Chapter 17
Mass Production of Natural Products from Microbes Derived from Sponges and Corals

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Abstract Lack of sufficient pure natural compounds hinders further drug developments. The optimization of fermentation conditions is essential to enhance the yield of metabolites. Microbial genome analysis reveals the presence of a large number of cryptic biosynthetic gene clusters, and different strategies are there to trigger these gene pathways for the extensive study of natural product chemistry. Hence, the advanced technologies play a crucial role to achieve efficient discovery and productivity of novel microbial bioactive compounds. This chapter provides an outline on the mass production of microbial natural products derived from marine sponges and corals.

Keywords Sponge · Corals · Microbes · Natural products · Mass production

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17.1 Introduction

Natural products are unique bioactive compounds, which led to the initiation of drug discovery [1]. Marine invertebrates are unexploited and significant resources in the marine environment to discover novel bioactive compounds. Marine sponges and corals harbor diverse microbial communities, such as actinobacteria, fungi, archaea, and viruses [2, 3]; their bioactive natural products are substantial in the pharmaceutical industries as antimicrobial, anticancer, and immunosuppressants [4]. The developments of innovative technologies have overcomed the hurdles for the discovery and characterization of microbial bioactive natural products. The mass production of microbial bioactive compounds is a significant aspect of achieving effective yield for structural elucidation, bioactivity studies, and pharmaceutical applications.

17.2 Cultured Microbes Derived from Sponges and Corals

17.2.1 Sponges

Sponges inhabit a range of marine and freshwater systems [5], which form a close association with phylogenetically diverse microorganisms [2, 3]. Moreover, the sponges have acquired symbiotic microbial flora through parental sponges, surrounding water, or from other sources [6–8]. Microorganisms derived from the marine sponges are best sources for bioactive natural products [4, 9]. Extensive research of the past two decades on sponge symbiotic microbial communities revealed their phylogenetic diversity and biogeography [10–12] and their vital role in host metabolism and health [13–15].

The cultured actinomycetes derived from the marine sponges are *Dietzia*, *Rhodococcus*, *Streptomyces*, *Salinispora*, *Marinophilus*, *Solwaraspora*, *Salinibacterium*, *Aeromicrobium marinum*, *Williamsia maris*, and *Verrucosispora* [12, 16]. Morphological variants of actinobacteria were isolated from the marine sponge *Haliclona* sp., in the South China Sea, e.g., *Streptomyces*, *Nocardiopsis*, *Micromonospora*, and *Verrucosispora* [17]. Moreover, the marine sponge-associated actinomycetes, like *Rhodococcus* sp. RV157 (*Dysidea avara*) and *Micromonospora* sp. RV43 (*Aplysina aerophoba*), were isolated from Mediterranean sponges, and *Actinokineospora* sp. EG49 (*Spheciospongia vagabunda*) were isolated from the Red Sea sponge, as well as *Nocardiopsis* sp. SBT366 (*Chondrilla nucula*), *Streptomyces* sp. SBT343 (*Petrosia ficiformis*), *Geodermatophilus* sp. SBT350 (*Chondrilla nucula*), *Streptomyces* sp. SBT345 (*Agelas oroides*), *Streptomyces* sp. SBT346 (*Petrosia ficiformis*), and *Micromonospora* sp. SBT373 (*Chondrilla nucula*) [18]. Diversity analysis of cultural actinomycetes associated with 8 species of marine sponges reported the 13 genera, including 5 genera as the first records belong to the 10 families and order *Actinomycetales* from the South China Sea and

the Yellow Sea [16]. 180 actinomycete strains including at least 14 new phylotypes within the genera *Micromonospora*, *Verrucosispora*, *Streptomyces*, *Salinispora*, *Solwaraspora*, *Microbacterium*, and *Cellulosimicrobium* were isolated from the Caribbean sponge and sediment samples [19]. The actinomycetes isolated from 15 species of sponges in the South China Sea consisted of 20 genera of 12 families, including the 3 rare genera, such as *Marihabitans*, *Polymorphospora*, and *Streptomonospora* [12]. The marine sponge *Mycale* sp. derived bacterial strains isolation reported from the genera *Actinobacteria*, *Bacteroidetes*, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Firmicutes* [20]. Particularly, 14 new actinobacterial strains were isolated from 3 Mediterranean sponges [21].

