Chapter 12
Biosynthesis of Antibiotics from Microbial Symbionts of Sponges and Corals

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Contents

Abstract Sponges and corals are significant sources for marine natural products. They have a pool of novel microorganisms. Due to low cost of gene sequencing, in recent years, several reports are available for novel compounds from sponge- and coral-associated microorganisms. Still, most of the biosynthesis mechanisms are not revealed. There are only few reports on the biosynthesis mechanism of antibiotics from sponge- and coral-associated microorganisms. The scanty amount of antibiotic was produced by most of the strains; hence it is important to explore the biosynthesis of antibiotics to improve the production. In this chapter, we cover the important reports of biosynthesis of antibiotics from microbial symbionts especially sponges and corals.

Keywords Sponges · Corals · Symbionts · Biosynthesis · Antibiotics

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12.1 Marine Invertebrates: A Treasure of Antibiotics

Due to a wide chemical diversity and potential activity, the natural products are best-selling drugs in clinical use [1]. The best example is Taxol (1, paclitaxel); it is isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. David Yuon said "Since 2006, the annual total sales of the natural raw materials paclitaxel injection and semi-synthetic paclitaxel injection paclitaxel reached $ 3.7 billion in international market" (http://www.articlesfactory.com/articles/marketing/paclitaxel-ranks-first-among-worlds-anti-cancer-drugs.html) [2].

The earth's surface covers ca. 70% of seawater with a wide biodiversity potential. So far around 100,000 species was reported in the world's ocean and day-by-day lot of new species is reporting by researchers. The phyla *Bryozoa*, *Coelenterata*, *Porifera*, and *Echinodermata* are exist only in aquatic region. These invertebrates don't have any physical protection (shells or spines), but still they are fighting against predators using biologically active secondary metabolites [3]. Previously, it was taught that the marine invertebrates only produce secondary metabolites, but now it's reported that the invertebrate-associated microbiome is a key producer of active secondary metabolites. Several new lead molecules have been discovered from marine invertebrates such as diterpene glycoside; eleutherobin, from the Australian soft coral *Eleutherobia* sp. [4]; discodermolide from the Caribbean sponge *Discodermia dissoluta* [5].

12.2 Important Enzymes Involved in the Biosynthesis of Secondary Metabolites

12.2.1 Polyketide Synthases (PKSs)

It is a multi-domain enzyme responsible for synthesis of polyketide compounds. Polyketide biosynthesis is similar to the fatty acid biosynthesis [6, 7]. It is classified into three types:

A. PKS-I
A.1. Iterative PKS s
A.1.1. Nonreducing PKSs (NR-PKSs)
A.1.2. Partially reducing PKSs (PR-PKSs)
A.1.3. Fully reducing PKSs (FR-PKSs)

A.2. Modular PKSs
B. PKS-II
C. PKS-III

PKS-I has several domains with known functions. It has three important modules such as starting module (AT-ACP), elongation modules (KS-AT-[DH-ER-KR]-ACP), and termination module (TE). The domains are acyltransferase (AT), acyl carrier

protein (ACP), ketosynthase (KS), ketoreductase (KR), dehydratase (DH), enoylreductase (ER), methyltransferase (MT), sulfhydrolase (SH), and thioesterase (TE).

Polyketides are group of secondary metabolites which have a unique structure and function. Their biological activities includes antimicrobial, antiparasitic, antitumor, etc. Examples for known polyketides include erythromycin A, avermectin, rifamycin, and lovastatin [8].

12.2.2 Nonribosomal Peptide Synthetases (NRPSs)

It is a multimodular enzyme, capable of synthesizing the nonribosomal peptide molecule, independent of ribosomal machinery. It is a class of secondary metabolites with a wide range of properties, like toxins, siderophores, pigments, antibiotics, etc. This enzyme is located at operon; hence its transcriptional or posttranscriptional regulation can be positive or negative [9, 10].

A peptide acts as a backbone in the amino acids inserted in a systemic manner by NRPS enzyme. Further, the module converted as domains leads to the nonribosomal peptide synthesis. One module contains three domains [11]:

1. Adenylation (A) domain
2. Peptidyl carrier protein (PCP) or thiolation (T) domain
3. Condensation (C) domain

The reaction is N- to C-terminal direction. The final peptide product size is 3–15 amino acids length; it is linear, cyclic, or branched cyclic form [12]. Figure 12.1 explains the biosynthesis of surfactin by NRPS enzyme [13].

