Published in final edited form as:

Chembiochem. 2013 May 27; 14(8): 955–962. doi:10.1002/cbic.201300147.

Bioactivity-guided genome mining reveals the lomaiviticin biosynthetic gene cluster in *Salinispora tropica*

Roland D. Kersten^[a], Prof. Dr. Amy L. Lane^[b], Dr. Markus Nett^[c], Taylor K. S. Richter^[a], Dr. Brendan M. Duggan^[d], Prof. Dr. Pieter C. Dorrestein^{[a],[d],[e]}, and Prof. Dr. Bradley S. Moore^{[a],[d]}

Bradley S. Moore: bsmoore@ucsd.edu

^[a]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093 (USA)

^[b]University of North Florida, Chemistry Department, 1 UNF Dr., Jacksonville, FL 32224 (USA)

^[c]Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena (Germany)

^[d]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA 92093 (USA)

^[e]Department of Pharmacology and Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California (USA)

Abstract

The use of genome sequences has become routine in guiding the discovery and identification of microbial natural products and their biosynthetic pathways. *In silico* prediction of molecular features, such as metabolic building blocks, physico-chemical properties or biological functions, from orphan gene clusters has opened up the characterization of many new chemo- and genotypes in genome mining approaches. Here, we guided our genome mining of two predicted enediynes pathways in *Salinispora tropica* CNB-440 by a DNA interference bioassay to isolate DNA-targeting enediynes polyketides. An organic extract of *S. tropica* showed DNA-interference activity that surprisingly was not abolished in genetic mutants of the targeted enediynes pathways, *ST_pks1* and *spo*. Instead we showed that the product of the orphan type II polyketide synthase pathway, *ST_pks2*, is solely responsible for the DNA-interfering activity of the parent strain. Subsequent comparative metabolic profiling revealed the lomaiviticins, glycosylated diazofluorene polyketides, as the *ST_pks2* products. This study marks the first report of the 59 open reading frame lomaiviticin gene cluster (*lom*) and supports the biochemical logic of their dimeric construction via a pathway related to the kinamycin monomer.

Keywords

polyketides; biosynthesis; genome mining; lomaiviticin; *Salinispora tropica*

Introduction

Microbial genome sequencing has increased dramatically over the past decade, providing access to a tremendous amount of information about the genetic capability of microorganisms to produce natural products.^[1] Bioinformatics analyses have revealed that

the number of putative natural product biosynthetic gene clusters often greatly exceeds the number of reported metabolites,^[2] suggesting that a large portion of nature's chemical potential remains unexplored. Realization of the chemistry encoded by orphan gene clusters may afford access to the next generations of drugs as well as insights into evolutionary benefits of the plethora of biosynthetic genes maintained within microbial genomes.

Researchers have developed a variety of approaches to discover natural products associated with orphan gene clusters.^[3,4] Such genome mining strategies include inactivation of selected gene clusters followed by comparative metabolic profiling of mutant and wild-type strains,^[5] prediction of physico-chemical properties from biosynthetic gene sequences,^[6] manipulation of genes regulating biosynthetic pathway expression,^[7] isotope labeling of predicted biosynthetic precursors in combination with isotope-guided fractionation,^[8] heterologous expression of orphan biosynthetic genes,^[9] and mass spectrometry-guided genome mining.^[10]

The discovery of novel natural products that impede DNA replication or function is of interest since DNA-interfering molecules have potential cancer chemotherapeutic properties,^[11,12] especially as drug conjugates linked to monoclonal antibodies.^[13] Hallmark examples of DNA-targeting natural products include the enediyne antibiotics.^[14] Their biosynthesis involves an iterative acting type I polyketide synthase, which assembles the distinctive core structure common to all members of this natural product family.^[15] Eneidyne feature two acetylenic groups that are linked via a conjugated double bond. They are differentiated into two subclasses possessing either 9-membered rings, as exemplified by neocarzinostatin, or 10-membered rings, such as calicheamicin and dynemicin (Figure 1A).^[15] These cytotoxic agents intercalate into chromosomal DNA in a sequence-specific manner and cause strand scission via a radical mechanism.^[16]

Actinomycetes are prevalent sources of DNA-interfering natural products, including enedynes.^[12,14] Genome sequenced *Salinispora* species devote approximately 10% of their genetic material to natural product biosynthesis, which includes several orphan enediyne polyketide synthase (ePKS) gene clusters.^[17,18] Although the 5.2-Mbp genome of the *Salinispora tropica* strain CNB-440 harbors at least 19 putative biosynthetic gene clusters,^[1] only five of these loci are linked with characterized natural products.^[18–22] Its untapped metabolic potential thus makes *S. tropica* a promising candidate for genomics-guided drug discovery. The *S. tropica* CNB-440 genome contains two ePKS gene clusters, the *ST_pks1* and *spo* loci. While the product of the former is not known, the *spo* gene set is associated with the biosynthesis of two chlorinated cyclopenta[*a*]indenes. Sporolides A and B were proposed to be degradation products of a reactive 9-membered enediyne precursor molecule (Figure 1A, Supplementary Figure 1A).^[22–24] While the potent proteasome inhibitor salinosporamide A was isolated from this strain and is currently in clinical trials for treatment of multiple myeloma,^[25] no DNA-interfering anticancer natural products have been reported from *S. tropica*.

