REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Biosynthetic activity of Marine Sponge Microbiota

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Abstract

Natural products played a key role in drug discovery. More than 50% of currently available FDA-approved drugs are either directly or indirectly derived from natural origin. However, novel therapeutic agents such as antimicrobial and anticancer drugs are continuously in demand. Marine environment is the most recent promising target for discovering new bioactive natural products. Sponges are sessile marine invertebrates which are known to be a rich source of structurally unique and chemically diverse secondary metabolites with potent biological activities. These metabolites have been frequently hypothesized to be of bacterial origin. More than 99 % of bacteria are challenging to be cultured under the frequently laboratory conditions so culture independent techniques such as metagenomics and metatranscriptomics have been used as effective tools to study the sponges associated bacterial community. These techniques also provide a mean of discovering new bioactive metabolites from the associated communities with the help of many computational and bioinformatics tools.

Keywords: Marine sponge; Natural products; Secondary metabolites; Biosynthetic gene cluster; polyketide synthase; non ribosomal peptide synthase.

1. Introduction

From the beginning of humanity, natural products have been a valuable source as a remedy for various diseases. They played a major role in drug discovery. As of September 2019, more than 50% of currently available FDA-approved drugs are either directly or indirectly derived from natural products **(Newman and Cragg 2020)**. However, novel therapeutic agents such as antimicrobial and anticancer drugs are continuously in demand. The prevalence of life-threatening microbial infections and resistance to current antimicrobial agents have increased dramatically. In addition, there is an ongoing high need to overcome the cancer recurrence associated with drug resistance, high toxicity and severe side effects of the current chemotherapeutic agents **(H. Zhang et al. 2020; Z. Zhang et al. 2020).** Besides, other medical needs are currently unmet **(Vuong 2021)**.

Marine environment can be considered the most recent promising source of novel bioactive natural products with structural and chemical features generally not found in terrestrial environment **(Romano et al. 2017)**. It hosts different organisms that have evolved to live in tough and challenging extreme conditions such as temperature, salinity, pressure, and illumination **(Karthikeyan, Joseph, and Nair 2022).** As a result, these organisms need more adaptive changes to survive in these hard conditions making them produce a special structure of bioactive substances **(Hamed et al. 2015)**.

Marine natural products are generally secondary metabolites. Unlike primary metabolites, they are not generated by regular metabolic pathways and do not have any primary function associated with the development, growth, or proliferation of its producing organism (A et al. 2014) but rather act as

essential factors to either attract, get rid of or kill other organisms and thus increase their chance of survival **(Petersen, Kellermann, and Schupp 2020)**.

Sponges (phylum Porifera) are sessile marine invertebrates which are known to be a rich source for the discovery of structurally unique secondary metabolites that has potent biological activities such as antimicrobial, anti-inflammatory, antitumor and general cytotoxicity **(Calcabrini et al. 2017; Carroll et al. 2019; Varijakzhan et al. 2021).**

This review illustrates the methods used for studying the diversity of the marine sponge associated microbial community and detecting their potential to produce bioactive natural products highlighting their importance as source for bioactive therapeutic natural products

2. Sponges microbiology

Sponges (Phylum: Porifera) are evolutionarily ancient metazoans that have existed for 700–800 million years. They are highly abundant not just in tropical oceans but also in temperate and freshwater **(Hentschel et al. 2002; Radjasa et al. 2007).** Marine sponges are found across different depths from intertidal zones to thousands of meters deep (Fusetani and Matsunaga 2002). Phylum Porifera is a highly diverse taxa among sessile multicellular invertebrates with about 8600 formally reported and 15,000 estimated species **(Orlić 2019)**.

