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(1) Juliá, F.; Yan, J.; Paulus, F.; Ritter, T. *J. Am. Chem. Soc.* **2021**, *143*, 12992, DOI: 10.1021/jacs.1c06632. (2) Jia, H.; Häring, A. P.; Berger, F.; Zhang, L.; Ritter, T. *J. Am. Chem. Soc.* **2021**, *143*, 7623, DOI: 10.1021/jacs.1c0260.
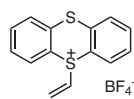
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ABOUT OUR COVER

Since time immemorial, the sea has been sustaining humans with its abundance of marine life as a food source. In the past few decades, marine life has also turned into a rich source of novel and bioactive natural products that have found important uses in combating disease and improving human health. This issue celebrates this marine research area that continues to experience rapid growth and that holds significant promise for the future.

Ocean Life (ca. 1859; watercolor, gouache, graphite, and gum arabic on off-white wove paper; 48.3 x 69.7 cm) is an apt illustration of the abundance of the sea and the diversity of marine life. This artwork was likely designed by James M. Sommerville (1825–1899) and executed by the French-born American teacher and artist Christian Schussele (1824–1879)—both associated with the Pennsylvania Academy of the Fine Arts—to be lithographically reproduced in a pamphlet that had the same title.

Detail from *Ocean Life*. Photo courtesy The Metropolitan Museum of Art, New York, NY

This artwork is a gift of Mr. and Mrs. Erving Wolf, in memory of Diane R. Wolf, 1977, to The Metropolitan Museum of Art, New York, NY.

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The Versatile Natural Product Enzymology of Marine Microbial Communities

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Abstract. Marine microbial communities are a wellspring of biochemical transformations, with discovery fueled by the ever-expanding development of biotechnological methodologies. Meta-omic strategies—from genome-based studies to enzymology and molecular-level investigations—have shed light on the involvement of microbial dark matter in the biosynthesis of bioactive metabolites. Herein, we describe the enzymology and biosynthesis of natural products derived from marine invertebrates, highlighting the diversity of this vast biochemical resource.

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1. Introduction

Marine natural products can derive from a variety of sources ranging from eukaryotic and prokaryotic microorganisms to diverse metazoans. Although these life forms represent a broad taxonomic range, marine bacteria have maintained a prominent role as extensive producers of bioactive specialized metabolites. Around 70% of all marine-derived bacterial natural products reported until 2020 were isolated from the genus *Streptomyces*.[1] While this abundance may suggest that efforts should be focused on studying the biosynthetic repertoire of this genus, there is mounting evidence that understudied producers and ecological niches are associated with novel chemistry and structural diversity. This is exemplified by groundbreaking work on other marine actinomycete groups spearheaded nearly twenty years ago,[2] and includes the discovery of the phase III anticancer candidate salinosporamide A from *Salinispora tropica*.[3]

    Early observations noted that many bacterial compounds bear resemblance to natural products isolated from chemically rich

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marine invertebrate groups (**Figures 1 and 2**). This significant overlap suggested that a multitude of substances previously attributed to macro-organisms may be assembled by members of their associated microbial community or obtained from a food source.[4-7] An example is the macrolide swinholide A that has been isolated from several sponges including *Theonella swinhoei* and an *Ircinia* species. A portion of the molecule, including the configurations of each asymmetric carbon, is shared by the cyanobacterial natural product scytophycin C from *Scytonema pseudohofmanni*.[8-12] This level of molecular and stereochemical conservation suggested the existence of a bacterial producer, and ultimately a free-living bacterial source was identified for the swinholides and related congeners.[10,11]
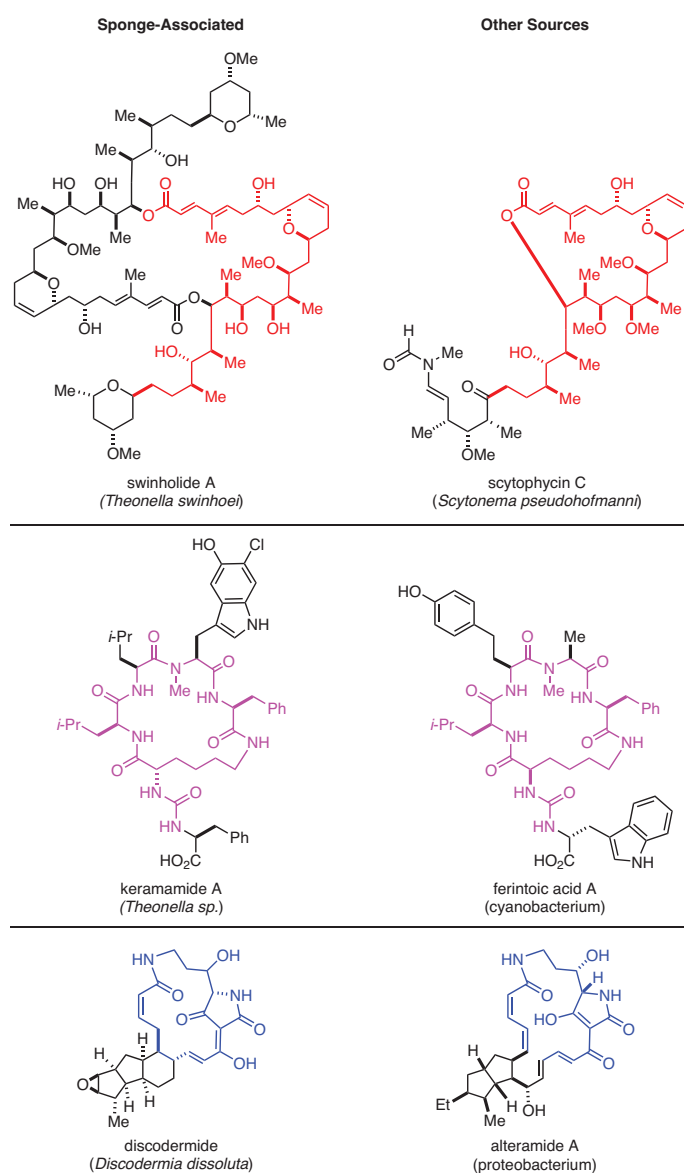
Structural overlap of this kind has been identified for various other sponge- and tunicate-associated natural products including those shown in Figures 1 and 2.

The formative discoveries and biosynthetic insights in marine and terrestrial actinomycetes and other bacteria[13,14] have led to a better understanding of metabolic contributions in marine invertebrates such as sponges, tunicates, bryozoans, and their symbionts. Invertebrate microbiomes can vary from a few detectable extra- or intracellular bacteria to spectacularly complex and abundant microbial consortia, the characterization of which is limited by the uncultivated nature of most symbiotic bacteria. Modern sequencing methods can reveal the genetic composition of these communities, thus providing insights into the molecular functions and enzymology involved. The technological capabilities in microbiome research have become increasingly more complex and powerful, facilitating the study of the most intricate systems at a molecular level and in a cultivation-independent manner. Earlier investigations of natural products in uncultivated microbiomes relied mostly on the construction and screening of large environmental DNA libraries,[15,16] gene targeting,[17] prediction of molecular structures from gene sequences,[18-20] and in situ hybridization. More recently, next-generation metagenomic and single-cell sequencing[21,22] have enabled the expansion of our knowledge in this area. Some of the first marine studies have been conducted on sponge holobionts due to their unparalleled chemical opulence. Key evidence for a biosynthetic role of symbiotic bacteria arose from the aforementioned observation that structurally similar, or even identical, molecules have been isolated from distantly related organisms, including other hosts. For example, the pederin family of defensive polyketides occurs in various distantly related sponges as well as insects.[15,17,23-25] The evolutionary relationship between the biosynthetic pathways in all producing holobionts facilitated the isolation of the biosynthetic gene clusters (BGCs) from DNA libraries, leading to evidence of prokaryotic origin and knowledge about the relevant producing enzymes.[26] Surprisingly, this work revealed a chemically rich sponge symbiont of the candidate genus "Entotheonella" that is responsible for generating numerous polyketides and peptides previously isolated from the examined sponge host. Of note, earlier mechanical bacterial enrichment and natural product extraction studies conducted on another sponge had suggested a biosynthetic role of "Entotheonella" for unrelated peptides.[27]

Strategies based on known enzymology in free-living bacteria provided insights into the biosynthesis of chlorinated dipeptides and polybrominated diphenyl ethers (PBDEs) in Dysideidae sponges harboring variants of the cyanobacterial symbiont *Hormoscilla* (formerly *Oscillatoria*) *spongeliae*.[28-30] For sponge dipeptides such as dysidenin, a candidate halogenase gene was identified based on a homologous sequence for a chlorinase involved in the biosynthesis of the structurally related barbamide from a culturable cyanobacterium (**Figure 3**).[28] The compounds were attributed to *H. spongeliae* through fluorescence-based cell sorting coupled to mass spectrometry.[29,30] In an

Figure 1. Structurally Related Natural Products from Sponges and Bacteria.
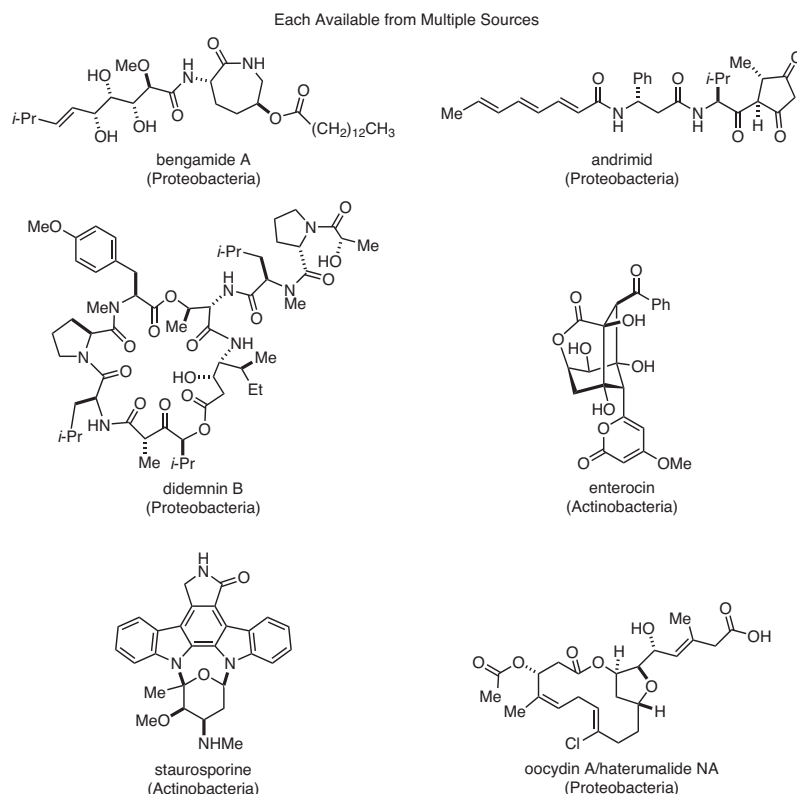
independent study, knowledge from a *Pseudoalteromonas* PBDE gene cluster[31] allowed for the identification of the PBDE genes in the sponge metagenome.[31,32] Here, the assignment to the symbiont *H. spongeliae* was accomplished by comparison to sequences of homologues deposited in GenBank® as well as the high representation of *H. spongeliae* in the 16S rRNA gene pool. Heterologous expression of BGC (biosynthetic gene clusters) portions in a cultivable cyanobacterium led to the production of the sponge-derived metabolites.[31,32]

While sponges are the most bountiful known marine source of symbiont-derived natural products, other chemically rich hosts do exist. Various compounds that are remarkably similar to those of bacterial origin are known from tunicates including enterocin, staurosporine, (also found in mollusk and flatworm host), and didemnin A.[33] The first example of an identified symbiotic producer from a tunicate was reported for the cytotoxic patellamides. These cyclic peptides were isolated from the tunicate *Lissoclinum patella* and were later confirmed to originate from the cyanobacterial symbiont *Prochloron didemni*.[34,35] In one case, *Prochloron* cells were isolated from *L. patella*, allowing for the extraction and sequencing of the genomic DNA.[34] A homology search of the draft genome revealed the coding sequence for the ribosomally synthesized patellamides. Subsequent functional expression in *E. coli* showed that it is possible to heterologously produce compounds from

an invertebrate symbiont in a laboratory host. A simultaneous effort applying a shotgun cloning approach to generate bacterial artificial chromosomes facilitated expression in *E. coli* and detection of heterologous patellamide production.[35] This foundation paved the way for follow-up studies surrounding the exchange of metabolites within the *L. patella* holobiome.[36]

For bryozoans, pioneering work revealed the bacterial producer of the cytotoxic bryostatins (**Figure 4**) from the bryozoan *Bugula neritina* and set the stage for further studies on the biosynthetic genes.[37] Degenerate PCR primers were used to amplify predicted polyketide synthase (PKS) gene regions in the animal, and in situ hybridization using PKS mRNA and 16S rRNA probes localized the PKS genes to a single bacterium with the proposed name "*Candidatus* Endobugula sertula." Follow-up studies involved isolation of the BGC from metagenomic and "*Ca*. E. sertula" enriched, DNA-derived cosmid libraries.[38-44]

One of the first metagenomic sequencing-based approaches was applied to the anticancer compound ET-743 from the mangrove-associated tunicate *Ecteinascidia turbinata*. The natural product shares a tetrahydroisoquinoline (THIQ) core with various compounds from cultivable bacterial sources including *Streptomyces lavendulae* (saframycin A), *Myxococcus xanthus* (saframycin Mx1), and *Pseudomonas fluorescens* (safracin B) (Figure 3).[45] First, a phylogenetic analysis of the bacterial diversity in the tunicate identified "*Candidatus*

Each Available from Multiple Sources

bengamide A
(Proteobacteria)

andrimid
(Proteobacteria)

didemnin B
(Proteobacteria)

enterocin
(Actinobacteria)

staurosporine
(Actinobacteria)

oocydin A/haterumalide NA
(Proteobacteria)

Figure 2. Biologically Active Compounds Available from Multiple Marine Invertebrate and Bacterial Sources.

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Endoecteinascidia frumentensis" as a probable producer due to its ubiquitous presence in ET-743-positive samples. An interdisciplinary approach was subsequently applied, which involved mass spectrometry (MS) based molecular analysis, cloning-independent next-generation sequencing, and metaproteomics. The raw sequencing reads were sorted based on sequence similarity to the BGCs for congeners of free-living bacteria. Further rounds of sequencing uncovered the complete "*Ca.* E. frumentensis" genome and additional ET-743 biosynthetic genes.[46]

These discoveries have led to the realization that marine invertebrate microbiomes have remarkable potential for diverse and new biochemical transformations. From peptides to polyketides and from early- to late-stage functionalization, the biochemistry occurring in marine microbial systems is among the most complex with an impressive diversity of structures,
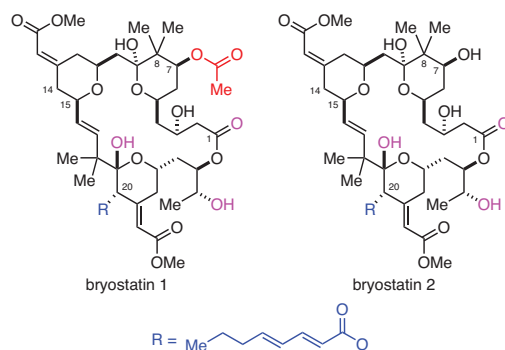
Figure 4. Bryostatins 1 and 2 Are Produced by a Gammaproteobacterial Symbiont of the Bryozoan *Bugula neritina*. The Octa-2,4-dienoate (Colored Blue) Part of the Molecules Is Specific to the Samples from Deep Collection Sites, While the Shorter Acetyl Ester Chain (Red Color) Is a Specific Moiety Found in Shallow Water Samples. Pharmacologically Important Elements Are Colored Pink. *(Ref. 42)*
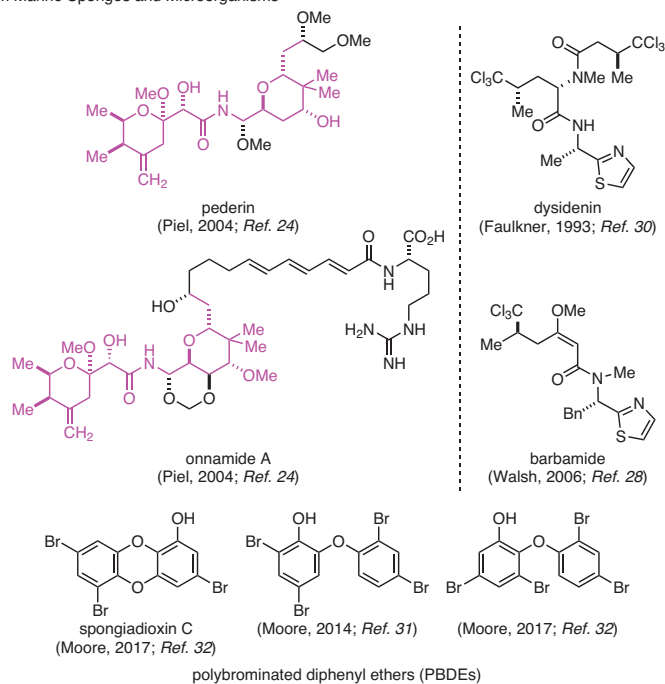
Figure 3. Molecules Derived from Marine Sponges, Tunicates, and Microorganisms. Pederin and Onnamide Are Produced by Related Enzymes, but Different Symbionts. The Biosynthetic Enzymes Responsible for Halogenation in Free-Living Bacteria Are Comparable to Those in Microbial Systems. The Patellamide Gene Cluster Was Identified by Isolation of the Producing Cyanobacterium. The Shared Core of ET-743 and Safracin B Indicate Homologous Biosynthetic Gene Clusters.

pathway types, novel biochemistry, and bioactivities (**Figure 5**). Herein, we describe the enzymology and biosynthesis of marine-invertebrate-derived natural products with the potential to impact human health and the environment. By covering a variety of host organisms and a broad chemical space, this review aims to highlight the biochemical diversity of this vast resource and provide a perspective on the future directions of the field.

