The Hidden Treasure: Marine Microbiome as Repository of Bioactive Compounds

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Abstract

Marine-associated microbiome is known as a hub for novel chemistry and biology by producing interesting pharmacophores. Thus, in the area of natural product drug discovery, contribution and attention toward marine natural product investigation is a growing trend. The rapid swift in exploring the sea for harvest untapped plethora of marine resources to investigate associated microorganisms such as bacteria, fungi, and cyanobacteria are facilitated by technological advances. This chapter discusses the importance of chemical diversity of the marine microbiome in the natural product drug discovery pipeline giving specific reported examples of promising marine-derived bioactive candidates, as well as intriguing strategies to ramp up the discovery of pharmacologically inspiring secondary metabolites out of the marine microbial biosynthesis process.

Keywords

Marine bacteria · Marine cyanobacteria · Marine fungi · Marine natural products · Secondary metabolites elicitation

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17.1 Introduction

Natural Products (NPs) continue to be one of the most inspiring sources for the development of new drugs due to their impressive chemical diversity and potent and selective biological activity. The structure of more than 450,000 NPs is available from a variety of different databases such as PubChem, REAXYS, ChEMBL, ZINC, NaprAlert, Natural Product Atlas, and SuperNatural II (Pereira 2019). The higher success rate of marine natural compounds (MNPs) (1 in 3500 MNPs against the industry-based average for synthetic compounds of (1 in 5–10,000 compounds) has led to the rejuvenation of interest in NP-like scaffolds for drug discovery campaigns. New approaches are required to combat the perceived disadvantages of NPs compared to synthetic drugs, such as the difficulty of access and supply, the complexity of NP chemistry and structure elucidation, and the slowness of working with NPs (Pereira and Aires-de-Sousa 2018).

MNP research is developing continuously with more new compounds added every year (Fig. 17.1). This rapid development in MNP discovery is associated with various technological advances (e.g., iChip, co-culture, OSMAC, and epigenetic manipulations) that have facilitated the exploration of this huge mine of chemical entities. To date, the global pharmaceutical pipeline from marine sources consists of thirteen approved drugs, ten of which are anticancer drugs (Table 17.1). Currently, there are about 23 marine natural products or antibody–drug conjugates in Phase I to Phase III clinical trials mainly in the area of cancer therapy (Jaspars et al. 2016; https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml). There are four marine natural products currently in Phase III clinical trials

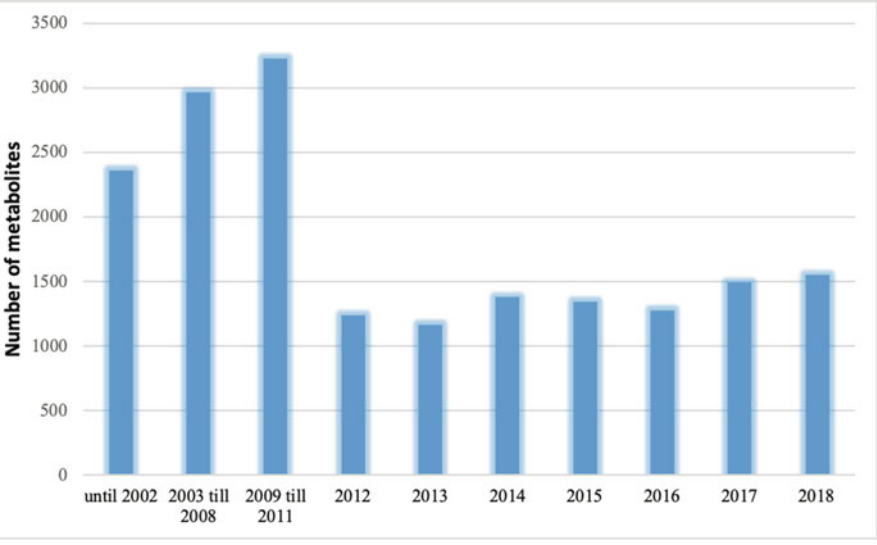


Fig. 17.1 Marine natural products discoveries till 2018 extracted from Marine Natural Products periodic reviews—Natural Product Reports—Royal Society of Chemistry

Table 17.1 Approved drugs from MNPs and derivatives (https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml)