Herein, we report the identification of DNA-targeting natural products from *S. tropica* CNB-440 via bioactivity-guided genome mining. Allelic-exchange mutagenesis of select biosynthesis genes, DNA interference assays and comparative metabolite profiling tracked the observed biological activity not to one of the anticipated enediyne pathways but exclusively to *ST_pks2*, an orphan biosynthetic gene cluster predicted to yield an anthracycline-type antibiotic. The metabolites associated with the *ST_pks2* locus were subsequently identified as the lomaiviticins, glycosylated diazofluorene polyketides with potent anticancer activity originally discovered from *Salinispora pacifica* strain DPJ-0019 (formerly *Micromonospora lomaivitiensis*).^[26–29] The discovery of the lomaivitin pathway provides the molecular logic for the construction of these complex organic molecules and, in

the long term, may help generate new bioactive derivatives by genetic engineering. Furthermore, the described genome mining approach may offer an effective strategy to mine for new DNA-interference natural products and their pathways with potential applicability as antitumor chemotherapies.

Results and Discussion

Bioinformatic prediction and biochemical detection of DNA-damaging chemotypes in *Salinispora tropica* CNB-440

The first step in the search for DNA-interfering chemotypes from the *S. tropica* CNB-440 genome was the bioinformatic analysis of candidate gene clusters. Initially, the two ePKS pathways in the *S. tropica* CNB-440 genome, *spo* and *ST_pksI*, were targeted. The *ST_pksI* locus was uncharacterized and not related to known enediyne molecules from *S. tropica*.

Proteins encoded by the *ST_pksI* genes exhibit strongest homology to enzymes required for the biosynthesis of dynemicin, a 10-membered enediyne.^[29] Interestingly, *ST_pksI* (Figure 1B, Supplementary Table 1) lacks ORFs for the synthesis of anthraquinone- or sugar-based groups that are typically clustered with *epks* genes and account for the extensive functionalization of the enediyne core. In dynemicin biosynthesis, genes encoding tailoring enzymes are chromosomally distinct from enediyne-associated genes,^[15] and an analogous scenario is plausible for the proposed *ST_pksI*-encoded enediyne as well.

The *spo* cluster (Figure 1C) includes all genes required for enediyne biosynthesis and is expected to yield a 9-membered enediyne product (Figure 1A). Most 9-membered enediynes are highly unstable relative to their 10-membered counterparts,^[23] and the *spo* enediyne product was proposed to readily undergo nonenzymatic Bergman-type cyclization in the presence of chloride anions, yielding sporolides A and B (Supplementary Figure 1A).^[22,23,30] Even though the enediyne origin of the sporolides has not been experimentally confirmed, previous *in vitro* studies supported an involvement of *spo* genes in the biosynthesis of their cyclohexenone residue.^[22]

To determine whether *S. tropica* produces DNA-damaging antibiotics, as predicted by bioinformatic analysis, the strain was fermented in a commonly employed seawater-based medium, and chemical extracts from multiple time points were evaluated for DNA-interfering natural products using the well-established biochemical prophage induction assay (BIA).^[31] DNA-damaging activity was most pronounced for methanol/ethyl acetate extracts of *S. tropica* cultures harvested 6–10 days after inoculation (Supplementary Figure 2A), suggesting that at least one of the biosynthetic clusters predicted to yield DNA-interfering enediyne compounds was expressed.

S. tropica enediyne genotypes are not responsible for biosynthesis of DNA-interfering chemotypes

To explore whether the candidate gene clusters *ST_pksI* and *spo* are associated with the observed DNA-damaging activity, a key biosynthetic gene from each cluster was inactivated and replaced with a gene for apramycin resistance (*aprR*) via PCR-targeted gene replacement.^[32] BIA activities were then compared between chemical extracts from individual mutants and the wild type strain (Supplementary Figure 2).

Elimination of the ePKS gene *spoE* from the *spo* cluster yielded a *spoE::apr^R* mutant incapable of sporolide production (Supplementary Figure 1B), confirming that the sporolides are indeed ePKS-derived products as originally suspected. However, chemical extracts from the *spoE::apr^R* mutant exhibited the same activity profile as the wild type strain in the BIA (Supplementary Figure 2C), indicating neither sporolides A and B nor their

proposed 9-membered enediyne precursor (Supplementary Figure 1A) were responsible for the observed DNA interference in the BIA. We suspect that this enediyne may decompose too rapidly for BIA detection due to the lack of a protective carrier. Most characterized gene clusters for 9-membered enediynes encode an apoprotein that sequesters the enediyne to enhance its stability and thus facilitate biological activity.^[15,23] However, no proteins with homology to characterized enediyne-stabilizing proteins were found within the *S. tropica* genome, suggesting that this organism lacks mechanisms for 9-membered enediyne stabilization and offering an explanation for the apparent instability of the proposed sporolide precursor. A similar transformation was hypothesized for the cyanosporasides from *Salinispora pacifica* CNS-143, which also have a cyclopenta[*a*]indene core structure derived from a putative 9-membered enediyne precursor.^[33]

From the *ST_pks1* gene cluster, PCR-targeted gene elimination of the putative 10-membered ePKS encoded by *strop0598* yielded a mutant with BIA activity equivalent to that of the wild type (Supplementary Figure 2B), thus eliminating *ST_pks1* as a candidate for biosynthesis of the observed DNA-interfering active metabolite. Further, LC-MS profiling of the mutant and wild type extracts revealed no evidence for enediyne natural products (data not shown), suggesting that the orphan *ST_pks1* gene cluster is either inactive under the selected fermentation conditions or expressed at low levels.

