Very Important Paper

Biosynthesis of Sinapigladioside, an Antifungal Isothiocyanate from *Burkholderia* Symbionts

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Sinapigladioside is a rare isothiocyanate-bearing natural product from beetle-associated bacteria (*Burkholderia gladioli*) that might protect beetle offspring against entomopathogenic fungi. The biosynthetic origin of sinapigladioside has been elusive, and little is known about bacterial isothiocyanate biosynthesis in general. On the basis of stable-isotope labeling, bioinformatics, and mutagenesis, we identified the sinapigladioside biosynthesis gene cluster in the symbiont and found that an isonitrile synthase plays a key role in the biosynthetic pathway. Genome mining and network analyses indicate that related gene clusters are distributed across various bacterial phyla including producers of both nitriles and isothiocyanates. Our findings support a model for bacterial isothiocyanate biosynthesis by sulfur transfer into isonitrile precursors.

Naturally occurring isothiocyanates constitute a small group of natural products from selected plants,[1] marine animals,[2] and bacteria. Owing to the reactive heterocumulene moiety, these compounds are endowed with diverse biological activities.[1,3] The best-known representatives of this group are the mustard oils of cruciferous vegetables that confer the typical pungent taste. These plant-derived isothiocyanates serve as defense compounds that are liberated upon cell damage. This triggered response involves specialized glycosidases (myrosinases) to cleave glucosinolate precursors, thus initiating a Lossen rearrangement into isothiocyanates for instance sulforaphane (**1**) and phenylethyl isothiocyanate (**2**; Figure 1).[4] A limited

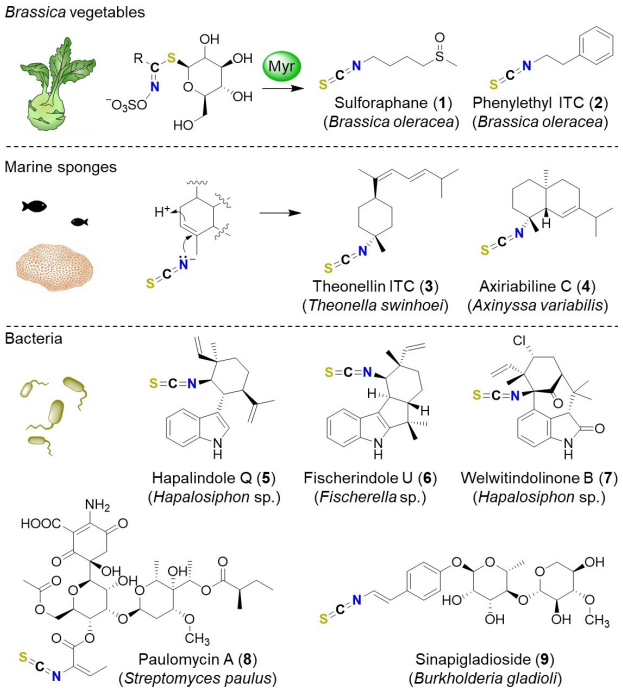

Figure 1. Structures of selected isothiocyanate-substituted natural products, and their biogenetic origin. Myr: myrosinase, ITC: isothiocyanate.

number of isothiocyanate-containing terpenes have been isolated from sponges and mollusks, such as theonellin isothiocyanate (**3**)[5] and axiriabiline C (**4**).[6] It has been proposed that these compounds result from the addition of naturally occurring inorganic thiocyanate to double bonds.[6,7] Apart from the plant- and animal-derived natural products, only a handful of isothiocyanates have been identified as bacterial metabolites. The hapalindole-type alkaloids from cyanobacteria like hapalindole Q (**5**),[8] fischerindole U (**6**),[9] and welwitindolinone B (**7**)[10] have a wide spectrum of antibacterial, antimycotic, insecticidal, and anticancer bioactivities.[11] Paulomycin A (**8**) from *Streptomyces paulus* NRRL 8115 is active against Gram-positive bacteria.[12]