12.2.3 Ribosomally Synthesized and Posttranslationally Modified Peptides (RiPPs)

It is recently identified as the major class of secondary metabolites with a wide variety of structural diversity due to extensive posttranslational modifications (PTMs) [14, 15]. The PTMs will play a major role in RiPP synthesis via expanded chemical functionalities, improved target recognition, and increased metabolic and chemical stability [14]. Figure 12.2 explains the general biosynthetic pathway of RiPPs, and Fig. 12.3 explains the biosynthesis of nisin A. Recently Tietz et al. [17] developed a new software called as RODEO (Rapid ORF Description and Evaluation Online) to identify the RiPP precursor peptides.

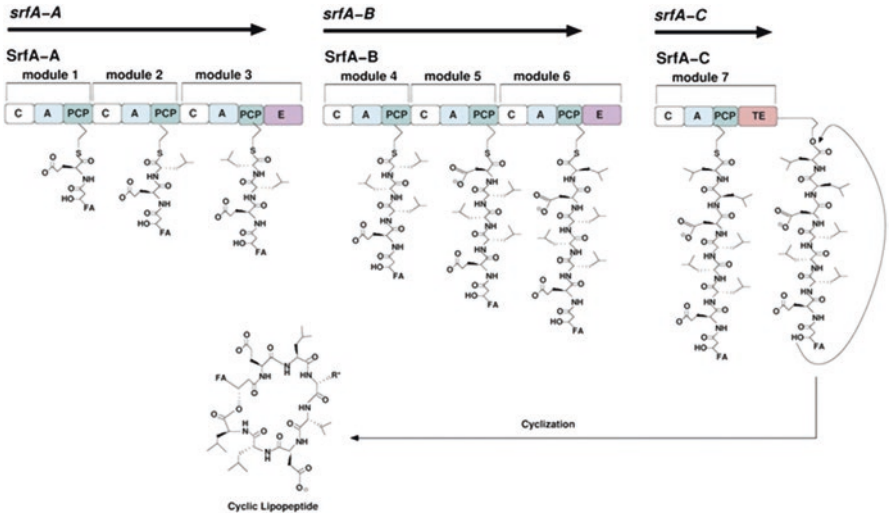

Fig. 12.1 Biosynthesis of surfactin [13]

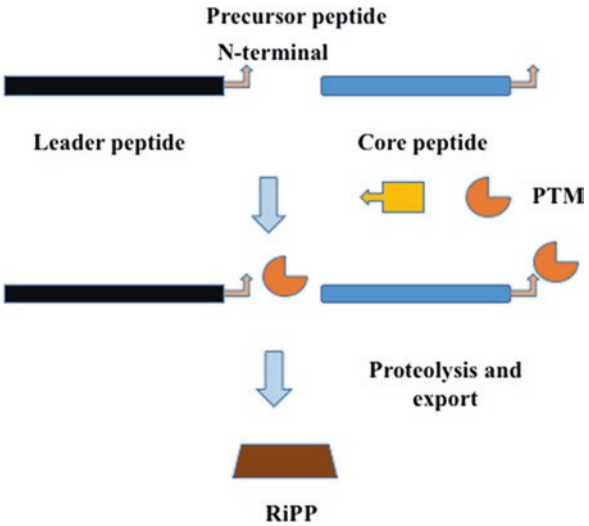

Fig. 12.2 General biosynthetic pathway of RiPPs

12.3 Revolution of Methods to Study the Biosynthesis of Natural Products

The classical method to study the biosynthesis of natural products is chemical degradation. In this method, compounds must be fully synthesized to assign the structure of compound. This method is vanished today because new technique arrivals.