Type II PKS pathway *ST_pks2* produces DNA-interfering compounds in *S. tropica* CNB-440

As no enediyne was responsible for causing DNA-interference in *S. tropica* extracts as originally suspected, we evaluated the other orphan biosynthetic pathways for the responsible agent. Another prominent class of DNA-targeting natural products from actinomycetes are aromatic polyketides such as the anthracycline anticancer agents.^[34] Aromatic polyketides are commonly biosynthesized by type II PKSs and are often glycosylated.^[35] Glycosylated anthracycline anticancer agents, such as daunomycin, can intercalate into chromosomal DNA with their polyketide moiety, while the glycosyl moiety binds the phosphoribose DNA backbone.^[34] The *S. tropica* CNB-440 genome contains two orphan type II PKS pathways, *ST_pks2* and *ST_pks3*. The *ST_pks3* locus (Supplementary Figure 3A, Supplementary Table 2) encodes the synthesis of a putative spore pigment polyketide related to the *whiE* dodecaketide product of unknown structure from *Streptomyces coelicolor* A3(2).^[36] We explored its function by gene elimination of the predicted *ST_pks3* β -ketosynthase (*strop2500*) and sporulation phenotyping on A1 agar. The *ST_pks3* mutant showed loss of spore pigmentation compared to wild type *S. tropica* confirming a predicted spore pigment product (Supplementary Figure 3B). The *ST_pks2* gene cluster, on the other hand, is predicted to encode production of a glycosylated aromatic polyketide, thereby making it a more promising alternative target in the search for a DNA-interfering molecule than the *ST_pks3* spore pigment.

To examine the function of the *ST_pks2* gene cluster (Figure 1D), we eliminated the putative β -ketosynthase gene *strop2223* to yield a *strop2223::apr^R* mutant. The organic extract of this mutant was inactive in the BIA (Supplementary Figure 2D), thereby correlating the DNA-interference activity observed from wild type *S. tropica* exclusively to the *ST_pks2* gene cluster and not to either of the enediyne-encoding gene clusters (*ST_pks1* and *spo*). In light of prior research correlating the observation of ePKS-encoding genes with BIA activity,^[37] we found this result surprising. This earlier work, however, did not confirm their observations with gene deletions or compound isolation, thus leaving open the possibility that non-enediyne polyketides were responsible for the observed biological activity.

Characterization of *ST_pks2* as the lomaiviticin biosynthetic gene cluster (*lom*)

To identify the DNA-interfering glycosylated polyketide predicted by bioinformatics analysis of the orphan *ST_pks2* gene cluster, we employed an LCMSⁿ-based comparative metabolite profiling strategy. In LCMSⁿ traces of the wild type extract, several masses were detected that were absent in the corresponding mutant sample (Figure 2A/B, Supplementary Figure 4). One compound with an exact mass of 1338.5319 Da showed candidate deoxysugar fragments (158.12 m/z – putative *N,N*-dimethyldideoxysugar; 145.09 m/z – putative *O*-methyldideoxysugar) in the MS/MS spectrum (Figure 2C), indicating a putative glycosylated natural product. The compound was isolated as a burgundy-red solid by MS-guided fractionation via reversed phase-flash column chromatography from a methanol/ethyl acetate extract of a culture of *S. tropica* CNB-440. NMR analysis enabled the characterization of the molecule as lomaiviticin C (Figure 2D, Supplementary Figure 5, Supplementary Table 3), a glycosylated aromatic polyketide recently described from *S. pacifica* strain DPJ-0019.^[26] In addition, other known derivatives, lomaiviticins A, D and E, were identified by LCMSⁿ (Supplementary Figure 6).^[27,28] In the β -ketosynthase mutant, no NDP-deoxysugar accumulation was detected (data not shown) as these NDP-species should have a generally short lifetime in cell metabolism due to their high energy content.

Lomaiviticin A was first isolated by Carter^[27] and is the derivative with most potent anticancer activity.^[26] Its C–C dimeric diazofluorene core is also found in its monomeric form in kinamycin natural products from *Streptomyces murayamaensis*.^[28,38] While the kinamycin gene cluster (*kin*) was previously reported by Gould in 1998,^[39] the lomaiviticin biosynthetic gene cluster (*lom*) remained unknown until now. The *lom* locus is predicted to comprise 59 ORFs, which includes all biosynthetic genes putatively involved in the construction of the diazofluorene core in kinamycin (Table 1, Supplementary Figure 7).^[28,39] The *lom* cluster contains three additional PKS genes adjacent to the minimal type II PKS genes for the incorporation of a propionate starter unit in contrast to a starter acetate unit in kinamycin (Supplementary Figure 7). Putative biosynthetic gene products include two glycosyltransferases and several deoxysugar biosynthetic enzymes that nicely correlate with the glycosylation pattern of the lomaiviticins (Supplementary Figure 8). In addition, the gene cluster contains a FAD-dependent monooxygenase gene, *strop2191*, that is homologous to ActVA-Orf4 from the actinorhodin biosynthetic gene cluster in *S. coelicolor* A3(2). ActVA-Orf4 catalyzes the C–C dimerization reaction of two benzoisochromanquinone monomers in actinorhodin biosynthesis.^[40] We thus suspect that its homolog, Strop2191, catalyzes the dimerization of kinamycin-like diazofluorene monomers in lomaiviticin biosynthesis. The dimerization could occur from C2 after a dihydroxylation at C3 and C4 and a hydroxylation at C10 as enzymatic hydroxylations might be sterically hindered in the dimer (Supplementary Figure 7). A homolog of Strop2191 is not present in the homologous *kin* cluster. These diazo-forming and dimerizing enzymes await characterization.

