)	615	Acyl-CoA dehydrogenase	Dehydrogenation (<i>Roseovarius nubinhibens</i> ISM)	6IJC_B	29%/48%
<i>bolR</i> (–10)	459	Citrate synthase	Citrate synthase (<i>Burkholderia thailandensis</i>)	4XGH_A	20%/18%
bolS (–11)	220	Methyltransferase	SAM–dependent methyltransferase (<i>Thiobacillus denitrificans</i>)	5EPE_A	16%/16%
bolT (–12)	357	Dioxygenase/ Decarboxylase	Clavaminate synthase- Protein ScoE Protein (<i>Arabidopsis</i> <i>thaliana</i>)	2Q4A_A/	30%/54%

 Table S8. Proteins encoded up- and downstream of bolH (continued).

Phylogenetic analysis of C domains from the bolagladin gene cluster

C domain amino acid sequences of the bolagladin biosynthesis gene cluster as well as sequences acquired from the NaPDos website^[4] were aligned using MAFFT^[5] with default settings. Maximum likelihood phylogeny was constructed using IQ-tree^[6]. Ultrafast bootstrapping (1000 iterations) analysis was performed. The neighbor-joining tree construction method showed similar tree topology. The phylogeny indicates the functionalities of the subjected C domains and allows a proposal for the biosynthetic logic of bolagladin.



Figure S2. Phylogenetic analysis of C domains from BolH and from the NaPDos website. C domains cluster into the canonical subfamilies (${}^{L}C_{L}$, ${}^{D}C_{L}$, starter, dual E/C, epimerization, heterocyclization domains and modified amino acid domain).

Phylogenetic analysis of the citrate synthase BolR

Characterized (bold) and non-characterized *Re-* and *Si*-citrate synthases amino acid sequences were downloaded from Uniprot database. Additionally, three proteins annotated as citrate synthases encoded in the genome of *B. gladioli* pv. *cocovenenans* were added, one of which was encoded in the bolagladin biosynthesis gene cluster. Citrate synthase proteins from the phomoidride^[7], maleidrides^[8] and squalestatin^[9] biosynthesis pathways were added. Malate synthesis amino acid sequences were added as outgroup. The

sequence alignment was performed using MAFFT 7 with L-INS-I, an iterative refinement method. Maximum likelihood phylogeny was constructed using IQ-tree. Ultrafast bootstrapping (1000 iterations) analysis was performed. The bolagladin citrate synthase (BoIR), as well as the CS-phomoidride, CS-maleidrides and CS-squalestatin clusters with *Si*-citrate synthases. *Re*-CS cluster next to 2-isopropylmalate (EC 2.3.3.13), citramalate synthases (EC 2.3.3.14) and are sometimes wrongly annotated such because they have a high sequence similarity. Notable, citramalate synthase or 2-isopropylmalate synthases are *Re*-face stereospecific with respect to C-2 of their substrates, 2-oxoglutarate and 2-oxo-3-methylbutyrate.^[10] Inspection of the multiple sequence alignment of BoIR shows conservation of reported, catalytically active residues His302, His350 and Asp356 (Figure S4).



Figure S3. Phylogenetic analysis of *Si*- and *Re*-citrate synthase (CS) proteins.

Citramalate synthase (CMS), homocitrate synthase (HCS) and 2-siopropylmalate synthase (IPMS) proteins are included. Malate synthase are used as outgroup. Alignment and construction of the phylogenetic tree was performed using MAFFT 7 respectively IQ-Tree. Malate synthases were chosen as an out group. Ultrafast bootstrapping (1000 iterations) analysis was performed. Red characters, *B. gladioli* pv. *cocovenenans* CS; green characters CS from other secondary metabolite pathways, bold characters in vitro characterized proteins.