Sponges are lacking muscular, nervous, respiratory, immune and digestive systems. Instead, they have a body with many tiny pores on their surface and canals within a body plan designed to effectively pump water **(Ereskovsky and Lavrov 2021)** which make marine sponges excellent filter feeders. Sponges are strongly associated with symbiotic microorganisms **(Mw et al. 2007).** They are considered as one of the most marine holobiont hosting diverse and complex microbial communities **(L. Pita et al. 2018)**. Developing sponge can acquire symbiotic microorganisms through vertical transmission of microorganisms through the gametes of the sponge by inclusion of the microbes in the oocytes or larvae or during filter feeding through selective absorption of particular microorganisms from the highly diverse microbes in the surrounding water column that passes through the sponge.

3. Approaches used in studying sponge associated microorganisms

3.1 Culture-dependent methods

Most traditional studies of biodiversity and sponge associated microbial community have depended on the isolation and cultivation of the microbes from sponges. However, the majority of bacteria are challenging to be cultured under the frequently laboratory conditions, therefore the culture-dependent method only provides limited information on the sponge community structure **(Dyda et al. 2018; Qaisrani et al. 2019)**.

3.2 Culture independent methods

In the former four decades, culture-independent molecular approaches, which exceed the need for isolation and laboratory cultivation of sponge associated microbes, have been developed. This novel approach has essentially revolutionized the field of environmental microbiology, as it is now feasible to investigate microorganisms, and their interactions with the environment and other organisms in situ **(Orlić 2019).** Omics-based culture independent techniques such as metagenomics and metatranscriptomics have been used as effective tools to get genomic and functional information on sponge symbionts **(T et al. 2010; F. L et al. 2012; R et al. 2012; Boparai and Sharma 2021; El Samak et al. 2023; Elsaeed et al. 2023).**

A term "metagenome" was first used by Handelsman et al., 1998 as "the genomes of the total microbiota found in nature", refers to using sequencing techniques to analyze all of the genomic DNA present in a sample to reveal the complete biodiversity of the sample microbial community including archaea, bacteria and eukaryotes in addition to the functional potential of this community **(J et al. 1998; Dd et al. 2017; Pérez-Cobas, Gomez-Valero, and Buchrieser 2020).** Currently, studying the biodiversity and structure of sponge microbial communities using high-throughput sequencing depends on two main methods: whole-genome shotgun (WGS) metagenomics and marker gene studies.

Marker gene analysis depends on the sequencing of a gene-specific region instead of all genomic DNA to study the diversity and composition of specific taxonomic groups exist in sponge microbial community. The main used marker genes are the 16S rRNA gene (to characterize the diversity of bacteria and archaea) **(Rj et al. 2007; Radwan et al. 2010)**, the internal transcribed spacer (ITS) region (to analyse the composition of the fungal community) **(Cl et al. 2012)** and the 18S rRNA (to explore the presence of eukaryotes) **(Bd et al. 2013).**

Both approaches combined with high-throughput sequencing technologies have been used widely to characterize microbial communities. However, the main advantage of WGS metagenomics against marker gene sequencing is that it gives the chance to characterize the genomic diversity of the analyzed community as well as the potential

functions that are present in the studied community.
Another strategy, metatranscriptomics, used to capture and sequence all of the RNA in a sample, providing a profile of all actively transcribed genes and also their relative abundance to allow the observation of gene expression patterns and functionality of microbial communities **(Moran et al. 2013; Niu et al. 2018)**.

4. Diversity and structure of sponge microbiota

Marine sponges host a wide range of microorganisms from many domains of life mainly bacteria. To date, more than 60 bacterial phyla, including newly discovered candidate phyla that do not have any cultured representative, have been reported from sponges**.** The most predominant sponge-associated microorganisms are represented in the phyla Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Nitrospirae and in the candidate phylum, Poribacteria which occurs almost exclusively in marine sponges **(Thomas et al. 2016; Moitinho-Silva et al. 2017; Orlić 2019; J. A. Taylor et al. 2021).**
Sponges associated microbial communities have a high degree of host specificity and temporal stability of the microbial symbionts, in spite of the continuous influx of seawater microorganisms resulting from filter-feeding process with low seasonal and inter-annual variation (Thomas et al. 2016). Surveys along different environmental conditions (e.g., geographical distance **(Lucía Pita et al. 2013)**, season **(Erwin et al. 2012; Pm et al. 2015)**, habitat **(Cárdenas et al. 2014)** and depth **(Steinert et al. 2016)** have reliably proved that sponges host species-specific and stable microbiomes at different bacterial taxonomic levels **(Steinert et al. 2017)** and prevalence levels **(Astudillo-García et al. 2017).**