Compound name	Marine organism	Chemical class	Molecular target	Disease associated
Lurbinectedin	Tunicate	Alkaloid	RNA Polymerase II	Cancer: metastatic small cell lung cancer
Belantamab Mafodotin-blmf	Mollusk/ cyanobacterium	ADC (MMAE)	BCMA	Relapsed/refractory multiple myeloma
Enfortumab vedotin	Mollusk/ cyanobacterium	ADC (MMAE)	Nectin-4	Cancer: metastatic urothelial cancer
Polatuzumab vedotin	Mollusk/ cyanobacterium	ADC (MMAE)	CD76b & microtubules	Cancer: non-Hodgkin lymphoma, chronic lymphocytic leukemia, lymphoma, B-cell lymphoma
Plitidepsin	Tunicate	Depsipeptide	eEF1A2	Cancer: multiple myeloma, leukemia, lymphoma
Trabectedin (ET-743)	Tunicate	Alkaloid	Minor groove of DNA	Cancer: soft tissue sarcoma and ovarian cancer
Brentuximab vedotin	Mollusk/ cyanobacterium	ADC (MMAE)	CD30 & microtubules	Cancer: anaplastic large T-cell systemic malignant lymphoma, Hodgkin's disease
Eribulin mesylate (E7389)	Sponge	Macrolide	Microtubules	Cancer: metastatic breast cancer
Omega-3-acid ethyl ester	Fish	Omega-3-fatty acids	Triglyceride-synthesizing enzymes (TSE)	Hypertriglyceridemia
Eicosapentaenoic acid ethyl ester	Fish	Omega-3-fatty acids	TSE	Hypertriglyceridemia
Omega-3-carboxylic acid	Fish	Omega-3-fatty acids	TSE	Hypertriglyceridemia
Ziconotide	Cone snail	Peptide	N-Type Ca channel	Pain: severe chronic pain
Vidarabine (Ara-A)	Sponge	Nucleoside	Viral DNA polymerase	Antiviral: Herpes simplex virus
Cytarabine (Ara-C)	Sponge	Nucleoside	DNA polymerase	Cancer: Leukemia

which include three anticancer compounds (plinabulin, lurbinectedin, and salinosporamide A) and one analgesic (tetrodotoxin). In phase II there are 12 MNPs and derivatives, from those three are small molecules: one anticancer (plocabulin) and two against Alzheimer's disease (bryostatin and DMXBA). All the remaining nine MNPs and their derivatives in the clinical trials (phase I) are recognized as anticancer antibody–drug conjugates.

It is worth noting that the availability of funding has had a great influence on the biological activity space of MNP, e.g., 10 out of the 13 approved MNPs drugs and 19 out of the 23 MNPs and derivatives in all clinical trial phases have anticancer activity. This is correlated with the National Institutes of Health (NIH)/National Cancer Institute (NCI) being the leading funding agency in the USA for MNP research over many years (Newman and Cragg 2016).

In this chapter, the importance of marine natural products from different sources with examples of potential bioactive molecules as well as advances in discovery strategies will be discussed.

17.2 The Current Status of Marine Microbe-Derived Drug Discovery

17.2.1 Marine Bacteria

Like other marine microorganisms, marine bacteria have evolved unique metabolic pathways that enable them to survive in harsh environments and to biosynthesize their own specialized metabolites that terrestrial bacteria may lack. The number of new metabolites originating from marine bacteria increased exponentially after the

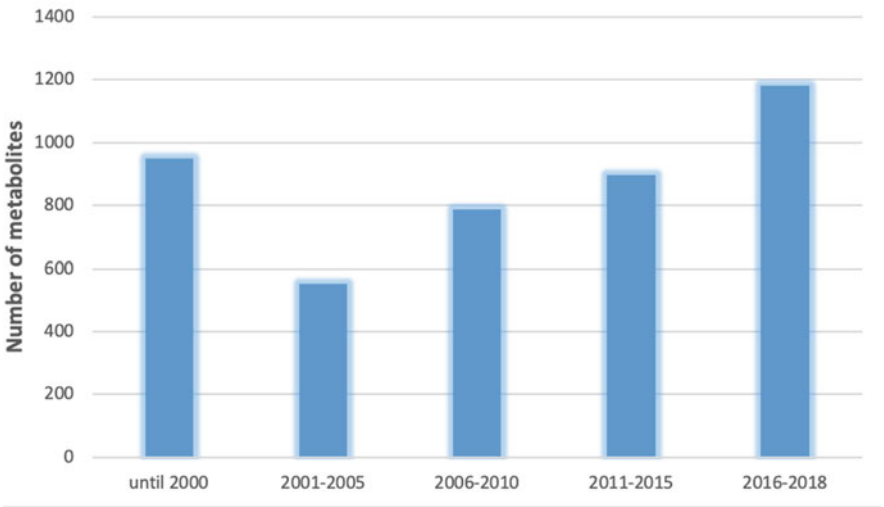


Fig. 17.2 Numbers of new marine bacteria-derived natural products from 2000 to 2018

year 2000 (Fig. 17.2) (Blunt et al. 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018; Carroll et al. 2019, 2020). Until the end of the year 2018, the continued interest in discovering novel bioactive compounds from marine bacteria led to the isolation of around 4500 metabolites, many of which showed promising broad-spectrum bioactivities, particularly anticancer and antimicrobial properties.

Marine actinomycetes are considered to be the major source of new chemical entities, notably *Streptomyces* that have accounted for about 38% of newly discovered marine bacteria-derived natural products. Marine bacteria are often isolated from marine sediments and from marine macro-organisms (e.g., sponges, corals, or algae) but also from extreme habitats such as the deep-sea and hypersaline lakes (Jones et al. 2019).