2. Unusual Enzymology from Microbiomes of Marine Invertebrates

2.1. RiPPs

2.1.1. Cyanobactins

Ribosomally synthesized and post-translationally modified peptides (RiPPs) represent a diverse natural product superfamily of genetically encoded peptides processed by maturation enzymes.[47] RiPP biosynthesis usually involves the modification of a precursor peptide region referred to as the core. In addition, precursors can contain N-terminal leader and/or C-terminal follower regions that are cleaved off after modification of the core to generate the final natural product. The cyanobactin family of RiPP natural products was first reported in studies on the cytotoxic patellamides, isolated from the tunicate *L. patella*, but were later found to be more widely distributed in various symbiotic and free-living cyanobacteria.[34,35] These RiPPs are modified by a small number of relatively conserved biosynthetic enzymes, but cover a broad range of structural diversity.[48] The suite of cyanobactin biosynthetic enzymes can include heterocyclases, cyclodehydratases, macrocyclases, proteases, prenyltransferases, and methyltransferases, among others. The cyanobactin precursors can contain multiple core peptides divided by conserved recognition sequences that serve to guide the biosynthetic enzymes (RSII and RSIII on the N- and C-terminus, respectively). By introducing various non-native cores into precursor peptides containing the recognition sequences, designer peptides or engineered libraries of cyanobactins can be constructed.[49,50] Additionally, modification enzymes have been combined from different pathways to generate new macrocyclic scaffolds.[51] For an overview of the biotechnological applications and synthetic biology potential of this diversity-generating approach, see the extensive review by Gu and Schmidt, 2017.[52] Among the unique chemistry observed in cyanobactin biosynthesis is the one involved in heterocyclization. Whereas a complex of three enzymes is utilized in ribosomal peptide heterocyclization in microcin B17 and streptolysin S,[53,54] only a single component is required for thiazoline and oxazoline formation in patellamide and trunkamide
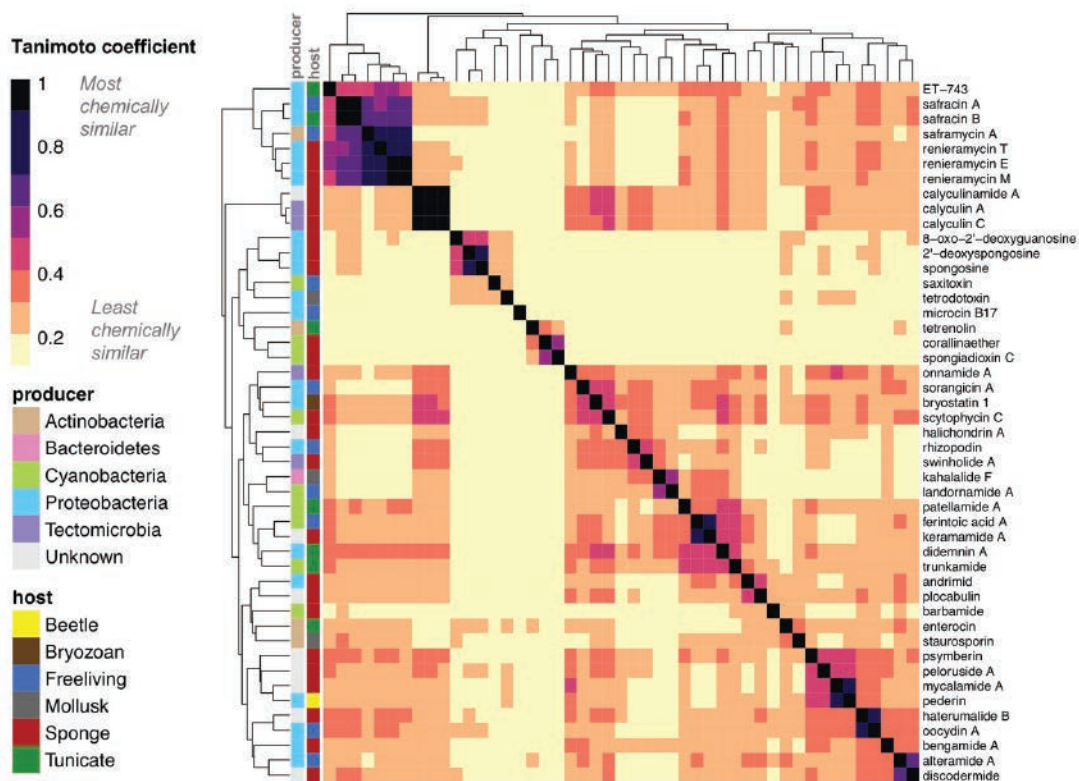
Figure 5. Natural Products Clustered by Chemical Similarity Rather Than by Taxonomy of the Microbial Producer or Its Host. Remarkable Chemical Resemblance Is Observed Across the Natural Product Repertoires of Free-Living and Host-Associated Microbes. This Heatmap Displays the Pairwise Chemical Similarity (Tanimoto Coefficient) with Single-Link Hierarchical Clustering—Ranging from Least Similar (Yellow) to Most Similar (Dark Violet)—of Selected Natural Products Covered in This Review. The Colored Side Bars Correspond to Taxonomic Information About the Bacterial Producer (If Known) and the Host (If Known).

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biosynthesis.[55] Based on detailed biochemical studies,[55] it was hypothesized that the heterocyclases act by properly orienting the peptide through suitable acid–base chemistry to facilitate nucleophilic attack on the carbonyl adjacent to the imminently cyclized amino acid cysteine or serine/threonine.

The patellamides are also noteworthy for their macrocyclic structures formed by the PatG macrocyclase domain that can manipulate inactivated peptide bonds (**Figure 6**).[34,56–59] The
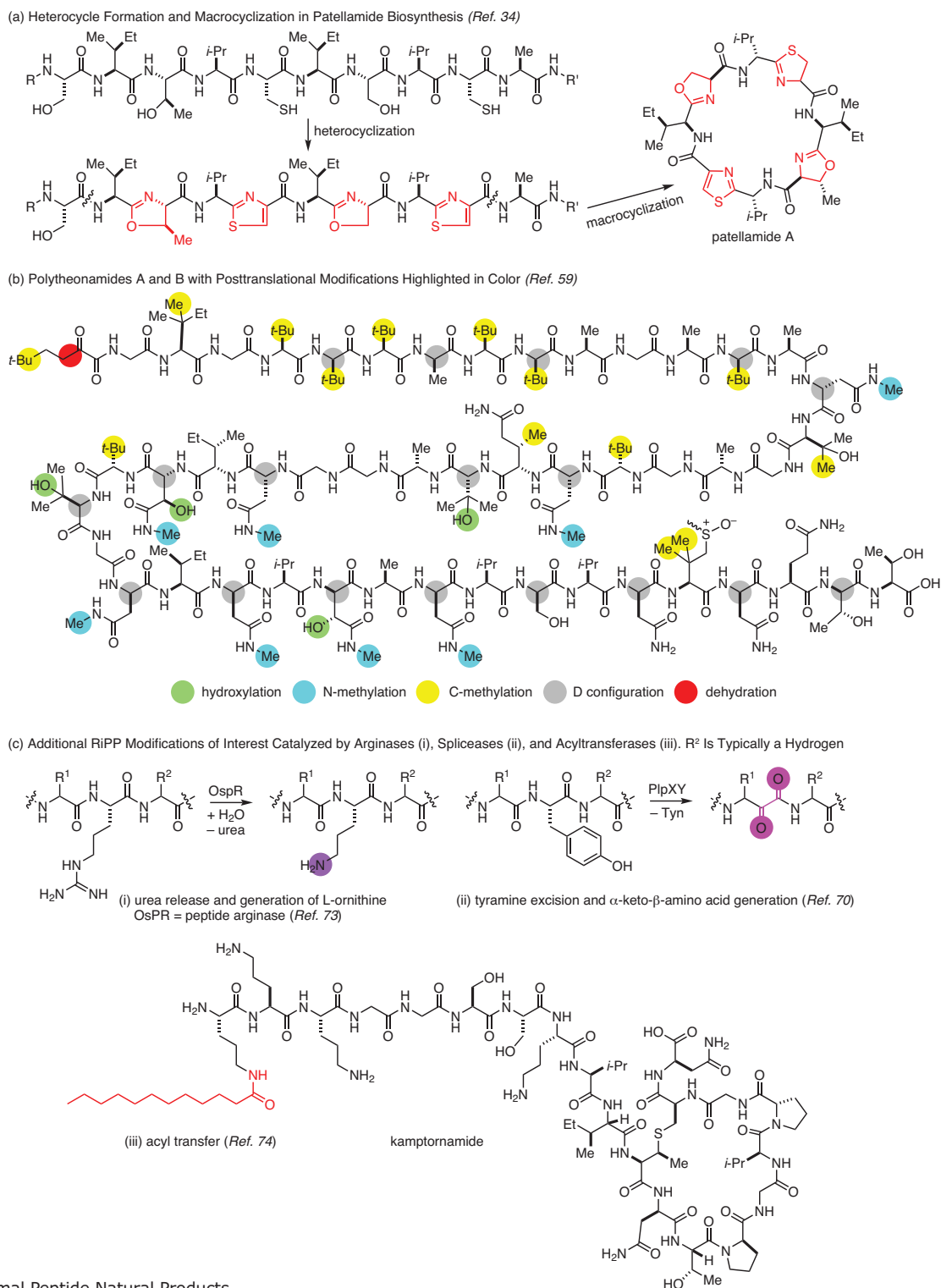

Figure 6. Ribosomal Peptide Natural Products.

enzyme catalyzes cyclization through proteolytic cleavage at the AYDG site followed by formation of an acyl–enzyme intermediate. A detailed structural analysis of the protein in complex with the AYDG peptide revealed the process for selective cyclization, as opposed to hydrolysis, through shielding of the active site from water.[56,57]

2.1.2. Polytheonamides

Further novel RiPP enzymology was discovered for the sponge-derived polytheonamides—peptides that display potent cytotoxic effects, are comprised of 48 amino acid residues, and possess some of the most complex structures known in natural products. Polytheonamides contain a large number of non-canonical amino acids that include 18 D-configured residues, amino acids with additional *C*-methyl branches, side-chain N-methylations, and hydroxylations (see Figure 6).[59] Because of these features, they were initially regarded as nonribosomal peptides, but isolation of the BGC revealed them as products of an extraordinary RiPP pathway located in the bacterial symbiont "*Ca.* E. factor". The ribosomal precursor PoyA features a leader region that resembles nitrile hydratases (nitrile hydratase leader peptide, NHLP), a signature of the proteusin family of RiPPs.[58,59] Polytheonamides are the most extensively modified ribosomal peptides known to date, with the *poy* BGC encoding just six enzymes that install forty-nine posttranslational modifications in the precursor PoyA.[59-64] These catalytic steps include (i) the unprecedented epimerization of the L to D form of all 18 D-configured amino acids by one enzyme, the radical *S*-adenosylmethionine (rSAM) epimerase PoyD; (ii) introduction of 8 Asn N-methylations by PoyE (rSAM-dependent), and (iii) dehydration by PoyF. Additionally, 17 C-methylation reactions at diverse sites—among them four that yield a terminal *tert*-butyl group—are catalyzed by the rSAM enzymes PoyB and PoyC. The rSAM superfamily catalyzes a variety of radical-mediated transformations in conjunction with a conserved [4Fe—4S] cluster. The LanM-like dehydratase PoyF has known homologues from other RiPP pathways, generally catalyzing the dehydration of serine and threonine to dehydroalanine and dehydrobutyrine.

The above modifications lead to the extreme structural complexity of polytheonamides and their orientation into a β-helical fold (β[6,3]), which acts as a transmembrane pore.[65-67] In eukaryotic cells, membrane insertion results in cytotoxicity due to rapid depolarization as well as decreased membrane potential. Investigations with synthetic analogues of polytheonamide B found that the hydrophobic 5,5-dimethyloxohexanoate N-terminal region increases the cytotoxicity, possibly by aiding the partitioning of the hydrophobic membrane.[68]

The insights into polytheonamide biosynthesis facilitated genome mining efforts for additional proteusin-type BGCs, revealing widespread homologues of rSAM epimerases and further unusual RiPP modifications in free-living bacteria. This is further explored in a recent review by Mitchell et al.[60,62,63,69-72] In the pathway for the new antiviral peptide landornamide A from a *Kamptonema* (*Oscillatoria*) sp. cyanobacterium, the peptide arginase OspR was identified that introduces ornithine residues

into natural and non-natural target peptides (Figure 6, Part (c-i)).[73] OspR is proposed to utilize a metal-bridging hydroxide in the active site to attack the guanidinium group of the RiPP arginine. Subsequent proton transfer and addition of water facilitates the loss of urea and the formation of L-ornithine. In another cyanobacterial (*Pleurocapsa*) BGC encoding proteusin and Nif11-type precursors, an orphan rSAM enzyme family was identified.[60,70] Expression in *E. coli* revealed an unusual peptide-splicing process involving backbone carbon–carbon bond cleavage and net excision of a tyramine equivalent from tyrosine (Figure 6, Part (c-ii)).[70] In this way, β-amino acids can be post-translationally incorporated into peptide products. Another RiPP mining study revealed that modification with fatty acids generates lipopeptides, of which one of the first examples was the *Kamptonema*-derived kamptornamide (Figure 6c-iii).[74] The biosynthetic pathways for these lipopeptides were all found to contain a GCN5-related *N*-acetyltransferase (GNAT) responsible for installation of the lipid moiety.[75] Recently, genome mining for polytheonamide-type pathways also resulted in the discovery of the cytotoxic congener aeronamide A from the culturable wastewater bacterium *Microvirgula aerodenitrificans*.[76] This work underscored that, once the BGC for an invertebrate-derived natural product is known, homology searches in sequenced bacterial genomes may reveal culturable production sources as an alternative to the often challenging heterologous expression of symbiont BGCs.

2.2. Polyketides

Within symbiotic bacteria in particular, an unusual type of multimodular polyketide synthase (PKS), the *trans*-acyltransferase (AT) PKS, is quite prevalent.[23,77-80] A multimodular PKS is a megaenzyme consisting of modules that are each responsible for the incorporation of a single building block into the growing polyketide chain. While elongation requires the activity of three domains (ketosynthase, KS; AT; acyl carrier protein, ACP), a characteristic of the *trans*-AT systems, as opposed to the canonical *cis*-AT systems, is that the AT is present as a distinct enzyme separate from the megasynthase. The ACP is covalently modified with a phosphopantetheine arm containing a free thiol group onto which the AT loads the acyl building blocks for polyketide biosynthesis. A β-keto thioester is then generated via a KS-catalyzed Claisen-like condensation. The minimal module architecture can be supplemented by a ketoreductase (KR) for β-hydroxy formation, a dehydratase (DH) for α,β-desaturation, and an enoylreductase (ER) for reduction of the double bond. In addition to the presence of discrete ATs, numerous peculiarities in architectural diversity exist for the *trans*-AT PKS systems including unusual domain orders and unique domains, non-elongating modules, domains acting across modules, modules split between two proteins, and diverse *trans*-acting modifying enzymes.[81]

2.2.1. Pederins

The pederin family of antitumor polyketides was one of the earliest reported *trans*-AT systems.[23,82] While pederin was

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isolated from rove beetles, many other members of this polyketide family have been isolated from sponges and their associated microbiomes, including onnamide A from *T. swinhoei*,[26,83,84] mycalamide A from *Mycale hen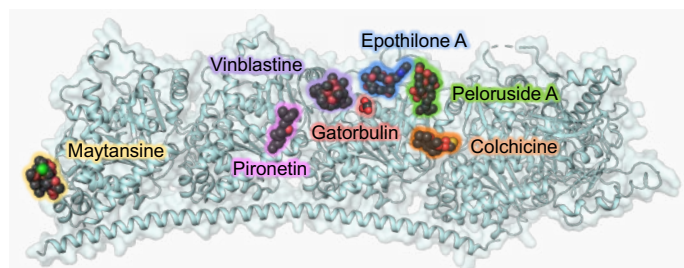tscheli*,[85,86] and psymberin from *Psammocinia* aff. *bulbosa*[17,87] and *Ircinia ramosa* (**Figure 7**).[88] An acyl hydrolase (AH) was identified in the pederin pathway and was found to act as a proofreading enzyme to remove stalled intermediates from PKS modules.[89] Now, homologues of this previously unassigned enzyme (PedC) are known to be widespread in *trans*-AT PKS systems. Another unusual pederin biosynthetic enzyme is the pyran synthase PedF[90,91] with homologues involved in the biosynthesis of the bryostatins (see Figure 4)[44] and sorangicin.[92] The enzyme facilitates stereoselective ring closure while accepting various substrates, indicating its usefulness for biocatalytic processes. Lastly, one of the most distinct molecular features of pederin and the related sponge compound mycalamide A is the presence of the oxidized terminus (highlighted in yellow in Figure 7). The traditional even number of carbons in the polyketide chain is broken with a single carbon terminus generated through a putative oxidative cleavage catalyzed by PedG. An analogous reaction has recently been demonstrated for the closely related enzyme OocK, a Baeyer–Villiger monooxygenase that catalyzes oxygen insertion in the complex polyketide oocydin.[93]
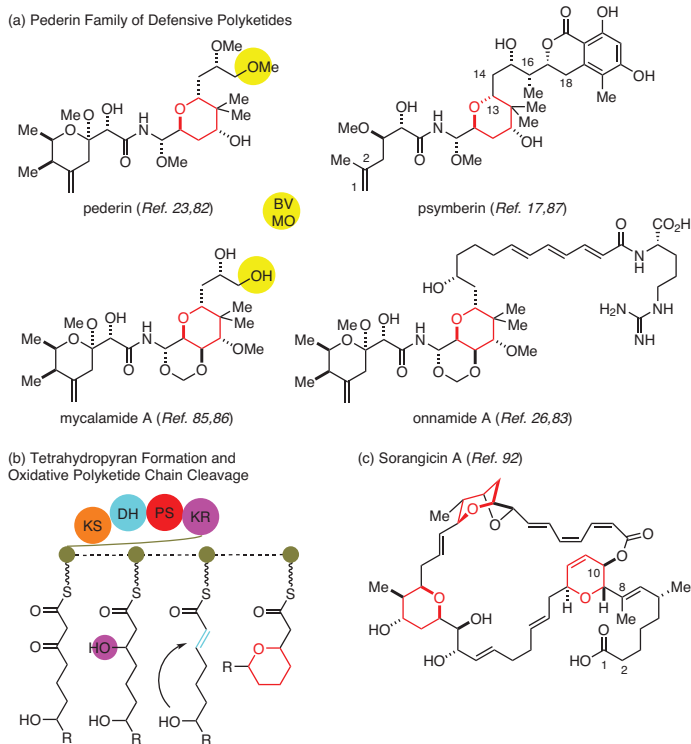
2.2.2. Pelorusides