We recently reported the discovery of sinapigladioside (**9**) as the first isothiocyanate from Gram-negative Proteobacteria.[13] **9** is an antifungal metabolite of the beetle symbiont *Burkholderia gladioli* HKI0739 (syn. *B. gladioli* Lv-StA) that is spread onto the eggs by female *Lagria* beetles to protect the beetle offspring from pathogens.[13b] Although the aglycone structure of **9** is reminiscent of plant-derived isothiocyanates, the biosynthesis of **9** likely follows a different route. Yet, the

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biogenetic origin of **9** has been cryptic. Furthermore, despite the knowledge of several bacterial isothiocyanates and the corresponding biosynthetic gene clusters in cyanobacteria and Actinobacteria, the bacterial route to this unusual heterocumulene moiety has remained elusive. Here we report the chemical and genetic analysis of sinapigladioside biosynthesis and present first insights into the formation of this rare bacterial isothiocyanate.

To reveal the biosynthetic origin of **9**, we performed stable-isotope labeling experiments with $^{13}C_1$-tyrosine, $^{13}C_3$-tyrosine, ^{15}N-tyrosine, and $^{13}C_6$-glucose. HRMS analyses of the culture broths indicated that the C1 atom of Tyr is lost *en route* to **9**. In contrast, the $^{13}C_3$ and ^{15}N labels are incorporated into the hydroxyphenylethyleneamine backbone of **9**, and the isothiocyanate carbon is derived from $^{13}C_6$-glucose (Figure 2A). This finding is remarkable as it points to a scheme that is similar to bacterial isonitrile formation.[14] Previous studies using environmental DNA,[15] and the analyses of the paerucumarin (**10**),[16] byelyankacin (**11**),[17] rhabduscin (**12**)[18] biosynthetic pathways in *Photorhabdus* and *Xenorhabdus* species have shown that two enzymes play essential roles in the formation of the α,β-unsaturated isonitrile: isonitrile synthase A (IsnA) for C1-transfer onto the nitrogen, and a Fe^{2+} α-ketoglutarate-dependent oxygenase (IsnB) for decarboxylation/desaturation by an E2 elimination (Figure 2B).[15,19] Yet, the biosynthesis of the non-ribosomally synthesized, isocyanide-lipopeptides SF2369 and SF2768 deviates from this biosynthetic scheme. Here, a non-heme iron(II)-dependent dioxygenase is responsible for an oxidative decarboxylation of glycine to create an isonitrile moiety.[19c,20]

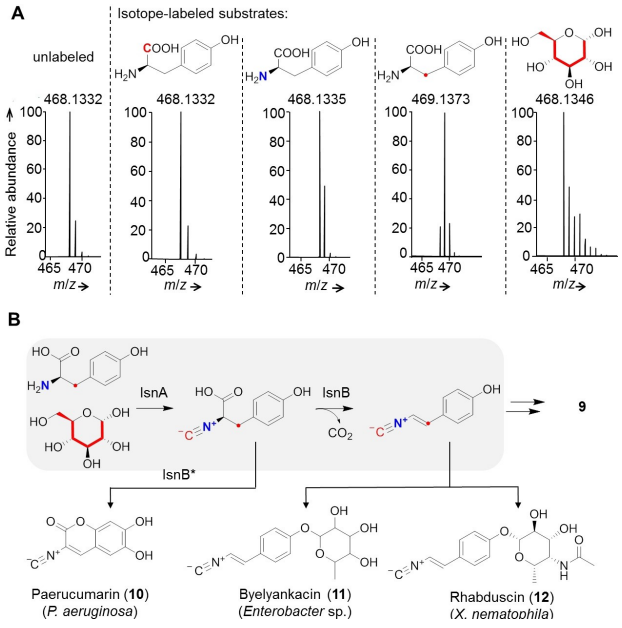

Figure 2. Investigation of the biosynthetic origin of **9** by using stable-isotope labeling experiments. A) Isotopic pattern of **9** *m/z* $[M-H]^-$ after culture supplementation with $^{13}C_1$-tyrosine, ^{15}N-tyrosine, $^{13}C_3$-tyrosine and ^{13}C-glucose. ^{13}C isotopes are labeled in red, marked by a red letter C or a red dot; ^{15}N isotopes are depicted as bold blue letters. B) Model of bacterial isonitrile formation, and structures of selected bacterial isonitriles.