Bg coco citrate ·	-292	FMA-SC	PSHVC	AALEA	OWLTA	ADTVSAP	RPPAA	EAAARA	A <mark>I</mark> D-2	AALEARO	QTIY <mark>GE</mark> +
Bg coco citrate ·	·142	IGALR	PKHGG	ANEVAL	FEIOSR	YR	-DADEA	EA	DIR-I	RRVENKE	VVIGE+
308J Salmonella	-242	· IGALR	PKHGG	ANEVS	EIQOR	YE	-TPDEA	EA	DIR-I	KRVENKE	VVIGE+
1A59 COLD ACTIV.	·213	IGALK	SPLHGG	ANEAV	HTFEE	GIRKDESI	DEAAT	RSKA		DATAOK	
3HWK Mycobacter ·	-255	· IGALK	RLHGG	ANEAVI	HDMIE	I G	-DPANA	RE	WLR-A	AKLARKE	KIMGF+
CISY PYRFU Pyro -	·216	· IGALK	PINGG	AVEEA	KQFME	I G	SPEK	/EE	WFF-I	KALQOK	KIMGA+
CISY THEAC Ther .	·215	LAALK	PLHGG	AAFAA	AQFDE	I K	-DPAM	ЕК	WFND	IINGK	(RL <mark>MG</mark> F+
2IFC Thermoplas ·	·215	LAALK	PLHGG	AAFAA	AQFDE	I K	-DPAM	ЕК	WFND	NIINGK	(RL <mark>MG</mark> F+
107X Sulfolobus	-210	LAALK	SPLHGG	AAFEAI	- FKQFIE	I G	-DPNR	QN	WFND	KVVNOKN	IRLM <mark>G</mark> F+
Bg coco 4315741 ·	-140	· IACLWO	SPAHGG	ANEAAI		I G	-SPDNI	PD	FIK-	VKDKNS	GVKLMGF+
2H12 Acetobacte ·	-264	· IAALWO	PAHGG	ANEAVI	KMLAR	I G	-KKENI	PA	FIA-	VKDKNS	GVKLMGF+
4G6B Escherichi ·	-256	· IA <mark>SLW</mark> G	PAHGG	ANEAAI	KMLEE	IS	-SVKH1	PE	FVR-I	RAKDKNI	SFRLMGF+
Squalestatin S1.	·310	ISAAS	PLHGG	ALEVCY	QGLEL	I G	-SVDNV	PA	YIA-2	AVKAKKE	RLFGY+
phiJ Fungal sp	-281	· ISAAY	PLHYG	AQEAGY	(RT <mark>L</mark> SE	I G	-SADRV	РН	FLE-	VKRRE	RLFGY+
bf12 ANF07286.1 ·	-276	LAAAY	SPLHFG	ATEAAI	IRALOE	I G	-SVERV	PD	FL <mark>E-</mark>	VKRGE	RKL <mark>FGY</mark> +
CISY CHICK Gall -	-266	MNGLAC	FPLHGL	ANOEVI	GWLAO	LOKAXXXAG	ADAS	RD	YIW-1	TLNSG	RVVPGY-
2CSC Gallus gal -	-266	MNGLAC	FPLHG L	ANOEVI	GWLAO	L <mark>OKAXXXA</mark> G	ADAS	.RD	YIW-I	TLNSG	RVVPGY+
4CSC Gallus gal ·	-266	MNGLAC	FLHGL	ANOEVI	GWLAQ	LQKAXXXAG	ADAS	RD	YIW-1	TLNSG	RV <mark>V</mark> PGY+
CISY PIG citrat .	-293	MNGLAC	FLHGL	ANOEVI	VWLTO	LQKEVGKDV	/SDEKI	RD	YIW-1	TLNSG	RV <mark>V</mark> PGY+
4CTS Sus scrofa ·	-266	MNGLAC	FLHGL	ANOEVI	VWLTO	LOKEVGKDV	7SDEKI	RD	YIW-1	TLNSG	RV <mark>V</mark> PGY+
2CTS Sus scrofa ·	-266	MNGLAC	FPLHGL	ANOEVI	VWLTO	LOKEVGKDV	7SDEKI	RD	YIW-1	TLNSG	RV <mark>V</mark> PGY+
5UZQ homo sapie ·	-289	MNGLAC	FPLHGL	ANOEVI	VWLTO	LOKEVGKDV	7SDEKI	RD	YIW-1	TLNSG	RV <mark>V</mark> PGY+
5UQO Neosartory ·	-294	·LLGLAC	FPLHGL	AAOEVI	RWILA	MODKIGTKE	TDDDV	RN	YLW-I	DTLKSG	RV <mark>V</mark> PGY+
6BON Aspergillu	-273	· LLGLAC	FPLHG L	AAQEVI	RWILA	MODKIGTKE	TDDDV	RN	YL <mark>W-</mark> I	DTLKSG	RV <mark>V</mark> PGY⊷
consensus · · · · ·	·361	· · · · · · · · · · · · · · · · · · ·	* *	*		••••••			•••		··· •*•
ب											
-											
۔ ب			_					_			
↓ Bg_coco_citrate·	·348	•G <mark>H</mark> PLF-	-TA <mark>D</mark> PR	PPHLR	RLLGEF	GLDGG	PYLSI	FDAS		-СЕНААА	ARRGLRPN-
⊷ ₽ Bg_coco_citrate Bg_coco_citrate	·348 ·188	•G <mark>H</mark> PLF- •GH <mark>PVY</mark> I	-TA <mark>D</mark> PR IIS <mark>D</mark> PR	PPHLRI NKVIKI	RLLGEF V <mark>A</mark> RKL	GLDGG SKDAGDT	PYLSI KIFE	FDAS AERLES	v	-CEHAAA	ARRGL <mark>RPN+</mark> DAKKMFPN+
← Bg_coco_citrate → Bg_coco_citrate → 308J_Salmonella →	·348 ·188 ·288	·G <mark>H</mark> PLF ·GHPVYI ·GHPVYI	-TA <mark>D</mark> PR TIS <mark>DPR</mark> TIADPR	PPHLRI NKVIKI HQVIKI	RLLGEF VARKL RVAKOL	GLDGG SKDAGDT SEEGGSL	-PYLSI -KLEEI -KMYHI	FDAS AERLES ADRLET	v	-CEHAAA MWI MWE	ARRGL <mark>RPN</mark> ↔ AKKMFPN↔ TKKMFPN
↓ Bg_coco_citrate · Bg_coco_citrate · 308J_Salmonella · 1A59_COLD_ACTIV ·	·348 ·188 ·288 ·268	-GHPLF- -GHPVYI -GHPVYI -GHRVYF	-TADPR IISDPR IIADPR NG <mark>D</mark> SR	PPHLR NKVIK HQVIK VPTMK	RLLGEF VARKL RVAKOL SALDAM	GLDGG SKDAGDT SEEGGSL IKHYDRP	-PYLSI -KLFEI -KMYHI -EMLGI	FDAS AERLES ADRLET YNGLEA	V V	-CEHAAA MWI MWE MEE	ARRGL <mark>RPN+</mark> DAKKMFPN+ CTKKMFPN+ CAKQIKPN+
♣J Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter -	·348 ·188 ·288 ·268 ·301	- GHPLF- - GHPVY1 - GHPVY1 - GHRVY2 - GHRVY2	-TADPR IISDPR IIADPR NGDSR NGDSR	PPHLR NKVIK HQVIK VPTMK VPTMK	RLLGEF VARKL RVAKOL SALDAM RALERV	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ	-FYLSI -KIFEI -KMYHI -EMLGI -RWLDI	FDAS AERLES ADRLET YNGLEA YQVLAA	V V A	-CEHAAA MWI MWE MEE MAS	ARRGLRPN+ DAKKMFPN+ CTKKMFPN+ CAKQIKPN+ SATGILPN+
<pre></pre>	·348 ·188 ·288 ·268 ·301 ·262	GHPLF GHPVYI GHPVYI GHRVYF GHRVYF GHRVYF	-TADPR IISDPR IIADPR (NGDSR RHGDSR (TYDPR	PPHLR NKVIK HQVIK VPTMK VPTMK ARIFK	RLLGEF VARKL RVAKOL SALDAM RALERV KYASKL	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK	-FYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI	FDAS AERLES ADRLET YNGLEA YQVLAA AERLER	V N NE RLVE	-CEHAAA MWI MWE MEE MAS WAS	ARRGIRPN+ DAKKMFPN+ TKKMFPN+ LAKQIKPN+ SATGILPN+ SKKGISIN+
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther -	·348 ·188 ·288 ·268 ·301 ·262 ·262	·GHPLF- ·GHPVYI ·GHPVYI ·GHRVYI ·GHRVYI ·GHRVYI ·GHRVYI	-TADPR TISDPR TIADPR MGDSR MGDSR TYDPR TYDPR	PPHLR NKVIK HQVIK VPTMK VPTMK ARIFK AKIFK	RLLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH-	-PYLSI -KLFEI -KMYHI -EMLGI -RWLDI -KLFEI -KVYEI	FDAS AERLIS ADRLIT YNGLIA YQVLAA AERLIT ATKLID	V A E LVE FGI	-CEHAAA MWI MWE MAS MAS YLS KAFG	ARRGIRPN+ DAKKMFPN+ TKKMFPN+ LAKOIKPN+ SATGILPN+ SKKGISIN+ SSKGIYPN+
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther - 2IFC_Thermoplas -	-348 -188 -288 -268 -301 -262 -262 -262	·GHPLF- ·GHPVYI ·GHPVYI ·GHRVYI ·GHRVYI ·GHRVYI ·GHRVYI ·GHRVYI	-TAD PR TISDPR TIAD PR (NGD SR (HGD SR (TYD PR (TYD PR (TYD PR	PPHLR NKVIK HQVIK VPTMK VPTMK ARIFK AKIFK AKIFK	RLLGEF VARKI RVAKOL SALDA RALERV KYASKI GIAEKI GIAEKI	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH-	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -KVYEI	FDAS AERLIS ADRLIT YNGLIA YQVLAA AERLIR ATKLID	V A L LVE)FGI)FGI	-CEHAAA MWI MWE MAS WASG KAFG	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGISIN SKGIYPN
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther - 2IFC_Thermoplas - 107X_Sulfolobus -	-348 -188 -288 -301 -262 -262 -262 -262 -257	GHPLF GHPV1 GHPV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1	-TAD PR IISD PR IIAD PR (NGD SR (HGD SR (TYD PR (TYD PR (TYD PR (TYD PR	PPHLR NKVIK HQVIK VPTMK VPTMK ARIFK AKIFK AKIFK	RLLGEF VARKL RVAKOL SALDA RALERV KYASKL SIAEKL SIAEKL KLALT	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR-	-FYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -KYFEI	FDAS AERLES ADRLET YNGLEA YQVLAA AERLER ATKLED ATKLED AQKLEE	V V LA LE VE PFGI DFGI DFGI	-CEHAAA MWI MWF MAS YLS KAFG KAFG KQFS	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGISIN SKGIYPN SKGIYPN
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther - 2IFC_Thermoplas - 107X_Sulfolobus - Bg_coco_4315741 -	-348 -188 -268 -301 -262 -262 -262 -262 -257 -188	GHPLF GHPV1 GHPV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1 GHRV1	TADPR TISDPR TIADPR NGDSR HGDSR TYDPR TYDPR TYDPR NYDPR	PPHLR NKVIK HQVIK VPTMK ARIFK AKIFK AKIFK AKIFK AKIFK	RLLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL KLALTL TCYEV	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE	PYLSI -KLFEI -KMYHI -EMLGI -RWLDI -KUFEI -KVYEI -RYFEI -RYFEI DPLFKI	FDAS AERLES ADRLET YNGLEA YQVLAA AERLER ATKLED ACKLEE AQKLEE	V 	-CEHAAA MWI MWI MAS YLS KAFG KAFG KQFS DYFV	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGISIN SKGIYPN SKGIYPN SKGIYPN