5. Bioactive natural products from marine sponges

Particularly among marine invertebrates, sponges are known as an important and a wealthy source of bioactive natural products **(Carroll et al. 2022)**. A lot of these products showed various biological activities, such as anticancer, antifungal, antibacterial, antiviral, anti-inflammatory, antioxidant, antimalarial and bio-surfactant activity **(Abdelmohsen et al. 2010; El Samak, Solyman, and Hanora 2018; Abraham et al. 2021; Carroll et al. 2022; Kamel, Hanora, and Solyman 2022).** Therefore, sponge-derived natural products have become an attractive subject for discovering new drug leads.
Searching for bioactive secondary metabolites produced from marine sponges have been started in the early 1950s when the nucleosides spongothymidine and spongouridine, the basis for the synthesis of the first marine-derived anticancer agent ara-C, and the antiviral drug ara-A, were isolated from the marine sponge *Cryptotethya crypta* **(Bergmann and Feeney 1951; P, Ra, and R 2002).**
Since this time, it was believed that the bioactive secondary metabolites were all produced by sponges themselves but later, it was hypothesized that they were often produced by the sponges microbial symbionts **(Hentschel et al. 2012; Esposito et al. 2015).** This hypothesis is supported by the bacterial like structure of various bioactive compounds isolated from marine sponges such as complex polyketides (PKSs) and non-ribosomal peptides (NRPSs), which are exclusively produced by microorganisms **(Varijakzhan et al. 2021)**.
Faulkner et al. was the first who supported that hypothesis through experimental investigation of the localization of natural products within sponge associated bacteria. For this target, sponge *Theonella swinhoei* associated bacterial cells were separated from sponge tissue by differential centrifugation and the obtained bacterial cells were analyzed chemically. As a result, the cytotoxic peptide theopalauamide and the macrolide swinholide A were proved to be produced by filamentous heterotrophic bacteria and heterotrophic unicellular bacteria, respectively **(Bewley, Holland, and Faulkner 1996).**
Cytarabine (ara-C) was the first real start of the sponge derived natural products to be applied as approved drug as it was approved from the U.S. Food and Drug Administration (FDA) as anticancer drug (Cytosar-U®, Alexan®, Udicil®) **(Anjum et al., 2016; Newman & Cragg, 2016; Sagar et al., 2010)**. This was followed by the anticancer Gemcitabine (Gemzar) (S. G et al. 2001) and the first intravenous antiviral Vidarabine (Vira-A®) **(Bertin et al. 2015; Brinkmann, Marker, and Kurtböke 2017)**. Cytarabine, Gemcitabine and Vidarabine were all derived from the Caribbean sponge Cryptotheca crypta **(Bergmann and Feeney 1951; P, Ra, and R 2002).**
Another sponge derived compound which moved through clinical trials to be FDA approved as anticancer drug was the macrolide, eribulin mesylate (Halaven®) a synthetic analog of the sponge natural product halichondrin B, isolated from the Japanese marine sponge *Halichondria okadai* and was approved for metastatic breast cancer chemotherapy **(Ledford 2010)**. Moreover, the current clinical pipeline contains many sponge derived drug candidates in different clinical trials in phase III, II, or I as illustrated in **Table 1.**

Table 1. List of sponges derived drug candidate in different clinical trials phases