Using the *isnA* and *isnB* sequences in a BLAST query,[21] we identified a putative sinapigladioside (*spg*) BGC in the symbiont genome (Figure 3A). The deduced gene products of *spgA* and *spgD* appear to be an isonitrile synthase A homolog (IsnA) and a fusion protein consisting of an N-terminal IsnA and a C-terminal IsnB domain, respectively. Multiple sequence alignments of IsnA and IsnB with SpgA and SpgD show high levels of conservation, thus indicating similar functionalities (Figures S2 and S3 in the Supporting Information).

To unequivocally link sinapigladioside biosynthesis to the *spg* gene locus, we disrupted the isonitrile synthase genes *spgA* and *spgD*. Gene knockouts were obtained by transforming *B. gladioli* with pJet1.2-based suicide plasmids that contain two sequence patches (~1,000 bp) homologous to the surrounding sequences of the respective target gene. A resistance cassette was inserted between the homologous sequences (kanamycin or apramycin), in this way disrupting the target gene by homologous recombination. Successful double-crossover events

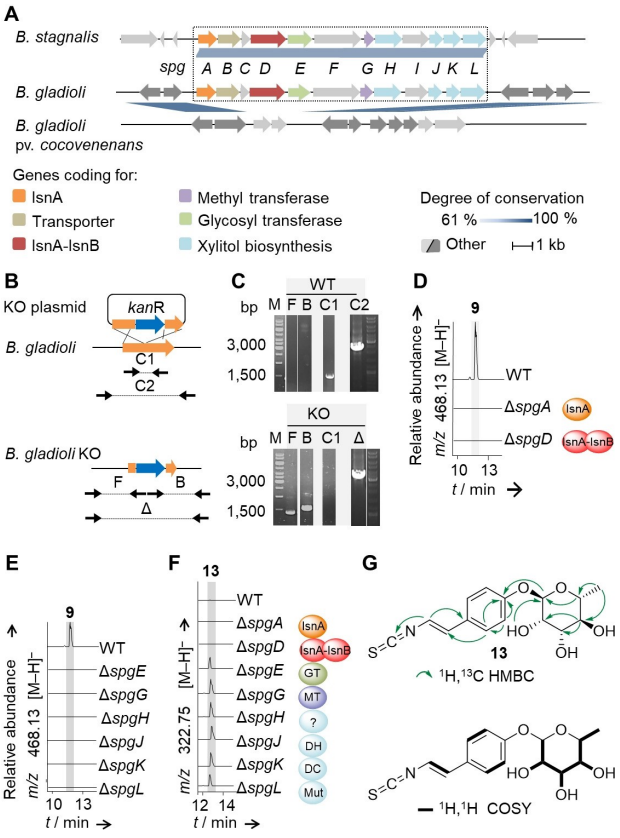

Figure 3. Molecular basis of sinapigladioside biosynthesis. A) Architecture of the *spg* gene cluster and orthologous gene clusters in *Burkholderia* strains. *B. gladioli* HKI0739 and *B. gladioli* pv. *agaricicola* contain identical *spg* gene clusters. B) Gene-knockout strategy using recombination between homologous regions flanking a kanamycin resistance cassette (*kanR*) encoded on a plasmid and a target gene (*spgA*, orange arrow). Mutations are detected using PCR with the indicated primers (black arrows) to amplify the front or back arms (F or B), the controls (C1 and C2) or the inserted *kanR* (Δ). C) Example gel electrophoresis picture of *B. gladioli* wild-type (WT) and mutant genotypes; M: DNA ladder. D)–F) Extracted ion chromatograms of **9** and **13**, respectively, in WT and mutant extracts; GT: glycosyl transferase, MT: methyl transferase, ?: unknown protein, DH: dehydrogenase, DC: decarboxylase, Mut: mutase. G) Key NMR correlations and structure of **13**.