Ascomycetous fungi, such as *Sordariomycetes*, *Dothideomycetes*, and *Eurotiomycetes*, are highly dominated in marine sponges [22]. Most of the marine sponges harbored some quite common fungal genera, such as *Acremonium*, *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, and *Trichoderma* [23, 24], and few rare genera, such as *Botryosphaeria*, *Epicoccum*, *Paraphaeosphaeria*, and *Tritirachium* [25]. Besides, fungal strains belonging to *Bartalinia* and *Volutella* from *Tethya aurantium* and *Schizophyllum*, *Sporidiobolus*, *Bjerkandera* (*Basidiomycota*), and *Yarrowia* (*Ascomycota*) were isolated from marine sponges [24, 26]. Cultured fungal strains from 10 species of marine sponges in the South China Sea belonged to the predominant genera, viz., *Aspergillus*, *Penicillium*, and *Volutella* and the others, such as *Ascomycete*, *Fusarium*, *Isaria*, *Plectosphaerella*, *Pseudonectria*, *Simplicillum*, and *Trichoderma* [27].

17.2.2 Corals

Corals are sessile marine invertebrates belonging to the phylum *Cnidaria*, living in the compact colonies of many identical individual polyps. Corals are categorized into stony and soft corals. Stony corals are mainly reef-building scleractinian corals, and soft corals include a range of species, like gorgonians and sea pens in the subclass of Alcyonaria or Octocorallia [28]. Corals involve a mutually beneficial symbiosis with photosynthetic dinoflagellate algae *Symbiodinium*. The dynamic relationship between the corals and microorganisms plays a significant role in the coral health [29–34]. Microorganisms associated with corals influence the coral host physiology as well as coral reef ecosystem, like pathogen resistance and biogeochemical cycling of critical nutrients [28, 31]. Fewer reports are available on the isolation of coral-associated microorganisms through the culture-dependent methods [35], whereas the culture-independent studies have revealed the diverse microflora associated with corals [36–44].

Green sulfur bacteria, such as *Alphaproteobacteria*, *Firmicutes* and *Planctomycetales* (*Montastraea annularis*), and *Gammaproteobacteria* and *Betaproteobacteria* (*M. cavernosa*), have been detected in corals [34], and *Alphaproteobacteria* and *Bacteroidetes* were found in the soft coral *Dendronephthya* sp. [36]. Predominant bacterial strains belonging to *Gamma-*, *Alpha-*, and

Betaproteobacteria, *Bacteroidetes*, *Firmicutes*, *Actinomycetales*, *Planctomycetes*, and *Chlorobi* were found to be associated with soft coral *Alcyonium antarcticum* [37]. Five new actinobacterial genera of *Cellulomonas*, *Dermacoccus*, *Gordonia*, *Serinicoccus*, and *Candidatus Microthrix* along with 19 common actinobacterial genera were reported from soft coral *Alcyonium gracllimum* and stony coral *Tubastraea coccinea* in the East China Sea [38].

Culture enrichment aided in the isolation of higher ascomycetes and basidiomycetes fungal taxa from the coral skeletons [39]. Cultured fungi belonging to genera of *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Microsphaeropsis*, *Paecilomyces*, *Phoma*, *Tilletiopsis*, *Gibberella*, *Isaria*, *Acremonium*, *Debaryomyces*, *Myrmecridium*, and *Nigrospora* were isolated from six species of gorgonians from the South China Sea [45]. Fungi associated with coral *Porites pukoensis* have been isolated, with *Aspergillus* being predominant, and the others consisted of *Penicillium*, *Cochliobolus*, *Acremonium*, *Rigidoporus*, *Gibberella*, *Eutypella*, *Didymellaceae*, and *Curvularia* [46]. To date, fungal spatial and functional relationship with corals is still poorly understood, and very few researchers have broadly explored the fungi associated with soft corals to isolate novel biologically active compounds [47].