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther - 2IFC_Thermoplas - 107X_Sulfolobus - Bg_coco_4315741 - 2H12_Acetobacte -	-348 -188 -288 -268 -301 -262 -262 -262 -257 -188 -312	- GHPLF - GHPVY1 - GHPVY1 - GHRVY2 - GHRVY2 - GHRVY2 - GHRVY2 - GHRVY2 - GHRVY2 - GHRVY2	TADPR TISDPR TIADPR NGDSR HGDSR TYDPR TYDPR TYDPR NYDPR NYDPR	PPHLR NKVIK VPTMK VPTMK ARIFK AKIFK AKIFK AKIFK AKIFK AKIM	RLLGEF VARKL SALDAM RALERV KYASKL SIAEKL SIAEKL SIAEKL CCYEV 2TCHEV	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE-GLHDI LTE-GIKDI	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -RUFEI -KVYEI -RVYEI -RYFEI DPLEDI	FDAS AERLSS ADRLSI YNGLSA YQVLAA AERLSE ATKLSI ATKLSI AQKLSE AQLSK	A A E F G G G A A A A A A A A A A A A A A A A	-CEHAAA MWI ME MAS YLS KAFG KAFG 	ARRGIRPN DAKKMFPN TKKMFPN AKOIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN
Bg_coco_citrate - Bg_coco_citrate - 308J_Salmonella - 1A59_COLD_ACTIV - 3HWK_Mycobacter - CISY_PYRFU_Pyro - CISY_THEAC_Ther - 2IFC_Thermoplas - 107X_Sulfolobus - Bg_coco_4315741 - 2H12_Acetobacte - 4G6B_Escherichi -	-348 -188 -288 -268 -301 -262 -262 -262 -257 -188 -312 -304	GHPLF GHPVYI GHPVYI GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2	TADPR TISDPR TIADPR NGDSR NGDSR TYDPR TYDPR TYDPR TYDPR NYDPR NYDPR NYDPR	PPHLR NKVIK HQVIK VPTMKS VPTMK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR	RLLGEF VARKU SALDAM RALERV KYASKI SIAEKI SIAEKI SIAEKI TCYEV TCHEV TCHEV	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE-GLHDI LTELGIKDI LKE-GTKD-	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -RVFEI -RVFEI -RVFEI -RYFEI DPLEN -DLEV	FDAS AERLS ADRLS YNGLS AVOUAA AERLS ATKLS ATKLS AVELS AVELS AMQLS AVELS	A E FGI	-CEHAAA MWI ME MAS YLS 	ARRGIRPN DAKKMFPN TKKMFPN AKOIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKGIYPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1	-348 -188 -288 -301 -262 -262 -262 -262 -257 -188 -312 -304 -356	GHPLF GHPVYI GHPVYI GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2 GHRVY2	TADPR TISDPR TADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR MYDPR	PPHLR NKVIK HQVIK VPTMKS VPTMK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR AKIMR ALTK	RLLGEF VARKU SALDAM RALERV KYASKI GIAEKI GIAEKI KLALTI TCYEV TCHEV LMEEH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE-GLHDI LTE-GIKDI LKELGTKD- REALDAN	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DELFKI DELLDI -DILEV -DILEV	FDAS AERLSS ADRLSI YNGLBA YQVLAA AERLSE ATKLSI ATKLSI AQKLSE AWQLSK AWQLSK AWQLSK AWQLSK	A 	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN AKOIKPN BATGILPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKKIYPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp.	-348 -188 -288 -301 -262 -262 -262 -262 -257 -188 -312 -304 -356 -327	GHPLF GHPVYI GHPVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI	TADPR TISDPR TADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR MYDPR TQPPR	PPHLR NKVIK HQVIK VPTMKS VPTMK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR ALIK AALIK	RLLGEF VARKU SALDAM RALERV KYASKI SIAEKI SIAEKI KLALTI TCYEV TCHEV SUCEEN SWLQEL	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEV -DILEV -DILEV -PILQI -PILQI	FDAS AERLS ADRLS ADRLS ADRLS ADRLS ADRLS ACLS ACLS ACLS AVELS AVELS AVELS AVELS AVELS	A 	-CEHAAA MWI MEH MAS YLS KAFG KAFG 	ARRGIRPN DAKKMFPN TKKMFPN AKGIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322	GHPLF GHPVYI GHPVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRTYI	TADPR TISDPR TADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR TEDPR	PPHLR NKVIK VPTMKS VPTMKS ARIFK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR ALIK INAVK VIPIK	RLLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV LMEEH GWLQEL KLLE	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEN DILEN -DILEN -DILEN -PLQI -PLMKI -PLIEI	FDAS AERLS ADRLS ADRLS ADRLS ADRLS ADRLS ACLS ACLS ACLS AVELS AVELS AVELS AVELS AVELS AVELS AVELS AVELS AVELS AVELS	V LE FGI-	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN AKGIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN ZSKIYPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322 -319	GHPLF GHPVYI GHPVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRTFZ GHRTYI	TADPR TISDPR TIADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR TEDPR GTDPR	PPHLR NKVIK HQVIK VPTMKS VPTMK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR ALIK LNAVK VIPIK YTCOR	ALLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV SWLQEL KLLE FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD	- EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEV -DILEV -DILEV -PILQI -PIMKI -PILII	FDAS AERL S ADRL S YNGL SA YQVLAA AERL S ATKL S ATKL S AVEL S A	V LE FGI-	-CEHAAA MWI MWE MAS YLS 	ARRGIRPN DAKKMFPN TKKMFPN AKQIKPN BATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN ZSKIYPN ZSKIYN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322 -319 -319	GHPLF GHPVYI GHPVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRTYI GHRIYI GHRIYI	TADPR TISDPR TIADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR GTDPR KTDPR KTDPR	PPHLR NKVIK HQVIK VPTMKS VPTMK AKIFK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR ALIK INAVK VIPIK YTCOR YTCOR	ALLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV SWLQEL KLLE FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEV -DILEV -DILEV -PILQI -PIMKI -PIFKI -PMFKI	FDAS AERL S ADRL S YNGL SA YQVI AA AERL S ATKL S ATKL S AVEL S	V LE DFGI DFGI DFGI DFGI DFGI TALE- I	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN AKGIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKKIYPN ZSKKIYPN ZSKKIYPN ZSKIKIN ZSKGIRAN ZSKGIRAN ZSKGIRAN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322 -319 -319 -319	GHPLF GHPVYI GHPVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVYI GHRVI GHRVI GHRVI GHRVI GHRVI	TADPR TISDPR TIADPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR KTPPR KTPPR KTPPR	PPH NKVIK HQVIK VPTMKS VPTMK ARIFK AKIFK AKIFK AKIFK AKIFK AKIFK ALIK INAVK VIPIK YTCOR YTCOR	ALLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV SWLQEL KLLE FALKH FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD	-EYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEV -DILEV -DILEV -PILQI -PIMKI -PMFKI -PMFKI -PMFKI	FDAS AERL S ADRL S ADRL S ADRL S ADRL S ADRL S AR AERL S ATKL S ATKL S ACL S AVEL S	V PFGI PFG	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN AKGILPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKGIYPN ZSKLYPN ZSKLYPN ZSKGIRN ZSKGIRN ZSKGIRN ZSKGIRN ZSKGIRN ZSKGIRN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal	-348 -188 -288 -268 -301 -262 -262 -262 -262 -257 -188 -312 -304 -356 -327 -322 -319 -319 -319 -319 -346	GHPLF GHPVYI GHPVYI GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVY GHRVE GHRVE	TADPR TISDPR TISDPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR GTDPR KTDPR KTDPR KTDPR KTDPR	PPH NKVIK HQVIK VPTMKS VPTMKS ARIFK AKIFK AKIFK AKIFK AKIFK AKIMR AKIMR ALIK INAVK VIPIK YTCOR