Compound name	Disease area	Company or Institution	Reference
Salinosporamide A (Marizomib)	Anticancer and Potent proteasome inhibitor	Bristol-Myers Squibb	**(National library of medicine(us) 2022)**
PM-10450 (Zalypsis®)	Anti-cancer	PharmaMar	**(Newman and Cragg 2014)**
Discodermolide	Anti-cancer	Novartis	**(Amos B. Smith and Freeze 2007)**
Hemiasterlin (E7974)	Anti-cancer	Eisai Inc.	**(K. G et al. 2009)**
PM-060184	Anti-cancer	PharmaMar	**(Conte et al. 2021)**
NVP-LAQ824 (Psammaplin derivative, Dacinostat)	Anti-cancer	Novartis Pharma	**(Conte et al. 2021)**

5.1 Chemical diversity of marine sponge natural products

Sponges secondary metabolites are characterized by high chemical diversity as they are grouped according to their chemical structures into many different classes such as alkaloids, terpenes, ribosomal peptides, polysaccharides, anthraquinones, polyketides and non-ribosomal peptides **(Varijakzhan et al. 2021)**.

Polyketides (PKs) and non-ribosomal peptides (NRPs) are two of the most important, diverse and largest natural product families **(Staunton and Weissman 2001; Süssmuth and Mainz 2017).** They are widely applied as pharmaceutical drugs for the treatment of different diseases such as the antibacterial (erythromycin and vancomycin), the antifungal (amphotericin and griseofulvin) (M and M 2018), the anti-parasitic avermectin **(Jf et al. 2017)**, and the anticancer drugs (epothilone, anthracycline, doxorubicin and bleomycin) (Washington and Wilson 1985; Altmann 2003; Ute Galm et al. 2005; Levine 2006; Li, Kim, and Blenis 2014; K. L and Rh 2016)**.**

PKs and NRPs are constructed from relatively simple chemical units which allow their high chemical diversity **(Süssmuth and Mainz 2017; Weissman and Leadlay 2005; Hertweck 2009)**. Their biosynthesis depends on complex enzyme machineries named polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs). PKSs condense small carboxylic acids, mainly acetate and propionate while NRPSs condense amino acids or sometimes other organic acids to form PKs or NRPs , respectively **(Staunton and Weissman 2001; Süssmuth and Mainz 2017)**. However, these simple beginnings give birth to a wide variety of medicinally valuable compounds, such as macrolides, polyethers, enediynes, and lactams **(Hertweck 2009; Süssmuth and Mainz 2017).**

5.1.1 PKSs

PKSs are classified into three different types (I–III) depending on their assembly line architecture and mode of action **(Hertweck 2009).**

A. Type1 modular PKS

Type-1 PKSs are large multi-modular assembly-line complexes primarily found in bacteria (Wang et al. 2020). They are composed of multiple catalytic domains which arranged into modules. Each module is responsible for adding one acyl building block into the polyketide chain and performing some types of modification **(Hertweck 2009; Keatinge-Clay 2012)**. PKS modules contain at least three main domains: an acyl carrier protein (ACP) domain, an acyltransferase (AT) domain and a ketosynthase (KS) domain. AT domain loads an (alkyl) malonyl extender unit onto the ppant thiol of the ACP then (KS) domain receives the developing polyketide chain from the ACP domain of the upstream module. After that, KS domain catalyzes a decarboxylative Claisen condensation reaction with the extender unit attached to the ACP domain of the downstream module, producing a β-keto chains. This chain may undergo some modifications by other accessory domains, *i.e.* the additional incorporation of a ketoreductase (KR) domain converts the keto-functionality into a β-OH group, which can be removed by a dehydratase (DH) domain to produce an alpha-beta unsaturated alkene, which can subsequently be reduced to a single bond in the presence of an enoyl-reductase (ER) domain. Also, C- and O-methyltransferases (MTs) domains can modify the growing polyketide chain. Elongation modules contain all three core domains (ACP, AT, and KS), while the loading module lacks a KS domain and the terminal module

have a thioestrase (TE) domain, which is responsible for only releasing the linear polyketide chain or releasing it with macrocyclization **(Alanjary et al. 2019).**

There are two phylogenetically distinct classes of modular PKSs, cis-AT PKSs and trans-AT PKSs **(Khosla et al. 2007)**. PKSs belong to cis-AT class commonly have AT domains incorporated into each module. On the other side, PKSs of trans-AT class mainly have a single standalone AT domain that provide a malonyl extender unit to each of the ACP domains in the assembly line **(Piel 2002; Cheng, Tang, and Shen 2003)**. About 40% of modular PKSs in bacteria were estimated to be grouped under the trans-AT class **(O'Brien et al. 2014)**. Metagenomic investigations have revealed that *trans*-AT polyketide synthases are often the source of the most potent and structurally diverse polyketides isolated from marine invertebrates **(Helfrich and Piel 2016)**.