were verified by colony PCR (Figure 3B and C). Comparative metabolic profiling of the wild type and the mutants showed that the deletion of either the isonitrile synthase A (Δ*spgA*) or the isonitrile synthase A–B fusion gene (Δ*spgD*) leads to the full abolishment of the production of **9** (Figure 3D). This indicates that the N-terminal IsnA domain of SpgD is not able to compensate an inactivation of SpgA. Accordingly, a sequence alignment showed that the IsnA domain of SpgD lacks conserved motifs that were identified in IsnA proteins, including SpgA (Figure S2). Thus, the IsnA domain of SpgD likely lost its function.

To test the protective, antifungal function of **9**, we inoculated beetle eggs with either wild type or Δ*spgD* mutant strains and exposed these, as well as symbiont-free eggs, to spores of entomopathogenic fungus *Purpureocillium lilacinum*. We noted that the mutant retained a strong protective effect (Figure S8). This finding is not surprising since the beetle symbionts are known to produce a blend of antifungal agents, including the glutarimide-containing polyketide gladiofungin[22] and the polyyne caryoynencin.[13b] These compounds seem to be sufficient to uphold the protection of the insect eggs against this fungal pathogen, even in the absence of sinapigladioside (**9**).

Interestingly, the *spg* gene locus is not limited to the beetle symbiont. A genome database search revealed that the genomes of two additional *Burkholderia* strains, the soil bacterium *Burkholderia stagnalis* MSMB735WGS and the mushroom pathogen *B. gladioli* pv. *agaricicola* HKI0676 (DSM4285), harbor orthologous gene loci (Figure 3A). The related pathovar *B. gladioli* pv. *cocovenenans*, a known contaminant of food fermentations,[23] lacks the *spg* gene locus, but the flanking regions upstream of *spgA* and downstream of *spgL* are present. Thus, it is also possible that *B. gladioli* pv. *cocovenenans* lost this gene locus. Alternatively, an insertion of *spg* via horizontal gene transfer into the genome of a common ancestor of *B. stagnalis*, *B. gladioli* pv. *agaricicola* and *B. gladioli* is conceivable but less likely (Figure 3A). Irrespective of the precise order of these events, the genome comparisons indicated the size (~15 kb) and the boundaries of the *spg* gene cluster.

In addition to the isonitrile synthase genes, the *spg* gene locus also codes for enzymes related to sugar biosynthesis and transfer, such as a dehydrogenase (SpgJ, DH), decarboxylase (SpgK, DC), a mutase (SpgL, Mut), an *O*-methyl transferase (SpgG, MT), a glycosyl transferase (SpgE, GT), and a transport-related protein of the major-facilitator-superfamily (SpgB). To learn more about the assembly of **9**, we systematically inactivated all structural *spg* genes in analogy to the homologous recombination approach described for *spgA* and *spgD*.

Metabolic profiling of the targeted mutant strains showed that individual deletions of *spgZ* (*araC* regulatory gene), *spgB* (major facilitator superfamily gene), *spgC* (tyrosine phosphatase gene), *spgF* (hypothetical membrane protein gene) and *spgI* (FAD-dependent oxygenase gene) have no influence on the production of **9**. In contrast, individual deletions of *spgE* (glycosyl transferase gene), *spgG* (*O*-methyl transferase gene), *spgH* (gene of unknown function), *spgJ* (dehydrogenase gene), *spgK* (decarboxylase gene), and *spgL* (mutase gene) led to mutant strains that accumulate a new compound (**13**) with *m/z* 322.0757 $[M-H]^-$ and a deduced molecular formula of $C_{15}H_{17}O_5NS$ (Figure 3E and 3F). We isolated compound **13** from the fermentation broth of a Δ*spgE*-mutant culture (3.5 L) by extraction, followed by size-exclusion chromatography (Sephadex LH-20) and preparative HPLC with a yield of 15.4 mg (4.4 mg L^{-1}). The structure of **13** was elucidated from 1D and 2D NMR data (Figures 3G and S12–S17) in combination with HR-ESI-MS^2 fragmentation. We detected all signals corresponding to the carbon backbone of **9**. Only the NMR signals for the xylose moiety are missing in both the ^1H and ^{13}C spectra.