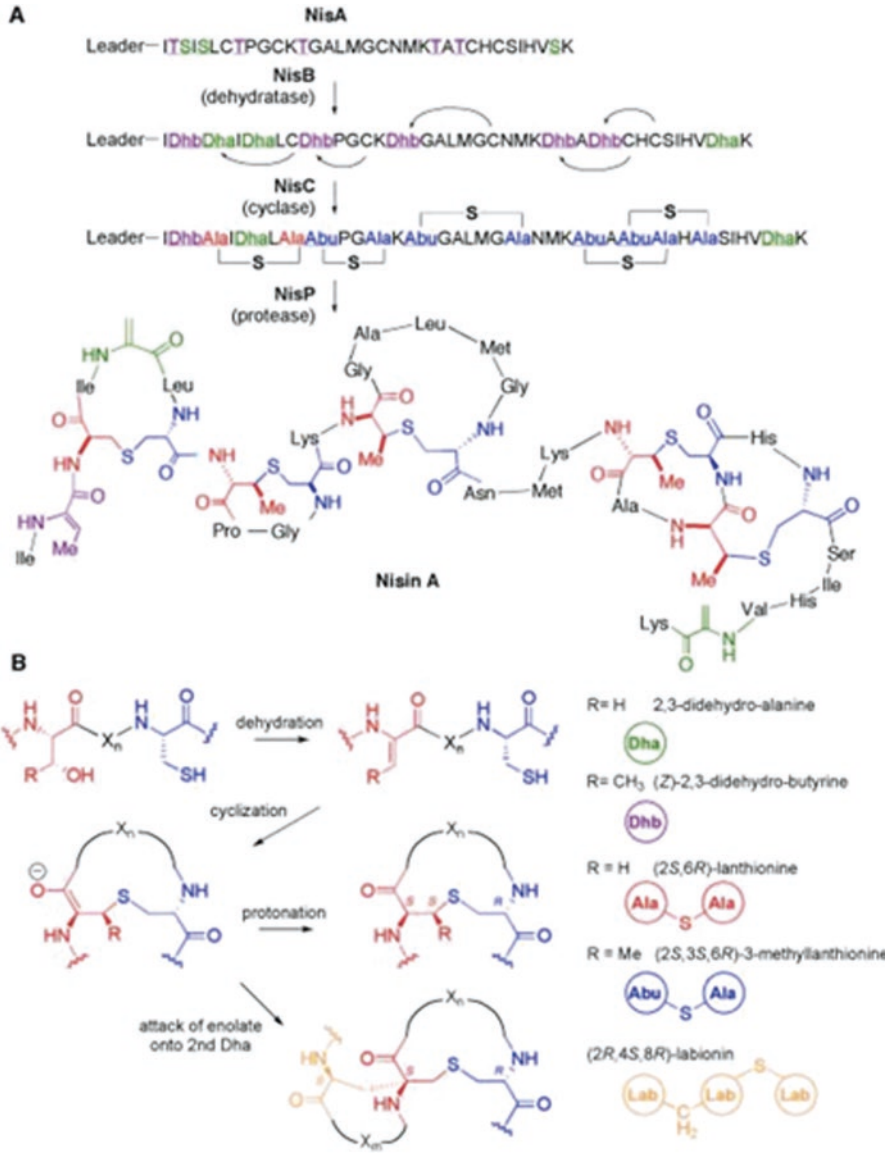

Fig. 12.3 (**a**) Biosynthesis of nisin A. (**b**) Generation of (Me)Lan and labionin motifs [16]

After the discovery of isotopes, the dimension of biosynthesis research was changed [18], and it led to the discovery of cholesterol biosynthesis [19].

The first metabolite investigated by isotope was polyketide compound [20]. The isotopes (1,2-^{13}C2) acetate and (1-^{13}C) or (2-^{13}C) acetate are sources for acetate units, chain direction, and modifications of PKS-derived natural products [21]. Bode et al. (2012) developed a method combination of isotope labeled with the