YTCOR YTCOR YTCOR	ALLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD PHD	-PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DILEV -DILEV -DILEV -PILQI -PIKI -PMFKI -PMFKI -PMFKI	FDAS AERL SS ADRL ST YNGL SA YQVU AA AERL SR ATKL SI ATKL SI AQKL SE AWGL SK AWGL SK AWGL SK AWGL YK VAQL YK VAQL YK VAQL YK	V PGI PGI PGI	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN TKKMFPN ATGILPN SKGISIN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKLYPN ZSKLYPN ZSKGIRN ZSRGIRN ZSRGIRN AANPMPN AANPMPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal CISY_PIG_citrat 4CTS_Sus_scrofa	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -327 -322 -319 -319 -319 -319 -346 -329	GHPLF GHPVYI GHPVYI GHRVY2 GHRV2 G	TADPR TISDPR TISDPR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MYDPR KTDPR KTDPR KTDPR KTDPR KTDPR	PPHLR NKVIK HQVIK VPTMKS VPTMKS ARIFK AKIFK AKIFK AKIFK AKIFK AKIFK INAVK VIPIK YTCOR YTCOR YTCOR YTCOR	ALLGEF VARKL RVAKOL SALDAM RALERV KYASKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD PHD PHD	-PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KVYEI -KVYEI -RYFEI DILEV -DILEV -PLQI -PL	FDAS AERL SS ADRL ST YNGL SA YQVU AA AERL SE ATKL SE ATKL SE AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK VACL YK VACL YK VACL YK VACL YK	V PGI PGI PGI	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN TKKMFPN AKGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN ZSKGIYPN ZSKGIYPN ZSKGIRN ZSRGIRN ZSRGIRN ZSRGIRN AANPMPN AANPMPN AANPMPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal CISY_PIG_citrat 4CTS_Sus_scrofa 2CTS_Sus_scrofa	-348 -188 -288 -268 -301 -262 -262 -262 -262 -257 -188 -312 -304 -356 -327 -322 -319 -319 -319 -319 -319 -319	GHPLF GHPVYI GHPVYI GHRVY2 GHRV2 GH	TADPR TISDPR TISDPR MGDSR MGDSR TYDPR TYDPR TYDPR TYDPR MYDPR MYDPR KTDPR KTDPR KTDPR KTDPR KTDPR KTDPR	PPHLR NKVIK HQVIK VPTMKS VPTMKS ARIFK AKIFK AKIFK AKIFK AKIFK AKIFK AKIFK INAVK VIPIK YTCOR YTCOR YTCOR YTCOR YTCOR	ALLGEF VARKL RVAKQL SALDAM RALERV KYASKL SIAEKL SIAEKL SIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH FALKH FALKH	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD PHD PHD PHD	-PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KVYEI -KVYEI -RYFEI DELEKI DELEKI DELEU -PLLQI -PLLQI -PLLQI -PLLQI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI	FDAS AERL SS ADRL ST YNGL SA YQVU AA AERL SE ATKL SE ATKL SE AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK VACL YK VACL YK VACL YK VACL YK VACL YK	V	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKKIYPN SRKIYPN AANPNPN AANPNPN AANPNPN AANPNPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal CISY_PIG_citrat 4CTS_Sus_scrofa 2CTS_Sus_scrofa 5UZQ_homo_sapie	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322 -319 -319 -319 -346 -319 -346 -319 -342	- GH GHPVYI - GHPVYI - GHRVYY - GHRVY - GHRVY - GHRVI - GHAVI - GHAVI - GHAVI - GHAVI - GHAVI	TADPR TISDPR TISDPR MGDSR MGDSR MGDSR TYDPR TYDPR TYDPR MYDPR MYDPR MTDPR KTDPR KTDPR KTDPR KTDPR KTDPR KTDPR	PPHLR NKVIK HQVIK VPTMKS VPTMKS ARIFK AKIF	ALLGEF VARKL RVAKQL SALDAM RALERV KYASKL SIAEKL SIAEKL SIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH FALKH FALKH	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD PHD PHD PHD PHD PHD	-PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KVYEI -KVYEI -RYFEI DELEKI DELEKI DELEKI -PLLQI -PLLQI -PLLQI -PLLQI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI	FDAS AERL SS ADRL ST YNGL AA AERL SR ATKL ST ATKL ST AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK AVEL SK VACL YK VACL YK VACL YK VACL YK VACL YK VACL YK VACL YK VACL YK	V A FGI FGI FGI FGI FGI FGI FGI FGI FGI FGI FGI	-CEHAAA MWI MWE MAS 	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIRN SRGIRN CRKLYPN ZRKLKIN SRGIRN SRGIRN AANPNPN AANPNPN AANPNPN AANPNPN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal 4CSC_Gallus_gal CISY_PIG_citrat 4CTS_Sus_scrofa 2CTS_Sus_scrofa 5UQQ_Neosartory 5UQQ_Neosartory	-348 -188 -288 -268 -301 -262 -262 -262 -262 -257 -188 -312 -304 -327 -322 -319 -319 -319 -346 -319 -342 -342 -342	- GH GHPVYI - GHPVYI - GHPVYI - GHRVYY - GHRVY - GHRVY - GHRVI - GHAVI - GHAVI	TADPR TISDPR TISDPR TISDPR TYDPR TYDPR TYDPR TYDPR TYDPR TYDPR TYDPR TEDPR TEDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR	PPHLR NKVIK HQVIK VPTMK VPTMK ARIFK AKIFK	ALLGEF VARKL RVAKQL SALDAM RALERV KYASKL SIAEKL SIAEKL SIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH FALKH FALKH FALKH FALKH	GLDGG SKDAGDT SEEGGSL IKHYDRP GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PHD PHD PHD PHD 	-PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -RVFEI -KVYEI -RYFEI DLEV -PLLDI -PLLDI -PLLQI -PLLQI -PLLQI -PLLQI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI	FDAS AERLES ADRLET YNGLEA YQUUAA AERLER ATKLEI ATKLEI AVELEK AVELEK AVELEK VACLYK VACLYK VACLYK VACLYK VACLYK VACLYK VACLYK	 A FGI	-CEHAAA MWI MWE MAS WAS WAS WAS WAS 	ARRGIRPN DAKKMFPN TKKMFPN CAKOIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIRN SRGIRN CRKLYPN CRKLYPN CRKLYPN CRKLYPN CRKLYPN CAKNPN PN AANPN PN AANPN PN AANPN PN CAKNPN PN CAKNPN PN CAKNPN CANNPN CANPNN CANNPN CANNPN CANNPNN CANNPNN CANNPNN CANNPNN CAN
Bg_coco_citrate Bg_coco_citrate 308J_Salmonella 1A59_COLD_ACTIV 3HWK_Mycobacter CISY_PYRFU_Pyro CISY_THEAC_Ther 2IFC_Thermoplas 107X_Sulfolobus Bg_coco_4315741 2H12_Acetobacte 4G6B_Escherichi Squalestatin_S1 phiJ_Fungal_sp. bf12_ANF07286.1 CISY_CHICK_Gall 2CSC_Gallus_gal 4CSC_Gallus_gal 4CSC_Gallus_gal CISY_PIG_citrat 4CTS_Sus_scrofa 2CTS_Sus_scrofa 5UQQ_Neosartory 6BON_Aspergillu	-348 -188 -288 -268 -301 -262 -262 -262 -262 -262 -262 -312 -304 -356 -327 -322 -319 -319 -319 -346 -319 -342 -347 -342 -347 -342	- GH GHPVYI - GHPVYI - GHPVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVYI - GHRVI - GHRVI	TADPR TISDPR TISDPR TADPR TYDPR TYDPR TYDPR TYDPR TYDPR TYDPR TYDPR TEDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR TTDPR	PPH NKVIK HQVIK VPTMK VPTMK ARIFK AKIFK AKIFK AKIFK AKIFK AKIFK AKIFK AKIFK AKIMR ALTK VIPIK YTCOR YTCOR YTCOR YTCOR YTCOR YTCOR YTCOR YTCOR YTCOR	ALLGEF VARKL RVAKQL SALDAM RALERV KYASKL GIAEKL GIAEKL GIAEKL TCYEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV TCHEV FALKH FALKH FALKH FALKH FALKH	GLDGG SKDAGDT SEEGGSL GTVRDGQ GDK SSKKPEVH- SSKKPEVH- IERNADAR- LNE GLHDI LTE GIKDI LKE GTKD- REA DAN DFDSKRE DSNATSN PGD PGD PGD PHD PHD PHD PHD PHD PHD PHD PHD PHD PHD PHD PHD PHD	PYLSI -KIFEI -KMYHI -EMLGI -RWLDI -KIFEI -KVYEI -RYFEI DLEV -PLLQI -PLLQI -PLLQI -PLLQI -PLLQI -PLLQI -PLKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI -PMFKI	FDAS AERI S ADRI S ADRI S ADRI S ADRI S ADRI S AUCI S ATKI S ATKI S AUCI S AUCI S AUCI S AUCI S AUCI S AUCI S AUCI S AUCI S AUCI S VACI S V	 	-CEHAAA MWI MWE MAS WAS WAS WAS WAS 	ARRGIRPN DAKKMFPN TKKMFPN TKKMFPN AKGIKPN SATGILPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIYPN SKGIRN SRGIRN TCRKIKIN SRGIRN SRGIRN AANPNPN AANPNPN AANPNPN AANPNPN AANPNPN AANPNPN CAKNPNPN CAKNPNPN CAKNPNPN CAKNPNPN