Consequently, **13** is a precursor of **9** lacking the D-xylose sugar moiety. The structure of **13** and the mutational analyses indicate that *spgH*, *spgJ*, *spgK* and *spgL* are involved in the biosynthesis or transfer of *O*-methylated D-xylose (Figure 3F and G). The lack of a second rhamnose-specific glycosyl transferase within the *spg* locus indicates that this enzyme is encoded elsewhere in the symbiont genome. Likewise, the *spg* gene cluster does not harbor any gene that could code for a sulfur transferase.

To identify a potential sulfur transferase, we evaluated whether isonitrile synthase genes from unrelated bacteria show a gene synteny with sulfur-transferase genes. Therefore, we created a sequence similarity network (SSN) and a genome neighborhood network (GNN) using either SpgA or SpgD as query and the EFI-EST[24] and EFI-GNT[25] webtools. In the SSN we identified multiple gene clusters containing isonitrile synthase genes, some of them could be linked to the isonitrile biosynthetic pathways of **10**,[16] **11**[17] and **12**,[26] and to the hapalindole-isonitrile (**14**–**17**) and -isothiocyanate (**5**–**7**) alkaloids[27] (Figure 4A). Additionally, we found a large number of related, yet uncharacterized, gene clusters in Proteobacteria and Actinobacteria, which may be producers of yet unknown isonitrile- or isothiocyanate-containing natural products. However, no conventional sulfur-transferase genes[28] could be identified in proximities to the isonitrile synthase genes.

The co-occurrence of isonitrile and isothiocyanate congeners of the hapalindole-type alkaloids is remarkable[8–10,29] and may suggest that the isonitrile moieties are converted to the corresponding isothiocyanates by cryptic sulfur transferases. Although we have not detected any isonitrile by HPLC-MS analysis of the culture broths, our labeling experiments and mutational analyses suggest that the isothiocyanate derives from an isonitrile intermediate (Figure 4B). Since the cryptic sulfur transferases for isothiocyanate formation are obviously encoded outside of isonitrile BGCs, it is possible that the sulfur is incorporated by housekeeping or detoxification enzymes. Although no sulfur transferase has been reported that produces an isothiocyanate from an isonitrile,[28] a family of enzymes is known to catalyze the pivotal detoxification reaction that converts cyanide to thiocyanate in organisms of all major phyla.[30] Such enzymes with rhodanese homology domains (RHOD; E.C. 2.8.1.1., thiosulfate: cyanide sulfur transferase) also play roles in the biosynthetic pathways to molybdopterin in *Escherichia coli*,[31] to thiouridine in *Thermus thermophilus*,[32] and to the thioquinolobactin siderophore in *Pseudomonas fluorescens*.[33] RHOD-encoding genes are, however, redundant