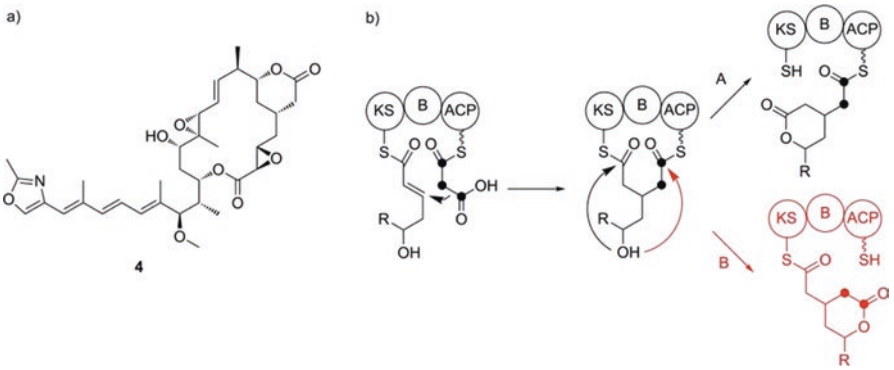

Fig. 12.4 Biosynthesis of rhizoxin using ^{13}C-labeled carbons [23]

bacterial strains and its transaminase mutants followed by MS analysis. Using this strategy, GameXPeptides, novel cyclopeptide structure was predicted in crude extract itself [22]. Figure 12.4 is an example for ^{13}C-labeled carbon used to study the biosynthesis of rhizoxin [23].

These techniques were used for terrestrial microbes and plants. But after the revolution of molecular biology techniques, the biosynthesis study went next level. The advantage and cost of DNA sequencing, lots of database with protein and gene information, and other advances in biological field expand our understanding of the biosynthesis of marine molecules [24].

The molecular techniques revealed the marine natural product "Dogma" from gene to products. It also differentiates the key steps and biosynthetic pathway that leads to the diverse structural diversity of marine natural products [25–27]. The molecular techniques only gave a clear picture on natural products from microbial origin, not from macroorganisms [28]. At first, genetic-level marine natural product biosynthesis was explained in actinomycetes and cyanobacteria. In 2000, Piel et al. explained the first marine actinomycete natural product biosynthesis (enterocins and wailupemycins). These compounds were isolated from *Streptomyces maritimus* (marine sediment) (Fig. 12.5) [29].

12.4 Biosynthetic Potential of Sponge-Associated Microbes

Most marine natural products are isolated from the marine sponges when compared to the other marine invertebrates [30]. So far, the natural products were reported from the class Demospongiae and particularly the 3 orders *Halichondrida*, *Poecilosclerida*, and *Dictyoceratida*. Many studies have proved that sponge-associated microbes are responsible for most of the natural product synthesis instead of sponges. In these, *Actinobacteria* and fungal division *Ascomycota* were potential producers of drugs. Sponges harbor large amount of gene diversity due to the

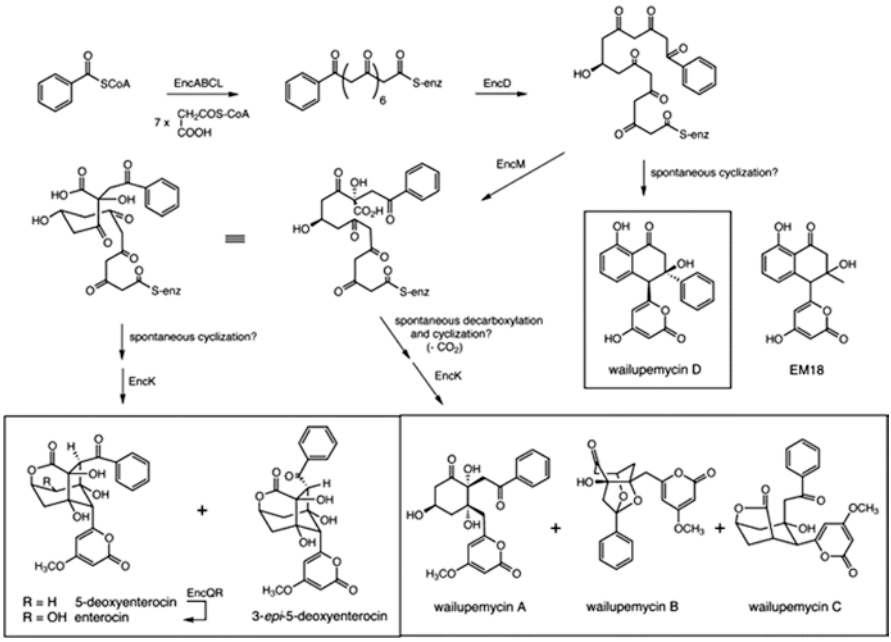

Fig. 12.5 Biosynthetic pathway of enterocins and wailupemycins [29]

localization of specific biosynthetic gene sequences [31]. Table 12.1 summarizes the list of marine natural product biosynthesis identified and characterized.