Conclusion

In this study, we identified the lomaiviticin biosynthetic gene cluster (*ST_pks2* = *lom*) in *Salinispora tropica* CNB-440 via bioactivity-guided genome mining. Motivated by *ST_pks1* and *spo*, two *S. tropica* enediyne gene clusters without characterized enediyne products, we analyzed organic extracts with the BIA, an assay for rapid screening of DNA-interfering chemotypes, to detect a *S. tropica* DNA-targeting chemotype. Although we did indeed measure a BIA-based activity, gene elimination experiments showed that this observed bioactivity was not caused by an enediyne pathway product. Instead we correlated the BIA bioactivity to the products of the type II PKS pathway, *ST_pks2*. Comparative metabolic profiling yielded the lomaiviticins as *ST_pks2* products and, thus, as the observed BIA-positive compounds. Our approach of using the BIA in combination with genome mining

and gene elimination may prove an effective strategy to identify new DNA-targeting chemo- and genotypes beyond enediyne scaffolds. The identified biosynthetic gene cluster for a highly complex and stereospecific natural product such as lomaiviticin A can open up semisynthetic and heterologous expression routes as alternatives for their challenging production via total synthesis.

Experimental Section

General

All chemicals were acquired from Fisher Scientific, Sigma Aldrich and Honeywell Burdick & Jackson, and solvents were of HPLC grade or higher. HPLC analyses were conducted with an Agilent 1200 HPLC system with diode array detection. LC-MS analyses were conducted with an Agilent 6530 Accurate-Mass Q-TOF MS (MassHunter software, Agilent) equipped with a Dual electrospray ionization source and with an Agilent 1260 LC system (ChemStation software, Agilent) with diode array detector. NMR data were acquired at the UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences NMR Facility on a 600MHz Varian NMR spectrometer (Topspin 2.1.6 software, Bruker) with a 1.7 mm cryoprobe.

Gene inactivation

Targeted biosynthetic genes within *S. tropica* gene clusters (*ST_pks1*, *ST_pks3*, *lom*, and *spo*) predicted to yield DNA-interfering natural products were inactivated by PCR-based mutagenesis following previously established methods.^[32] Briefly, the apramycin resistance (*aac(3)IV*) cassette from pIJ773 was PCR amplified and extended using primer sequences (Supplementary Table 4). Extended antibiotic resistance cassettes were introduced by electroporation into *Escherichia coli* BW25113/pKD20 carrying appropriate pCCFOS-based fosmids BHXS2039 (for *ST_pks1* genes), BHXS0939 (for *ST_pks3* genes), BHXS5407 (for *lom* genes), and BHXS4676 (for *spo* genes). The mutated fosmid was then transferred into *S. tropica* CNB-440 by conjugation from *E. coli* S17-1, and gene replacement confirmed by colony PCR and sequencing of PCR products. Gene replacement experiments were minimally carried out in duplicate.

S. tropica fermentation and biochemical prophage induction assay (BIA)

Wild type *S. tropica* CNB-440 as well as *spoE::apr^R*, *strop2223::apr^R*, *strop2500::apr^R*, and *strop0598::apr^R* mutants were cultured at 28 °C in Fernbach flasks containing A1 medium (1 L, 1% starch, 0.4% yeast extract, 0.2% peptone, 0.1% calcium carbonate, 3% InstantOcean® sea salt). Inoculation occurred with A1 medium starter cultures (10 mL) in falcon tubes (50 mL). Flasks were shaken at 225 rpm, and time point samples (50 mL) were drawn every two days starting on day 4. Each sample (50 mL) was evenly divided into two portions. The first portion was clarified by centrifugation at 5000 rpm for 10 min to provide a supernatant sample. The remaining mycelial pellet from this portion was suspended in methanol (MeOH, 25 mL), stirred for 30 min at room temperature, and cell debris subsequently removed by centrifugation. The second portion of *S. tropica* fermentation (25 mL) was extracted three times with an equal volume of ethyl acetate. Supernatant samples, as well as MeOH and EtOAc extracts, were concentrated to dryness *in vacuo*. Samples were re-suspended at 1 mg/mL in their respective extraction solvents. The biochemical prophage induction assay (BIA) was performed as previously described.^[31] Briefly, extract (10 µL) was applied to agar plates seeded with *E. coli* ATCC 33312 and incubated for 5 h at 37 °C. Soft agar containing Fast Blue RR salt (1.2 mg/mL) and 6-bromo-2-naphthyl-β-D-galactopyranoside (0.4 mg/mL) was added onto the plate and color development was observed within 15 min. Cisplatin was included as a positive reference (test concentration: 5 mg/mL and 2-fold serial dilutions thereof). The fermentation medium and solvents were also

assayed alone and negative for BIA activity. Every assay was run at least in duplicate. For sporulation phenotyping, wild type *S. tropica* CNB-440 and *strop2500::apr^R* mutant were inoculated on an A1 agar plate (18 g agar/L A1 medium) and incubated for 14 days at 28°C.