17.2.1.1 Early Discoveries of Marine Bacterial Natural Products

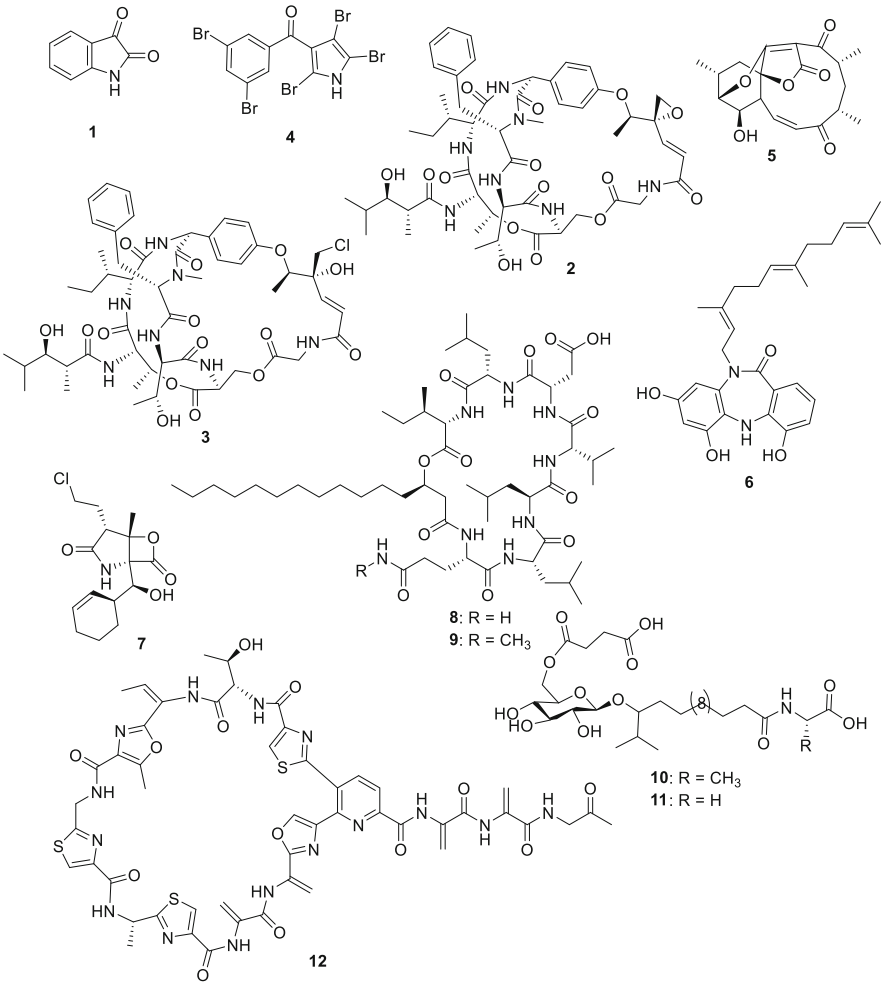
Isatin (**1**) was one of the earliest reported antimicrobial and anticancer metabolites. This compound is produced by several bacterial strains that colonize the surface of embryos of the shrimp *Palaemon macrodactylus*, which protects these embryos against the pathogenic fungus *Lagenidium callinectes* (Gil-Turnes et al. 1989). The *Alteromonas luteoviolaceus*-derived metabolite possesses broad-spectrum antimicrobial activity and its biosynthetic pathway has been fully elucidated (Laatsch 2017). The research interest in marine actinomycetes started to expand from the beginning of the 1990s when the unusual bicyclic depsipeptides salinamide A and B (**2** and **3**) were reported from a *Streptomyces* sp. that was isolated from the jellyfish *Cassiopeia xamachana*. Both depsipeptides exhibited moderate antibiotic activity but they had potent in vivo anti-inflammatory potential (Moore et al. 1999; Trischman et al. 1994). In 1994, the highly brominated pyrrole antibiotic pentabromopseudiline (**4**) was among the first reported marine bacteria-derived metabolites. The growing interest in marine actinomycetes as producers of bioactive compounds continued into this century and only in the period 2000–2002 nearly 250 new compounds have been reported from this group of microorganisms. During the same period, the number of reported new metabolites produced by terrestrial actinomycetes did not exceed 150 (Laatsch 2017). Although the exploration of marine actinomycetes as a source for new bioactive metabolites was at an early stage, numerous interesting compounds have been isolated during the period of 2000–2005. For example, the novel polycyclic polyketide antibiotic abyssomicin C (**5**) was reported from a marine *Verrucosispora* (Riedlinger et al. 2004). This unusual compound interferes with the biosynthesis of *p*-aminobenzoic acid and inhibits the biosynthesis of folic acid at an earlier stage than do the traditional sulfa drugs (Bister et al. 2004). As a result, abyssomicin C and its analogs showed antibacterial activity toward a broad spectrum of pathogenic bacteria including those that are multiple antibiotic resistant (Rath et al. 2005). Diazepinomicin (**6**) is another example of a unique farnesylated dibenzodiazepinone isolated from a marine-derived *Micromonospora* (Charan et al. 2004). This compound exhibits a wide spectrum of biological activities ranging from antibacterial to anticancer. In 2006, diazepinomicin (*aka* ECO-4601) has been submitted for clinical trials as an

anticancer agent for many types of tumors and has successfully completed phase 1 trials (https://go.drugbank.com). Furthermore, a novel β-lactone-γ-lactam metabolite, salinosporamide A (NPI-0052, **7**), was reported from a new obligate marine actinomycete, *Salinispora tropica* (Feling et al. 2003). This halogenated metabolite is an orally active proteasome inhibitor and can induce apoptosis in multiple myeloma cancer cells with a unique mode of action (Chauhan et al. 2005). In 2007, NPI-0052 (**7**) was submitted for clinical trials as an anticancer agent for multiple myeloma and its evolution through phase 2 clinical trials is ongoing at the time of writing of this chapter (https://clinicaltrials.gov/ct2/show/NCT00461045).