In microbial biodiversity, ca. 99% of microorganisms are unculturable. The novel chemical entity will be discovered followed by an identification of novel bacterial species. In the future, using in situ cultivation methods and growth factor for unculturable microbes, we can discover new secondary metabolites [62].

So far only five biosynthetic gene clusters were identified from uncultured microorganisms associated with marine organisms such as psymberin from uncultivated prokaryotic symbiont of *Psammocinia* aff. *bulbosa* (sponge), bryostatin from uncultivated prokaryotic symbiont of *Bugula neritina* (bryozoan), patellamide from *Prochloron didemni*, uncultivated cyanobacterial symbiont of *Lissoclinum patella* (ascidian), and onnamide/theopedrin from uncultivated prokaryotic symbiont of *Theonella swinhoei* (sponge). Out of five, three biosynthetic gene clusters were reported from sponge symbionts.

In 2004, Piel et al. reported the first genetic evidence of natural products from uncultured sponge-associated microbes. The PKS-NRPS hybrid gene was responsible for biosynthesis of onnamide/theopedrin which was isolated from *Theonella swinhoei* (sponge). The compound structure is similar with pederin which is originally isolated from *Paederus fuscipes*. Piel's group identified the PKS gene responsible for the onnamides and theopederins from a complex metagenome. These two compounds' gene clusters have unique property compared to pederin gene cluster. The pederin gene-encoding type I PKS megasynthases don't have a sequence of acyltransferase (AT) domains, but it is present in other two gene clusters (Fig. 12.6) [57].

Table 12.1 List of marine natural products' gene identified and characterized (2000–2016) [31]

Year published	Molecule	Organism	Molecule type class	References
2016	Thalassospiramide lipopeptides	*Rhodospirillaceae* strains	PKS-NRPS hybrid	Zhang et al. 2016 [32]
2016	Ammosamides A–C, pyrroloquinoline alkaloids	*Streptomyces* sp. CNR-698	NRPS	Jordan and Moore 2016 [33]
2016	Tetrabromopyrrole	*Pseudoalteromonas* sp.	Halogenase	Gamel et al. 2016 [34]
2015	Unusual thiotetronic acid	*Salinispora*	PKS-NRPS hybrid	Tang et al. 2015 [35]
2014	Polybrominated diphenyl ethers	*Pseudoalteromonas* spp.	Halogenase	Agarwal et al. 2014 [36]
2013	Novel cyanosporasides C–F	*Salinispora pacifica* CNS-143 and *Streptomyces* sp. CNT-179	PKS	Lane et al. 2013 [37]
2013	Thalassospiramide C Thalassospiramide F	Marine α-proteobacterium *Thalassospira* sp. CNJ-328	PKS-NRPS hybrid	Ross et al. 2013 [38]
2012	Didemnin	Marine α-proteobacteria *Tistrella mobilis*	PKS-NRPS hybrid	Xu et al. 2012 [39]
2011	Ansalactam A	*Streptomyces* sp.	PKS	Wilson et al. 2011 [40]
2010	ML-449	*Streptomyces* sp.	PKS	[41]
2010	Rifamycin/saliniketal	*Salinispora arenicola*	PKS	[42]
2010	Tirandamycin	*Streptomyces* sp.	PKS/NRPS	[43]
2010	TP-1161	*Streptomyces* sp.	Ribosomal peptide	[44]
2009	BE-14106	*Streptomyces* sp.	PKS	[45]
2009	Psymberin	Uncultivated prokaryotic symbiont of *Psammocinia* aff. *bulbosa* (sponge)	PKS	[46]
2008	Cyclomarin/ cyclomarazine	*Salinispora arenicola*	NRPS	[47]
2008	Napyradiomycin	*Streptomyces aculeolatus* NRRL 18422 and CNQ525	Polyketide/ terpenoid	[48]
2007	Bryostatin	Uncultivated prokaryotic symbiont of *Bugula neritina* (bryozoan)	PKS	[49]
2007	Hectochlorin	*Lyngbya majuscula*	PKS/NRPS	[50]
2007	Salinosporamide	*Salinispora tropica*	PKS/NRPS	[51]
2007	Sporolide	*Salinispora tropica*	Polyketide (enediyne)	[51]
2005	Patellamide	*Prochloron didemni*, uncultivated cyanobacterial symbiont of *Lissoclinum patella* (ascidian)	Ribosomal peptide	[52]