The peloruside biosynthetic pathway from a bacterial symbiont of the sponge *Mycale hentscheli* provides further evidence for an unusual module architecture of *trans*-AT pathways. Within the chemically rich sponge, almost all members of the microbiome jointly contribute to the chemical diversity.[86,94] The derived compounds include members of three distinct polyketide families: mycalamide-type poisons,[85] translation-inhibiting pateamines,[95] and microtubule-inhibiting pelorusides.[96,97] Members of the latter compound family have attracted much attention as potential drug candidates. Microtubules are critical components for cell processes and are considered some of the most significant and promising targets for the successful development of anticancer therapies.[98,99] Since the recognition of the tubulin dimer as a promising anticancer target, an assortment of new microtubule targeting agents (MTAs) have been discovered, many of which are natural products (**Figure 8**). Their various binding sites have formed the basis for differentiation of MTAs; including the maytansine,[100] colchicine,[101] pironetin,[102] and vinca alkaloid microtubule destabilizing agents (MDAs);[103] and the taxane-[104] and laulimalide-family[105] microtubule stabilizing agents (MSAs)—all from natural sources such as plants and marine environments. Even just recently, the discovery of cyanobacterial gatorbulin-1 has added a seventh binding site for microtubule-inhibition candidates.[106] Mainly targeted for anticancer drug discovery purposes, the taxane site is affected by ligands such as the clinically approved paclitaxel (Taxol) and its analogue docetaxel (Taxotere®),[107] along with the natural products epothilones,[108,109] zampanolide,[110] dictyostatin,[111] taccalonolides,[112] and discodermolide.[113] The sponge-associated natural products laulimalide[114,115] and peloruside A[96] are promising non-taxane-site MSAs that have demonstrated potent inhibition against the growth of multidrug-resistant (MDR) cancer cells in addition to synergistic effects with Taxol and the epothilones.[116] Due to the small amounts of isolated material, research efforts have been invested into *M. hentscheli* mariculture[117,118] and total chemical synthesis.[119] With the recent identification of the peloruside A BGC, further investigation into the biosynthetic machinery is expected to inform on production methods that are based on synthetic biology.[86,94]

Figure 7. Tetrahydropyran Formation (Red) and Oxidative Polyketide Chain Cleavage (Yellow) in Pederin and Related Polyketides. Domains Include Ketosynthase (KS), Dehydratase (DH), Pyran Synthase (PS), and Ketoreductase (KR).

Figure 8. Various Sites Targeted for Microtubule Inhibition by Natural Products (PDB Codes: 4TV8, 1SA0, 5FNV, 5J2T, 4O4L, 7ALR).

The assignment of the *pel* locus to peloruside production was based on the knowledge that phylogenetically related KS domains featuring similar sequence patterns accept intermediates carrying similar chemical moieties around the thioester.[18] Partial intermediate structures can therefore be predicted from KS sequences (and vice versa). The automated structure prediction program TransATor[19] was successful in predicting the linearized version of the peloruside structure.[86] However, due to the highly aberrant architectures of the second and third modules, the prediction substantially differed for the exocyclic moiety. These modules feature a series of non-elongating KSs (KS[0]), a condensation (C) domain that normally occurs in non-ribosomal peptide synthetases (NRPSs) to generate amide bonds, and two internal TE domains, which are usually positioned at the C-termini of PKSs and NRPSs to catalyze thioester hydrolysis or macrocyclization (**Figure 9**).[86]

2.2.3. Calyculins

Another mixed *trans*-AT PKS/NRPS system with unusual architecture is that involved in the biosynthesis of calyculin. Calyculins A–H, incorporating a spiroketal structural feature at C-19, were isolated from the sponge *Discodermia calyx*, and all congeners displayed cytotoxic activity as protein phosphatase 1 and 2a inhibitors.[120-125] The similarly potent IC_{50}'s (0.9–6 nM) for these eight antitumor agents indicate that the contribution of the tetraene moiety and presence or absence of a methyl group at C-31 are negligible factors in determining the biological activity.[123] The isolation of the calyculins—in addition to other cytotoxic metabolites such as the calyculinamides—from another sponge, *Lamellomorpha strongylata*, provided further evidence for microbes as the calyculin source (**Figure 10**).[126] Metagenome mining ultimately led to the identification of the microbial symbiont harboring the biosynthetic gene cluster, a member of the candidate genus "Entotheonella".[127]

Identified from the sponge metagenome of *D. calyx*, this BGC was consistent with the expected structure of the secondary metabolite (see Figure 10).[127] With 29 KS domains and five adenylation (A) domains, this cluster is the largest hybrid cluster discovered to date. In the proposed biosynthesis, the starter serine is permethylated on the hydroxyl and amino groups by two methyltransferase domains to generate the trimethylseryl unit that constitutes one end of calyculin. It is worth noting that the number of carbons in calyculin from the *trans*-olefin to the terminal nitrile does not follow the typical rule of C_2 elongation cycles in polyketide biosynthesis. It is proposed that the standalone oxidation domain CalD performs an α-hydroxylation of the β-hydroxyl substrate bound to the acyl carrier protein to produce a diol intermediate.[127] Subsequent dehydration of the diol with a dehydratase would generate an enol, leading to a spontaneous tautomerization to the α-ketothioester intermediate. Chain shortening could occur via a Baeyer–Villiger-type oxidation of the α-ketothioester to a mixed anhydride and Claisen condensation on the ester
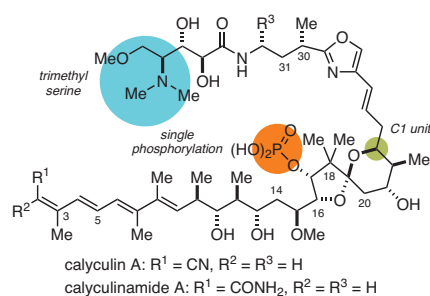
Figure 10. Molecular Comparison of Calyculin A and Calyculinamide A. *(Ref. 126,127)*
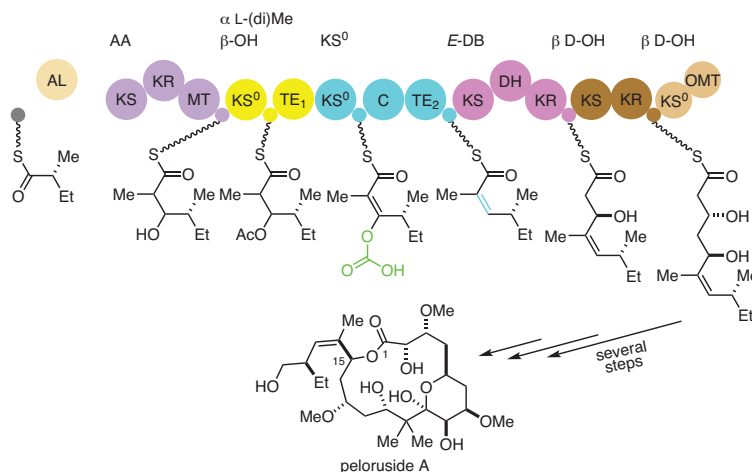
Figure 9. First Three Proteins of the Peloruside Biosynthetic Pathway, PelA, B, and C, and Proposed Biosynthetic Pathway. PelC Contains Unusual Module Architectures Such as an Internal Thioesterase and a Condensation Domain. Predicted Substrates Are Described above the KS Domains. The Modules Are Indicated by Color and Include the Following Domains: Acyl Ligase (AL); Ketosynthase (KS); Non-elongating KS (KS[0]); Ketoreductase (KR); *O*-Methyltransferase (*O*MT); Thioesterase (TE); Condensation (C); Dehydratase (DH). Smaller Spheres Are Used to Denote the Carrier Protein (ACP/PCP). Above Each KS Is the Substrate Prediction by TransATor. *(Ref. 19,86)*

carbonyl. An alternative mechanism could involve a Favorskii rearrangement, as reported in ambruticin and enterocin biosynthesis.[128,129] The phosphorylation of the calyculins, which involves the incorporation of pyrophosphate into calyculin A to generate phosphocalyculin A, led to the reassignment of the final product to the diphosphorylated analogue. Interestingly, disruption of the sponge tissue releases dephosphorylation enzymes that cleave the pyrophosphate, generating the 1000x more potent toxin calyculin A.

2.2.4. Bryostatins

The bryostatins represent another *trans*-AT system with unusual domain architecture (**Figure 11**). Initial loading,[41] acyl transfer,[130] and β-branching[131,132] were demonstrated for this pathway. The β-branching cassette is a piece of biosynthetic machinery resembling features of terpene biosynthesis. It employs a 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase-type enzyme (HMGS) to catalyze a two-carbon addition to the β-carbonyl of the polyketide.[133] In bryostatin biosynthesis, the nascent intermediate undergoes dehydration catalyzed by an enoyl-CoA hydratase (BryT, ECH_1) to form an α,β double bond followed by O-methylation to generate the vinylic methyl ester (see Figure 11).[132,134] Combination assays of BryT, BryA module 3, and *S*-adenosylmethionine in the presence of HMG-CoA led to dehydration followed by O-methylation. Interestingly, this enzymatic machinery requires a strict order of catalysis: BryU (ACP) donation of the acetyl unit to the β-carbonyl, dehydration catalyzed by BryT, and O-methylation by BryA. In other β-branching cassettes, ECH_1 and ECH_2 act concomitantly to catalyze dehydration and decarboxylation,[135] respectively, while the *bry* pathway involves a single ECH (with homology to standard ECH_1) followed by O-methylation.

Of significance, bryostatins 1 and 2 are agonists of protein kinase C,[136] with the high potency of bryostatin 1 causing this
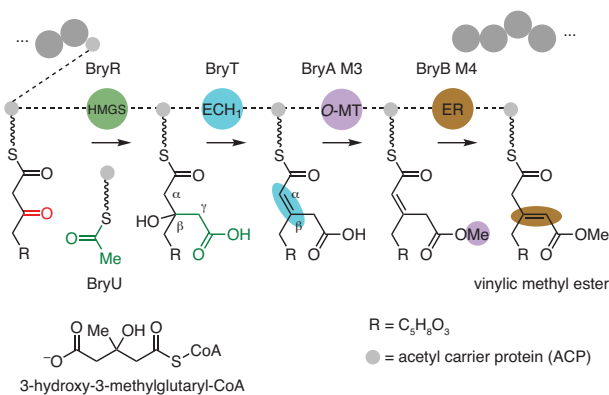
Figure 11. β-Branching Step in Bryostatin Biosynthesis Including Extension by a 3-Hydroxy-3-methylglutaryl-CoA Synthase-like Enzyme (HMGS) Using an Acetyl Unit from a Free-Standing ACP (Small Gray Circles), Dehydration by the Enoyl CoA Hydratase (ECH_1), Methylation by the *O*-Methyltransferase (*O*-MT), and Isomerization by a Putative Enoylreductase (ER)-like Domain. *(Ref. 132)*

molecular family to be among the most extensively studied marine natural products in clinical trials (see Figure 4). Among the various uses in the biomedical realm, bryostatin has demonstrated great potential as a combination chemotherapy agent, particularly with paclitaxel for esophageal cancer, as well as in treatments of fragile X syndrome, Alzheimer's disease, and HIV.

2.3. Nonribosomal Peptides

Much like the above-described PKSs, nonribosomal peptide synthetases (NRPSs) are also organized into multidomain megaenzymes.[137] NRP biosynthesis can incorporate proteinogenic amino acids in addition to other building blocks, which are selected and activated by the A domain. The C domain catalyzes peptide-bond formation with the building blocks tethered to a peptidyl carrier protein (PCP). Additional accessory domains, such as epimerases (E), can diversify the peptide scaffold. Furthermore, the N-terminus of the peptide can be modified with an acyl group, providing many biological benefits such as protection from degradation, polarity modulation, and the possibility of membrane insertion. In general, the biosynthesis is terminated by a TE (thioesterase) to form either a linear or cyclized peptide product.

2.3.1. Ecteinascidins

Various antitumor ecteinascidins have been isolated from *E. turbinata*, a colonial tunicate which grows on mangrove roots throughout the Caribbean and Mediterranean.[138,139] A bacterial symbiont "*Ca.* Endoecteinascidia frumentensis" was later identified as the ecteinascidin producer.[45,139,140] Their tetrahydroisoquinoline (THIQ) core has since gained a reputation as a significant drug scaffold for antiviral, immunosuppressive, and antitumor regiments. Ecteinascidin-743 (ET-743), the most recognized and studied of the ecteinascidins, contains three THIQ units and is most active against lung cancer, melanoma, and breast cancer cell lines.[141] While earlier studies hypothesized that ET-743 acted through binding to microtubules,[142] later ones found it to alkylate the minor groove of DNA (**Figure 12**), causing distortions and demonstrating high activity in cellular assays.[143] The perturbation of the cell cycle induced by the binding of ET-743 is not entirely unusual for DNA-interacting drugs, but detailed observations led to the hypothesis that this molecule might modulate DNA by a different means compared to the minor groove binder tallimustine[144], or major groove binder cisplatin.[145] The success of ET-743 ultimately led to its development and commercialization as Yondelis® (common name trabectedin) by PharmaMar and Johnson & Johnson, with U.S. Food and Drug Administration (FDA) approval for the treatment of various forms of sarcoma. The commercialization of ET-743 was made possible by early, purely synthetic methods,[146-148] while presently it is semi-synthetically manufactured from the natural product cyanosafracin B.[149]

Advances in (meta)genomic sequencing technology also facilitated the discovery of novel biosynthetic machinery involved in the production of ecteinascidins.[45,46,150] The chemical structure

of the ecteinascidins is similar to that of the THIQ-containing secondary metabolites saframycin A,[151] saframycin Mx1,[152] safracin A,[153] naphthyridinomycin,[154] and quinocarcin.[155,156] Similar pieces of biosynthetic machinery were proposed to be
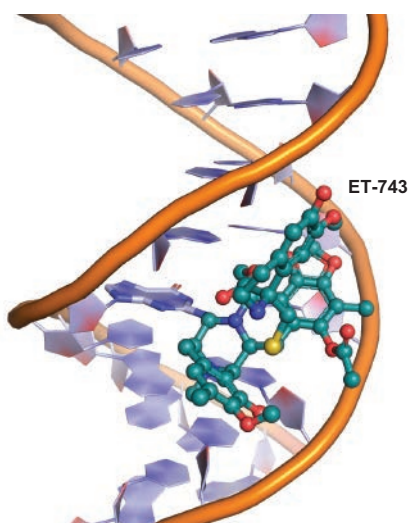

Figure 12. Model of ET-743 Bound to the Minor Groove of a DNA Double Helix. Simulations Indicate That Three ET-743 Ligands Can Alkylate Adjacent Binding Sites to Stabilize a New Conformation of DNA.

involved in the generation of the α-hydroxyethyl moiety that is shared with naphthyridomycin and quinocarcin.[157] Additionally, isotopic feeding studies with cell-free extracts of *E. turbinata* identified the diketopiperazine as the first committed precursor to ET-743.[158] The BGC was identified by comparison with the similar pathways mentioned above.[159-161] The ecteinascidin (*etu*) and saframycin (*sfm*) biosynthetic loci encode architecturally similar NRPS enzymes with a C domain-like Pictet-Spenglerase responsible for the formation of the THIQ core (**Figure 13**).[45,162] Other unusual ET-743 biosynthetic components include an E3 component of pyruvate dehydrogenase complex, EtuP3, responsible for generating the glycolyl-A-ACP extender unit for the NRPS. Additionally, the pathway hinted at a potential overlap between primary and secondary metabolisms.[46]