17.2.1.2 Recently Discovered Marine Bacterial Natural Products

With the advances in structural biology and fermentation processes marine bacteria have gained much attention in the fields of bioremediation and biotechnology (Andryukov et al. 2019). Moreover, extensive investigation of the biosynthetic pathways of marine-derived bacteria revealed that more than 70% of the secondary metabolites they produced were non-ribosomal peptides (NRPs), polyketides (PKs), and mixed PKS-NRPS (Pinu et al. 2017; Wang and Lei 2018). Most of these classes of metabolites have antimicrobial and anticancer potential. Terrestrial actinomycete-derived metabolites also produced a large number of NRPs and PKs but marine bacteria produce a greater chemical diversity of these molecules. Hence, the focus on exploring metabolic pathways of NRPs and PKs in marine bacteria, particularly in actinomycetes, has dramatically increased in recent years (Andryukov et al. 2019).

Over the last decade, lipopeptides were amongst the most frequently reported marine bacteria-derived metabolites with promising antimicrobial potential. Halobacillin (**8**) and methylhalobacillin (**9**) are examples of two cyclic lipopeptides obtained from bacteria isolated from deep-sea sediments (Zhou and Guo 2012). Both metabolites showed high efficacy against the growth of human colon tumor cells (IC_{50} 0.98 μg/mL) (Mondol et al. 2013). Polyketides are another large class of microbial natural products that have provided many successful pharmaceutical products. Marine bacteria have further extended the chemical space of polyketides with several novel compounds (Tareq et al. 2012). The antibiotics ieodoglucomides A and B (**10** and **11**) show broad-spectrum antibacterial activity (MIC ~8 μg/mL) and selective cytotoxicity against human lung cancer cells (IC_{50} 17 and 25 μg/mL, respectively). Both polyketides were recovered from the marine sediment-derived *Bacillus licheniformis* (Tareq et al. 2012). The antibiotic TP-1161 (**12**) is another thiopeptide-polyketide that was isolated from the marine actinomycete *Nocardiopsis* sp. (Engelhardt et al. 2010). This unusual metabolite shows in vitro antibacterial activity against a panel of Gram-positive bacteria (with MICs varying from 0.25 to 4 μg/mL). Additionally, TP-1161 is able to inhibit the growth of vancomycin-resistant bacterial strains, including *Enterococcus faecalis* and *Enterococcus faecium* at MIC = 1 μg/mL (Engelhardt et al. 2010).



17.2.2 Marine Fungi

Fungi form part of marine microbiological communities and are present as saprotrophs, parasites, or symbionts in all ecosystems. There is considerable interest in marine fungi due to the structural diversity of their natural products. Despite the discovery of several interesting bioactive compounds from marine fungi, the number of these products that were isolated has increased only slowly over a long period of time. To date, only ~1100 species of fungi have been described from marine environments, although estimates of the total number of fungal species range from 1.5 to 5 million (Jones et al. 2019). Researchers discovered that the same marine fungal species obtained from distant geographical locations produce different

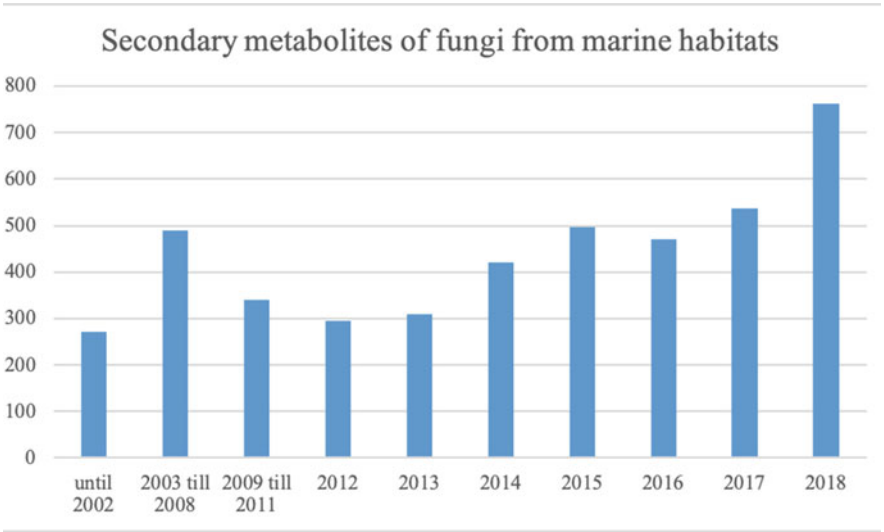


Fig. 17.3 Secondary metabolites discovered from marine fungi

metabolites. Until 2002, only 270 natural products were reported from marine fungi. Thereafter, this number increased and reached 1120 by the end of 2010 (Rateb and Ebel 2011). The discovery of more new marine fungal bioactive compounds continued to increase and in the year 2018 alone 760 new compounds reported (Fig. 17.3). This data indicates that by the end of 2018 the total number of newly discovered natural marine fungal products reached approximately 4400.