17.3 Natural Products from Microbes Derived from Sponges and Corals

The discovery of microbes associated with marine sponges and corals has led to their intense exploitation for an untapped resource of the novel bioactive compounds, for example, polyketides, terpenoids, alkaloids, and non-ribosomal peptides [48–50], which might be ample candidates for the invention of new drug leads for cancer, infectious diseases, and lipid metabolic disorders or as immunosuppressants. Marine *Actinobacteria*, e.g., *Streptomyces*, *Micromonospora*, *Microthrix parvicella*, and *Acidimicrobium*, and particularly obligate marine actinomycetes, *Salinispora tropica* and *Salinispora arenicola*, are the producers of bioactive microbial metabolites [51–53]. Marine sponge-derived fungi, especially endophytic, produce the most of marine natural products among the marine fungi [54]. Some metabolites isolated from microorganisms associated with sponges and corals are summarized in Table 17.1.

Table 17.1 Some metabolites isolated from the sponge- and coral-associated microorganisms [28, 55]

Host	Microorganism	Family	Compounds	Reference
Sponge				
Bacteria:				
Petrosia ficiformis	*Streptomyces* sp. SBT348	*Actinobacteria*	Petrocidin A	[56]
			2,3-Dihydroxybenzoic acid	
			2,3-Dihydroxybenzamide	
			Maltol	
Unidentified	*Brevibacillus* sp.		Ulbactins F and G	[57]
Dysidea tupha	*Streptomyces* sp. RV15	*Actinobacteria*	Naphthacene glycoside SF2446A2	[58]
Haliclona sp.	*Pseudomonas fluorescens* H40, H41	*Proteobacteria*	Diketopiperazine	[59]
	Pseudomonas aeruginosa H51			
Unidentified	*Nocardiopsis* sp. 13–33-15 & 13–12-13	*Actinobacteria*	1,6-Dihydroxyphenazine	[60]
			1,6-Dimethoxyphenazine	
Spheciospongia vagabunda	*Micrococcus* sp. EG45	*Actinobacteria*	Microluside A	[61]
Spongia officinalis	*Streptomyces* sp. MAPS15	*Actinobacteria*	2-Pyrrolidone	[62]
Xestospongia testudinaria	*Serratia marcescens* IBRL USM 84	*Proteobacteria*	Prodigiosin	[63]
Callyspongia spp.	*Pseudomonas* spp. RHLB 12	*Proteobacteria*	Chromophore compound	[64]
Haliclona oculata	*Bacillus licheniformis* T6-1	*Firmicutes*	Fluorophore compound	[64]
Halichondria panicea	*Streptomyces* sp. HB202	*Actinobacteria*	Streptophenazines G and K	[65]
Polymastia boletiformis, Axinella dissimilis, and Haliclona simulans	*Pseudovibrio* sp. W64, W69, W89, W74	*Proteobacteria*	Tropodithietic acid	[66]
Dysidea avara	*Nocardiopsis* sp. RV163	*Actinobacteria*	1,6-Dihydroxyphenazine (produced from co-culture)	[67]

(continued)

Table 17.1 (continued)

Host	Microorganism	Family	Compounds	Reference
Acanthostrongylophora ingens	*Micromonospora* sp. M42	*Actinobacteria*	Manzamine A	[68]
Haliclona simulans	*Streptomyces* sp. SM8	*Actinobacteria*	Kitamycins A and B	[69]
			Antimycins A2, A3, A7, A8, A11, and A17	
Spheciospongia vagabunda	*Actinokinespora* sp. EG49	*Actinobacteria*	Actinosporin A	[70]
Unidentified	*Kocuria palustris* F-276,310;	*Actinobacteria*	Kocurin	[15]
	Kocuria marina F-276,345			
	Micrococcus yunnanensis F-256, 446			
Haliclona simulans	*Bacillus subtilis* MMA7	*Firmicutes*	Subtilomycin	[71]
Aplysina aerophoba	*Micromonospora* sp. RV115	*Actinobacteria*	Diazepinomicin	[72]
Axinella polypoides	*Streptomyces axinellae* Pol001T	*Actinobacteria*	Tetromycins 1, 2, 3, 4, and B	[73]
Halichondria sp.	*Bacillus licheniformis* SAB1	*Firmicutes*	Indole	[74]
			3-Phenylpropionic acid	
			4,41-Oxybis(3-phenylpropionic acid)	
Aplysina polypoides	*Streptomyces* sp. 34	*Actinobacteria*	Valinomycin	[75]
Axinella aerophoba	*Streptomyces* sp. 22	*Actinobacteria*	Valinomycin	[75]
Tedania sp.	*Streptomyces* sp. 11	*Actinobacteria*	Staurosporine	[75]
Tethya sp.	*Streptomyces* sp. T03	*Actinobacteria*	Butenolide	[75]
Aplysina fistularis	*Streptomyces* sp. Hedaya48	*Actinobacteria*	Saadamycin	[76]
			5,7-Dimethoxy-4-pmethoxylphenylcoumarin	
Halichondria panicea	*Streptomyces* sp. HB202	*Actinobacteria*	Mayamycin	[77]
Dysidea arenaria	*Streptomyces rochei* MB037	*Actinobacteria*	Borrelidin, BC194	[78]