2.3.2. Keramamides

Marine-sourced cyclic NRPs have a broad range of biological activities.[163] One of many examples of NRPs is the keramamide family. Keramamide A was isolated from marine sponges of the genus *Theonella* and was the first instance of a 6′-chloro-5′-hydroxy-*N*-methyl-tryptophan-containing peptide natural product (**Figure 14**).[164,165] Around the same time, orbiculamide A was also found to be associated with *Theonella* sp.,[166] adding 2′-bromo-5′-hydroxy-tryptophan to the repertoire of halogenated amino acid building blocks. The various substitution patterns on the indole moiety of these molecules
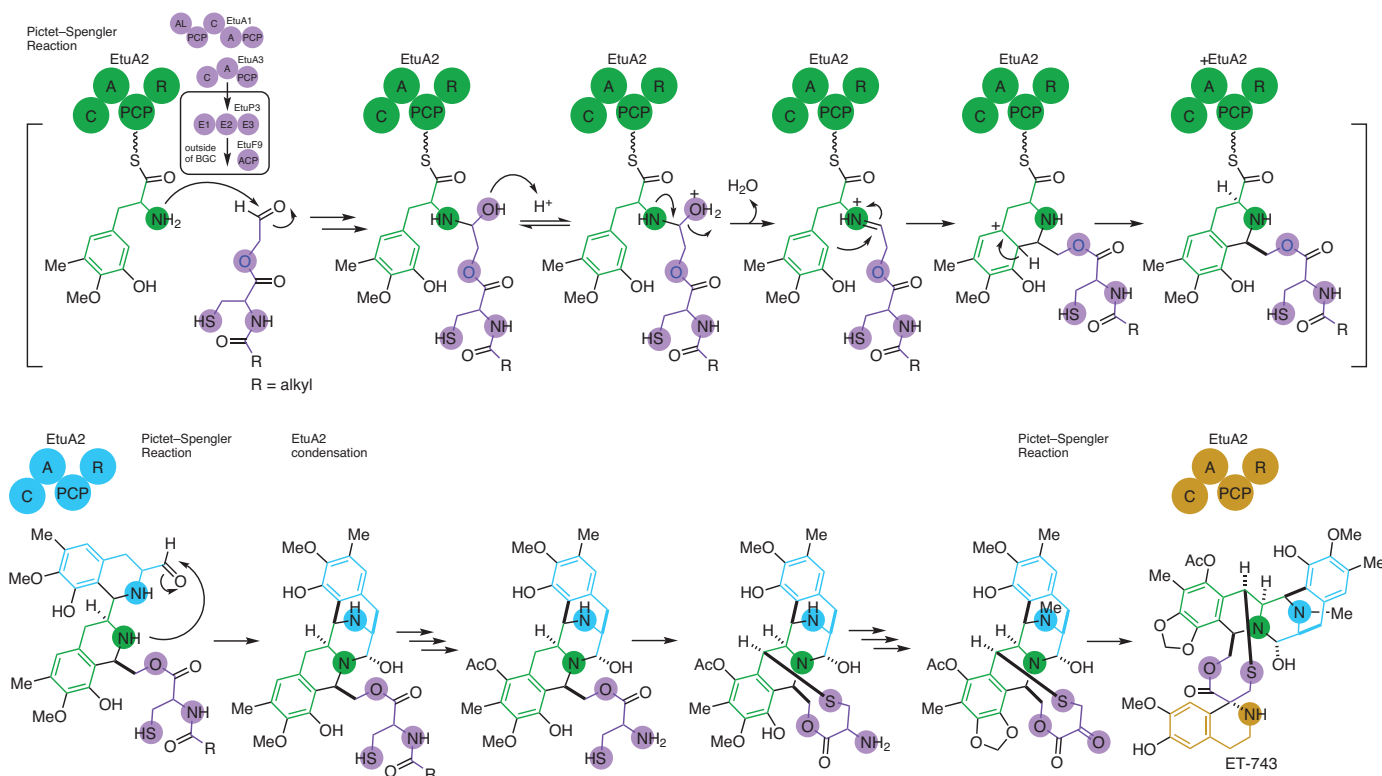
Figure 13. Condensed Biosynthetic Pathway for ET-743. An Initial Starter Originating in Primary Metabolism Is Followed by Three Pictet–Spengler Reactions (Mechanism in Brackets). *(Ref. 45,46)*

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has been shown to dictate their biological properties.[167] These cyclic peptides demonstrated inhibitory activity against sarcoplasmic reticulum Ca^{2+}-ATPase, while the keramamides B–D analogues are capable of inhibiting the superoxide-generation response of human neutrophils induced with a chemotactic peptide.[164] Furthermore, new peptide features were discovered in the analogue keramamide F,[168] including isoserine and (O-methylseryl)thiazole, and the historically rare feature of a sulfate ester in keramamides M and N,[169] contributing to cytotoxic effects against human epidermoid carcinoma KB cells and murine lymphoma L1210 cells. Identification of the keramamide and orbiculamide (*ker*) BGC in the multiproducer "E. factor"[26,170] suggested that a PKS module is responsible for a net one-carbon extension to generate the α-keto-β-amino acid unit present in many keramamides and orbiculamide A, a qualitatively similar process to the extension in calyculins discussed above.

2.3.3. Kahalalides

Some defensive symbioses involve more than two partners, generating a beneficial network for the organisms. This is the case for the tripartite interaction between the mollusk *Elysia rufescens*, alga *Bryopsis* sp., and bacterium "*Ca.* Endobryopsis kahalalidefaciens", all of which harbor the cytotoxic kahalalides (**Figure 15**).[171] Given the presence of nonproteinogenic amino acids in addition to the incorporation of a fatty acid— moieties often biosynthesized by microbes—a bacterium was hypothesized to be the origin of the kahalalides.[171] Delving into the details of kahalalide production provided strong evidence that the toxins are indeed produced by an NRPS in a bacterial symbiont of the alga.[171] Incorporating an initial condensation domain for linkage of the fatty acid to the N-terminal valine and 8 epimerization domains within a total of 13 NRPS modules, this assembly line aligns perfectly with the structure of kahalalide F. In addition to the NRPS for kahalalide F, many other related NRPS BGCs were discovered within the genome of "Ca. E. kahalalidefaciens", eight of which were linked to known kahalalide variants. Of relevance to megaenzyme engineering, the authors remarked on the possibility of NRPS evolution through inter-pathway recombination, mixing and matching to generate the kahalalides and more. Further analysis ascertained that the metabolic machinery responsible for production of the kahalalide amino acid constituents is not coded for in the "Ca. E. kahalalidefaciens" genome, indicating that they are sourced through a collaboration with the host. The remarkable biosynthetic potential of this candidate species broadens avenues in the peptide chemical space, and future enzymology work is likely to be just as fruitful.

2.3.4. Renieramycins

In addition to the previously highlighted ecteinascidins, the sponge- and nudibranch-associated renieramycins form another group of THIQ-containing natural products.[172] Of most prominence are renieramycins M, E, and T (**Figure 16**)[173] due to their nanomolar IC_{50} values against various cancer cell lines. The presence of only two THIQ moieties and various oxidation patterns on the ring system differentiate the renieramycins from the tunicate-derived ET-743. A logical biosynthetic hypothesis would involve an NRPS-based Pictet–Spenglerase with variation in the enzyme(s) responsible for dioxolane formation. This
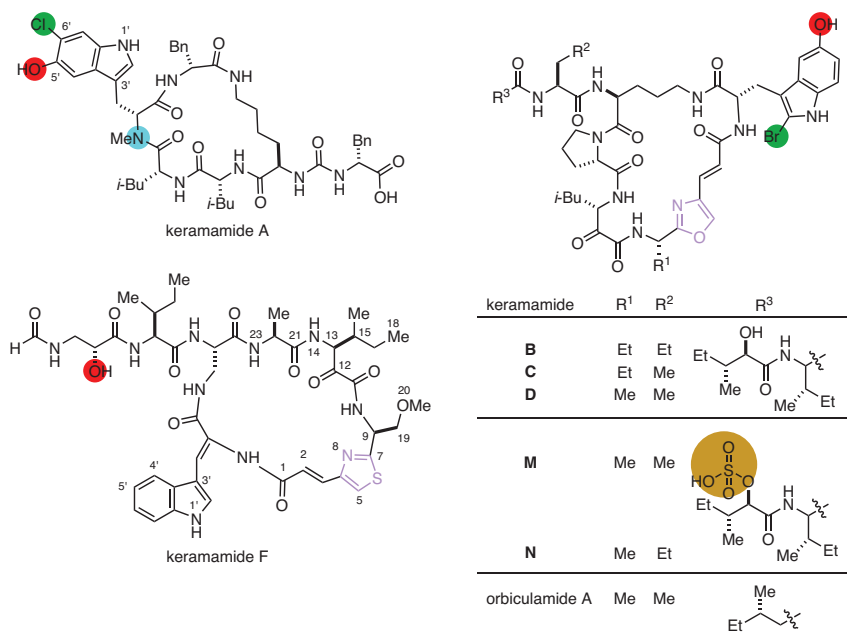
Figure 14. The Keramamide Family of Cyclic NRPs from *Theonella* sp. *(Ref. 164–169)*

distinction could preclude the introduction of a third THIQ (as in ET-743 biosynthesis) and lead to the bis-THIQ observed in renieramycin M.

Just as in the case of the kahalalides, the renieramycins were hypothesized to be involved in another tripartite symbiotic system between a nudibranch, sponge, and bacterial producer. Indeed, deep metagenomic sequencing of four renieramycin-positive sponges revealed the *ren* BGC from which activity for a single tyrosine-methyltransferase was observed through heterologous expression in *E. coli*.[173] Comparison of the genome to a small plasmid containing the *ren* BGC facilitated the identification of an intracellular bacterial producer, "*Ca.* Endohaliclona renieramycinfaciens". This symbiont and the ET-743 producer "*Ca.* E. frumentensis" are distantly related and form intracellular symbiotic relationships with very different marine invertebrates. Both, however, have managed to base their defensive symbioses on the biosynthesis of structurally similar THIQ molecules. While this itself is an intriguing evolutionary quandary, further comparative investigations into the biosynthetic enzymology of renieramycins, safracins, and ecteinascidins could provide a roadmap of evolving pathways responsible for the formation of the THIQ-containing natural products.

2.4. Other
2.4.1. Saxitoxin Polyketide-like Synthase
Several neurotoxins with the ability to modulate voltage-gated ion channels have been isolated from marine invertebrates.[174] Examples with suspected microbial origin are tetrodotoxin (e.g.,

from the ovaries of pufferfish) and tarichatoxin (from newt eggs).[175] A bacterial source has been identified for saxitoxin first isolated from the siphon of the Alaska butterclam.[175,176] Saxitoxin and its derivatives are paralytic shellfish toxins and stand out owing to their presence in both marine and freshwater environments and to being found in producers that inhabit two kingdoms of life (prokaryotic cyanobacteria in freshwater and eukaryotic dinoflagellates in marine waters).[174] The biosynthetic gene cluster for saxitoxin was discovered within various cyanobacteria (**Figure 17**).[177-180] Importantly, filter-feeding bivalves and fish accumulate masses of these molecules, which can result in potentially fatal human illnesses following ingestion.[174]

While most marine neurotoxins target the sodium channel, the saxitoxins also function on potassium and calcium channels, with differing mechanisms of action among the three targets. Saxitoxin's high affinity for the sodium channel is facilitated by the guanidinium moiety, allowing it to effectively block the flow of sodium ions into nerve and muscle cells, which results in death via respiratory paralysis.[181] Addition of saxitoxins and related molecules in vitro stabilizes the protein for imaging by high-resolution single-particle cryo-electron microscopy and has greatly expanded our understanding of voltage-gated sodium ion channels.[182-184] It is worth noting that the level of oxidation on the tricyclic scaffold has a great impact on the biological activity of the saxitoxins.[185-187]

While saxitoxins have demonstrated detrimental effects on human health, several metazoans have evolved mechanisms for
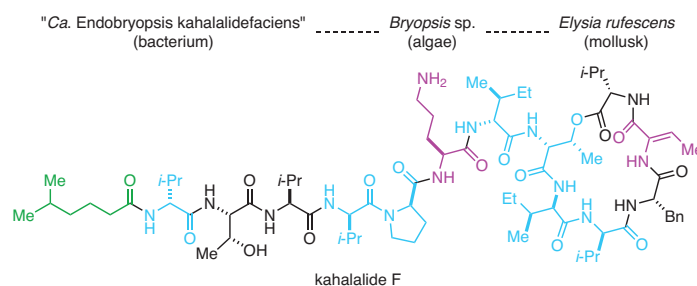

Figure 15. Kahalalide Biosynthesis Occurs in a Bacterial Symbiont of an Algal Food Source for Snails. The Fatty Acid Component Is Shown in Green, D-Amino Acids in Blue, and Nonproteinogenic Amino Acids in Purple. *(Ref. 171)*
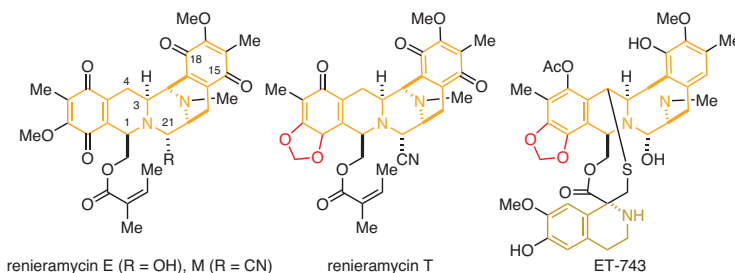
Figure 16. The Sponge-Derived Renieramycins Share a Common Core (Orange) with the Tunicate-Derived Ecteinascidins (e.g., ET-743). *(Ref. 173)*

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evading this fate. For example, frogs show resistance to saxitoxin poisoning through the production of a high-affinity saxitoxin-binding protein saxiphilin,[188–191] while saxitoxin-binding proteins have also been identified in pufferfish,[192,193] cockles,[194] and crabs.[195] These toxin-binding proteins are thought to constitute a less-studied mode of resistance, and the crystal structures of the saxiphilin complex with saxitoxin have facilitated the identification of a core molecule recognition motif.[196]

The saxitoxin BGC encodes a PKS-like protein (SxtA) responsible for the formation of two C–C bonds, two decarboxylation steps, and ultimately stereospecific protonation (see Figure 17).[197] The protein contains four domains: an *S*-adenosyl-methionine (SAM)-dependent methyltransferase (MT), a GCN5-related *N*-acetyltransferase (GNAT), an ACP, and an 8-amino-7-oxononanoate synthase (AONS) domain. Detailed in vitro enzymatic studies determined that saxitoxin biosynthesis is initiated with the loading of malonyl-CoA onto the ACP, with subsequent methylation (MT-catalyzed) and decarboxylation (GNAT). The resultant acyl group is then transferred to the α-carbon of arginine by the AONS and decarboxylation leads to formation of the linear saxitoxin precursor. Prior to cyclization, an amidino group is transferred to the ethyl ketone substrate by the amidinotransferase SxtG. Various oxidation steps decorate the scaffold, with SxtA acting at an early stage on the linear form. SxtT and GxtA catalyze late-stage oxidation steps, all with exquisite stereo- and site-selectivity.[198] The intermediary steps hypothesized to be involved in cyclization
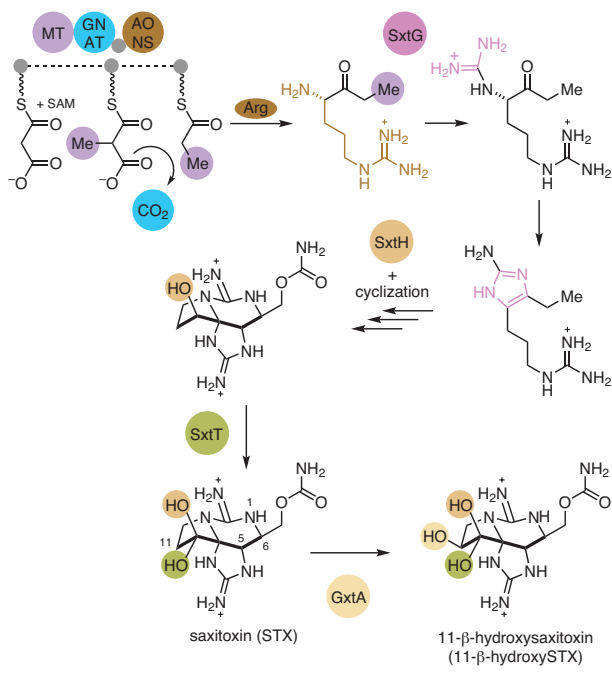
to form the tricyclic scaffold have yet to be revealed.[179,180,199,200] In addition to the already demonstrated substrate scope, the structural analyses have provided further basis for engineering the enzymes for biocatalytic utility.[201]

2.4.2. Polybrominated Diphenyl Ethers

Brominated secondary metabolites are common in the marine realm where bromide is naturally abundant.[202] In particular, marine invertebrates are a source of numerous potentially hazardous halogenated natural products including polybrominated diphenyl ethers. Present in a range of marine organisms—including plants,[203] algae,[204–206] invertebrates,[29,207–209] and even mammals[210-212]—these molecules were speculated to be of bacterial origin (**Figure 18**; see also Figure 3). Their similarity to pseudilin and other halogenated pyrroles produced by gammaproteobacterial *Pseudoalteromonas* spp. provided further evidence for this hypothesis. Elegant (meta)genomics analyses led to the identification of the BGC for these brominated marine pyrroles/phenols, and the chemical space of this molecular family was expanded through reconstitution of the BGC.[31,32,213] These compounds are structurally similar to polybrominated flame retardant chemicals, polychlorinated DDT, and industrial coolants (PCB)—all persistent environmental hazards that were once widely used. The knowledge gained about the biosynthesis of the natural analogues in marine systems provides a genetic fingerprint for monitoring production of these toxins in future populations.