(continued)

Table 12.1 (continued)

Year published	Molecule	Organism	Molecule type class	References
2004	Curacin	*Lyngbya majuscula*	PKS/NRPS	[53]
2004	Jamaicamide	*Lyngbya majuscula*	PKS/NRPS	[54]
2004	Lyngbyatoxin	*Lyngbya majuscula*	Nonribosomal peptide/ terpenoid	[55]
2004	Nodularin	*Nodularia spumigena*	PKS/NRPS	[56]
2004	Onnamide/theopedrin	Uncultivated prokaryotic symbiont of *Theonella swinhoei* (sponge)	PKS/NRPS	[57]
2003	Barbamide	*Lyngbya majuscula*	PKS/NRPS	[58]
2002	Eicosapentaenoic acid	*Photobacterium profundum*	PKS	[59]
2002	Griseorhodin	*Streptomyces* sp.	PKS	[60]
2000	Docosahexaenoic acid	*Moritella marina*	PKS	[61]
2000	Enterocin/ wailupemycin	*Streptomyces maritimus*	PKS	[29]

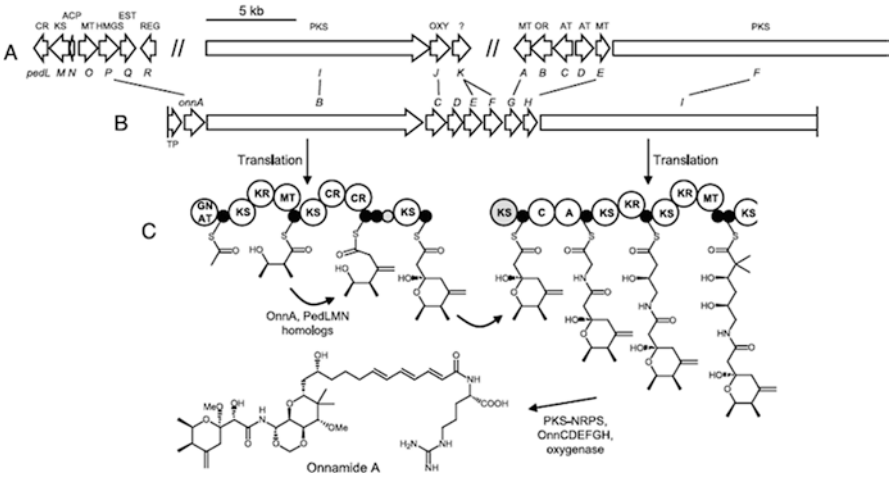

Fig. 12.6 Biosynthesis of onnamide A [57]

Until the microbes grow in laboratory condition, the natural products from microbial origin is remain a mystery, but the molecular evidence support the microbial biosynthesis. Recently, psymberin metabolites were reported from uncultured microbial symbiont of the sponge *Psammocinia* aff. *bulbosa*. Its structure analogs and genes are significantly similar to the theopederins, onnamides, and pederins [46, 63]. These findings show the importance of polyketide biosynthesis of prokaryotic symbionts.

12.5 Future Perspectives

Sponge- and coral-associated symbionts can produce diverse group of secondary metabolites, but its biosynthesis process was not explored fully. In recent decade, due to the revolution of genome mining and other molecular techniques, the marine natural product biosynthesis was revealed. In the future, direct cloning and heterologous expression of large biosynthetic pathways will lead to the next level of biosynthesis study. Recently, several studies showed the heterologous expression of biosynthetic gene cluster of actinomycetes [64, 65]. The discovery of biosynthetic potential of marine microbes will be the new era in pharmaceutical industry. Only less reports are available on biosynthetic potential of marine microbes, in the case of sponge- and coral-associated microbes. Hence, the researchers should turn their attention toward the biosynthesis of antibiotics from sponge- and coral-associated microbes.

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