Comparative metabolite profiling of wild type and mutant *S. tropica* chemical extracts by LC-MS

Crude MeOH/EtOAc extracts of wild type and mutant *S. tropica* strains were filtered through Acrodisc MS PTFE Syringe filters (Pall Inc.) and adjusted in concentration (0.2 mg/mL). For HPLC analysis, 25 µl was injected on a Luna reversed phase C18 (5 µm, 4.6 × 100 mm) HPLC column and subjected to a 10–100% MeCN (0.1% TFA) / 0.1% TFA gradient (15 min, 0.7 mL/min). For LCMSⁿ analysis, crude extract (2 µg) was injected onto a Phenomenex Luna C18 reverse phase HPLC column (5µm, 150×4.6mm) and was analyzed with an Agilent 6530 Accurate-Mass LC/MS with an Agilent 1260 LC system under the following LC conditions (1–3 min – 10% MeCN (0.1% TFA), 3–23 min – 10–100% MeCN (0.1% TFA), 23–25 min – 100% MeCN (0.1% TFA), 0.7 mL/min). Q-TOF MS settings during the LC gradient were as follows: Acquisition -Mass range 300–1500 m/z, MS scan rate 1/sec, MS/MS scan rate 2/sec, fixed collision energy 20eV, Source -Gas Temperature 300°C, gas flow 11 L/min, Nebulizer 45 psig, Ion polarity positive, Scan source parameters – VCap 3000, Fragmentor 100, Skimmer1 65, OctopoleRFPeak 750. The MS was autotuned with Agilent tuning solution in positive mode before each measurement. LC(DAD) data were analyzed with ChemStation software (Agilent), and MS data were analyzed with MassHunter software (Agilent).

Isolation and characterization of *ST_pks2* product, lomaiviticin C

Lomaiviticin C was isolated by MS-guided fractionation from an A1 medium culture (1L) of wild type *S. tropica* CNB-440 after 10 d incubation. The cells of 500 ml of the culture were extracted with MeOH for 30 min under stirring, and the remaining supernatant and culture were extracted twice with EtOAc. The crude organic extract was concentrated *in vacuo*, resuspended in MeOH (2 mL), and loaded on a reversed phase C18 silica gel column for flash-column chromatography. Lomaiviticin C was further purified as previously described^[26] using LC-MS for identification of lomaiviticin C-containing fractions. Purified lomaiviticin C was dissolved in MeOD-d₄ and subjected to NMR structure elucidation (¹H, DQF-COSY, ¹H-¹³C HMBC, NOESY). NMR data were analyzed with Topspin 2.1.6 software (Bruker).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Paul R. Jensen and William Fenical for providing *S. tropica* CNB-440. This work was supported by a postdoctoral fellowship from the German Academic Exchange Service (DAAD) to M.N. and an NIH IRACDA postdoctoral fellowship (GM068524) to A.L.L. This work was supported by grants from the NIH (GM085770 to B.S.M., GM097509 to P.C.D. and instrument grant 1-S10-RR031562-01A1).

References

1. Nett M, Ikeda H, Moore BS. Nat Prod Rep. 2009; 26:1362–1384. [PubMed: 19844637]
2. Challis GL. J Med Chem. 2008; 51:2618–2628. [PubMed: 18393407]
3. Corre C, Challis GL. Nat Prod Rep. 2009; 26:977–986. [PubMed: 19636446]
4. Winter JM, Behnken S, Hertweck C. Curr Opin Chem Biol. 2011; 15:22–31. [PubMed: 21111667]