Fungi:				
Halichondria okadai	*Trichoderma harzianum* OUPS-111D-4	*Ascomycota*	Tandyukisins B–D	[79]
Hymeniacidon perleve	*Aspergillus versicolor* MF359	*Ascomycota*	5-Methoxydihydrosterigmatocystin	[80]
Unidentified	*Aspergillus sydowii* ZSDS1-F6	*Ascomycota*	(Z)-5-(Hydroxymethyl)-2-(60)- methylhept-20-en-20-yl)-phenol	[81]
Melophus sp.	*Penicillium* sp. FF001	*Ascomycota*	Citrinin	[82]
Axinella corrugata	*Penicillium* sp.	*Ascomycota*	Dipeptide cis-cyclo(leucyl-tyrosyl)	[83]
Xestospongia testudinaria	*Stachybotrys chartarum* MXH-X73	*Ascomycota*	Stachybotrin D	[84]
Xestospongia testudinaria	*Aspergillus* sp.	*Ascomycota*	(Z)-5-(Hydroxymethyl)- 2-(61 -methylhept-21 – en-21 -yl)phenol	[85]
			Aspergiterpenoid A	
			(−)-5-(Hydroxymethyl)- 2-(21,61, 61 -trimethyltetrahydro2H-pyran-2-yl)phenol	
			(−)-Sydonic acid	
Callyspongia sp.	*Epicoccum* sp. JJY40	*Ascomycota*	Pyronepolyene C-glucoside iso-D8646-2-6	[86]
Psammocinia sp.	*Aspergillus insuetus*	*Ascomycota*	Insuetolides A	[87]
			Strobilactone A	
			(E,E)-6-(60,70-Dihydroxy20,40-octadienoyl)-strobilactone A	
Unidentified	*Aspergillus clavatus* MFD15	*Ascomycota*	1H-1,2,4-Triazole-3-carboxaldehyde 5-methyl	[88]
Petrosia sp.	*Aspergillus versicolor*	*Ascomycota*	Averantin	[89]
			Nidurufin	
Unidentified	*Trichoderma* sp. 05FI48	*Ascomycota*	Trichoderins A, A1, and B	[90]

(continued)

Table 17.1 (continued)

Host	Microorganism	Family	Compounds	Reference
Phakellia fusca	*Pestalotiopsis maculans* 16F-12		Xylariterpenoids H, I, J, and K	[91]
Corals				
Muricella abnormaliz	*Aspergillus* sp.	*Ascomycota*	Penilumamides B–D	[92]
			22- *O* -(*N* -me-L-valyl)afl aquinolone B	
			22- *O* -(*N* me-L-valyl)-21-epi- afl aquinolone B	
			Afl aquinolones A and D	[93]
Sarcophyton sp.	*Eurotium rubrum*	*Ascomycota*	Eurothiocins A and B	[94]
Dichotella gemmacea	*Aspergillus* sp.	*Ascomycota*	Aspergilones A and B	[95]
Sarcophyton tortuosum	*Chondrostereum* sp.	*Ascomycota*	Chondrosterins F–H	[96]
Dichotella gemmacea	*Aspergillus* sp.	*Ascomycota*	17-Epinotoamides Q and M	[97]
			Cordyols D and E	
Sarcophyton sp.	*Pestalotiopsis* sp.	*Ascomycota*	(±)-Pestalachlorides C and D	[98]
Sarcophyton sp.	*Acrogenotheca elegans*		Phenylalanine derivative 4′-OMeasperphenamate	[99]
			Aspochalasin A1	
			Cytochalasin Z24	
Sarcophyton sp.	*Alternaria* sp.	*Ascomycota*	Tetrahydroaltersolanols C–F	[100]
			Dihydroaltersolanol A	
			Alterporriols N–R	