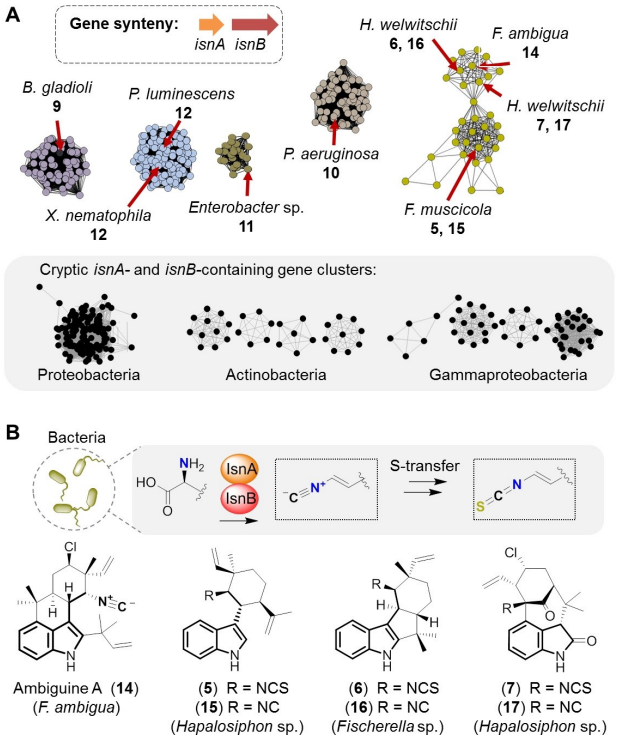

Figure 4. Genome mining for bacterial isonitrile synthases, and correlation with isothiocyanate producers. A) Sequence similarity network of *isnA*- and *isnB*-containing gene clusters showing the distribution of bacterial isonitrile synthase genes in different bacterial phyla, including producers of isothiocyanates and isonitriles. B) Biosynthetic model for isothiocyanate formation in bacteria via isonitriles, and structures of **5**–**7** and **14**–**17**. The tryptophan-derived indolethyl backbone is drawn in bold.

in the genome of *B. gladioli* (Table S2), thus hampering the creation of a mutant incapable of isothiocyanate formation.

Taken together, on the basis of isotope labeling, bioinformatics, and mutational analyses, we present a model for the biosynthesis of the antibacterial isothiocyanate metabolite sinapigladioside in *Burkhoderia* species (Figure 5). Our results support a scheme in which an isonitrile intermediate is converted into the isothiocyanate, likely by detoxifying RHOD proteins.[34] Network analyses and the reported co-occurrence of isonitrile and isothiocyanate metabolites suggest that this pathway, which clearly deviates from previously reported routes

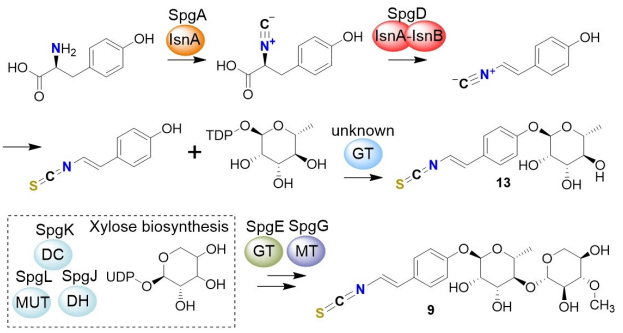

Figure 5. Model for sinapigladioside (**9**) biosynthesis in *B. gladioli*.

to thiocyanates, is widespread among Proteobacteria and Actinobacteria.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biosynthesis · *Burkholderia* · genome mining · isothiocyanate · natural products