Figure S4. Extract of the multiple sequence alignment of citrate synthase amino acid sequences confirms conservation of catalytically active residues in BolR (marked with red boxes).

Strains containing the bolagladin gene cluster

Organism	Origin	Accession number
<i>B. gladioli</i> pv. cocovenenans HKI0521	Contaminant in fungal culture in the fermentation of coconut patties	DSM11318
<i>Burkholderia gladioli</i> AU26456	Sputum, <i>Homo sapiens</i>	NZ_PVGZ01000021.1
<i>Burkholderia gladioli</i> AU30473	Sputum, <i>Homo sapiens</i>	NZ_PVHI01000143.1
<i>Burkholderia gladioli</i> AU29541	Sputum, <i>Homo sapiens</i>	NZ_PVHE01000070.1
Burkholderia gladioli 3723STDY6437372	<i>Homo sapiens</i> , cystic fibrosis, USA	NZ_UWYX01000001.1
Burkholderia gladioli BSR3	Diseased rice sheath (South Korea)	NC_015376.1
<i>Burkholderia gladioli</i> 3723STDY6437373	Clinical; Pathogen (United Kingdom)	NZ_UWYW01000003.1
Burkholderia gladioli Co14	Fermented corn meal (Heilongjiang Province)	NZ_CP033431.1
<i>Burkholderia gladioli</i> AU0032	Sputum, <i>Homo sapiens</i>	NZ_PVFC01000046.1
<i>Burkholderia gladioli</i> MSMB1756	Soil	NZ_LOZK01000085.1
Burkholderia BCC238	Clinical	NZ_UZVS01000001.1
<i>Burkholderia gladioli</i> pv. <i>gladioli</i> FDAARGOS_188	-	NZ_CP022210.1
<i>Burkholderia gladioli</i> NCTC12378	Plant (<i>Gladiolus</i> species)	NZ_UARB01000021.1
<i>Burkholderia gladioli</i> pv. <i>gladioli</i> KACC 11889	Gladiolus, (South Korea)	NZ_CP022006.1
<i>Burkholderia gladioli</i> ATCC 10248	Plant	NZ_CP009322.1
<i>Burkholderia gladioli</i> NBRC 13700	Free living	NZ_BBJG01000123.1
<i>Burkholderia gladioli</i> ATCC 25417	Leaves, <i>Gladiolus</i>	NZ_KN150850.1

Table S9. Genomes of *Burkholderia* spp. that contain the bolagladin NRPS gene cluster.

Gene cluster comparison using EasyFig2.3

The sequence similarity of *bol* gene clusters is visualized using the tool Easyfig 2.3.^[11] Color code represents the sequence similarity values as indicated (Figure S5).



Figure S5. Comparison of *bol* gene clusters from *Burkholderia gladioli* (*Bg*.) spp. isolated from different sources. Genes shared by all listed *bol* gene clusters are represented by blue arrows, others are depicted in black.

Genetic manipulation of Burkholderia gladioli pv. cocovenenans HKI0521

Burkholderia gladioli was genetically manipulated by homologous recombination to inactivate genes putatively involved in the biosynthesis of bolagladin. Homologous regions encoded on a suicide plasmid were recombined with the genome of the bacteria leading to the integration of a kanamycin resistance cassette and thereby rendering the target gene inactive.

Construction of the knockout plasmids

All experiments have been performed according to manufacturer's recommendations if not stated otherwise. Genomic DNA isolation of bacteria was performed using PureTM DNA-Isolation kit (Epicentre Biotechnologies). PCRs have been performed as follows: *Burkholderia gladioli* HKI0521genomic DNA, 2x master mix of KAPA2G Robust HotStart ReadyMix PCR kit (Merck KGaA) and 35 cycles of 95 °C for 30 s, 60 °C for 30 s kb⁻¹, and a final extension time at 72 °C for 30 s kb⁻¹. The following cloning strategies have been used to construct the knockout plasmid:

I: Amplification of homologous regions and subsequently restriction to introduce a kanamycin resistance cassette, construction of pJB02, pJB04, pJB06 and pJB14. Primers listed in table S10 have been used in a PCR to amplify homologous regions from *Burkholderia gladioli* genomic DNA. The PCR product was blunt-end ligated into pJET1.2 linear vector (CloneJET PCR Cloning Kit, Thermo Fisher Scientific). The resulting plasmids as well as the kanamycin resistance cassettes amplified from pGEM-Kan^[12] with the listed primers have been digested with the indicated restriction enzymes (Table S10).

Plasmid	Name and sequence $5' \rightarrow 3'$	Product size and	Digestion
(target gene)		name	enzyme
pJB02	JB01, ccagttcgtcctcgctgtag	2,299 bp,	<i>Eco</i> RI
(NRPS gene, <i>bolH</i>)	JB02, cctctacctgctggccttc	homologous region	
	JB03, cgtggaattcgtaagcttaggctgctgcc	1,182 bp,	-
	JB04, cgtggaattctcagaagaactcgtcaag	KanR	
pJB04	JB07, cttctcgtcgagcttgttcc	2,371 bp,	Sacl
(fatty acid ligase gene,	JB08, gcctcgactacgaggtgttc	homologous region	
bolO)	BD247, cggacgagctcgtaagcttaggctg	1,162 bp,	-
	BD248, cgcgcgagctctcagaagaactcgtcaag	KanR	
pJB06	JB11, cgcacatcgacatgaagc	2,071 bp	<i>Bam</i> HI
(desaturase gene,	JB12, tctcaagcagctgttcatcg	homologous region	
bolF)	BD357, ctcgggatccgtaagcttaggctgctgcc	1,162 bp KanR	-
	BD358, tcaaggatcctcagaagaactcgtcaag		
pJB14	BD406, gaacttgtggcggtgcag	2,032 bp	Sphl
(fatty acid ligase gene,	BD407, gcgatgttcgacggctatc	homologous region	
bolB)	BD408, gccggcatgctcagaagaactcgtcaag	1,088 bp, KanR	-
	BD409, aagcgcatgctcagccaatcggatgaatg		

 Table S10. Detailed information on plasmids constructed using cloning strategy I.

II: NEBuilder mediated fusion of two homologous regions with a resistance cassette and subsequent pJet1.2 cloning, construction of pJB10, pJB12, and pBD87.

Primers listed in table S11 were used to amplify homologous regions from genomic DNA of *Burkholderia gladioli* pv. *cocovenenans* respectively the kanamycin resistance cassette from pGEM-Kan. In a subsequent step a NEBuilder three-fragments ligation have been performed (New England Biolabs, Frankfurt am Main) according to the manufacture's

recommendations. Finally, the ligated DNA fragments have been blunt-end ligated into pJET1.2 linear vector yielding the final knockout plasmids.