The broad-spectrum antibiotic cephalosporin C (**13**) has been known since 1955 and has been obtained from the fungus *Acremonium chrysogenum* that was isolated from seawater sampled near a sewage outfall of the Sardinian coast (Abraham 1979). The tubulin depolymerizing agent diketopiperazine halimide (**14**) was isolated from the fungus *Aspergillus* sp. (Fenical et al. 1998). This molecule was later used for the development of the closely related synthetic analog plinabulin (NPI-2358) (**15**) which is currently in the clinical trial phase. Plinabulin is the lead asset of BeyondSpring Pharmaceuticals and is currently in the late stage III clinical phase and its intended use will be to avoid chemotherapy-induced neutropenia in non-small-cell lung- and brain tumors (https://clinicaltrials.gov/ct2/results?term=plinabulin&pg=1).

The small contribution that marine fungi have made up-to-date to the discovery of new drug leads may be attributed to the fact that the chemical investigation of these microorganisms for the production of promising bioactive metabolites has been virtually neglected between 1980 and 1992. By the end of the 1980s, only 15 secondary metabolites had been reported as being derived from marine fungi (Bugni and Ireland 2004).

17.2.2.1 Anti-infective Marine Fungal Natural Products

Neoechinulin B (**16**), an indole prenylated diketopiperazine isolated from the marine fungus *Eurotium rubrum*, exhibits a strong inhibitory effect against the H1N1 virus in infected cells of the MDCK cell line and inhibited clinical isolates of amantadine-, oseltamivir-, and ribavirin-resistant influenza. The limited toxicity of Neoechinulin B, its wide-spectrum activity against drug-resistant clinical viral isolates, and decreased drug resistance induction made it a strong candidate for its possible use for the treatment of clinically resistant viral isolates (Chen et al. 2015). Chemical investigation of the gorgonian coral-derived fungus *Aspergillus terreus* SCSGAF0162 led to the isolation of the cyclic tetrapeptide asperterrestide A (**17**) that inhibits the replication of M2-resistant influenza strain A/WSN/33 H1N1 in MDCK cells (He et al. 2013). Screening of the *Stachybotrys chartarum* MXH-X73 marine sponge-associated fungus led to the isolation of phenylspirodrimane stachybotrin D (**18**), which inhibits HIV-1 replication by inhibiting reverse transcriptase without being toxic for humans. In addition, evaluation of phenylspirodrimane stachybotrin D revealed similar inhibitory effects on the replication of wild and multiple non-nucleoside reverse transcriptase inhibitor (NNRTI)-resistant HIV-1 strains to HIV-1 (Ma et al. 2013).

Chemical analysis of the marine-derived fungus *Stagonosporopsis cucurbitacearum* resulted in the isolation of the pyridone alkaloid didymellamide A (**19**), which showed promising antifungal activity against the azole-resistant and sensitive *Candida albicans*, *C. glabrata*, and *C. neoformans* (Haga et al. 2013). Sesquiterpene penicibilaene B (**20**), isolated from *Penicillium bilaiae* MA-267 recovered from mangrove rhizospheric soil, exhibited selective action against the plant pathogenic fungus *Colletotrichum gloeosporioides* (Meng et al. 2014).