[1] a) M. Traka, R. Mithen, *Phytochem. Rev.* **2009**, *8*, 269–282; b) J. W. Fahey, A. T. Zalcmann, P. Talalay, *Phytochemistry* **2001**, *56*, 5–51.
[2] M. J. Garson, J. S. Simpson, *Nat. Prod. Rep.* **2004**, *21*, 164–179.
[3] a) K. Srivastava, A. Bhatt, N. Singh, R. Khare, R. Shukla, D. Chaturvedi, R. Kant, *Chem. Biol. Interfaces* **2020**, *10*, 35–50; b) L. Romeo, R. Lori, P. Rollin, P. Bramanti, E. Mazzon, *Molecules* **2018**, *23*, 624.
[4] a) L. Rask, E. Andréasson, B. Ekbom, S. Eriksson, B. Pontoppidan, J. Meijer, *Plant Mol. Biol.* **2000**, *42*, 93–114; b) G. S. Stoewsand, *Food Chem. Toxicol.* **1995**, *33*, 537–543.
[5] S. C. Mao, Y. W. Guo, R. van Soest, G. Cimino, *Helv. Chim. Acta* **2007**, *90*, 588–593.
[6] X. W. Li, S. H. Chen, F. Ye, E. Mollo, W. L. Zhu, H. L. Liu, Y. W. Guo, *Tetrahedron* **2017**, *73*, 5239–5243.
[7] C. M. Kondempudi, R. Singanaboina, N. Manchala, V. G. Gunda, V. R. Janapala, V. Yenamandra, *Chem. Pharm. Bull.* **2009**, *57*, 990–992.
[8] R. E. Moore, C. Cheuk, X. Q. G. Yang, G. M. Patterson, R. Bonjouklian, T. A. Smitka, J. S. Mynderse, R. S. Foster, N. D. Jones, J. K. Swartzendruber, *J. Org. Chem.* **2002**, *52*, 1036–1043.
[9] A. Park, R. E. Moore, G. M. Patterson, *Tetrahedron Lett.* **1992**, *33*, 3257–3260.
[10] K. Stratmann, R. E. Moore, R. Bonjouklian, J. B. Deeter, G. M. Patterson, S. Shaffer, C. D. Smith, T. A. Smitka, *J. Am. Chem. Soc.* **1994**, *116*, 9935–9942.
[11] S. S. Swain, S. K. Paidesetty, R. N. Padhy, *Biomed. Pharmacother.* **2017**, *90*, 760–776.
[12] a) J. Li, Z. Xie, M. Wang, G. Ai, Y. Chen, *PLoS One* **2015**, *10*, e0120542; b) A. D. Argoudelis, T. A. Brinkley, T. F. Brodasky, J. A. Buege, H. F. Meyer, S. A. Mizsak, *J. Antibiot.* **1982**, *35*, 285–294.
[13] a) L. V. Flórez, M. Kaltenpoth, *Environ. Microbiol.* **2017**, *19*, 3674–3688; b) L. V. Flórez, K. Scherlach, P. Gaube, C. Ross, E. Sitte, C. Hermes, A. Rodrigues, C. Hertweck, M. Kaltenpoth, *Nat. Commun.* **2017**, *8*, 15172; c) L. V. Flórez, K. Scherlach, I. J. Miller, A. Rodrigues, J. C. Kwan, C. Hertweck, M. Kaltenpoth, *Nat. Commun.* **2018**, *9*, 1–10; d) S. C. Waterworth, L. V. Flórez, E. R. Rees, C. Hertweck, M. Kaltenpoth, J. C. Kwan, *mBio.* **2020**, *11*, e02430–19..
[14] X. Zhang, L. Evanno, E. Poupon, *Eur. J. Org. Chem.* **2020**, *2020*, 1917-1917.
[15] a) S. F. Brady, J. Clardy, *Angew. Chem. Int. Ed.* **2005**, *44*, 7045–7048; *Angew. Chem.* **2005**, *117*, 7207–7210; b) S. F. Brady, J. D. Bauer, M. F. Clarke-Pearson, R. Daniels, *J. Am. Chem. Soc.* **2007**, *129*, 12102–12103; c) S. F. Brady, J. Clardy, *Angew. Chem. Int. Ed.* **2005**, *44*, 7063–7065; *Angew. Chem.* **2005**, *117*, 7225–7227.
[16] M. F. Clarke-Pearson, S. F. Brady, *J. Bacteriol.* **2008**, *190*, 6927–6930.