S. tortuosum	*Chondrostereum* sp.	*Ascomycota*	Chondrosterins A–E	[101]
Scleronephthya sp.	*Micromonospora* sp.		Jadomycin B	[102]
Cladiella sp.	*Aspergillus versicolor*	*Ascomycota*	Cottoquinazoline D	[103]
D. gemmacea	*Curvularia lunatus*	*Ascomycota*	Cochliomycins A–C	[104]
Annella sp.	*Aspergillus sydowii*	*Ascomycota*	Aspergillusenes A and B	[105]
			(+)-(7S)-7-O-Methylsydonic acid	
			Aspergillusones A and B	
D. gemmacea	*Aspergillus* sp.	*Ascomycota*	(+)-Methyl sydowate	[106]
			7-Deoxy-7,14-didehydrosydonic acid	
			7-Deoxy-7,8-didehydrosydonic acid	

17.4 Mass Production of Natural Products from Cultured Microbes Derived from Sponges and Corals

The microorganisms are able to synthesize a vast number of primary and secondary metabolites. However the quantities produced are very low for the industrial scale in the view of the industrial biotechnologists [107]; hence, the mass production efficiency of the microbial bioactive metabolites needs to be improved.

17.4.1 Fermentation Optimization

The optimization of fermentation condition depends on the type of microbial strain and target metabolite [56–58, 62, 82–85, 92], since the standard conditions may not favor the expression of a majority of microbial biosynthetic pathways [70, 108, 109]. The fermentation optimization includes fermentation method and production medium (carbon and nitrogen sources), along with the physical-chemical factors which include salt concentration, pH, temperature, agitation, aeration, incubation time, and competition/interaction between microorganisms [110–114].The solid substrates are widely used for mass production of fungal metabolites, but not much preferred for actinomycetes and bacteria [92, 115–117].

The traditional method of one parameter each a time for factorial optimization might not produce accurate results, so the statistical methods are helpful in this aspect. The widely used statistical tools for the optimization of critical factors of mass production culture conditions are Plackett-Burman (PB) design and response surface methodology [62, 112, 113]. The PB design method is useful to select the critical control factors through the evaluation of the relative importance of bioprocess culture conditions and nutrients on the biomass and metabolite yield in liquid culture. The variables include the medium components, e.g., carbon and nitrogen sources, pH, temperature, incubation time, inoculum concentration, agitation, and aeration [111–114]. Response surface methodology (RSM) is useful to elucidate the interaction of selected critical variables of the bioprocess medium and selection of optimized conditions for the enhanced production of biomass and metabolite yield.

Different factors may hinder or induce the rate of biosynthesis of a novel or known marine microbial natural product or biomass during the mass production. The production medium, physicochemical factors, fermentation conditions, and carbon and nitrogen sources influence the efficient mass production and recovery of microbial natural products [70, 111–113, 117, 118]. The ideal conditions for growth and biosynthesis of secondary metabolites are not indeed the same, and even each organism obliges contrarily. The physiological and chemical regulators vary with diverse microorganisms and different metabolic pathways. Therefore, the individual optimal zones are required to improve the qualitative and quantitative secondary metabolite production. For instance, effective yield of antitrypanosomal active metabolite was observed from ISP2 medium with calcium alginate beads [70]. The

sponge-associated fungus *Aspergillus carneus* was able to produce 3 new and 14 known compounds in the rice medium without sea salt than the rice medium with sea salt and modified Czapek medium [116]. Higher yield of (+)-terrein was achieved from the optimized mass production of *Aspergillus terreus* strain PF26 derived from a marine sponge than the un-optimized culture conditions [112]. Two new and one known lumazine peptides, along with a new cyclic pentapeptide, were isolated from the static, submerged fermentation of gorgonian-derived fungus *Aspergillus* sp. XS-20090B15. Further, L-methionine induced the isolation of new penilumamide B in comparison with traditional culture [93].

17.4.2 Efficient Finding and Preparation

Microorganisms are ubiquitous, and they thrive under different environmental conditions. Diverse habitats will influence different class of bioactive metabolites. Moreover, the production of microbial bioactive compounds will be affected by microbial strain selection, production mediums, fermentation conditions, microbial or chemical elicitors or inducers used, and the balance between biosynthesis and biotransformation during the mass production [70, 111–119].