The biosynthesis of the brominated diphenyl ethers involves a brominase (Bmp2) capable of mono-, di-, and tribrominating pyrrolyl-*S*-ACP.[31] Bmp2 is an FAD-dependent halogenase that catalyzes bromination via a well-characterized mechanism requiring an FAD reductase partner protein. In addition, phenolic bromination involves the more unusual halogenase
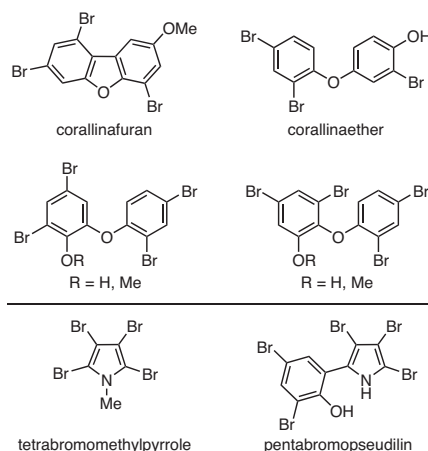

Figure 17. The Saxitoxin Biosynthetic Gene Cluster Encodes the Early-Acting PKS-like Protein SxtA with a Methyltransferase (MT), GCN5-Related *N*-Acetyltransferase (GNAT), and 8-Amino-7-oxononanoate Synthase (AONS), as well as Standalone Amidinotransferase SxtG and Oxidases SxtH, SxtT, and GxtA. (*Ref. 177–180,197,198*)


Figure 18. Polybrominated Natural Products Permeate a Broad Phylogenetic Spectrum from Marine Bacteria to Algae, Invertebrates, and Even Mammals. Polybrominated Diphenyl Ethers Were Found to Be Produced by a Symbiont of Sponges. (*Ref. 31,32,213*)

Bmp5, which operates through a new mechanism to catalyze decarboxylation and halogenation in the absence of a flavin reductase partner. A cytochrome P450 enzyme then couples the pyrrole and phenolic portions. Homologues of Bmp2 were also subsequently identified in another marine symbiont,[214] while genes of Bmp5 homologues (oxygenases) were found in other bacterial BGCs, indicating a higher prevalence. Thus, the identification of the *bmp* gene locus facilitates the mining of metagenomic data sets for other sources of hydroxylated BDEs. The enzymes from the *bmp* pathway were utilized to expand the chemical space of diphenyl ethers, as well as provide a hypothesis for the production of dibenzo-*p*-dioxins, which are naturally occurring hazards to human health.[213]

3. Interesting Pathways Remaining to Be Biosynthetically Explored

3.1. Halichondrins

The halichondrins are another example of tubulin-binding natural products from sponge microbiomes. These molecules were first isolated from *Halichondria okadai* Kadota by bioassay-guided fractionation,[215] and subsequently from the unrelated sponges of the genera *Axinella*[216] and *Lissodendoryx* sp.[217,218] While various analogues were effective cytotoxic agents (homohalichondrin A, B, C; norhalichondrins A, B, C), the most profound activity was observed for halichondrin B. Both halichondrin B and homohalichondrin B arrest cells in mitosis
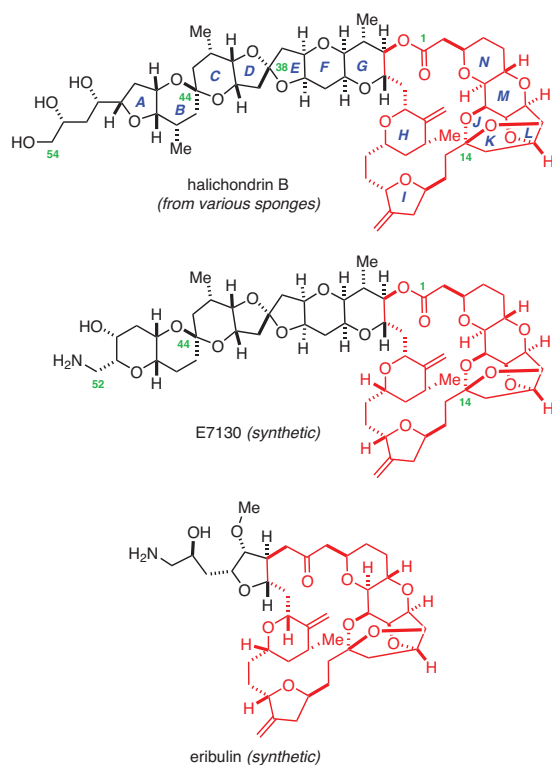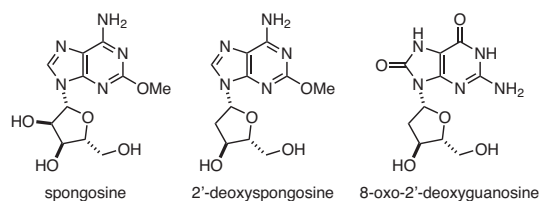
and inhibit tubulin polymerization,[219] while halichondrin B is a noncompetitive inhibitor of known molecules that bind the vinca alkaloid site of tubulin (described above). Furthermore, halichondrin B inhibited tubulin-dependent GTP hydrolysis and nucleotide exchange at the GTP site of tubulin.

The first total synthesis of halichondrin B and norhalichondrin B was achieved in 1992 and required more than one hundred steps to successfully install all thirty chiral centers.[220] Further evaluation by Eisai Research Laboratories and the Kishi group at Harvard University determined that the Eastern half of the molecule made up a core scaffold worth developing as a drug lead.[221] After the synthesis of around two hundred analogues, the drug candidate eribulin was discovered, with subsequent approval by the U.S. FDA in 2010 for advanced metastatic breast cancer and 2016 for soft tissue sarcoma (**Figure 19**).[221] More recent efforts have led to the development and total synthesis of the halichondrin-based E7130, which uniquely targets the tumor microenvironment and enhances the effects of combination therapies.[221] Based on the ladder-type polyether structure of halichondrins, the producer might be a dinoflagellate, but various bacterial polyethers are also known.[222]

3.2. Nucleoside Natural Products

Interest in sponge natural products started in the early 1950s with the isolation of the bioactive pyrimidine bases spongouridine (Ara-U) and spongothymidine (Ara-T), and the purine base analogue spongosine.[223,224] More recent work involved the LC-MS/MS-based screening of extracts of bacterial isolates from the marine sponge *Tectitethya crypta*, leading to the identification of a microbial producer of nucleoside natural products (**Figure 20**).[225] This approach is distinct from those described in preceding sections because it relies heavily on chemical analysis as opposed to genome mining. These efforts identified the sponge-associated *Vibrio harveyi* strain as a producer of spongosine, 2'-deoxyspongosine, and 8-oxo-2'-deoxyguanosine. The genome sequence ultimately provided further evidence for the bacterial source, with a BGC that encodes an adenylosuccinate synthetase, an oxidoreductase, an *O*-MT, and a phosphatase being likely responsible for the production of spongosine. A proposed biosynthesis involves the formation of adenosine monophosphate from adenylosuccinate via an adenylosuccinate lyase, followed by C-2 hydroxylation with the oxidoreductase, methylation

Figure 19. Molecular Structures of Halichondrin B and Synthetic Analogues/Drug Candidates Eribulin and E7130. *(Ref. 221)*

Figure 20. Anti-inflammatory Nucleoside Natural Products from a Sponge-Associated Microbe. *(Ref. 225)*

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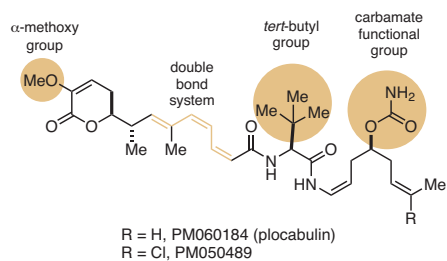Amy E. Fraley, Serina L. Robinson, and Jörn Piel*

involving SAM and *O*-MT, and dephosphorylation catalyzed by a phosphatase. Apart from this example, the biosynthesis of nucleoside-specialized metabolites in marine invertebrates remains unexplored. Previously, enzymatic studies on the biosynthesis of nucleoside antibiotics focused mainly on free-living *Streptomyces* strains.[226]

3.3. Plocabulin

In line with the previous discussion of tubulin-inhibiting natural products, a new class of sponge-associated polyketides was found to bind to the maytansine site on β-tubulin.[227,228] Isolated from the sponge *Lithoplocamia lithistoides*, the plocabulins (PM050489 and PM060184) demonstrated subnanomolar antitumor activity on various human cancer cell lines (**Figure 21**).[229] Various studies have further characterized the bioactivity of these natural products,[230–232] yet their biosynthesis remains unexplored. The plocabulin structure indicates biosynthesis by a hybrid PKS-NRPS system. Counterintuitively, the biosynthesis seems to proceed from the dihydropyrone to the methyl terminus. This is indicated by the orientation of the two amide bonds, assuming N-to-C incorporation of amino acids.

4. Conclusion

Marine microbial communities are a wellspring of biochemical transformations and bioactive natural products, with discovery fueled by the ever-expanding development of biotechnological methodologies. Meta-omic strategies from genome-based studies to enzymology and molecular-level investigations have shed light on the involvement of microbial dark matter in the biosynthesis of bioactive metabolites. Sponges are noteworthy sources, hosting symbiotic systems with extraordinary biosynthetic potential. Cases where complex metabolites are isolated from both a marine invertebrate and an unrelated source have led to initial hypotheses of bacterial sources for macro-organism-derived compounds. While first studies were based on cosmid and fosmid libraries, metagenomic sequencing has facilitated further advancements in the field, leading to the discovery of symbiont-derived BGCs from a variety of invertebrates. As we gain further understanding of these biosynthetic systems, the synthetic biology toolbox can be expanded to spawn novel chemistry. Not only can we access these complex bioactive metabolites, but our understanding of their biosyntheses can facilitate their derivatization through molecular manipulation as well as biocatalyst development for environmentally benign chemical transformations.

6. References

(1) Cullen, C. M.; Aneja, K. K.; Beyhan, S.; Cho, C. E.; Woloszynek, S.; Convertino, M.; McCoy, S. J.; Zhang, Y.; Anderson, M. Z.; Alvarez-Ponce, D.; Smirnova, E.; Karstens, L.; Dorrestein, P. C.; Li, H.; Gupta, A. S.; Cheung, K.; Powers, J. G.; Zhao, Z.; Rosen, G. L. *Front. Microbiol.* **2020**, *11*, Article No. 136. (DOI: 10.3389/fmicb.2020.00136)

(2) Mincer, T. J.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Appl. Environ. Microbiol.* **2002**, *68*, 5005.

(3) Jensen, P. R.; Moore, B. S.; Fenical, W. *Nat. Prod. Rep.* **2015**, *32*, 738.

(4) König, G. M.; Kehraus, S.; Seibert, S. F.; Abdel-Lateff, A.; Müller, D. *ChemBioChem.* **2006**, *7*, 229.

(5) De la Calle, F. *Microb. Biotechnol.* **2017**, *10*, 1293.

(6) Flórez, L. V.; Biedermann, P. H. W.; Engl, T.; Kaltenpoth, M. *Nat. Prod. Rep.* **2015**, *32*, 904.

(7) McCauley, E. P.; Piña, I. C.; Thompson, A. D.; Bashir, K.; Weinberg, M.; Kurz, S. L.; Crews, P. *J. Antibiot.* **2020**, *73*, 504.

(8) Kitagawa, I.; Kobayashi, M.; Katori, T.; Yamashita, M.; Tanaka, J.; Doi, M.; Ishida, T. *J. Am. Chem. Soc.* **1990**, *112*, 3710.

(9) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5300.

(10) Andrianasolo, E. H.; Gross, H.; Goeger, D.; Musafija-Girt, M.; McPhail, K.; Leal, R. M.; Mooberry, S. L.; Gerwick, W. H. *Org. Lett.* **2005**, *7*, 1375.

(11) Tao, Y.; Li, P.; Zhang, D.; Glukhov, E.; Gerwick, L.; Zhang, C.; Murray, T. F.; Gerwick, W. H. *J. Org. Chem.* **2018**, *83*, 3034.

(12) Humisto, A.; Jokela, J.; Liu, L.; Wahlsten, M.; Wang, H.; Permi, P.; Machado, J. P.; Antunes, A.; Fewer, D. P.; Sivonen, K. *Appl. Environ. Microbiol.* **2018**, *84*, Article No. e02321-17. (DOI: 10.1128/AEM.02321-17)

(13) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666.

(14) Hopwood, D. A. *Chem. Rev.* **1997**, *97*, 2465.

(15) Hrvatin, S.; Piel, J. *J. Microbiol. Methods* **2007**, *68*, 434.

(16) Kallifidas, D.; Brady, S. F. Reassembly of Functionally Intact Environmental DNA-Derived Biosynthetic Gene Clusters. In *Natural Product Biosynthesis by Microorganisms and Plants*, Part C; Hopwood, D. A., Ed.; Methods in Enzymology Series, Vol.

Figure 21. The Sponge-Derived Microtubule-Depolymerizing Polyketide Plocabulin Contains Unusual Features for Polyketide Biosynthesis. *(Ref. 227–229)*

517; Abelson, J. N., Simon, M. I., Series Eds.; Academic Press, 2012; Chapter 11, pp 225–239.

(17) Fisch, K. M.; Gurgui, C.; Heycke, N.; van der Sar, S. A.; Anderson, S. A.; Webb, V. L.; Taudien, S.; Platzer, M.; Rubio, B. K.; Robinson, S. J.; Crews, P.; Piel, J. *Nat. Chem. Biol.* **2009**, *5*, 494.

(18) Nguyen, T.; Ishida, K.; Jenke-Kodama, H.; Dittmann, E.; Gurgui, C.; Hochmuth, T.; Taudien, S.; Platzer, M.; Hertweck, C.; Piel, J. *Nat. Biotechnol.* **2008**, *26*, 225.

(19) Helfrich, E. J. N.; Ueoka, R.; Dolev, A.; Rust, M.; Meoded, R. A.; Bhushan, A.; Califano, G.; Costa, R.; Gugger, M.; Steinbeck, C.; Moreno, P.; Piel, J. *Nat. Chem. Biol.* **2019**, *15*, 813.

(20) Blin, K.; Shaw, S.; Kloosterman, A. M.; Charlop-Powers, Z.; van Wezel, G. P.; Medema, M. H.; Weber, T. *Nucleic Acids Res.* **2021**, *49*, W29.

(21) Grindberg, R. V.; Ishoey, T.; Brinza, D.; Esquenazi, E.; Coates, R. C.; Liu, W.; Gerwick, L.; Dorrestein, P. C.; Pevzner, P.; Lasken, R.; Gerwick, W. H. *PLoS One* **2011**, *6* (4), e18565. (DOI: 10.1371/journal.pone.0018565)

(22) Robinson, S. L.; Piel, J.; Sunagawa, S. *Nat. Prod. Rep.* **2021**, *38*, 1994.

(23) Piel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 14002.

(24) Piel, J.; Hui, D.; Wen, G.; Butzke, D.; Platzer, M.; Fusetani, N.; Matsunaga, S. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 16222.

(25) Piel, J.; Butzke, D.; Fusetani, N.; Hui, D.; Platzer, M.; Wen, G.; Matsunaga, S. *J. Nat. Prod.* **2005**, *68*, 472.

(26) Wilson, M. C.; Mori, T.; Rückert, C.; Uria, A. R.; Helf, M. J.; Takada, K.; Gernert, C.; Steffens, U. A. E.; Heycke, N.; Schmitt, S.; Rinke, C.; Helfrich, E. J. N.; Brachmann, A. O.; Gurgui, C.; Wakimoto, T.; Kracht, M.; Crüsemann, M.; Hentschel, U.; Abe, I.; Matsunaga, S.; Kalinowski, J.; Takeyama, H.; Piel, J. *Nature* **2014**, *506*, 58.

(27) Bewley, C. A.; Holland, N. D.; Faulkner, D. J. *Experientia* **1996**, *52*, 716.

(28) Galonić, D. P.; Vaillancourt, F. H.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 3900.

(29) Unson, M. D.; Holland, N. D.; Faulkner, D. J. *Mar. Biol.* **1994**, *119*, 1.

(30) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349.

(31) Agarwal, V.; El Gamal, A. A.; Yamanaka, K.; Poth, D.; Kersten, R. D.; Schorn, M.; Allen, E. E.; Moore, B. S. *Nat. Chem. Biol.* **2014**, *10*, 640.

(32) Agarwal, V.; Blanton, J. M.; Podell, S.; Taton, A.; Schorn, M. A.; Busch, J.; Lin, Z.; Schmidt, E. W.; Jensen, P. R.; Paul, V. J.; Biggs, J. S.; Golden, J. W.; Allen, E. E.; Moore, B. S. *Nat. Chem. Biol.* **2017**, *13*, 537.

(33) Xu, Y.; Kersten, R. D.; Nam, S.-J.; Lu, L.; Al-Suwailem, A. M.; Zheng, H.; Fenical, W.; Dorrestein, P. C.; Moore, B. S.; Qian, P.-Y. *J. Am. Chem. Soc.* **2012**, *134*, 8625.

(34) Schmidt, E. W.; Nelson, J. T.; Rasko, D. A.; Sudek, S.; Eisen, J. A.; Haygood, M. G.; Ravel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 7315.

(35) Long, P. F.; Dunlap, W. C.; Battershill, C. N.; Jaspars, M. *ChemBioChem* **2005**, *6*, 1760.

(36) Donia, M. S.; Fricke, W. F.; Partensky, F.; Cox, J.; Elshahawi, S. I.; White, J. R.; Phillippy, A. M.; Schatz, M. C.; Piel, J.; Haygood, M. G.; Ravel, J.; Schmidt, E. W. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, E1423.

(37) Haygood, M. G.; Davidson, S. K. *Appl. Environ. Microbiol.* **1997**, *63*, 4612.

(38) Davidson, S. K.; Allen, S. W.; Lim, G. E.; Anderson, C. M.; Haygood, M. G. *Appl. Environ. Microbiol.* **2001**, *67*, 4531.

(39) Lopanik, N.; Lindquist, N.; Targett, N. *Oecologia* **2004**, *139*, 131.

(40) Lopanik, N. B.; Targett, N. M.; Lindquist, N. *Appl. Environ. Microbiol.* **2006**, *72*, 7941.

(41) Hildebrand, M.; Waggoner, L. E.; Liu, H.; Sudek, S.; Allen, S.; Anderson, C.; Sherman, D. H.; Haygood, M. *Chem. Biol.* **2004**, *11*, 1543.

(42) Hale, K. J.; Hummersone, M. G.; Manaviazar, S.; Frigerio, M. *Nat. Prod. Rep.* **2002**, *19*, 413.

(43) Linneman, J.; Paulus, D.; Lim-Fong, G.; Lopanik, N. B. *PLoS One* **2014**, *9* (10), e108783.

(44) Sudek, S.; Lopanik, N. B.; Waggoner, L. E.; Hildebrand, M.; Anderson, C.; Liu, H.; Patel, A.; Sherman, D. H.; Haygood, M. G. *J. Nat. Prod.* **2007**, *70*, 67.