B. Type 1 iterative PKS

This type is more dominant in fungi. It is a mono-modular type so the same set of domains is used multiple times for each round of polyketide elongation and processing during a single polyketide biosynthesis **(Hertweck 2009)**.

C. Type 11 PKS

Type II PKSs are multi-enzyme complexes formed of mono-functional proteins. They are found mainly in bacteria and produce diverse aromatic polyketides **(Hertweck et al. 2007; Wang et al. 2020).**

D. Type III PKS

Type III PKSs consist of a single protein with multiple modules. they are also iterative and have been found in bacteria, fungi and plants **(D. Yu et al. 2012)**. Microbial type III PKSs have many interesting features over that of plant type III PKSs. Moreover, they actually produce many compounds with significant biological functions and important pharmaceutical activities **(Katsuyama and Ohnishi 2012)**.

5.1.2 NRPSs

NRPSs, similar to modular type 1 PKSs, are large modular multifunctional enzymes. Each module catalyzes the incorporation and modification of a certain amino acid and sequentially extends the peptide in an assembly line fashion in order to synthesize peptides from different non-proteinogenic amino acids without using the cell ribosomal machinery and mRNAs **(Soltani 2016; Alanjary et al. 2019)**. Each module consists of multiple catalytic domains at least three main domains that form a minimal module: the adenylation (A) domain, the peptidyl carrier protein (PCP) domain and the condensation (C) domain.

The A domain picks, activates and transfers a certain amino acid to the PCP domain which holds the amino acid building blocks via a 4'-phosphopantetheine prosthetic arm. Successively, the C domain catalyzes the peptide bond formation of the amino acid attached to the PCP domain of the same module and the other amino acid on the PCP domain of the upstream module. The formed growing peptide chain can be further modified by the action of additional accessory domains such as epimerization (E) domain which is responsible for the incorporation of D-amino acids through the epimerization of the C_α center of the S-aminoacyl-PCP. Another additional domain is methyltransferases (MT) domain which is responsible for the C- or N-methylation. In addition, heterocyclization may occur due to cyclization (Cy) domains, while redox-active (Ox, Red) domains are able to determine their redox state **(Kim et al. 2019; Alanjary et al. 2019; Setyahadi 2020).**

The biosynthesis of both NRPs and type 1 PKs proceeds according to the co-linearity rule where the amino acid or acyl units sequence of the peptide or the polyketide product can be predicted depending on the organization and order of modules **(Kim et al. 2019; Alanjary et al. 2019; Setyahadi 2020).**

5.1.3 Hybride PKS and NRPS

The remarkable similarities between PKSs and NRPSs enable the formation of hybrid clusters that contain domains of both classes. The hybrid PKS-NRPS clusters increase the diversity of the secondary metabolites produced by microorganisms **(I et al. 2012)**.

5.2 Polyketides and non-ribosomal peptides from marine sponges

Many different macrolides as well as cyclic and linear peptides which are synthesized by PKSs or/and NRPS were isolated from sponges. Swinholide A was the first symmetric 44-membered macrolide to be isolated from the Red Sea marine sponge *Theonella swinhoei* **(Carmely and Kashman 1985)**. It showed antifungal activity and potent cytotoxicity against different tumor cells. Many derivatives of swinholide A were also isolated from the same sponge. These derivatives differ from the parent compound of swinholide A in the carbon backbone as in swinholide I, misakinolide A and Hurghadolide A. Swinholide I and hurghadolide A exhibited in vitro cytotoxic activity against human colon adenocarcinoma (HCT-116) with IC50 values of 5.6 and 365 nM, respectively. In addition, they caused disruption of the actin cytoskeleton at concentrations of 70 and 7.3 nM, respectively. Furthermore, both compounds showed antifungal activity against *Candida albicans*. Misakinolide A is also considered a highly active antitumor macrolide **(Kato et al. 1987; SakaiRyuichi, HigaTatsuo, and KashmanYoel 2006; Dt and Sl 2006)**.