5. Song LJ, Barona-Gomez F, Corre C, Xiang LK, Udway DW, Austin MB, Noel JP, Moore BS, Challis GL. *J Am Chem Soc.* 2006; 128:14754–14755. [PubMed: 17105255]
6. Eustaquio AS, Nam SJ, Penn K, Lechner A, Wilson MC, Fenical W, Jensen PR, Moore BS. *Chembiochem.* 2011; 12:61–64. [PubMed: 21154492]
7. a) Lautru S, Deeth RJ, Bailey LM, Challis GL. *Nat Chem Biol.* 2005; 1:265–269. [PubMed: 16408055] b) Ishida K, Lincke T, Behnken S, Hertweck C. *J Am Chem Soc.* 2010; 132:13966–13968. [PubMed: 20853892] c) Laureti L, Song L, Huang S, Corre C, Leblond P, Challis GL, Aigle B. *Proc Natl Acad Sci USA.* 2011; 108:6258–6263. [PubMed: 21444795]
8. Gross H, Stockwell VO, Henkels MD, Nowak-Thompson B, Loper JE, Gerwick WH. *Chem Biol.* 2007; 14:53–63. [PubMed: 17254952]
9. Blasiak LC, Clardy J. *J Am Chem Soc.* 2010; 132:926–927. [PubMed: 20041686]
10. Kersten RD, Yang YL, Xu Y, Cimermancic P, Nam SJ, Fenical W, Fischbach MA, Moore BS, Dorrestein PC. *Nat Chem Biol.* 2011; 7:794–802. [PubMed: 21983601]
11. La Ferla B, Airoidi C, Zona C, Orsato A, Cardona F, Merlo S, Sironi E, D'Orazio G, Nicotra F. *Nat Prod Rep.* 2011; 28:630–648. [PubMed: 21120227]
12. a) Newman DJ, Cragg GM. *Curr Opin Invest Dr.* 2009; 10:1280–1296. b) Olano C, Mendez C, Salas JA. *Nat Prod Rep.* 2009; 26:628–660. [PubMed: 19387499]
13. a) Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, Hallett W, Tsou HR, Upešlaciš J, Shochat D, Mountain A, Flowers DA, Bernstein I. *Bioconjug Chem.* 2002; 13:47–58. [PubMed: 11792178] b) Ducry L, Stump B. *Bioconjug Chem.* 2010; 21:5–13. [PubMed: 19769391]
14. Galm U, Hager MH, Van Lanen SG, Ju JH, Thorson JS, Shen B. *Chem Rev.* 2005; 105:739–758. [PubMed: 15700963]
15. Liang ZX. *Nat Prod Rep.* 2010; 27:499–528. [PubMed: 20336235]
16. Smith AL, Nicolaou KC. *J Med Chem.* 1996; 39:2103–2117. [PubMed: 8667354]
17. Penn K, Jenkins C, Nett M, Udway DW, Gontang EA, McGlinchey RP, Foster B, Lapidus A, Podell S, Allen EE, Moore BS, Jensen PR. *ISME J.* 2009; 3:1193–1203. [PubMed: 19474814]
18. Udway DW, Zeigler L, Asolkar RN, Singan V, Lapidus A, Fenical W, Jensen PR, Moore BS. *Proc Natl Acad Sci USA.* 2007; 104:10376–10381. [PubMed: 17563368]
19. Roberts AA, Schultz AW, Kersten RD, Dorrestein PC, Moore BS. *FEMS Microbiol Lett.* 2012; 335:95–103. [PubMed: 22812504]
20. Eustáquio AS, McGlinchey RP, Liu Y, Hazzard C, Beer LL, Florova G, Alhamadsheh MM, Lechner A, Kale AJ, Kobayashi Y, Reynolds KA, Moore BS. *Proc Natl Acad Sci U S A.* 2009; 106:12295–12300. [PubMed: 19590008]
21. Miyanaga A, Janso JE, McDonald L, He M, Liu H, Barbieri L, Eustáquio AS, Fielding EN, Carter GT, Jensen PR, Feng X, Leighton M, Koehn FE, Moore BS. *J Am Chem Soc.* 2011; 133:13311–13313. [PubMed: 21815669]
22. McGlinchey RP, Nett M, Moore BS. *J Am Chem Soc.* 2008; 130:2406–2407. [PubMed: 18232689]
23. Jean M, Tomasi S, Van de Weghe P. *Org Biomol Chem.* 2012; 10:7453–7456. [PubMed: 22898710]
24. Buchanan GO, Williams PG, Feling RH, Kauffman CA, Jensen PR, Fenical W. *Org Lett.* 2005; 7:2731–2734. [PubMed: 15957933]
25. a) Gulder TA, Moore BS. *Angew Chem Int Ed.* 2010; 49:9346–9367. b) Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. *Angew Chem Int Ed.* 2003; 42:355–357.
26. Woo CM, Beizer NE, Janso JE, Herzon SB. *J Am Chem Soc.* 2012; 134:15285–15288. [PubMed: 22963534]
27. He H, Ding WD, Bernan VS, Richardson AD, Ireland CM, Greenstein M, Ellestad GA, Carter GT. *J Am Chem Soc.* 2001; 123:5362–5363. [PubMed: 11457405]
28. Herzon SB, Woo CM. *Nat Prod Rep.* 2012; 29:87–118. [PubMed: 22037715]
29. a) Konishi M, Ohkuma H, Tsuno T, Oki T, VanDuyne GD, Clardy J. *J Am Chem Soc.* 1990; 112:3715–3716. b) Gao Q, Thorson JS. *FEMS Microbiol Lett.* 2008; 282:105–114. [PubMed: 18328078]

30. Perrin CL, Rodgers BL, O'Connor JM. J Am Chem Soc. 2007; 129:4795–4799. [PubMed: 17378569]
31. Elespuru RK, White RJ. Cancer Res. 1983; 43:2819–2830. [PubMed: 6221794]
32. a) Gust B, Challis GL, Fowler K, Kieser T, Chater KF. Proc Natl Acad Sci USA. 2003; 100:1541–1546. [PubMed: 12563033] b) Eustáquio AS, Pojer F, Noel JP, Moore BS. Nat Chem Biol. 2008; 4:69–74. [PubMed: 18059261]
33. Lane AL, Nam SJ, Fukuda T, Yamanaka K, Kauffman CA, Jensen PR, Fenical W, Moore BS. J Am Chem Soc. 2013; 135:4171–4174. [PubMed: 23458364]
34. Rabbani A, Finn RM, Ausio J. Bioessays. 2005; 27:50–56. [PubMed: 15612030]
35. Fujii I, Ebizuka Y. Chem Rev. 1997; 97:2511–2523. [PubMed: 11851468]
36. Yu TW, Shen Y, McDaniel R, Floss HG, Khosla C, Hopwood DA, Moore BS. J Am Chem Soc. 1998; 120:7749–7759.
37. Zazopoulos E, Huang KX, Staffa A, Liu W, Bachmann BO, Nonaka K, Ahlert J, Thorson JS, Shen B, Farnet CM. Nat Biotechnol. 2003; 21:187–190. [PubMed: 12536216]
38. a) Ito S, Matsuya T, Omura S, Otani M, Nakagawa A. J Antibiot. 1970; 23:315–317. [PubMed: 5458310] b) Gould SJ, Tamayo N, Melville CR, Cone MC. J Am Chem Soc. 1994; 116:2207–2208.
39. Gould SJ, Hong ST, Carney JR. J Antibiot. 1998; 51:50–57. [PubMed: 9531987]
40. Taguchi T, Ebihara T, Furukawa A, Hidaka Y, Ariga R, Okamoto S, Ichinose K. Bioorg Med Chem Lett. 2012; 22:5041–5045. [PubMed: 22765902]

Figure 1. Candidate DNA-targeting genotypes based on genome mining of *Salinispora tropica* CNB-440. A) Representative 10-membered enediyne, dynemicin A, and predicted enediyne precursor of sporolides A/B. Red –enediyne scaffold. B) *ST_pks1* gene cluster with predicted 10-membered enediyne polyketide biosynthetic machinery. C) *spo* (sporolide A/B) gene cluster with a predicted 9-membered enediyne biosynthetic machinery. D) *ST_pks2* gene cluster with a predicted glycosylated anthracycline product.