Plasmid (target gene)	Name and sequence of primers used $5' \rightarrow 3'$	Product size and name
pJB10	JB27, ctcctttggattcctgatcg	1,241 bp, homologous
(Thiolation domain	JB28, ctaagcttacagatcaacctcacgcagg	region
gene, <i>bolC</i>)	JB29, aggttgatctgtaagcttaggctgctgc	1,182 bp, KanR
	JB30, tcgtgttctgtcagaagaactcgtcaagaag	
	JB31, gttcttctgacagaacacgatcagctcg	1,471 bp, homologous
	JB32, acgtgctcgacaacagctac	region
pJB12	JB43, gtcgaagccggtctcgtag	1,296 bp, homologous
(Desaturase gene,	JB44, gatggacgagctcatgaaggcggagaagtc	region
bolL)	JB45, ccttcatgagctcgtccatcgtaagcttag	1,182 bp, KanR
	JB46, atagcgattcccgatcgatgtcagaagaac	
	JB47, catcgatcgggaatcgctatcgcaaggaag	1,079 bp, homologous
	JB48, aggacatccacctcgacaag	region
pBD87	BD424, gcgatagccgtcgaacatc	1,206 bp, homologous
(Ketosynthase III	BD425, ctaagcttacctacgagaccggcttcgac	region
gene, <i>bolM</i>)	BD426, ggtctcgtaggtaagcttaggctgctgc	1,162 bp, KanR
	BD427, ccagccgttctcagaagaactcgtcaagaag	
	BD428, gttcttctgagaacggctgggtatgcac	946 bp, homologous region
	BD429, gacttctccgccttcatgag	

 Table S11. Detailed information plasmids constructed using cloning strategy II.

III: Four fragment NEBuilder fusion of two homologous regions, a resistance cassette, and a vector backbone. Primers listed in table S12 were used to amplify two homologous regions from genomic DNA of *Burkholderia gladioli* pv. *cocovenenans* respectively the kanamycin resistance cassette from pGEM-Kan. The plasmid pGL42a_T251A^[13] was digested with *Xhol* and *Xbal*. In a subsequent step a NEBuilder four-fragment ligation has been performed according to the manufacture's recommendations yielding the final knockout plasmids (Table S12).

Dia a se i d		Dirich of
Plasmid (terget gone)	Name and sequence of primers used $5^{\circ} \rightarrow 3^{\circ}$	Product
(larger gene)		SIZE
		and
		name
pBD92	BD459, tatagggagagcggccgccagatcttccggatggcagccggaggtgtagagcag	1,498 bp,
(Decarboxylase	BD460, ctgcggactggctttctacgtgttcaatcactagtgatggacgaggacttcatgc	hr
gene, <i>bolN</i>)	JK582, gaattcgattctggtcggtc	1,151 bp,
	JK583, actagtgattgaacacgtag	KanR
	BD461, tctggggttcgaaatgaccgaccagaatcgaattccgggtcgtacacgtagcag	1,371 bp,
	BD462, tttccatggcagctgagaatattgtaggagatcttgcggattcgacaagatgattg	hr
pBD93	BD476, tatagggagagcggccgccagatcttccggatggctcggaagtgccgaaatac	1,272 bp,
(Ketosynthase	BD477, ctgcggactggctttctacgtgttcaatcactagtaagggatgcagccatttatc	hr
III gene, <i>bolP</i>)	JK582, gaattcgattctggtcggtc	1,151 bp,
	JK583, actagtgattgaacacgtag	KanR
	BD478, tctggggttcgaaatgaccgaccagaatcgaattccgacacgctcgactggtg	1,421 bp,
	BD479, tttccatggcagctgagaatattgtaggagatcttggctcttcatgtcgatcagc	hr
pBD95 (Citrat	BD459, tatagggagagcggccgccagatcttccggatggctctgggaaggcaccaactac	1,387 bp,
synthase gene,	BD460, ctgcggactggctttctacgtgttcaatcactagtcacggtcggcgtgatatag	hr
bolR)	JK582, gaattcgattctggtcggtc	1,151 bp,
	JK583, actagtgattgaacacgtag	KanR
	BD461, tctggggttcgaaatgaccgaccagaatcgaattccctacctgagcctgttcg	1,434 bp,
	BD462, tttccatggcagctgagaatattgtaggagatcttatgtaggccatctcctgg	hr
pBD107	BD555, tatagggagagcggccgccagatcttccggatggccgcagggctacctctacttc	1, 505
(acyl-CoA-	BD556, ctgcggactggctttctacgtgttcaatcactagtgacatccgattgctggtag	bp, hr
dehydrogenase,	JK582, gaattcgattctggtcggtc	1,151 bp,
bolQ)	JK583, actagtgattgaacacgtag	KanR
	BD557, tctggggttcgaaatgaccgaccagaatcgaattctcctgacccaggtgctctac	1,201 bp,
	BD558, tttccatggcagctgagaatattgtaggagatcttcgaggaagatctccagttcg	hr

Table S12. Detailed information on the plasmids constructed using cloning strategy III; hr, homologous region; KanR, Kanamycin resistance cassette.

Gene knockout of Burkholderia gladioli pv. cocovenenans HKI0521

Burkholderia gladioli pv. cocovenenans HKI0521 cells were washed three times with one volume of 300 mM sucrose in dH₂O. Cells have been spun down at 6,000 x g for 5 min. Afterwards the cells have been suspended in 100 μ L aliquots. 2 μ L of a plasmid have been added to the cell solution and afterwards a pulse of 2.5 kV has been applied. Cells have been suspended in MGY media and recovered for 3–4 h at 30 °C and 120 rpm. Afterwards, cell solution was plated onto NAG agar containing 50 μ g mL⁻¹ kanamycin and incubated at 30 °C until colonies appeared. Colonies grown after transformation with knockout plasmids have been subjected to a colony PCR to detect mutations. The templates were colony material that have been picked with a pipette tip and transferred into 100 μ L dH₂O, incubated for 5 min at 99 °C and centrifuged at 18,000 × g for 1 min. 5 μ L of the template have been added to 5 μ L of 2x master mix of KAPA2G Robust HotStart ReadyMix PCR kit (Merck KGaA) with the appropriate primers.

Three separate PCRs have been performed to confirm homologous recombination in each mutant that has been created. The PCR were named front arm (FA), back arm (BA) and wild type control (WT). The primer pairs and expected products are listed (Table S13 and Table S14). The PCRs FA and BA only yield a product if the recombination of the front and respectively back homologous region with the genome took place. The WT PCR was used to exclude that wild type cells were present in the tested colony. As an additional control all PCRs were run in parallel with wild type cell material as template.