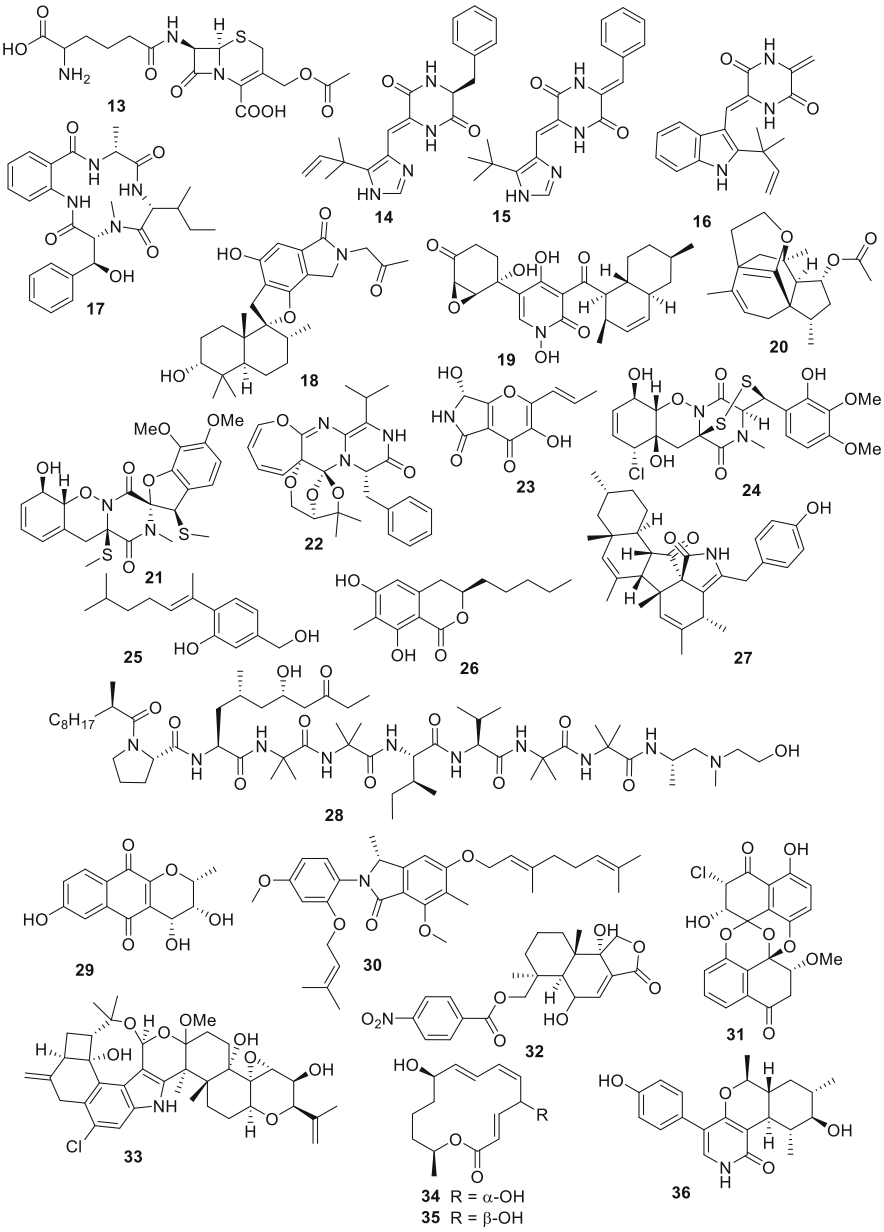
Chemical analysis of the sponge-derived fungus *Penicillium adametzioides* AS-53 resulted in the discovery of the peniciadametizine A derivative of dithiodiketopiperazine (**21**), which exhibited selective antifungal activity against plant pathogenic fungus *Alternaria brassicae* (Liu et al. 2015). The alkaloid varioxepine A (**22**) was isolated from the marine alga-derived endophytic fungus *Paecilomyces variotii* and showed a potent inhibitory effect against the plant pathogen *Fusarium graminearum* (Zhang et al. 2014).

The chemical characterization of *Penicillium brocae* MA-231 isolated from a mangrove plant resulted in the isolation of pyranonigrin A (**23**) with a clear antimicrobial activity against several Gram-positive and -negative pathogenic bacteria (Meng et al. 2015). The bisthiodiketopiperazine derivative adametizine A (**24**) isolated from *Penicillium adametzioides* AS-53, a marine sponge-derived fungus, showed strong inhibitory activity against *Staphylococcus aureus*, *Aeromonas hydrophila*, *Vibrio* spp. *V. harveyi*, and *V. parahaemolyticus* (Liu et al. 2015). Aspergillusene A (**25**), a sesquiterpene isolated from the sponge-associated fungus *Aspergillus sydowii* ZSDS1-F6 displayed promising antimicrobial activity against *Klebsiella pneumoniae* and *Aeromonas hydrophila* (Wang et al. 2014). The isocoumarin derivative penicisimpin A (**26**) isolated from the mangrove

plant-derived fungus *Penicillium simplicissimum* MA-332 exhibited strong activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *V. parahaemolyticus*, and *V. harveyi* (Xu et al. 2016). Diaporthalasin (**27**), a pentacyclic cytochalasin isolated from the marine-derived fungus *Diaporthaceae* sp. PSU-SP2/4 displayed strong antibacterial activity against both *S. aureus* and methicillin-resistant *S. aureus* (Khamthong et al. 2014). The aminolipopeptide trichoderin A (**28**) isolated from the marine sponge-derived *Trichoderma* sp. exhibited potent anti-mycobacterial activity against *Mycobacterium smegmatis*, *M. bovis* BCG, and *M. tuberculosis* H37Rv (Pruksakorn et al. 2010).