Figure 2.

Identification of the *ST_pks2* (*lom*) gene cluster product, lomaiviticin C, from extracts of wild type *Salinispora tropica* by LCMS-based comparative metabolic profiling. A) Comparative metabolic profiling identified a compound at 670.27 m/z with abolished production in the *ST_pks2* mutant (shown by corresponding base peak chromatogram (BPC)). B) MS spectrum of the *ST_pks2* product. C) MS/MS spectrum of the *ST_pks2* product showed deoxysugar-specific fragments (red) indicating a glycosylated natural product. D) The identified *ST_pks2* product is lomaiviticin C.

Table 1

Organization of the lomaiviticin biosynthetic gene cluster (*lom*) from *Salinispora tropica* CNB-440.

Gene	Size [aa]	Predicted function	Closest homolog [similarity/identity, (%/%)]	Reference	Kinamycin homolog
<i>strop2/172</i>	524	Drug resistance transporter	Drug resistance transporter [Salinispora arenicola CNS-205] (84/90)	ABY98201	
<i>strop2/173</i>	161	Hypothetical protein	Hypothetical protein [Salinispora arenicola CNS-205] (75/86)	ABY98202	
<i>strop2/174</i>	276	ABC transporter	ABC transporter [Catenulispora acidiphila DSM 44928] (56/39)	ACU70166	
<i>strop2/175</i>	316	ABC transporter	ABC transporter [Streptomyces svicens ATCC 29083] (60/74)	EDY54089	
<i>strop2/176</i>	171	Bleomycin resistance protein	Bleomycin resistance protein [Streptomyces pristinaespiralis ATCC 25486] (63/79)	EDY62258	
<i>strop2/177</i>	258	Hypothetical protein	Alpha/beta hydrolase [Streptomyces sp. Mg1] (49/62)	EDX26142	
<i>strop2/178</i>	278	Transcriptional activator	Transcriptional activator [Streptomyces coelicoflavus ZG0656] (68/52)	EHN80233	
<i>strop2/179</i>	113	Hypothetical protein	Hypothetical protein [Lodderomyces elongisporus NRRL YB-4239] (63/40)	XP_001524904	
<i>strop2/180</i>	281	AraC-family transcriptional regulator	AraC-family transcriptional regulator [Micromonospora sp. ATCC 39149] (61/45)	ZP_04603952	
<i>strop2/181</i>	477	NDP-hexose 2,3-dehydratase	3-dehydratase [Streptomyces coelicoflavus ZG0656] (81/72)	EHN77727	
<i>strop2/182</i>	221	Hypothetical protein	Hypothetical protein [Streptomyces coelicoflavus ZG0656] (79/63)	EHN77694	
<i>strop2/183</i>	148	Carboxymuconolactone decarboxylase	Carboxymuconolactone decarboxylase [Streptomyces coelicoflavus ZG0656] (73/58)	EHN77723	
<i>strop2/184</i>	275	Glutamine amidotransferases	Peptidase C26 [Streptomyces coelicoflavus ZG0656] (77/67)	EHN77724	
<i>strop2/185</i>	590	Aromatic ring hydroxylase	XiaK [Streptomyces sp. SCSIO 02999] (61/47)	AFK78077	
<i>strop2/186</i>	488	Protoporphyrinogen oxidase	Protoporphyrinogen oxidase [Streptomyces coelicoflavus ZG0656] (73/62)	EHN77710	
<i>strop2/187</i>	368	ParB domain protein nuclease	Nuclease, partial [Streptomyces coelicoflavus ZG0656] (79/67)	HN77721	
<i>strop2/188</i>	503	Anthrone hydroxylase	FAD-binding monooxygenase [Streptomyces coelicoflavus ZG0656] (78/71)	EHN77709	KinO2 (60/47)
<i>strop2/189</i>	490	Anthrone hydroxylase	FAD-binding monooxygenase [Streptomyces coelicoflavus ZG0656] (75/66)	EHN77708	
<i>strop2/190</i>	263	3-oxoacyl-ACP reductase	Short-chain dehydrogenase/reductase [Streptomyces coelicoflavus ZG0656] (82/71)	EHN77698	
<i>strop2/191</i>	290	Putative dimerase	NmrA family protein [Streptomyces coelicoflavus ZG0656] (76/67)	EHN77697	
<i>strop2/192</i>	205	DSBA oxidoreductase	DSBA oxidoreductase [Streptomyces hygroscopicus ATCC 53653] (71/57)	ZP_07297742	
<i>strop2/193</i>	109	Polyketide synthesis cyclase	Cyclase [Streptomyces antibioticus] (85/75)	CAG14964	KinI (82/70)
<i>strop2/194</i>	261	Polyketide ketoreductase	Short-chain dehydrogenase/reductase [Streptomyces bingchengensis BCW-1] (85/77)	YP_004965103	KinE (80/71)
<i>strop2/195</i>	345	Polyketide O-methyltransferase	O-methyltransferase [Streptomyces coelicoflavus] (78/63)	EHN77696	