Mutant strain	PCR	Name and sequence of used primers 5' \rightarrow 3'	Product size
	FA	JB05, tatcgttccattcgctcaag TIISSD_B_fwd, cgttggctacccgtgatatt	1,360 bp
Bg_coco_pJB02 (Δ <i>bolH</i>)	BA	JB06, ctacaacctgccgaagatgc TIISS_A_rv, agtgacaacgtcgagcacag	2,224 bp
	WT	JB05, tatcgttccattcgctcaag JB06, ctacaacctgccgaagatgc	2,919 bp
	FA	JB09, gcgttaccaggtccttgatg TIISS_A_rv, agtgacaacgtcgagcacag	2,134 bp
Bg_coco_pJB04 (Δ <i>bolO</i>)	BA	JB10, tgtcacgtcatgggagattc TIISSD_B_fwd, cgttggctacccgtgatatt	1,505 bp
	WT	JB09, gcgttaccaggtccttgatg JB10, tgtcacgtcatgggagattc	2,880 bp
	FA	JB13, gaaggccagcaggtagagg TIISSD_B_fwd, cgttggctacccgtgatatt	1,038 bp
Bg_coco_pJB06 (Δ <i>bolF</i>)	BA	JB14, ctgctcaagtacggcgacac TIISS_A_rv, agtgacaacgtcgagcacag	2,180 bp
	WT	JB13, gaaggccagcaggtagagg JB14, ctgctcaagtacggcgacac	2,459 bp
	FA	JB33, gtgtcgccgtacttgagcag TIISS_A_rv, agtgacaacgtcgagcacag	2,363 bp
Bg_coco_pJB10 (Δ <i>bolC</i>)	BA	JB34, gcctcgactacgaggtgttc TIISSD_B_fwd, cgttggctacccgtgatatt	2,045 bp
	WT	JB33, gtgtcgccgtacttgagcag JB34, gcctcgactacgaggtgttc	4,818 bp
	FA	BD430, gagtaggtgcggtgataggc TIISS_A_rv, agtgacaacgtcgagcacag	2,198 bp
Bg_coco_BD87 (Δ <i>bolM</i>)	BA	BD431, tctgctcctcgaagaaccac TIISSD_B_fwd, cgttggctacccgtgatatt	1,090 bp
	WT	BD430, gagtaggtgcggtgataggc BD431, tctgctcctcgaagaaccac	2,876 bp
	FA	BD410, aggctcatgcgtgtgtctc TIISS_A_rv, agtgacaacgtcgagcacag	1,773 bp
Bg_coco_pJB14 (Δ <i>bolB</i>)	BA	BD411, gatggacgaggacttcatgc TIISSD_B_fwd, cgttggctacccgtgatatt	1,403 bp
	WT	BD410, aggctcatgcgtgtgtctc BD411, gatggacgaggacttcatgc	2,511 bp
	FA	BD463, tcagcccgtacatcaggtag TIISS_A_rv, agtgacaacgtcgagcacag	1,968 bp
Bg_coco_pBD92 (Δ <i>bolN</i>)	BA	BD462, ccctgacaaacgacttttcc TIISSD_B_fwd, cgttggctacccgtgatatt	1,558 bp
	WT	BD463, tcagcccgtacatcaggtag BD462, ccctgacaaacgacttttcc	3,483 bp
	FA	BD480, gatcaatccgcagttgaagg TIISS_A_rv, agtgacaacgtcgagcacag	2,225 bp
Bg_coco_pBD93 (Δ <i>bolP</i>)	BA	BD481, cgaggaagatctccagttcg TIISSD_B_fwd, cgttggctacccgtgatatt	1,902 bp
	WT	BD480, gatcaatccgcagttgaagg BD481, cgaggaagatctccagttcg	3,646 bp

Table S13. Primer pairs and expected product sizes for colony PCRs to test for homologous recombination.

Table S14. Primer pairs and expected product sizes for colony PCRs to test for homologous recombination. (continued)

	FA	BD559, tcggaagtgccgaaatactg	2,560 bp
		TIISS_A_rv, agtgacaacgtcgagcacag	
	BA	BD560, tcgtctccatgtcattccac	1,722 bp
		TIISSD_B_fwd, cgttggctacccgtgatatt	
	WT	BD559, tcggaagtgccgaaatactg	3,610 bp
		BD560, tcgtctccatgtcattccac	
	FA	BD492, gcatgctgatcgacatgaag	2,174 bp
		TIISS_A_rv, agtgacaacgtcgagcacag	-
Bg_coco_pBD95	BA	BD493, aagttgcgcaggtacatcac	1,693 bp
(ΔbolR)		TIISSD_B_fwd, cgttggctacccgtgatatt	
	WT	BD492, gcatgctgatcgacatgaag	3,432 bp
		BD493, aagttgcgcaggtacatcac	

All mutant strains were cultivated on 20 mL PDA agar overnight at 30 °C, extracted, analyzed using LC-MS and their metabolic profiles compared to the wild type.

Bioactivity assays

Antibiotic assays

Bolagladin B (2 mg mL⁻¹, in DMSO) was tested against *Bacillus subtilis*, *Escherichia co*li, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium vaccae*, *Candida albicans* and *Penicillium notatum* as described previously.^[14] Ciprofloxacin (5 µg mL⁻¹, in dH₂O) or amphotericin B (10 µg mL⁻¹, in DMSO and MeOH) was used as a positive control for test against bacteria respectively fungi (Table S15).

 Table S15. Inhibitory effects of bolagladin B (2) against selected strains.

	Zone of inhibition in mn	ı		
Bacterial strains tested	Bolagladin B (2)	Ciprofloxacin	DMSO	
	(1 mg mL ⁻¹)	(5 µg mL⁻¹)	DIVISO	
Bacillus subtilis 6633 B1	13 p	28	11 p	
Escherichia coli 458 B4	15 p	24/31p	13	
Pseudomonas aeruginosa SG 137 B9	0	27/34 p	13 p	
Staphylococcus aureus 134/94 R9	14 5	0	11 p	
(MRSA)	14 p	0	пр	
Enterococcus faecalis 1528 R10	47	16	10 5	
(VRE)	17	10	iz p	
Mycobacterium vaccae 10670 M4	18	22 p	13 p	
Fundal strains tested	Bolagladin B (2)	Amphotericin B	DMSO	
	(1 mg mL ⁻¹)	(10 mg mL ⁻¹)		
Candida albicans H8	14 p	20	0	
Penicillium notatum JP36 P1	15 p	18 p	12 p	

p – partial inhibition

Swarming assays

MGY+M9 medium containing 1.5% agar was prepared, sterilized, and diluted with 1:1 water (0.7% MGY agar plates). 10 μ L of culture with an OD₆₀₀ of 0.4, that was inoculated from an overnight *B. gladioli* pv. *cocovenenans* wild type and Δ *bolH* culture was pipetted in the middle of a 0.7% MGY agar plate and incubated at 30 °C. Similar swarming behavior was observed within 14 days of incubation.

Drop-collapsing assay^[15]

Droplets of water (20 μ L) were placed on a hydrophobic surface (Parafilm 'M'). Bacterial cells of a single colony were transferred with a toothpick and resuspended in one droplet (Figure S6). The positive control, water *B. gladioli* HKI0739 (Bg739) droplets, resulted in a collapsed droplet. The control water, no additive and neither the *B. gladioli* pv. *cocovenenans* (*Bg.* pv. *coco*) nor the *B. gladioli* pv. *cocovenenans* Δ *bolO* lead to a collapse of the droplet. For visualization purposes 0.0025% crystal violet were added to the droplet. It had no influence on the shape of the droplet.



Figure S6. Droplet collapsing assay to test for surface activity of wild type *versus* mutant strains. ΔbolO, B. gladioli pv. cocovenenans ΔbolO; Bg739, B. gladioli HKI0739; Bg. pv. coco, B. gladioli pv. cocovenenans).

CAS agar test

To test for siderophore production of *B. gladioli* pv. *cocovenenans* we used CAS agar plates.^[16] 100 μ L of an overnight culture was pipetted onto the agar and incubated for a varying periods at 30 °C. A discoloration and halo formation colonies indicates siderophore activity. No or minor differences were observed when comparing the halo formation of *B. gladioli* pv. *cocovenenans* wild type, *B. gladioli* pv. *cocovenenans* Δ *bolH* or *B. gladioli* pv. *cocovenenans* Δ *bolR* (producer of **3** and **4**) colonies (Figure S7). The discoloration likely occurs due to the production of gladiobactin. Spotting of pure compound did not result in discoloration of the agar.



Figure S7. *B. gladioli* pv. *cocovenenans* or bolagladin incubated on CAS agar plates at 30 °C, respectively, 20 °C for 24 h. Spotting of 2 = bolagladin B (**2**) 0.1 mg ml⁻¹; 3 = bolagladin B (**2**) 0.5 mg ml⁻¹. EDTA was used as positive control. No noteworthy differences have been observed between B. gladioli pv. cocovenenans wild type (WT), *B. gladioli* pv. *cocovenenans* $\Delta bolP$ (no bolagladin production), *B. gladioli* pv. *cocovenenans* $\Delta bolR$ (producer of **3** and **4**).

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Physicochemical data

Bolagladin A (1)



(–)ESI HR-MS: *m/z* 822.4163 [M–H]⁻ (calcd. C₃₉H₆₀N₅O₁₄ 822.4142)



Figure S8. (+)ESI MS/MS spectrum of bolagladin A (1).

Table S16. NMR data of bolagladin A (1).