A conventional method of natural product discovery depends on bioassay or chemotypes. Natural product discovery programs through traditional way are not supportive, time-consuming, laborious, and need more resources. Recent technologic advances have simplified the screening and efficient production of microbial bioactive natural products in addition to proposing the unique opportunity for re-establishment of microbial natural products as a more significant source of drug leads. The bacterial and fungal genome sequence information show the link between known natural products and the genes encoding their biosynthesis as analyzed by various software tools, such as antiSMASH, SMURF, CLUSEAN, ClustScan, and so on. Moreover, gene clusters and chemistry of the compounds progressively exploit to classify known natural products to discover new ones. Further, biosynthetic pathways responsible for the production of specific natural products enable a better understanding of mechanisms or interactions during the metabolite production under culture condition [120, 121].

The biosynthetic potential-based strain prioritization may help for natural product discovery, through pathway-specific probes [120] and high-throughput real-time PCR [121]. Moreover, the optimized mass production methods [94, 95, 112–117] and analytical approach of collective LC-MS and UV profile of each active extract help the systematic analysis, early de-replication, and screening with an LC-MS library to known or novel compounds [63–66, 122–125]. Comparative study has showed the utility of standard solvent partitioning (SSP) and accelerated solvent extraction methods (ASE) related to overall yields, solvent consumption, processing time, and chemical stability of both fractions [121]. In the past two decades, the excellent applications of combinatorial chemistry and high-throughput screening (HTS) technologies, genome sequencing, proteomics, metabolomics, and other

methods have changed the entire scenario of finding natural products and the ways of harnessing its intricacies [126].

17.4.3 Activating Silent Gene Cluster

There are an increased number of cryptic or orphan pathways discovered; they are new sources to mine novel bioactive natural products. The developments in our understanding of microbial genome sequence, cluster arrangements, and metabolic pathways, and growth conditions, help to improve the natural product yield. Complete genome sequencing and mining are an alternate approach for the exploration of known or novel microbial species to analyze their metabolic potential [127]; however, these biosynthetic pathways are sometimes silent [128] or rarely expressed under standard laboratory conditions [129].

The traditional screening method incudes the selection of indigenous strain, followed by strain improvements through a series of mutational selection for the enhanced growth and metabolite yield [130]. It was suggested that new environments led to discover new microbial species to isolate novel bioactive natural products [131]. Thus, cryptic biosynthetic gene clusters could be activated by changing the cultural conditions. The OSMAC (one strain many compounds) principle is to mine and discover the new bioactive compounds through different approaches [132, 133].

17.4.3.1 Microbial Co-culture

Microorganisms show an active interspecies interaction with each other for available nutrients, space, and other resources for their existence in natural environments. Besides, the interaction may be beneficial or detrimental; the coexistence may incur production of novel bioactive secondary metabolites [134]. Therefore, microbial coexistence under laboratory conditions may induce activation of cryptic biosynthetic gene clusters which led to the innovative prospects. The co-culture strategy helps us to study the interspecies interactions responsible for the production of novel compounds with diverse structure and distinct bioactivities, such as antimicrobial and anticancer compounds [135]. Besides, this strategy has other benefits in comparison with pure cultures, such as in finding novel compounds or enhancing the yield of biological molecule, increase in the growth rate, and better utilization of mixed substrates. For example, based on the investigations of interspecies metabolic diversity of sponge-derived *S. arenicola* and *S. pacifica*, the *S. pacifica* induced the production of new rifamycins O and W from *S. arenicola* and known rifamycins and saliniketals [136]. Three new and ten known compounds isolated from sponge-derived *Actinokineospora* sp. EG49 and *Nocardiopsis* sp. RV163 were the results of co-culture induced biosynthesis [67, 137]. A novel

keyicin, a poly-nitroglycosylated anthracycline, was produced by the co-culture of marine ascidian-associated *Micromonospora* sp. Strain WMMB235 and marine sponge-associated *Rhodococcus* sp. Strain WMMA185. The biosynthetic gene cluster analysis of both strains and sequencing results of keyicin BGC confirm that the compound is from the *Micromonospora* sp. [126]. Though many researchers have conducted experiments on co-culture and synergistic microbial interactions, via coax between two or more than two microorganisms, but in reality, the challenges and questions related to the methods are still unanswered [138, 139].