(45) Rath, C. M.; Janto, B.; Earl, J.; Ahmed, A.; Hu, F. Z.; Hiller, L.; Dahlgren, M.; Kreft, R.; Yu, F. A.; Wolff, J. J.; Kweon, H. K.; Christiansen, M. A.; Håkansson, K.; Williams, R. M.; Ehrlich, G. D.; Sherman, D. H. *ACS Chem. Biol.* **2011**, *6*, 1244.

(46) Schofield, M. M.; Jain, S.; Porat, D.; Dick, G. J.; Sherman, D. H. *Environ. Microbiol.* **2015**, *17*, 3964.

(47) Montalbán-López, M.; Scott, T. A.; Ramesh, S.; Rahman, I. R.; van Heel, A. J.; Viel, J. H.; Bandarian, V.; Dittmann, E.; Genilloud, O.; Goto, Y.; Burgos, M. J. G.; Hill, C.; Kim, S.; Koehnke, J.; Latham, J. A.; Link, A. J.; Martinez, B.; Nair, S. K.; Nicolet, Y.; Rebuffat, S.; Sahl, H.-G.; Sareen, D.; Schmidt, E. W.; Schmitt, L.; Severinov, K.; Süssmuth, R. D.; Truman, A. W.; Wang, H.; Weng, J.-K.; van Wezel, G. P.; Zhang, Q.; Zhong, J.; Piel, J.; Mitchell, D. A.; Kuipers, O. P.; van der Donk, W. A. *Nat. Prod. Rep.* **2021**, *38*, 130.

(48) Gu, W.; Dong, S.-H.; Sarkar, S.; Nair, S. K.; Schmidt, E. W. *Methods Enzymol.* **2018**, *604*, 113.

(49) Donia, M. S.; Hathaway, B. J.; Sudek, S.; Haygood, M. G.; Rosovitz, M. J.; Ravel, J.; Schmidt, E. W. *Nat. Chem. Biol.* **2006**, *2*, 729.

(50) Ruffner, D. E.; Schmidt, E. W.; Heemstra, J. R. *ACS Synth. Biol.* **2015**, *4*, 482.

(51) Houssen, W. E.; Bent, A. F.; McEwan, A. R.; Pieiller, N.; Tabudravu, J.; Koehnke, J.; Mann, G.; Adaba, R. I.; Thomas, L.; Hawas, U. W.; Liu, H.; Schwarz-Linek, U.; Smith, M. C. M.; Naismith, J. H.; Jaspars, M. *Angew. Chem., Int. Ed.* **2014**, *53*, 14171.

(52) Gu, W.; Schmidt, E. W. *Acc. Chem. Res.* **2017**, *50*, 2569.

(53) Lee, S. W.; Mitchell, D. A.; Markley, A. L.; Hensler, M. E.; Gonzalez, D.; Wohlrab, A.; Dorrestein, P. C.; Nizet, V.; Dixon, J. E. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 5879

(54) Milne, J. C.; Roy, R. S.; Eliot, A. C.; Kelleher, N. L.; Wokhlu, A.; Nickels, B.; Walsh, C. T. *Biochemistry* **1999**, *38*, 4768.

(55) McIntosh, J. A.; Schmidt, E. W. *ChemBioChem* **2010**, *11*, 1413.

(56) Koehnke, J.; Bent, A.; Houssen, W. E.; Zollman, D.; Morawitz,

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F.; Shirran, S.; Vendome, J.; Nneoyiegbe, A. F.; Trembleau, L.; Botting, C. H.; Smith, M. C. M.; Jaspars, M.; Naismith, J. H. *Nat. Struct. Mol. Biol.* **2012**, *19*, 767.

(57) Brás, N. F.; Ferreira, P.; Calixto, A. R.; Jaspars, M.; Houssen, W.; Naismith, J. H.; Fernandes, P. A.; Ramos, M. J. *Chem.—Eur. J.* **2016**, *22*, 13089.

(58) Haft, D. H.; Basu, M. K.; Mitchell, D. A. *BMC Biol.* **2010**, *8*, Article No. 70. (DOI: 10.1186/1741-7007-8-70)

(59) Freeman, M. F.; Gurgui, C.; Helf, M. J.; Morinaka, B. I.; Uria, A. R.; Oldham, N. J.; Sahl, H.-G.; Matsunaga, S.; Piel, J. *Science* **2012**, *338*, 387.

(60) Morinaka, B. I.; Vagstad, A. L.; Helf, M. J.; Gugger, M.; Kegler, C.; Freeman, M. F.; Bode, H. B.; Piel, J. *Angew. Chem., Int. Ed.* **2014**, *53*, 8503.

(61) Morinaka, B. I.; Verest, M.; Freeman, M. F.; Gugger, M.; Piel, J. *Angew. Chem., Int. Ed.* **2017**, *56*, 762.

(62) Fuchs, S. W.; Lackner, G.; Morinaka, B. I.; Morishita, Y.; Asai, T.; Riniker, S.; Piel, J. *Angew. Chem., Int. Ed.* **2016**, *55*, 12330.

(63) Freeman, M. F.; Helf, M. J.; Bhushan, A.; Morinaka, B. I.; Piel, J. *Nat. Chem.* **2017**, *9*, 387.

(64) Helf, M. J.; Freeman, M. F.; Piel, J. *J. Ind. Microbiol. Biotechnol.* **2019**, *46*, 551.

(65) Hamada, T.; Sugawara, T.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 719.

(66) Hamada, T.; Matsunaga, S.; Yano, G.; Fusetani, N. *J. Am. Chem. Soc.* **2005**, *127*, 110.

(67) Iwamoto, M.; Shimizu, H.; Muramatsu, I.; Oiki, S. *FEBS Lett.* **2010**, *584*, 3995.

(68) Matsuoka, S.; Shinohara, N.; Takahashi, T.; Iida, M.; Inoue, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 4879.

(69) Mitchell, D.; Precord, T.; Hudson, G.; Mahanta, N.; Burkhart, B.; Gerlt, J. *FASEB J.* **2022**, *36*, 10.1096/fasebj.2022.36.S1.0I185.

(70) Morinaka, B. I.; Lakis, E.; Verest, M.; Helf, M. J.; Scalvenzi, T.; Vagstad, A. L.; Sims, J.; Sunagawa, S.; Gugger, M.; Piel, J. *Science* **2018**, *359*, 779.

(71) Vagstad, A. L.; Kuranaga, T.; Püntener, S.; Pattabiraman, V. R.; Bode, J. W.; Piel, J. *Angew. Chem., Int. Ed.* **2019**, *58*, 2246.

(72) Bösch, N. M.; Borsa, M.; Greczmiel, U.; Morinaka, B. I.; Gugger, M.; Oxenius, A.; Vagstad, A. L.; Piel, J. *Angew. Chem., Int. Ed.* **2020**, *59*, 11763.

(73) Mordhorst, S.; Morinaka, B. I.; Vagstad, A. L.; Piel, J. *Angew. Chem., Int. Ed.* **2020**, *59*, 21442.

(74) Hubrich, F.; Bösch, N. M.; Chepkirui, C.; Morinaka, B. I.; Rust, M.; Gugger, M.; Robinson, S. L.; Vagstad, A. L.; Piel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119*, Article No. e2113120119.

(75) Kozakai, R.; Ono, T.; Hoshino, S.; Takahashi, H.; Katsuyama, Y.; Sugai, Y.; Ozaki, T.; Teramoto, K.; Teramoto, K.; Tanaka, K.; Abe, I.; Asamizu, S.; Onaka, H. *Nat. Chem.* **2020**, *12*, 869.

(76) Bhushan, A.; Egli, P. J.; Peters, E. E.; Freeman, M. F.; Piel, J. *Nat. Chem.* **2019**, *11*, 931.

(77) Albertini, A. M.; Caramori, T.; Scoffone, F.; Scotti, C.; Galizzi, A. *Microbiology* **1995**, *141*, 299.

(78) Cheng, Y.-Q.; Tang, G.-L.; Shen, B. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3149.

(79) El-Sayed, A. K.; Hothersall, J.; Cooper, S. M.; Stephens, E.;

Simpson, T. J.; Thomas, C. M. *Chem. Biol.* **2003**, *10*, 419.

(80) Piel, J. *Nat. Prod. Rep.* **2010**, *27*, 996.

(81) Helfrich, E. J. N.; Piel, J. *Nat. Prod. Rep.* **2016**, *33*, 231.

(82) Piel, J.; Wen, G.; Platzer, M.; Hui, D. *ChemBioChem* **2004**, *5*, 93.

(83) Sakemi, S.; Ichiba, T.; Kohmoto, S.; Saucy, G. *J. Am. Chem. Soc.* **1988**, *110*, 4851.

(84) Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369.

(85) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *J. Am. Chem. Soc.* **1988**, *110*, 4850.

(86) Rust, M.; Helfrich, E. J. N.; Freeman, M. F.; Nanudom, P.; Field, C. M.; Rückert, C.; Kündig, T.; Page, M. J.; Webb, V. L.; Kalinowski, J.; Sunagawa, S.; Piel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 9508.

(87) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951.

(88) Pettit, G. R.; Xu, J.-P.; Chapuis, J.-C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149.

(89) Jensen, K.; Niederkrüger, H.; Zimmermann, K.; Vagstad, A. L.; Moldenhauer, J.; Brendel, N.; Frank, S.; Pöplau, P.; Kohlhaas, C.; Townsend, C. A.; Oldiges, M.; Hertweck, C.; Piel, J. *Chem. Biol.* **2012**, *19*, 329.

(90) Pöplau, P.; Frank, S.; Morinaka, B. I.; Piel, J. *Angew. Chem., Int. Ed.* **2013**, *52*, 13215.

(91) Wagner, D. T.; Zhang, Z.; Meoded, R. A.; Cepeda, A. J.; Piel, J.; Keatinge-Clay, A. T. *ACS Chem. Biol.* **2018**, *13*, 975.

(92) Irschik, H.; Kopp, M.; Weissman, K. J.; Buntin, K.; Piel, J.; Muller, R. *ChemBioChem* **2010**, *11*, 1840.

(93) Meoded, R. A.; Ueoka, R.; Helfrich, E. J. N.; Jensen, K.; Magnus, N.; Piechulla, B.; Piel, J. *Angew. Chem., Int. Ed.* **2018**, *57*, 11644.

(94) Storey, M. A.; Andreassend, S. K.; Bracegirdle, J.; Brown, A.; Keyzers, R. A.; Ackerley, D. F.; Northcote, P. T.; Owen, J. G. *mBio.* **2020**, *11*, Article No. e02997-19.

(95) Northcote, P. T.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1991**, *32*, 6411.

(96) West, L. M.; Northcote, P. T.; Battershill, C. N. *J. Org. Chem.* **2000**, *65*, 445.

(97) Hood, K. A.; West, L. M.; Rouwé, B.; Northcote, P. T.; Berridge, M. V.; Wakefield, S. J.; Miller, J. H. *Cancer Res.* **2002**, *62*, 3356.

(98) Field, J. J.; Kanakkanthara, A.; Miller, J. H. *Bioorg. Med. Chem.* **2014**, *22*, 5050.

(99) Guo, H.; Li, X.; Guo, Y.; Zhen, L. *Med. Chem. Res.* **2019**, *28*, 927.

(100) Kupchan, S. M.; Komoda, Y.; Court, W. A.; Thomas, G. J.; Smith, R. M.; Karim, A.; Gilmore, C. J.; Haltiwanger, R. C.; Bryan, R. F. *J. Am. Chem. Soc.* **1972**, *94*, 1354.

(101) Leung, Y. Y.; Hui, L. L. Y.; Kraus, V. B. *Arthritis Rheum.* **2015**, *45*, 341.

(102) Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. *Cancer Lett.* **1998**, *126*, 29.

(103) Pauwels, O.; Kiss, R.; Pasteels, J.-L.; Atassi, G. *J. Pharm. Phamacol.* **1995**, *47*, 870.

(104) Miller, M. L.; Ojima, I. *Chem. Rec.* **2001**, *1*, 195.

(105) Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J.

J. Org. Chem. **1988**, *53*, 3644.

(106) Matthew, S.; Chen, Q. Y.; Ratnayake, R.; Fermaintt, C. S.; Lucena-Agell, D.; Bonato, F.; Prota, A. E.; Lim, S. T.; Wang, X. M.; Diaz, J. F.; Risinger, A. L.; Paul, V. J.; Oliva, M. A.; Luesch, H. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, Article No. e2021847118.

(107) Pazdur, R.; Kudelka, A. P.; Kavanagh, J. J.; Cohen, P. R.; Raber, M. N. *Cancer. Treat. Rev.* **1993**, *19*, 351.

(108) Altmann, K.-H.; Höfle, G.; Müller, R.; Mulzer, J.; Prantz, K. *The Epothilones: An Outstanding Family of Anti-Tumor Agents: From Soil to the Clinic;* Kinghorn, A. D., Falk, H., Kobayashi, J., Eds.; Progress in the Chemistry of Organic Natural Products Series, Vol. 90; Springer-Verlag: Vienna, Austria, 2009.

(109) Altmann, K.-H.; Pfeifer, B.; Arseniyadis, S.; Pratt, B. A.; Nicolaou, K. C. *ChemMedChem* **2007**, *2*, 396.

(110) Chen, Q.-H.; Kingston, D. G. I. *Nat. Prod. Rep.* **2014**, *31*, 1202.

(111) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Boyd, M. R.; Schmidt, J. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1111.

(112) Li, J.; Risinger, A. L.; Mooberry, S. L. *Bioorg. Med. Chem.* **2014**, *22*, 5091.

(113) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912.

(114) Bennett, M. J.; Chan, G. K.; Rattner, J. B.; Schriemer, D. C. *Cell Cycle* **2012**, *11*, 3045.

(115) Bennett, M. J.; Barakat, K.; Huzil, J. T.; Tuszynski, J.; Schriemer, D. C. *Chem. Biol.* **2010**, *17*, 725.

(116) Prota, A. E.; Bargsten, K.; Northcote, P. T.; Marsh, M.; Altmann, K.-H.; Miller, J. H.; Díaz, J. F.; Steinmetz, M. O. *Angew. Chem., Int. Ed.* **2014**, *53*, 1621.

(117) Page, M. J.; Northcote, P. T.; Webb, V. L.; Mackey, S.; Handley, S. J. *Aquaculture* **2005**, *250*, 256.

(118) Page, M. J.; Handley, S. J.; Northcote, P. T.; Cairney, D.; Willan, R. C. *Aquaculture* **2011**, *312*, 52.

(119) Brackovic, A.; Harvey, J. E. *Chem. Commun.* **2015**, *51*, 4750.

(120) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 2780.

(121) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *J. Org. Chem.* **1988**, *53*, 3930.

(122) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *Drugs Exp. Clin. Res.* **1988**, *14*, 723.

(123) Matsunaga, S.; Fujiki, H.; Sakata, D.; Fusetani, N. *Tetrahedron* **1991**, *47*, 2999.

(124) Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1991**, *32*, 5605.

(125) Fagerholm, A. E.; Habrant, D.; Koskinen, A. M. P. *Mar. Drugs* **2010**, *8*, 122.

(126) Dumdei, E. J.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *J. Org. Chem.* **1997**, *62*, 2636.

(127) Wakimoto, T.; Egami, Y.; Nakashima, Y.; Wakimoto, Y.; Mori, T.; Awakawa, T.; Ito, T.; Kenmoku, H.; Asakawa, Y.; Piel, J.; Abe, I. *Nat. Chem. Biol.* **2014**, *10*, 648.

(128) Julien, B.; Tian, Z.-Q.; Reid, R.; Reeves, C. D. *Chem. Biol.* **2006**, *13*, 1277.

(129) Teufel, R.; Miyanaga, A.; Michaudel, Q.; Stull, F.; Louie, G.; Noel, J. P.; Baran, P. S.; Palfey, B.; Moore, B. S. *Nature* **2013**, *503*, 552.

(130) Lopanik, N. B.; Shields, J. A.; Buchholz, T. J.; Rath, C. M.; Hothersall, J.; Haygood, M. G.; Håkansson, K.; Thomas, C. M.; Sherman, D. H. *Chem. Biol.* **2008**, *15*, 1175.

(131) Buchholz, T. J.; Rath, C. M.; Lopanik, N. B.; Gardner, N. P.; Håkansson, K.; Sherman, D. H. *Chem. Biol.* **2010**, *17*, 1092.

(132) Slocum, S. T.; Lowell, A. N.; Tripathi, A.; Shende, V. V.; Smith, J. L.; Sherman, D. H. *Methods Enzymol.* **2018**, *604*, 207.

(133) Campobasso, N.; Patel, M.; Wilding, I. E.; Kallender, H.; Rosenberg, M.; Gwynn, M. N. *J. Biol. Chem.* **2004**, *279*, 44883.

(134) Miller, I. J.; Vanee, N.; Fong, S. S.; Lim-Fong, G. E.; Kwan, J. C. *Appl. Environ. Microbiol.* **2016**, *82*, 6573.

(135) Walker, P. D.; Weir, A. N. M.; Willis, C. L.; Crump, M. P. *Nat. Prod. Rep.* **2021**, *38*, 723.

(136) Ramsdell, J. S.; Pettit, G. R.; Tashjian, A. H. *J. Biol. Chem.* **1986**, *261*, 17073.

(137) Süssmuth, R. D.; Mainz, A. *Angew. Chem., Int. Ed.* **2017**, *56*, 3370.

(138) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512.

(139) Pérez-Matos, A. E.; Rosado, W.; Govind, N. S. *Antonie Van Leeuwenhoek* **2007**, *92*, 155.

(140) Moss, C.; Green, D. H.; Perez, B.; Velasco, A.; Henriquez, R.; McKenzie, J. D. *Mar. Biol.* **2003**, *143*, 99.

(141) Valoti, G.; Nicoletti, M. I.; Pellegrino, A.; Jimeno, J.; Hendriks, H.; D'Incalci, M.; Faircloth, G.; Giavazzi, R. *Clin. Cancer Res.* **1998**, *4*, 1977.

(142) García-Rocha, M.; García-Gravalos, M. D.; Avila, J. *Br. J. Cancer* **1996**, *73*, 875.