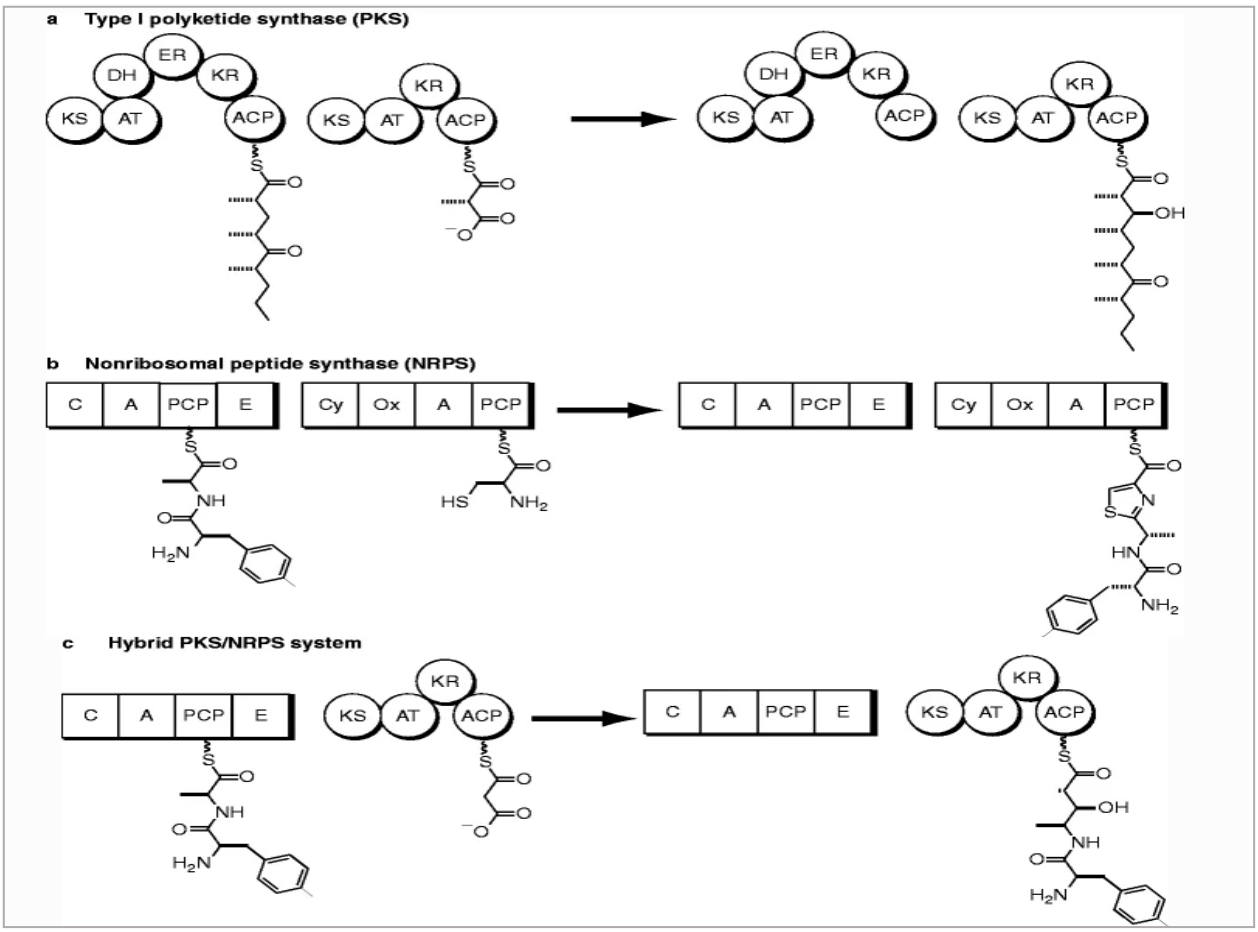

Figure 1. Hypothetical examples of the modular organization in a) PKSs, b) NRPs and c) hybrid PKS/NRPSs **(Sherman et al. 2012).**