[17] S. Takahashi, H. Iwai, K. Kosaka, T. Miyazaki, Y. Osanai, N. Arao, K. Tanaka, K. Nagai, A. Nakagawa, *J. Antibiot.* **2007**, *60*, 717.
[18] a) J. M. Crawford, R. Kontnik, J. Clardy, *Curr. Biol.* **2010**, *20*, 69–74; b) N. J. Tobias, H. Wolff, B. Djahanschiri, F. Grundmann, M. Kronenwerth, Y. M. Shi, S. Simonyi, P. Grun, D. Shapiro-Ilan, S. J. Pidot, T. P. Stinear, I. Ebersberger, H. B. Bode, *Nat. Microbiol.* **2017**, *2*, 1676–1685; c) N. J. Tobias, B. Mishra, D. K. Gupta, R. Sharma, M. Thines, T. P. Stinear, H. B. Bode, *BMC Genom.* **2016**, *17*, 537.
[19] a) W. C. Chang, D. Sanyal, J. L. Huang, K. Ittiamornkul, Q. Zhu, X. Liu, *Org. Lett.* **2017**, *19*, 1208–1211; b) C.-P. Yu, Y. Tang, L. Cha, S. Milikisiyants, T. I. Smirnova, A. I. Smirnov, Y. Guo, W. C. Chang, *J. Am. Chem. Soc.* **2018**, *140*, 15190–15193; c) N. C. Harris, D. A. Born, W. Cai, Y. Huang, J. Martin, R. Khalaf, C. L. Drennan, W. Zhang, *Angew. Chem. Int. Ed.* **2018**, *57*, 9707–9710; *Angew. Chem.* **2018**, *130*, 9855–9858; d) J. L. Huang, Y. Tang, C. P. Yu, D. Sanyal, X. Jia, X. Liu, Y. Guo, W. C. Chang, *Biochemistry* **2018**, *57*, 1838–1841.
[20] N. C. Harris, M. Sato, N. A. Herman, F. Twigg, W. Cai, J. Liu, X. Zhu, J. Downey, R. Khalaf, J. Martin, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 7025–7030.
[21] S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, *Nucleic Acids Res.* **1997**, *25*, 3389–3402.
[22] S. P. Niehs, J. Kumpfmüller, B. Dose, R. F. Little, K. Ishida, L. V. Florez, M. Kaltenpoth, C. Hertweck, *Angew. Chem. Int. Ed.* **2020**.
[23] T. Coenye, B. Holmes, K. Kersters, J. R. W. Govan, P. Vandamme, *Int. J. Syst. Evol. Microbiol.* **1999**, *49*, 37–42.
[24] R. Zallot, N. Oberg, J. A. Gerlt, *Biochemistry* **2019**, *58*, 4169–4182.
[25] J. A. Gerlt, *Perspect Sci.* **2016**, *9*, 24–32.
[26] J. M. Crawford, C. Portmann, X. Zhang, M. B. J. Roeffaers, J. Clardy, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10821–10826.
[27] a) M. L. Micallef, D. Sharma, B. M. Bunn, L. Gerwick, R. Viswanathan, M. C. Moffitt, *BMC Microbiol.* **2014**, *14*, 213; b) K. Ittiamornkul, Q. Zhu, D. S. Gkotsi, D. R. M. Smith, M. L. Hillwig, N. Nightingale, R. J. M. Goss, X. Liu, *Chem. Sci.* **2015**, *6*, 6836–6840; c) M. L. Hillwig, H. A. Fuhrman, K. Ittiamornkul, T. J. Sevco, D. H. Kwak, X. Liu, *ChemBioChem* **2014**, *15*, 665–669.
[28] K. L. Dunbar, D. H. Scharf, A. Litomska, C. Hertweck, *Chem. Rev.* **2017**, *117*, 5521–5577.
[29] S. Mo, A. Krunic, G. Chlipala, J. Orjala, *J. Nat. Prod.* **2009**, *72*, 894–899.
[30] D. Bordo, P. Bork, *EMBO Rep.* **2002**, *3*, 741–746.
[31] W. Zhang, A. Urban, H. Mihara, S. Leimkühler, T. Kurihara, N. Esaki, *J. Biol. Chem.* **2010**, *285*, 2302–2308.
[32] N. Shigi, S. I. Asai, K. Watanabe, *FEBS Lett.* **2016**, *590*, 4628–4637.
[33] A. M. Godert, M. Jin, F. W. McLafferty, T. P. Begley, *J. Bacteriol.* **2007**, *189*, 2941–2944.
[34] R. Cipollone, P. Ascenzi, P. Tomao, F. Imperi, P. Visca, *J. Mol. Microbiol. Biotechnol.* **2008**, *15*, 199–211.

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