17.4.3.2 Epigenetic Regulators

Putative biosynthetic gene regulators for the production of bioactive secondary metabolites of particular interest have been proved to be unique in different ways from previously understood models of gene regulation. The epigenetic regulators act as a signaling molecule by the regulation of putative biosynthetic genes and induce a variety of responses in microbes, for example, N-acetyl-D-glucosamine (GlcNAc), suberoylanilide hydroxamic acid (SAHA), DNA methyltransferase inhibitor (5-AZA), proteasome inhibitor (Bortezomib), and sodium citrate. The N-acetyl-D-glucosamine-mediated elicitation toward three sponge-derived actinomycetes led to the induced production of 3-formylindole and guaymasol in *Micromonospora* sp. RV43, the siderophore bacillibactin, and surfactin antibiotic in *Rhodococcus* sp. RV157 and improved the production of minor metabolites, actinosporins E–H in *Actinokineospora* sp. EG49 [140].

The influence of SAHA on *Aspergillus terreus* strain PF26 associated with a marine sponge in the biosynthesis of (+)-terrein was investigated. The epigenetic modifier shows the higher impact on (+)-terrein production than the control by stimulating the biosynthesis of the precursor, 6-hydroxymellein [141]. Optimized precursor-directed mutasynthesis has produced higher yield of BC194, a derivative of borrelidin from the *Streptomyces rochei* MB037 derived from the marine sponge *Dysidea arenaria* [78]. Bortezomib, a protease inhibitor, has induced the production of new bergamotene derivatives (xylariterpenoids H–K) from *Pestalotiopsis maculans* 16F-12 derived from marine sponge [91].

17.4.3.3 Gene Engineering

Majority of microbial natural product biosynthetic gene clusters (BGCs), relatively under standard laboratory conditions, are either transcriptional silent or expressed at deficient level, so these are the significant challenges for the discovery of novel natural products [142]. Analysis of microbial genes responsible for the biosynthesis of secondary metabolites usually depends on gene knockout and heterologous expression. Hence, the BGC identification and manipulation are accessible from the complete genome sequencing [128, 143]. For this purpose, some sponge- and

coral-associated microorganisms are yet to be cultivated to study their true biosynthetic potential for microbial natural product discovery [130, 144].

The actinomycetes, especially the genus *Streptomyces*, harbor dozens of BGCs per genome [145]. Recently, advanced activation of cryptic or silent BGCs was carried out through the genetic approaches, such as either to unlock the suppression of BGC gene expression in the native hosts [146] or directly bypass the regulatory system by refactoring and reconstructing controlling elements in BGCs in the heterologous hosts [147–149]. Heterologous microbial hosts are an unusual choice, to bypass the task of removing introns and stitching genes by PCR to ensure the correct expression in the model hosts, such as *E. coli*, yeasts, and filamentous fungi [150]. Eukaryotic microorganisms have large and complex gene networks. The complexity and lack of understanding of the physiology of filamentous fungi, compared to bacteria, have delayed rapid development of these organisms as highly efficient hosts for homologous or heterologous gene expression [151].The fungal biosynthetic gene clusters mRNA processing will be complicated for heterologous gene expression.

17.5 Summary and Future Perspectives

The microorganisms associated with marine sponges and corals are the primary sources of marine bioactive natural products, which are least studied and under exploration for the discovery of novel drug leads. Marine microbial bioactive natural products, which are majorly from *Streptomyces* and filamentous fungi, include terpenoids, polyketides, alkaloids, non-ribosomal peptides, phenazines, indolocarbazoles, sterols, butenolides, and cytochalasins. Optimized mass production studies are helpful to achieve high yield of microbial bioactive compounds. Lack of sufficient yield of the pure natural compounds hinders the analysis, structural elucidation, biological activity assays, and further drug developments. So, to achieve a higher yield of the compounds, further developments are required for mass production studies as well as to reduce the labor and other requirements. These aspects are helpful for the upcoming researchers to take up further challenges to produce the novel bioactive marine microbial natural products with pharmaceutical development potentials, such as antimicrobials, antituberculosis, and anticancer compounds.

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