Theonellamides are bicyclic peptides isolated from the marine sponge *Theonella swinhoei*. Theonellamides A–G were found to be potent antifungal agents and showed potent cytotoxicity against different cancer cell lines **(Shigeki Matsunaga and Fusetani 1995; Shigeki Matsunaga et al. 2002; Youssef et al. 2014)**. Theonellamide I was shown to possess cytotoxicity against HeLa cell line **(Fukuhara et al. 2018)**. Theonellamide F displayed antifungal properties against different pathogenic fungal strains, such as *Candida sp.*, *Aspergillus sp.*, and *Trichophyton sp.* **(Shigeki Matsunaga et al. 2002)**. Another cyclic peptide, microsclerodermins, exhibited potent antifungal activity. They were isolated from marine sponges of the genera Microscleroderma as well as Theonella **(Schmidt and Faulkner 1998)**.

Discodermin A, a tetra-decaspeptide; , and its analouges, discodermin B, C, D and E were isolated from the Japanese sponge Discodermia kiiensis **(S. Matsunaga, Fusetani, and Konosu 1984; Shigeki Matsunaga, Fusetani, and Konosu 1985).** All of them have antimicrobial activity. In addition, discodermins A–D are potent inhibitors of enzyme PLA2 while discodermin E showed cytotoxicity **(Ryu, Matsunaga, and Fusetani 1994; Ebada and Proksch 2012; Negi, Kumar, and Rawat 2017).** The cytotoxic peptides **,** discokiolides and lipodiscamides have also been isolated from *Discodermia kiiensis* **(Tan, Wakimoto, and Abe 2014; Wakimoto 2023).**

A proline-rich cyclopeptide, Callyaerins A, was isolated from marine sponge *Callyspongia aerizusa* and displayed potent antifungal activity against *Candida albicans* **(Vitali 2018)**. In addition, the compound woodylides A and C, a linear polyketide from marine sponge Plakortis simplex, exhibited moderate antifungal activity against *Cryptococcus neoformans*, with IC_{50} values of 3.67 µg/mL and 10.85 µg/mL, respectively **(H.-B. Yu et al. 2012).**

The macrolides Altohyrtin A-C and 5-desacetylaltohyrtin A have been isolated from the Okinawan marine sponge *Hyrtios altum*. They

exhibited highly potent cytotoxicity against KB cells with IC50 values of 0.02, 0.4 and 0.3 ng/ml, respectively **(Kobayashi, Aoki, Sakai, Kawazoe, et al. 1993; Kobayashi, Aoki, Sakai, Kihara, et al. 1993).**

5.3 Approaches for discovering marine natural products

5.3.1 Traditional isolation methods

Traditional strategies are used for marine sponges' natural product discovery. These strategies involve sponge metabolic extraction followed by isolation of pure compounds by different separation techniques such as TLC, column chromatography and HPLC. These pure compounds are then used for the determination of structure and screening for biological activity. **(Sasidharan et al. 2011)**. However, the supply problem is considered as a major limitation of this method. The majority of natural products produced from marine sponges are hypothesized to be produced by the sponge associated bacteria leading to their production in inadequate low amounts especially as more than 99% of these associated bacteria are uncultrable. The insufficient production of several marine natural products with promising pharmaceutical applications has led to ending their progression into clinical phases because the stage of clinical trial needs a considerable amount of drug mass; usually kilogram amounts, while the traditional isolation techniques produce approximately up to 10 μg **(Donia et al. 2011; Tsukimoto et al. 2011)**. In addition, over harvesting from marine animals to produce sufficient amounts is not allowed since this can lead to extinction of marine species and damage essential coastal reefs.