(143) Zewail-Foote, M.; Hurley, L. H. *J. Med. Chem.* **1999**, *42*, 2493.

(144) Broggini, M.; Coley, H. M.; Mongelli, N.; Pesenti, E.; Wyatt, M. D.; Hartley, J. A.; D'Incalci, M. *Nucleic Acids Res.* **1995**, *23*, 81.

(145) Rice, J. A.; Crothers, D. M.; Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 4158.

(146) Corey, E. J.; Gin, D. Y.; Kania, R. S. *J. Am. Chem. Soc.* **1996**, *118*, 9202.

(147) Martinez, E. J.; Corey, E. J. *Org. Lett.* **2000**, *2*, 993.

(148) Cuevas, C.; Pérez, M.; Martín, M. J.; Chicharro, J. L.; Fernández-Rivas, C.; Flores, M.; Francesch, A.; Gallego, P.; Zarzuelo, M.; de la Calle, F.; García, J.; Polanco, C.; Rodríguez, I.; Manzanares, I. *Org. Lett.* **2000**, *2*, 2545.

(149) Cuevas, C.; Francesch, A. *Nat. Prod. Rep.* **2009**, *26*, 322.

(150) Le, V. H.; Inai, M.; Williams, R. M.; Kan, T. *Nat. Prod. Rep.* **2015**, *32*, 328.

(151) Arai, T.; Takahashi, S.; Nakahara, S.; Kubo, A. *Experientia* **1980**, *36*, 1025.

(152) Irschik, H.; Trowitzsch-Kienast, W.; Gerth, K.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **1988**, *41*, 993.

(153) Ikeda, Y.; Idemoto, H.; Hirayama, F.; Yamamoto, K.; Iwao, K.; Asoa, T.; Munakata, T. *J. Antibiot.* **1983**, *36*, 1279.

(154) Kluepfel, D.; Baker, H. A.; Piattoni, G.; Sehgal, S. N.; Sidorowicz, A.; Singh, K.; Vézina, C. *J. Antibiot.* **1975**, 28, 497.

(155) Tomita, F.; Takahashi, K.; Shimizu, K. *J. Antibiot.* **1983**, *36*, 463.

(156) Takahashi, K.; Tomita, F. *J. Antibiot.* **1983**, *36*, 468.

(157) Peng, C.; Pu, J.-Y.; Song, L.-Q.; Jian, X.-H.; Tang, M.-C.; Tang,

74

The Versatile Natural Product Enzymology of Marine Microbial Communities
Amy E. Fraley, Serina L. Robinson, and Jörn Piel*

G.-L. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 8540.

(158) Jeedigunta, S.; Krenisky, J. M.; Kerr, R. G. *Tetrahedron* **2000**, *56*, 3303.

(159) Li, L.; Deng, W.; Song, J.; Ding, W.; Zhao, Q.-F.; Peng, C.; Song, W.-W.; Tang, G.-L.; Liu, W. *J. Bacteriol.* **2008**, *190*, 251.

(160) Pospiech, A.; Bietenhader, J.; Schupp, T. *Microbiology* **1996**, *142*, 741.

(161) Velasco, A.; Acebo, P.; Gomez, A.; Schleissner, C.; Rodríguez, P.; Aparicio, T.; Conde, S.; Muñoz, R.; de la Calle, F.; Garcia, J. L.; Sánchez-Puelles, J. M. *Mol. Microbiol.* **2005**, *56*, 144.

(162) Koketsu, K.; Watanabe, K.; Suda, H.; Oguri, H.; Oikawa, H. *Nat. Chem. Biol.* **2010**, *6*, 408.

(163) Agrawal, S.; Acharya, D.; Adholeya, A.; Barrow, C. J.; Deshmukh, S. K. *Front. Pharmacol.* **2017**, *8*, Article No. 828.

(164) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Ishibashi, M.; Takahashi, K.; Ogura, M.; Nagasawa, S.; Nakamura, T.; Hirota, H.; Ohta, T.; Nozoe, S. *J. Am. Chem. Soc.* **1991**, *113*, 7812.

(165) Kobayashi, J.; Sato, M.; Ishibashi, M.; Shigemori, H.; Nakamura, T.; Ohizumi, Y. *J. Chem. Soc., Perkin Trans.* **1991**, 2609.

(166) Fusetani, N.; Sugawara, T.; Matsunaga, S. *J. Am. Chem. Soc.* **1991**, *113*, 7811.

(167) Junk, L.; Kazmaier, U. *Angew. Chem., Int. Ed.* **2018**, *57*, 11432.

(168) Itagaki, F.; Shigemori, H.; Ishibashi, M.; Nakamura, T.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 5540.

(169) Tsuda, M.; Ishiyama, H.; Masuko, K.; Takao, T.; Shimonishi, Y.; Kobayashi, J. *Tetrahedron* **1999**, *55*, 12543.

(170) Lackner, G.; Peters, E. E.; Helfrich, E. J. N.; Piel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E347. (10.1073/pnas.161623411)

(171) Zan, J.; Li, Z.; Tianero, M. D.; Davis, J.; Hill, R. T.; Donia, M. S. *Science* **2019**, *364*, Article No. eaaw6732.

(172) Fang, Y.; Li, H.; Ji, B.; Cheng, K.; Wu, B.; Li, Z.; Zheng, C.; Hua, H.; Li, D. *Eur. J. Med. Chem.* **2021**, *210*, 113092.

(173) Tianero, M. D.; Balaich, J. N.; Donia, M. S. *Nat. Microbiol.* **2019**, *4*, 1149.

(174) Cusick, K. D.; Sayler, G. S. *Mar. Drugs* **2013**, *11*, 991.

(175) Kao, C. Y.; Nishiyama, A. *J. Physiol.* **1965**, *180*, 50.

(176) Schantz, E. J.; Ghazarossian, V. E.; Schnoes, H. K.; Strong, F. M.; Springer, J. P.; Pezzanite, J. O.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 1238.

(177) Kellmann, R.; Michali, T. K.; Neilan, B. A. *J. Mol. Evol.* **2008**, *67*, 526.

(178) Kellmann, R.; Mihali, T. K.; Jeon, Y. J.; Pickford, R.; Pomati, F.; Neilan, B. A. *Appl. Environ. Microbiol.* **2008**, *74*, 4044.

(179) Mihali, T. K.; Carmichael, W. W.; Neilan, B. A. *PLoS One* **2011**, *6*, e14657.

(180) Mihali, T. K.; Kellmann, R.; Neilan, B. A. *BMC Biochem.* **2009**, *10*, DOI:10.1186/1471-2091-10-8.

(181) Stevens, M.; Peigneur, S.; Tytgat, J. *Front. Pharmacol.* **2011**, *2*, Article No. 71.

(182) Shen, H.; Li, Z.; Jiang, Y.; Pan, X.; Wu, J.; Cristofori-Armstrong, B.; Smith, J. J.; Chin, Y. K. Y.; Lei, J.; Zhou, Q.; King, G. F.; Yan, N. *Science* **2018**, *362*, eaau2596.

(183) Shen, H.; Liu, D.; Wu, K.; Lei, J.; Yan, N. *Science* **2019**, *363*, 1303.

(184) Pan, X.; Li, Z.; Zhou, Q.; Shen, H.; Wu, K.; Huang, X.; Chen, J.; Zhang, J.; Zhu, X.; Lei, J.; Xiong, W.; Gong, H.; Xiao, B.; Yan, N.

Science **2018**, *362*, eaau2486.

(185) Walker, J. R.; Novick, P. A.; Parsons, W. H.; McGregor, M.; Zablocki, J.; Pande, V. S.; Du Bois, J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 18102.

(186) Koehn, F. E.; Ghazarossian, V. E.; Schantz, E. J.; Schnoes, H. K.; Strong, F. M. *Bioorg. Chem.* **1981**, *10*, 412.

(187) Usup, G.; Leaw, C.-P.; Cheah, M.-Y.; Ahmad, A.; Ng, B.-K. *Toxicon* **2004**, *44*, 37.

(188) Mahar, J.; Lukács, G. L.; Li, L.; Hall, S.; Moczydlowski, E. *Toxicon* **1991**, *29*, 53.

(189) Doyle, D. D.; Wong, M.; Tanaka, J.; Barr, L. *Science* **1982**, *215*, 1117.

(190) Morabito, M. A.; Moczydlowski, E. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 2478.

(191) Llewellyn, L. E.; Bell, P. M.; Moczydlowski, E. G. *Proc. R. Soc. Lond. B* **1997**, *264*, 891.

(192) Yotsu-Yamashita, M.; Sugimoto, A.; Terakawa, T.; Shoji, Y.; Miyazawa, T.; Yasumoto, T. *Eur. J. Biochem.* **2001**, *268*, 5937.

(193) Yotsu-Yamashita, M.; Yamaki, H.; Okoshi, N.; Araki, N. *Toxicon* **2010**, *55*, 1119.

(194) Takati, N.; Mountassif, D.; Taleb, H.; Lee, K.; Blaghen, M. *Toxicon* **2007**, *50*, 311.

(195) Lin, H.; Zhang, C.; Liao, J.; Yang, F.; Zhong, S.; Jiang, P.; Chen, X.; Nagashima, Y. *Toxicon* **2015**, *99*, 51.

(196) Yen, T.-J.; Lolicato, M.; Thomas-Tran, R.; Du Bois, J.; Minor, D. L., Jr. *Sci. Adv.* **2019**, *5*, eaax2650.

(197) Chun, S. W.; Hinze, M. E.; Skiba, M. A.; Narayan, A. R. H. *J. Am. Chem. Soc.* **2018**, *140*, 2430.

(198) Lukowski, A. L.; Ellinwood, D. C.; Hinze, M. E.; DeLuca, R. J.; Du Bois, J.; Hall, S.; Narayan, A. R. H. *J. Am. Chem. Soc.* **2018**, *140*, 11863.

(199) Shimizu, Y.; Norte, M.; Hori, A.; Genenah, A.; Kobayashi, M. *J. Am. Chem. Soc.* **1984**, *106*, 6433.

(200) Kellmann, R.; Neilan, B. A. *J. Phycol.* **2007**, *43*, 497.

(201) Lukowski, A. L.; Liu, J.; Bridwell-Rabb, J.; Narayan, A. R. H. *Nat. Commun.* **2020**, *11*, Article No. 2991.

(202) Wagner, C.; El Omari, M.; König, G. M. *J. Nat. Prod.* **2009**, *72*, 540.

(203) Gaul, S.; Bendig, P.; Olbrich, D.; Rosenfelder, N.; Ruff, P.; Gaus, C.; Mueller, J. F.; Vetter, W. *Mar. Pollut. Bull.* **2011**, *62*, 2463.

(204) Kitamura, M.; Koyama, T.; Nakano, Y.; Uemura, D. *Chem. Lett.* **2005**, *34*, 1272.

(205) Kuniyoshi, M.; Yamada, K.; Higa, T. *Experientia* **1985**, *41*, 523.

(206) Malmvärn, A.; Zebühr, Y.; Kautsky, L.; Bergman, Å.; Asplund, L. *Chemosphere* **2008**, *72*, 910.

(207) King, G. M.; Giray, C.; Kornfield, I. *Mar. Biol.* **1995**, *123*, 369.

(208) Calcul, L.; Chow, R.; Oliver, A. G.; Tenney, K.; White, K. N.; Wood, A. W.; Fiorilla, C.; Crews, P. *J. Nat. Prod.* **2009**, *72*, 443.

(209) Löfstrand, K.; Malmvärn, A.; Haglund, P.; Bignert, A.; Bergman, Å.; Asplund, L. *Environ. Sci. Pollut. Res.* **2010**, *17*, 1460.

(210) Vetter, W.; Scholz, E.; Gaus, C.; Müller, J. F.; Haynes, D. *Arch. Environ. Contam. Toxicol.* **2001**, *41*, 221.

(211) Marsh, G.; Athanasiadou, M.; Athanassiadis, I.; Bergman, Å.; Endo, T.; Haraguchi, K. *Environ. Sci. Technol.* **2005**, *39*, 8684.

(212) Teuten, E. L.; Xu, L.; Reddy, C. M. *Science* **2005**, *307*, 917.

(213) Agarwal, V.; Moore, B. S. *ACS Chem. Biol.* **2014**, *9*, 1980.

(214) Donia, M. S.; Fricke, W. F.; Ravel, J.; Schmidt, E. W. *PLoS One.* **2011**, *6*, e17897.

(215) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701.

(216) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A.; Rützler, K. C. *J. Med. Chem.* **1991**, *34*, 3339.

(217) Litaudon, M.; Hickford, S. J. H.; Lill, R. E.; Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *J. Org. Chem.* **1997**, *62*, 1868.

(218) Hickford, S. J. H.; Blunt, J. W.; Munro, M. H. G. *Bioorg. Med. Chem.* **2009**, *17*, 2199.

(219) Bai, R.; Paull, K. D.; Herald, C. L.; Malspeis, L.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1991**, *266*, 15882.

(220) Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162.

(221) Kawano, S.; Ito, K.; Yahata, K.; Kira, K.; Abe, T.; Akagi, T.; Asano, M.; Iso, K.; Sato, Y.; Matsuura, F.; Ohashi, I.; Matsumoto, Y.; Isomura, M.; Sasaki, T.; Fukuyama, T.; Miyashita, Y.; Kaburagi, Y.; Yokoi, A.; Asano, O.; Owa, T.; Kishi, Y. *Sci. Rep.* **2019**, *9*, Article No. 8656. (DOI: 10.1038/s41598-019-45001-9)

(222) Liu, T.; Cane, D. E.; Deng, Z. *Methods Enzymol.* **2009**, *459*, 187.

(223) Bergmann, W.; Feeney, R. J. *J. Am. Chem. Soc.* **1950**, *72*, 2809.

(224) Bergmann, W.; Feeney, R. J. *J. Org. Chem.* **1951**, *16*, 981.

(225) Bertin, M. J.; Schwartz, S. L.; Lee, J.; Korobeynikov, A.; Dorrestein, P. C.; Gerwick, L.; Gerwick, W. H. *J. Nat. Prod.* **2015**, *78*, 493.

(226) Shiraishi, T.; Kuzuyama, T. *J. Antibiot.* **2019**, *72*, 913.

(227) Prota, A. E.; Bargsten, K.; Diaz, J. F.; Marsh, M.; Cuevas, C.; Liniger, M.; Neuhaus, C.; Andreu, J. M.; Altmann, K.-H.; Steinmetz, M. O. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 13817.

(228) Pera, B.; Barasoain, I.; Pantazopoulou, A.; Canales, A.; Matesanz, R.; Rodriguez-Salarichs, J.; García-Fernandez, L. F.; Moneo, V.; Jiménez-Barbero, J.; Galmarini, C. M.; Cuevas, C.; Peñalva, M. A.; Díaz, J. F.; Andreu, J. M. *ACS Chem. Biol.* **2013**, *8*, 2084.

(229) Martín, M. J.; Coello, L.; Fernández, R.; Reyes, F.; Rodríguez, A.; Murcia, C.; Garranzo, M.; Mateo, C.; Sánchez-Sancho, F.; Bueno, S.; de Eguilior, C.; Francesch, A.; Munt, S.; Cuevas, C. *J. Am. Chem. Soc.* **2013**, *135*, 10164.

(230) Galmarini, C. M.; Martin, M.; Bouchet, B. P.; Guillen-Navarro, M. J.; Martínez-Diez, M.; Martinez-Leal, J. F.; Akhmanova, A.; Aviles, P. *BMC Cancer* **2018**, *18*, Article No. 164. (DOI: 10.1186/s12885-018-4086-2)

(231) Pantazopoulou, A.; Galmarini, C. M.; Peñalva, M. A. *Sci. Rep.* **2018**, *8*, Article No. 8616. (DOI: 10.1038/s41598-018-26736-3)

(232) Wang, Y.; Wozniak, A.; Wellens, J.; Gebreyohannes, Y. K.; Guillén, M. J.; Avilés, P. M.; Debiec-Rychter, M.; Sciot, R.; Schöffski, P. *Transl. Oncol.* **2020**, *13*, 100832.

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Micromapping kits for proximity labeling of proteins

Photocatalytic Target Identification

In spite of the widespread use of Photoaffinity Labeling (PAL), often when the orientation of the alkyl diazirine is not optimal, >99% of the in situ generated carbene reacts only with water, leading to minimal cross-linking and significantly complicating analysis. To address this shortcoming of conventional PAL probes, Professor MacMillan's group at Princeton University developed a catalytic cross-linking method in which the small-molecule ligand is conjugated to an iridium photocatalyst and the aryl diazirine is activated by irradiation with blue light to form the carbene. The photosensitization process is catalytic, allows temporal control of the labeling, and leads to a higher concentration of labeled peptides. By tethering these iridium catalysts to the small molecule under investigation, this method provides an extremely effective way for small-molecule target identification.

Introduced in collaboration with Professor David MacMillan's laboratory and Dexterity Pharma, the following all-in-one proximity labeling kits allow the identification of protein interactions within 4 nm of the protein.

Atlas Kits—Proprietary photocatalysts for antibodies; all components needed to get started (including solvent); enough material for 10 / 20 reactions (AtlasKit A / B).

Dione Kit (DIONEKit-A)—Allows small-molecule intracellular target identification via photocatalytic proximity labeling. Kit contains photocatalysts for capturing the interactions with amino groups on almost any small-molecule ligand.

Hyas Kit—Allows small-molecule intracellular target identification via photocatalytic proximity labeling. Kit contains photocatalysts for capturing the interactions with carboxylic acids on almost any small-molecule ligand.

ATLASKIT-A & ATLASKIT-B
Everything needed for antibody labeling.

To find out more about how these kits can help you in your research, visit our **AtlasKit** and **DioneKit** web pages.

16S NGS Assay for Degraded and Low Biomass DNA: A Guide

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Keywords. 5R-PLEX; SMURF; low biomass; NGS library prep; 16S rRNA; metagenomics; tumor microbiome; ultra-sensitive; bacterial profiling; degraded FFPE DNA.