17.2.2.2 Anticancer Marine Fungal Natural Products

Diaporthalasin (**27**), a pentacyclic cytochalasin isolated from *Diaporthaceae* sp., a marine fungus (SP-SP2/4 PSU) demonstrated substantial antibacterial activity against both *S. aureus* and methicillin resistant *S. aureus* (Khamthong et al. 2014). Trichoderin A (**28**) aminolipopeptide was isolated from a marine sponge-derived *Trichoderma* sp. and showed potent anti-mycobacterial activity against *M. smegmatis*, *M. bovis* BCG, and *M. tuberculosis* H37Rv (Pruksakorn et al. 2010). Chemical investigation of the sponge-derived fungus *Stachylidium* sp. resulted in the isolation of a phthalimidine derivative mariline A1 (**30**) with potent inhibitory activity against the human leukocyte elastase (Almeida et al. 2012). Chloropreussomerin A (**31**) was the first chlorinated metabolite in the preussomerin family and was obtained from the fungus *Lasiodiplodia theobromae* ZJ-HQ1, an endophyte isolated from a mangrove plant. Chloropreussomerin A showed a potent in vitro cytotoxicity against several human cancer cell lines (Chen et al. 2016a). Chemical screening of the marine fungus *Aspergillus ochraceus* Jcma1F17 resulted in the isolation of a member of an unusual class of nitrobenzoyl sesquiterpenoid: 6β,9α-dihydroxy-14-p-nitrobenzoylcinnamolide (**32**), which exhibited strong cytotoxicity against 10 cancer cell lines (Fang et al. 2014). The discovery of 20, structurally diverse, complex indole-diterpene compounds resulted from genome mining of the fungus *Mucor irregularis* QEN-189, which was isolated from mangrove plants. Among them, rhizovarin B (**33**) showed good activity against the human A-549 and HL-60 cancer cell lines (Gao et al. 2016). A mangrove-derived endophytic fungus *Pestalotiopsis microspora* led to the isolation of the macrolides pestalotioprolides E (**34**) and F (**35**), which show strong cytotoxicity against the murine lymphoma cell line L5178Y while in addition, pestalotioprolide F shows potent activity against the human ovarian cancer cell line A2780 (Liu et al. 2016). Chaunolidone A (**36**), a pyridinone derivative isolated from the marine fungus *Chaunopycnis* sp. (CMB-MF028) showed selective and potent inhibition of human non-small-cell lung carcinoma cells (NCI-H460) (Shang et al. 2015).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17.2.3 Marine Cyanobacteria

Cyanobacteria are an ancient group of oxygenic phototrophs equipped with a wide range of cellular strategies, physiological capacities, as well as other adaptations that allow their global colonization of a wide range of habitats. They thrive under diverse and often extreme range of environmental conditions (e.g., in marine environments, hypersaline lakes, terrestrial environments, freshwater lakes, and thermal springs) (Kurmayer et al. 2016; Mazard et al. 2016; Whitton and Potts 2012). More than 90 genera of cyanobacteria produce compounds with potential bioactivities, most of which belong to the orders Oscillatoriales, Nostocales, Chroococcales, and Synechococcales. In terms of their molecular diversity and relative bioactivity, the majority of the cyanobacterial orders remain poorly explored. The metabolites of cyanobacteria with potential bioactivity belong to about 10 different chemical classes (Demay et al. 2019).

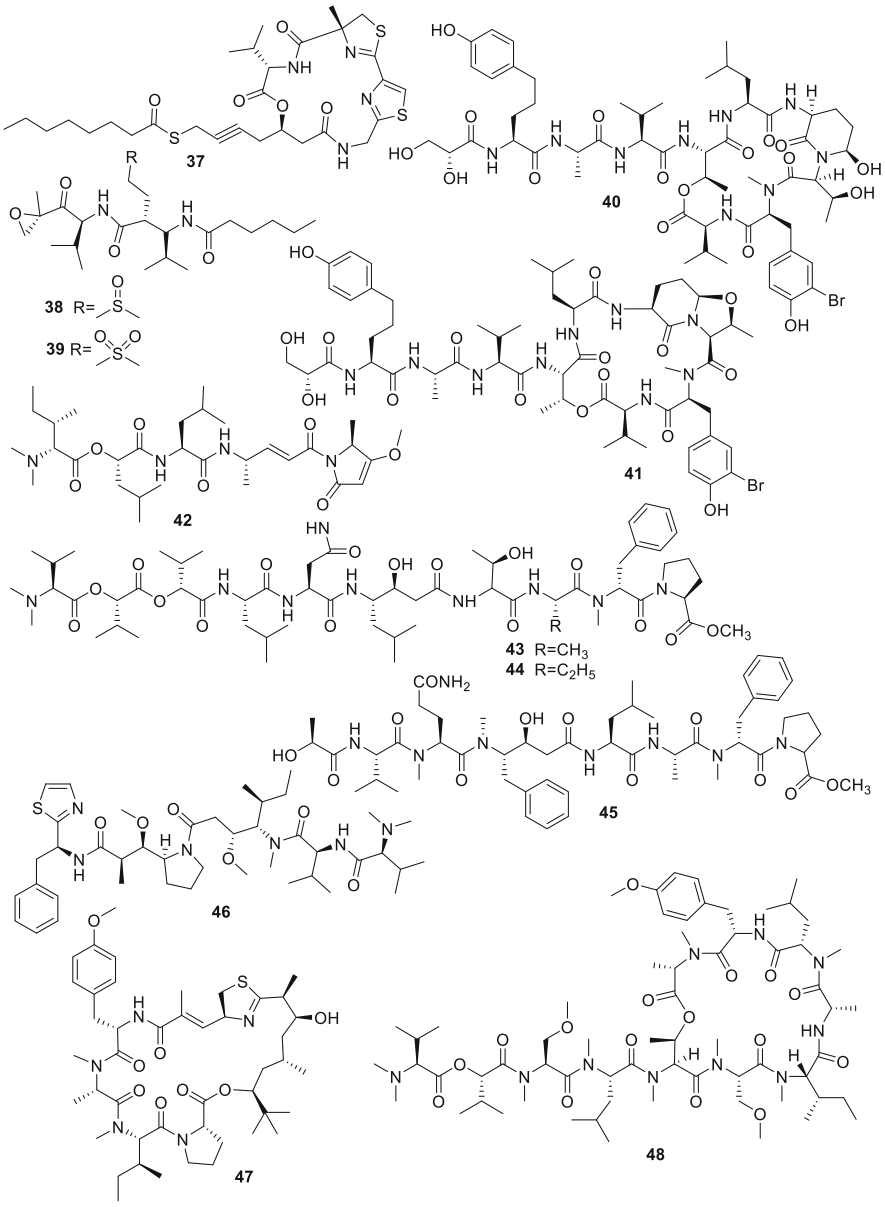
In the marine environment, cyanobacteria occur as free-living organisms but also live associated with a variety of hosts (e.g., fungi, ascidians, corals, and protists). Up to the end of 2019, about 550 secondary metabolites were reported from diverse genera of marine cyanobacteria such as *Lyngbya*, *Moorea*, *Symploca*, and *Oscillatoria*. The biosynthetic pathways of cyanobacteria show unusual mechanistic and enzymatic features that result in the production of bioactive compounds with a variety of chemical structures (Tan and Phyo 2020). Several pharmacological trends have been observed amongst the various marine cyanobacterial secondary metabolites. An important number of molecules possess either potent cytotoxic, neuromodulating, or anti-infective properties (Aráoz et al. 2010; Costa et al. 2012; Niedermeyer 2015; Rivas and Rojas 2019). These compounds show potency and selectivity against human drug targets, including cancer, inflammation, and neurodegenerative disorders. As such, these cyanobacterial secondary metabolites are considered prolific drug leads for drug discovery and development. For instance, cyanobacteria-derived compounds and their synthetic analogs have been reported as Antibody–Drug Conjugates (ADCs) and include dolastatin 10, auristatin E, and OKI-179. These ADCs have undergone or are currently undergoing clinical trials for the treatment of cancer diseases (Newman and Cragg 2014, 2017). Here we will discuss some of the mechanisms of the actions of ADCs.