5.3.2 Mining sponge metagenome for natural products biosynthetic gene clusters (BGCs)

As a result of the limitations of traditional methods, development of techniques to both discover and supply sufficient compounds for biological assay and pharmaceutical applications is required. On this aspect, metagenomics provide a mean of discovering new bioactive metabolites from bacterial communities associated with marine sponges **(Banik and Brady 2010; Trindade et al. 2015)**. The enzymatic machineries responsible for the biosynthesis of these metabolites are usually encoded by many locally clustered genes within the genome of the producing microorganism known as BGCs **(Chen et al. 2020)**. Metagenomics allow the identification of these BGCs responsible for the biosynthesis of bioactive natural products within the marine sponge metagenome. This metagenomics-BGCs mining approach would enable the cloning of these BGCs captured directly from sponge metagenome and subsequently constructing heterologous expression systems of these BGCs in easily cultured bacteria allowing the sustainable production of sponge-derived natural products **(Ar, J, and T 2018)**. For example, approximately 13,000 kg of the marine bryozoan *Bugula neritina* are required to obtain only 18 g of the cyclic polyketides bryostatins for anti-cancer clinical trials but later, the bryostatin biosynthetic genes have been discovered and characterized through metagenomic BGC mining approach and the uncultivated marine symbiotic bacteria "*Candidatus* Endobugula sertula" has been expected to be its likely natural source so heterologous expression of this biosynthetic gene cluster has the potential of producing the bioactive bryostatins in large enough amounts for development into a pharmaceutical **(M. W. Taylor et al. 2007; S et al. 2007; Trindade-Silva et al. 2010)**

On the other side, the cloning and expression approach of BGCs captured directly from sponge metagenome can also overcome the limitation of the silent or cryptic BGCs of cultrable associated bacteria as most of the BGCs present in genomes of cultured sponge symbiotic bacteria are silent or cryptic under standard laboratory growth conditions so identification and activation of these BGCs would allow the production of their encoded natural product **(Mao et al. 2018).**

Many computational and bioinformatic tools have been designed for the identification of the BGCs responsible for the biosynthesis of bioactive natural products within the marine sponge metagenome. The majority of them use the searching tools, Basic Local Alignment Search Tool (BLAST) or profile hidden Markov models (HMMs) as a base to identify the BGCs responsible for natural product biosynthesis **(Ren et al., 2020)**. These tools include NAPDOS "Natural Product Domain Seeker", antiSMASH "antibiotics and secondary metabolites analysis shell', NP.searcher and ClustSca **(H, C, and H 2020)**.

Many publicly available online databases can facilitate the metagenome mining analysis of BGCs. The antiSMASH database is a repository of antiSMASH-annotated BGCs from more than 20,000 bacterial genomes and includes above 150,000 BGCs. The BGC family database BiG-FAM is a database of 29,955 GCFs covering the global diversity of 1,225,071 BGCs detected within 209,206 publicly available microbial genomes and metagenome-assembled genomes (MAGs) **(Kautsar et al. 2021).** The "Minimum Information about a Biosynthetic Gene Cluster" (MIBiG) database contains annotated BGCs with known functions and the secondary metabolites they produce. This database is especially useful in

identifying BGCs in sequenced genomes that can produce the same or similar sets of compounds according to sequence homology, therefore allow detecting novel BGCs. The MIBiG till now contains 2021 manually curated BGCs **(Kautsar et al. 2020).**

6. Conclusion

Marine sponge hosts highly diverse microbial communities. The sponges associated bacterial community is the largest source of marine natural products with diverse structure and potent therapeutic activities. Omics based techniques such as meagenomics and metatranscriptomics are the most effective and informative tools for studying the sponge associated microbes and can be used for detecting BGCs responsible for the biosynthesis of natural products.

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