Gene	Size [aa]	Predicted function	Closest homolog [similarity/identity, (%/%)]	Reference	Kinamycin homolog
<i>strop2196</i>	503	Anthrone hydroxylase	SaqE [Micromonospora sp. Tu 6368] (70/59)	ACP19351	KinOR (60/47)
<i>strop2197</i>	319	Polyketide aromatase	SaqL [Micromonospora sp. Tu 6368] (71/61)	ACP19357	KinD (67/55)
<i>strop2198</i>	479	FAD-dependent monooxygenase	Putative FAD-dependent monooxygenase [Streptomyces albaduncus] (61/52), JagF	CBH32087	
<i>strop2199</i>	492	Anthrone hydroxylase	Oxygenase-like protein [Streptomyces murayamaensis] (73/63)	AAO65343	KinO1 (73/63)
<i>strop2200</i>	247	Anthrone oxidase	JadG [Streptomyces venezuelae] (62/46)	AAV52247	KinG (68/52)
<i>strop2201</i>	627	Hypothetical protein	Hypothetical protein [Streptomyces ambofaciens] (71/61)	CAK51011	
<i>strop2202</i>	118	4Fe-4S ferredoxin	Putative ferredoxin [Streptomyces ambofaciens ATCC 23877] (83/75)	CAI78079	
<i>strop2203</i>	264	Hypothetical protein	Hypothetical protein [Salinispora tropica CNB-440] (78/65)	YP_001159312	
<i>strop2204</i>	500	Glutamine synthetase	Glutamine synthetase [uncultured bacterium BAC AB649/1850] (75/65)	AEE65491	
<i>strop2205</i>	484	Amidase	Putative amidase [Streptomyces coelicoflavus ZG0656] (73/63)	EHN77687	
<i>strop2206</i>	428	Adenylosuccinate lyase	Putative adenylosuccinate lyase [Streptomyces coelicoflavus ZG0656] (80/70)	EHN77655	
<i>strop2207</i>	137	N-acetyltransferase GCN5	Putative acetyltransferase [Streptomyces ambofaciens ATCC 23877] (79/72)	CAI78074	
<i>strop2208</i>	523	ABC transporter-like protein	Nova [Streptomyces coelicoflavus ZG0656] (82/69)	EHN77703	
<i>strop2209</i>	106	Dioxygenase	Dioxygenase [Thermobifida fusca YX1] (76/73)	YP_290041	
<i>strop2210</i>	136	Bleomycin resistance protein	Bleomycin resistance protein [Salinispora arenicola CNS-205] (97/92)	YP_001537194	
<i>strop2211</i>	263	Bleomycin resistance protein	Bleomycin resistance protein [Nesterenkonia sp. FI] (61/42)	ZP_09541316	
<i>strop2212</i>	520	Secreted peptidase	TAP domain-containing protein [Streptomyces coelicoflavus ZG0656] (72/61)	EHN77722	
<i>strop2213</i>	380	Glycosyltransferase	Glycosyl transferase [uncultured bacterium] (57/41)	AEM44235	
<i>strop2214</i>	371	Polyketide O-methyltransferase	O-methyltransferase [Ktedonobacter racemifer DSM 44963] (66/56)	ZP_06965007	
<i>strop2215</i>	313	O-methyltransferase	NanM [Streptomyces nanchangensis] (77/60)	AAP42862	
<i>strop2216</i>	328	NDP-hexose 4-ketoreductase	4-ketoreductase [Streptomyces sp. TP-A0274] (68/56)	BAC55215	
<i>strop2217</i>	199	NDP-hexose 3,5-epimerase	3,5-epimerase [Streptomyces sp. TP-A0274] (77/67)	BAC55217	
<i>strop2218</i>	335	NDP-hexose 3-ketoreductase	Putative 3-ketoreductase [Streptomyces galliae] (68/55)	AAF73453	
<i>strop2219</i>	244	Glycosyltransferase (auxiliary)	Hypothetical protein [Salinispora arenicola CNS-205] (88/80)	YP_001537195	
<i>strop2220</i>	344	Glycosyltransferase	Glycosyl transferase [Streptomyces cyanogenus] (55/42)	AAD13553	
<i>strop2221</i>	401	NDP-hexose 3,4-dehydratase/ aminotransferase	3,4-dehydratase-like protein [Streptomyces sp. KCTC 0041BP] (74/61)	ABB52533	
<i>strop2222</i>	333	dTDP-glucose-4,6-dehydratase	Putative dTDP-glucose-4,6-dehydratase [Actinoplanes missouriensis 431] (79/69)	YP_005462170	
<i>strop2223</i>	423	Minimal type II PKS, KS	Beta-ketoacyl synthase [Streptomyces acidiscabies 84-104] (82/71)	ZP_10452176	KinA (79/66)

Gene	Size [aa]	Predicted function	Closest homolog [similarity/identity, (%/%)]	Reference	Kinamycin homolog
<i>strop22224</i>	423	Minimal type II PKS, AT	Putative chain length factor [Streptomyces ravidus] (76/62)	CBH32808	KinB (73/59)
<i>strop22225</i>	84	Minimal type II PKS, ACP	ACP [Thermomonospora curvata DSM 43183] (73/48)	YP_0033300377	KinC (62/47)
<i>strop22226</i>	327	Propionate starter unit, AT	AknF [Streptomyces galilaeus] (64/53)	BAB72049	
<i>strop22227</i>	322	Propionate starter unit, KS	Putative modular polyketide synthase [Kitasatospora setae KM-6054] (45/30)	BAJ32815	
<i>strop22228</i>	107	Propionate starter unit, ACP	Actinorhodin polyketide dimerase, ACP [Thermobifida fusca YX] (71/51)	YP_289281	
<i>strop22229</i>	160	AraC family transcriptional regulator	AraC family transcriptional regulator [Salinispora arenicola CNS-205] (91/87)	YP_001537196	
<i>strop22230</i>	290	Glucose-1-phosphate thymidyl- transferase	G1P thymidyltransferase [Thermomonospora curvata DSM 43183] (87/72)	YP_0033300347	