Abstract. Microbiome profiling of low biomass and degraded DNA samples is considered most challenging for amplicon-based metagenomics. Here we introduce the 5R-PLEX technology, which allows the ultra-sensitive detection of ~1 pg bacterial DNA in high host DNA environment and highly degraded DNA to reach species-level taxonomy profiling. 5R-PLEX targets five short variable regions along the 16S rRNA that are computationally combined to reconstruct one coherent, high-resolution microbial profile via a unique 5R-PLEX algorithm in the M-CAMP™ platform. The 5R-PLEX technology has been tested successfully on mock bacterial communities, as well as clinical skin and FFPE tumor DNA samples, and offers a complete and cost-effective solution for microbiome metagenomics analysis of such challenging sample types.

Outline

1. Introduction

The 16S ribosomal RNA gene (16S rRNA) is a common bacterial marker that is used as a target for microbial community profiling in microbiome metagenomic studies.[1] The gene is composed of nine variable regions (V1–V9) interspersed between conserved regions. The most common 16S rRNA NGS assays target one or two regions (e.g., V3–V4) using a single set of primers.[2] However, this approach usually results in a poor bacterial detection and classification rate when the DNA input is of low quality or when applying this technique to extremely low biomass samples. In fact, most amplicon sequencing methods lead to only a partial sequencing of the 16S gene and result directly in the loss of specificity as well as in a higher ambiguity with only a partial accounting of bacterial diversity. While some computational methods attempt to overcome these limitations by producing short reads that originate from one long amplified region, there are some drawbacks to these solutions, namely the lack of long amplicons from fragmented and damaged DNA samples.[1] Examples of challenging samples include: (i) formalin-fixed, paraffin-embedded (FFPE) tissue,[1] (ii) cancerous tumor tissue,[3] (iii) degraded or damaged DNA, (iv) low biomass samples, and (v) fossil-derived and ancient DNA.
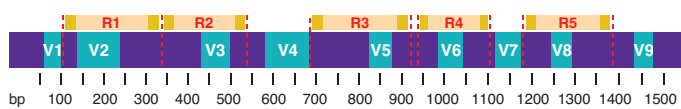
2. What Is 5R-PLEX Technology?

The 5R-PLEX technology is designed for challenging biological samples, where the standard 16S sequencing protocols fail to provide complete data. 5R-PLEX targets five short variable

regions along the 16S rRNA gene (V2, V3, V5, V6, and V8) that are co-amplified in a multiplexed PCR within a single tube (**Figure 1**).

After sequencing, the output data is uploaded to the M-CAMP™ platform (Microbiome Computational Analysis for Multi-omics Profiling) and analyzed using the unique 5R-PLEX algorithm. This new analysis approach combines the amplified regions computationally to reconstruct one coherent, high-resolution microbial profile (**Figure 2**).[4]

3. 5R-PLEX Technology Enhances High-Resolution Bacterial Detection in the Most Challenging Microbiome Applications

The 5R-PLEX technology has been applied to high-molecular-weight (HMW) and highly degraded (Deg) bacterial mock community DNA mix[5,6] and low biomass (**Figure 3**). The samples tested mimic the low biomass found in formalin-fixed, paraffin-embedded (FFPE) tissue. The standard 16S V3-V4 amplicon library prep resulted in an insufficient number of reads and poor taxonomy classification from the degraded DNA. Amplification of the same degraded DNA sample by 5R-PLEX technology yielded high-recovery microbial profiling in degraded DNA (with input as low as 1 picogram).

Figure 1. The 5R-PLEX Technology Targets Five Variable Regions of the 16S rRNA Gene (Light Beige) That Are Interspersed between Conserved Regions (Dark Blue), and Results in Higher Sensitivity and Improved Taxonomical Resolution.
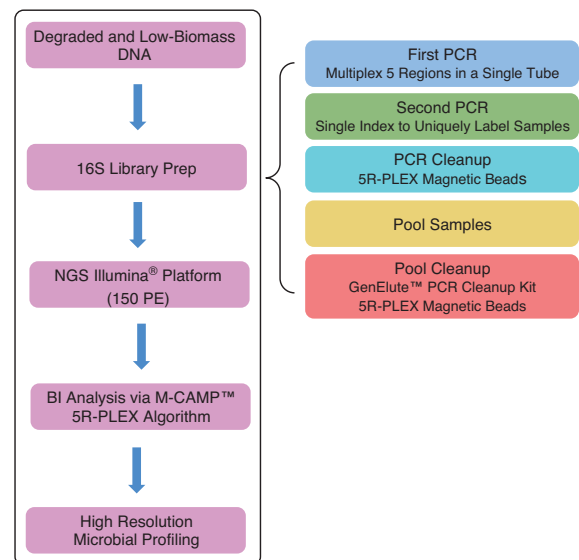
Figure 2. The 5R-PLEX Workflow, from Library Prep to Comprehensive Data Analysis. *(Ref. 4)*
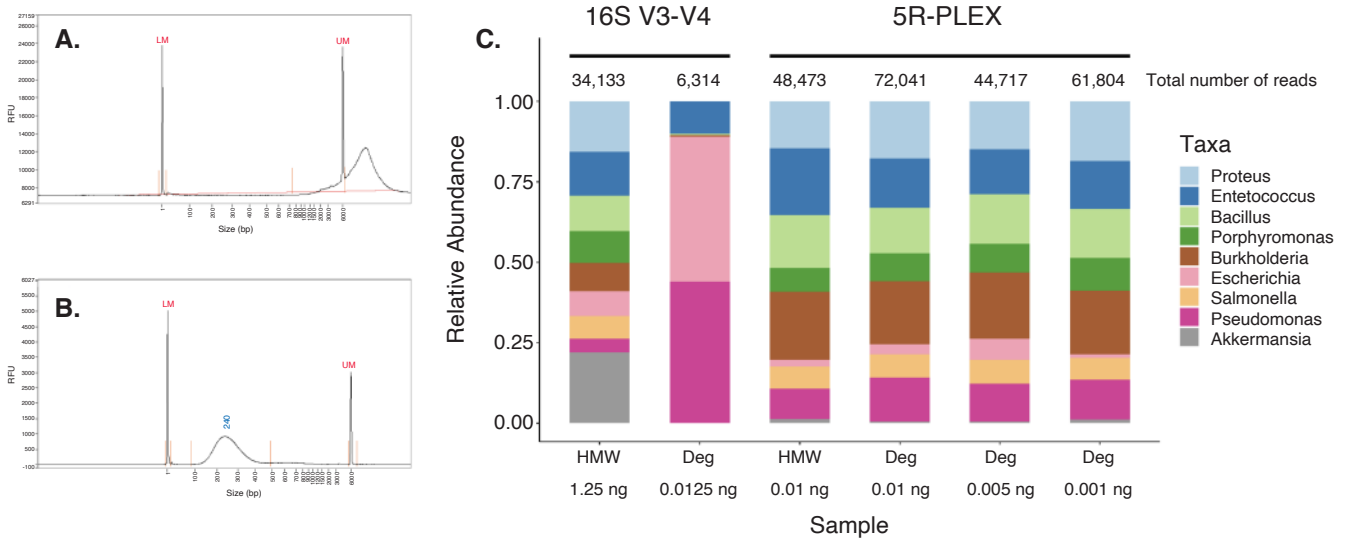
Figure 3. The 5R-PLEX Kit Targets 5 Variable Regions to Provide Highly Sensitive Detection Compared to the Most Utilized Method (16S V3-V4). Input DNA Was a Mix of 10 Bacterial Species, Which Was either HMW (A) or Degraded (240 bp Average Size; (B)). Differential Concentrations Were Tested with the 5R-PLEX Kit vs the Standard 16S V3-V4 Assay (C); Equal Amounts Were Pooled and Sequenced with Illumina® MiSeq® V2 (2 x 150 bp Reads).

4. Conclusion

High-resolution microbial profiling of challenging biological samples can now be performed thanks to the 5R-PLEX kit which allows an ultra-sensitive multiplexed NGS assay to be carried out. It offers the advantage of targeting 5 variable regions of the 16S rRNA gene in a single primer pool. Coupled with a comprehensive bioinformatic analysis through the M-CAMP™ platform, one coherent microbial profile can be reconstructed via advanced statistical and comparative analysis as well as background signal subtraction.

5. Acknowledgment

6. References

(1)   Fuks, G.; Elgart, M.; Amir, A.; Zeisel, A.; Turnbaugh, P. J.; Soen, Y.; Shental, N. *Microbiome* **2018**, *6*, Article No. 17, DOI 10.1186/s40168-017-0396-x.

(2)   Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F. O. *Nucleic Acids Res*. **2013**, *41* (1), Article No. e1, DOI: 10.1093/nar/gks808.

(3)   Nejman D.; Livyatan, I.; Fuks, G.; Gavert, N.; Zwang, Y.; Geller, L. T.; Rotter-Maskowitz, A.; Weiser, R.; Mallel, G.; Gigi, E.; et al. *Science* **2020**, *368*, 973, DOI: 10.1126/science.aay9189.

(4)   Microbiome Computational Analysis for Multi-omics Profiling (M-CAMP™); MilliporeSigma, 2022; https://m-camp.info/microbiome/ (accessed 2022-11-02).

(5)   (a)   *Microbial Community DNA Mix* (MBD0026-0.3UG); MilliporeSigma, 2022; https://www.sigmaaldrich.com/US/en/product/sigma/mbd0026 (accessed 2022-11-02). (b) See also: DNA Standards for Metagenomic Research into Microbiome Communities; MilliporeSigma, 2022; https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/next-gen-sequencing/metagenomic-research-into-microbiome-communities (accessed 2022-11-02).

(6)   For additional information, see: (a) Hornung, B. V. H.; Zwittink, R. D.; Kuijper, E. J. *FEMS Microbiol. Ecol.* **2019**, *95*, Article No. fiz045, DOI: 10.1093/femsec/fiz045. (b) Tourlousse, D. M.; Narita, K.; Miura, T.; Sakamoto, M.; Ohashi, A.; Shiina, K.; Matsuda, M.; Miura, D.; Shimamura, M.; Ohyama, Y.; et al. *Microbiome* **2021**, *9* (95), DOI: 10.1186/s40168-021-01048-3.

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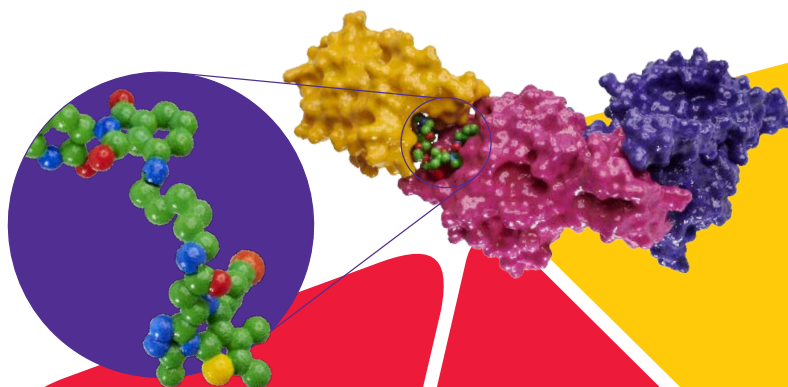
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Shira Lezer holds a Ph.D. degree in life sciences from the Weizmann Institute in Rehovot, Israel. Before joining Merck KGaA, Darmstadt, Germany, she served as Chief Technology Officer in a biotech company that specializes in human molecular genetics diagnostics. Until very recently, Shira led the R&D team of the Microbiome Genomics Technologies department at the Israel site of Merck KGaA, Darmstadt, Germany. Dr. Lezer and her team were responsible for developing advanced high-throughput multi-omics techniques for microbiome profiling, with specialized unique technologies for tumor microbiome and other challenging sample types. **M**

Microbiome Research: An Overview

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Microbiome research studies microbial populations in a defined environment. Pioneered by the NIH Human Microbiome Project (HMP) in 2007, the human microbiome and its associated genomes, transcriptomes, and metabolites have become a major focus in the field. The first stage of the research aimed to determine the composition of a "healthy" versus a "disease related" microbiome.[1] An important finding was that the taxonomic composition of the microbiome, on its own, is not well correlated with the host phenotype.[2] Further studies of the data from "healthy" microbiomes revealed a similarity in metabolic pathways.[2] This led to the second stage of HMP research which has focused on learning the biology of the microbiome or microbiota and how it interacts with the host.[3] It appears that the microbial community may mediate the effect of environmental factors on human health and disease.[4]

The most established human microbiome site is the gut, which has been shown to have the largest microbial community followed by the oral microbiome site. Other sites include the skin, vagina, and lungs. Not surprisingly, the gut microbiota is thought to be the most influential on human health.[5] Overall, a healthy-gut microbial community is characterized by stable microbiome functional cores, a high taxa diversity, and a high microbial gene richness.[6] Chronic metabolic disorders are often associated with a decline in microbiome diversity, and low microbial gene richness is linked to a relative increase in adiposity, insulin resistance, inflammation, and the imbalance of lipids such as cholesterol and triglycerides.[6] Some common conditions that can be related to this are obesity, type 2 diabetes, heart disease, and non-alcoholic fatty liver disease.[6,7,8] Another disease that is associated with the microbiome is cancer. Studies have shown that the microbiota, specifically the gut colonizers, but also intratumor microbes, regulate tumor progression through immunomodulation.[9] This

is not surprising as the microbiome is known to be involved in the maturation of both the innate and the adaptive immune systems and that bacterial metabolites may be driving this maturation.[10] Hopefully, further research and discovery of the pathways and mechanisms that drive microbiome-associated illnesses will lead eventually to the discovery of new therapies for these diseases.

Analysis of multi-omics data (e.g., metagenomics, proteomics, metatranscriptomics, metabolomics, and culturomics) as well as integrated host data analysis are anticipated to provide a more comprehensive picture of the human microbiome. However, establishing experimental designs that incorporate biology, chemistry, bioinformatic, and statistical methods remains a fundamental challenge in microbiome research.[4] With the potential of microbiome research to tackle some of humanity's most common diseases, we have taken up the challenge of providing tools to scientists that help them overcome some of the complexities they encounter in their microbiome studies. Metagenomic and metabolomic standards, specialized growth media, and a bioinformatic platform are examples of such tools. Further development by us of new tools and research solutions is ongoing.

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References

(1) Proctor, L. M.; Creasy, H. H.; Fettweis, J. M.; Lloyd-Price, J.; Mahurkar, A.; Zhou, W.; Buck, G. A.; Snyder, M. P.; Strauss, J. F., III; Weinstock, G. M.; et al. *Nature* **2019**, *569*, 641, DOI: 10.1038/s41586-019-1238-8.

(2) Huttenhower, C.; Gevers, D.; Knight, R.; Abubucker, S.; Badger, J. H.; Chinwalla, A. T.; Creasy, H. H.; Earl, A. M.; FitzGerald, M. G.; Fulton, R. S.; et al. *Nature* **2012**, *486*, 207, DOI: 10.1038/nature11234.

(3) Proctor, L. M.; Sechi, S.; DiGiacomo, N. D.; Fettweis, J. M.; Jefferson, K. K.; Strauss, J. F., III; Rubens, C. E.; Brooks, J. P.;

Girerd, P. P.; Huang, B.; et al. *Cell Host Microbe* **2014**, *16*, 276, DOI: 10.1016/j.chom.2014.08.014.

(4) Knight, R.; Vrbanac, A.; Taylor, B. C.; Aksenov, A.; Callewaert, C.; Debelius, J.; Gonzalez, A.; Kosciolek, T.; McCall, L.-I.; McDonald, D.; et al. *Nat. Rev. Microbiol.* **2018**, *16*, 410, DOI: 10.1038/s41579-018-0029-9.

(5) Hou, K.; Wu, Z.-X.; Chen, X.-Y.; Wang, J.-Q.; Zhang, D.; Xiao, C.; Zhu, D.; Koya, J. B.; Wei, L.; Li, J.; et al. *Signal Transduction Targeted Ther.* **2022**, 7, Article No. 135, DOI: 10.1038/s41392-022-00974-4.

(6) Fan, Y.; Pedersen, O. *Nat. Rev. Microbiol.* **2021**, *19*, 55, DOI: 10.1038/s41579-020-0433-9.

(7) Kolodziejczyk, A. A.; Zheng, D.; Shibolet, O.; Elinav, E. *EMBO Mol. Med.* **2019**, *11*, e9302, DOI: 10.15252/emmm.201809302.

(8) Scheithauer, T. P. M.; Rampanelli, E.; Nieuwdorp, M.; Vallance, B. A.; Verchere, C. B.; van Raalte, D. H.; Herrema, H. *Front. Immunol.* **2020**, *11*, Article No. 571731, DOI: 10.3389/fimmu.2020.571731.

(9) Sepich-Poore, G. D.; Zitvogel, L.; Straussman, R.; Hasty, J.; Wargo, J. A.; Knight, R. *Science* **2021**, *371*, eabc4552, DOI: 10.1126/science.abc4552.

(10) Jain, T.; Sharma, P.; Are, A. C.; Vickers, S. M.; Dudeja, V. *Front. Immunol.* **2021**, *12*, Article No. 622064, DOI: 10.3389/fimmu.2021.622064.

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Shirley Satanower completed her M.Sc. and Ph.D. studies in the field of microbiology in Dr. Ehud Banin's laboratory at Bar-Ilan University in Israel. Her research focused on biofilm formation and the type III secretion system of *P. aeruginosa*. She later continued to work as a scientist and R&D manager in a probiotic start-up company developing a proprietary delivery method for live bacteria. She has since had several roles including coordinating a start-up accelerator, managing environmental aspects in the food industry, and as a project manager of R&D projects in Algatech, a company that produces commercial-scale microalgae. Dr. Satanower is now the R&D manager of the microbiome products department at Sigma-Aldrich Israel, where her focus is on providing high-quality microbiome tools for research. Ⓜ