		Bolagladin A	(1)
	Position	¹³ C	¹ H (<i>J</i> in Hz)
L-Serine 1	1	169.4	-
	2	55.0	4.32 m
	3	61.0	3.68 m
			3.57 m
	NH	-	7.22 d (8.0)
L-Homoserine	1	1	-
	2	51.5	4.22 m
	3	n. d.	1.80 m
	4	57.8	3.45 m
			3.37 m
	NH	-	8.56 d (7.7)
D-Valine	1	171.7	-
	2	59.6	4.07 t (<i>10.0</i>)
	3	29.0	1.23 m
	4	19.0	0.81 d (6.5)
	5	19.5	0.81 d (6.5)
	NH	-	7.29 d (8.9)
L-Serine 2	1	169.9	-
	2	53.1	4.54 m
	3	65.3	4.23 m
	NH	-	8.33 d (7 <i>.3</i>)
Dehydro-β-alanine	1	166.7	-
	2	103.6	5.72 d (<i>14.0</i>)
	3	135.2	7.72 dd (<i>13.8; 11.3</i>)
	NH	-	10.53 d (<i>11.1</i>)
Fatty acid	1	164.4	-
	2	121.7	5.87 d (<i>11.4</i>)
	3	149.8	6.21 dt (<i>11.3;</i> 7.5)
	I	1	

4	28.7	2.63 m
		1.35 m
5-9	Not assigned	Not assigned
10	27.1	1.96 m
11	130.2	5.30 m
12	129.9	5.30 m
13	27.1	1.96 m
14	Not assigned	Not assigned
15	Not assigned	Not assigned
16	33.9	1.70 m
17	80.2	-
18	39.0	2.63
19	172.3	-
20	174.0	-
17-OCH₃	51.3	3.13 s



Figure S9. ¹H NMR spectrum of bolagladin A (1).



Figure S10. ¹³C NMR spectrum of bolagladin A (1).



Figure S11. H,H-COSY spectrum of bolagladin A (1).



Figure S12. HSQC spectrum of bolagladin A (1).



Figure S13. H,C-HMBC spectrum of bolagladin A (1).



(–)ESI HR-MS: *m*/z 836.4315 [M–H]⁻ (calcd. C₄₀H₆₂N₅O₁₄ 836.4299)



Figure S14. (-)ESI MS/MS spectrum of bolagladin B (2).



Figure S15. (+)ESI MS/MS spectrum of bolagladin B (2).



Figure S16. (+)ESI MS/MS spectrum of bolagladin B (2), mass range *m/z* 300–500.



Figure S17. (+)ESI MS/MS spectrum of the fragment *m*/*z* 450 amu derived from bolagladin B (2).

 Table S17. NMR data of bolagladin B (2).

		Bolagladin B (2)	
	Position	¹³ C	¹ H (<i>J</i> in Hz)
L-Serine 1	1	168.8	-
	2	54.4	4.33 m
	3	60.7	3.67 m
			3.57 m
	NH	-	7.20 d (7.8)
L-Homoserine	1	171.0	-
	2	51.2	4.22 m
	3	33.5	1.78 m
	4	57.3	3.45 m
			3.37 m
	NH	-	8.62 d (7.7)
D-Isoleucine	1	171.8	-
	2	57.5	4.11 t (<i>10.1</i>)
	3	34.1	1.73 m
	4	24.2	1.42 m
			1.04 m
	5	10.2	0.80 t (7. <i>4</i>)
	6	15.0	0.77 d (6.7)
	NH	-	7.38 brd
L-Serine 2	1	169.5	-
	2	52.5	4.56 m
	3	64.8	4.22 m
	NH	-	8.45 brd
Dehydro-β-alanine	1	166.2	-
	2	103.1	5.76 d (<i>14.0</i>)
	3	134.7	7.72 dd (<i>14.0; 11.2</i>)
	NH	-	10.73 d (<i>10.8</i>)
	l	I	

Fatty acid	1	163.9	-
	2	121.3	5.92 d (<i>11.5</i>)
	3	149.1	6.20 dt (<i>11.5;7.5</i>)
	4	28.3	2.63 m
			1.35 m
	5-9	Not assigned	Not assigned
	10	26.6	1.92 m
	11	129.8	5.30 m (<i>10; 6</i>)
	12	129.3	5.30 m (<i>10; 6</i>)
	13	26.5	1.92 m
	14	29.2	1.25 m
	15	22.4	1.23 m
			1.17 m
	16	33.9	1.73 m
	17	79.8	-
	18	39.0	2.63
	19	171.2	-
	20	173.2	-
	17-0CH ₃	50.9	3.16 s
	l	l	



Figure S18. ¹H NMR spectrum of bolagladin B (2).



Figure S19. ¹³C NMR spectrum of bolagladin B (2).



Figure S20. DEPT-135 NMR spectrum of bolagladin B (2).



Figure S21. H,H-COSY spectrum of bolagladin B (2).



Figure S22. HSQC spectrum of bolagladin B (2).



Figure S23. H,C-HMBC spectrum of bolagladin B (2).

Bolagladin M749 (3)



(–)ESI HR-MS: *m*/z 748.4157 [M–H]⁻ (calcd. C₃₇H₅₈N₅O₁₁ 748.4138)



Figure S24. Selected diagnostic ¹³C NMR signals of bolagladin A (1) and bolagladin M749 (3).

It should be noted that the signals for C18 and C19 in bolagladin A (1) are overlapping with either the solvent signal (C18) or amide carbon signals (C19).

		Bolagladin M749 (3)	
	Position	¹³ C	¹ H (<i>J</i> in Hz)
L-Serine 1	1	169.3	-
	2	54.4	4.33 m
	3	60.7	3.67 m
			3.57 m
	NH	-	7.19
L-Homoserine	1	171.2	-
	2	51.2	4.24 m
	3	33.5	1.73 m
	4	57.3	3.45 m
			3.37 m
	NH	-	8.62 d (7.7)
D-Isoleucine	1	171.8	-
	2	57.3	4.12 t (<i>10.0</i>)
	3	33.9	1.73 m
	4	24.2	1.46 m
			1.04 m
	5	10.2	0.80 t
	6	15.0	0.77 d
	NH	-	7.23 brd
L-Serine 2	1	169.4	-
	2	52.5	4.57 m
	3	64.9	4.23 m
	NH	-	8.34 d
Dehydro-β-alanine	1	166.2	-
	2	103.2	5.73 d
	3	134.7	7.72 dd (14.0; 11.2)
	NH	-	10.53 d (<i>10.8</i>)
Fatty acid	1	163.9	-

Table S18. NMR data of bolagladin M749 (3).¹

2	121.2	5.87 d (<i>11.5</i>)
3	149.1	6.20 dt (<i>11.5;7.5</i>)
4	28.4	2.63 m
		1.35 m
5-9	Not assigned	Not assigned
10	26.6	1.95 m
11	129.7	5.30 m
12	129.5	5.30 m
13	26.6	1.95 m
14	29.2	1.25 m
15	22.0	1.12-1.25 m
16	24.2 ^a	1.46 m ^a
17	31.6ª	2.10-2.30 m ^a
18	-	-
19	-	-
20	174.4 ^a	-
17-OCH₃	-	-

¹ Data deduced from the NMR spectra of a bolagladin M749-enriched fraction



Figure S25. (-)ESI MS/MS spectrum of bolagladin M749 (3).



Figure S26. (+)ESI MS/MS spectrum of bolagladin M749 (3).









Bolagladin M765 (4)







Figure S29. (+)ESI MS/MS spectrum of bolagladin M765 (4).



Figure S30. (+)ESI MS/MS spectrum of bolagladin M765 (4) mass range m/z 300-500.



Figure S31. (+)ESI MS/MS spectrum of the fragment *m*/z 360 amu derived from bolagladin M765 (4).



(–)ESI HR-MS: *m*/z 838.4479 [M–H]⁻ (calcd. C₄₀H₆₄N₅O₁₄, 838.4455)

Figure S32. (+)ESI MS/MS spectrum of bolagladin M839 (5).



Figure S33. (+)ESI MS/MS spectrum of bolagladin M839 (5) mass range *m/z* 300–500.

Bolagladin M824 (6)





Figure S34. (+)ESI MS/MS spectrum of bolagladin M824 (6).



Figure S35. (+)ESI MS/MS spectrum of bolagladin M824 (6) mass range *m/z* 300–500.