Largazole (**37**), a cyclic depsipeptide, is a highly potent inhibitor of histone deacetylase (HDAC) class I, originally discovered as a secondary metabolite of the marine cyanobacterium *Symploca* sp. from Key Largo, USA. Largazole possesses a variety of unusual structural features, including the attachment of a 4-methylthiazoline unit to a thiazole and a 3-hydroxy-7-mercaptohept-4-enoic acid unit. In comparison with paclitaxel, actinomycin D and doxorubicin exhibited potent inhibition of growth of transformed human mammary epithelial cells (MDA-MB-231) with a GI_{50} of 7.7 nM and exhibited exquisite antiproliferative activity against transformed fibroblastic osteosarcoma U2OS (GI_{50} 55 nM) over non-transformed fibroblasts NIH3T3 (GI_{50} 480 nM) (Taori et al. 2008). Carmaphycins A (**38**) and B (**39**) are potent novel proteasome inhibitors isolated in low yield from organic extracts of *Symploca* sp. from Carmabi beach, Curaçao. They were evaluated against

Saccharomyces cerevisiae 20S proteasome and have comparable IC_{50} of ~2.5 nM. Such inhibitory effect is similar to that of epoxomicin and salinosporamide A (Pereira 2012).

The largamide D derivative (**41**) is generated by intramolecular largamide D condensation (**40**). This molecule, compared to largamide D, demonstrated an 11- and 33-fold decrease in activity against chymotrypsin and elastase, respectively. For serine protease inhibition, the Ahp moiety is necessary and any structural or conformational changes to this unit will influence the activity (Luo et al. 2016). Gallinamide A (**42**) is one of the most potent and selective marine cyanobacterial antimalarial compounds reported to date, with an EC_{50} of 74 nM when tested against *Plasmodium falciparum* strain 3D7. *Plasmodium falciparum*-infected red blood cells treated with nanomolar concentrations of (**42**) have a swollen food vacuole phenotype. Using fluorescent probes based on rhodamine fluorophore-tagged molecules, it was discovered that (**42**) is a specific inhibitor of plasmodial cysteine proteases, falcipains 2, 2′, and 3. Moreover, for antimalarial activity, the methoxypyrrolinone unit in gallinamide A is critical (Stolze et al. 2012).

Grassystatins A (**43**) and B (**44**) displayed potent inhibitory activity against aspartyl proteases cathepsins D and E with an average IC_{50} of 16.9 nM and 0.62 nM, respectively. Moreover, tasiamide B (**45**) is a statin-containing linear depsipeptide that displayed a potent activity against cathepsins D and E, with IC_{50} of 50 nM and 9.0 nM, respectively (Al-Awadhi et al. 2017; Kwan et al. 2009; Tan et al. 2013; Turk 2006). One of the earliest examples of potent microtubule inhibitors reported from marine cyanobacteria is the dolastatin class of molecules, especially dolastatin 10 (**46**) (Poncet 1999). The apratoxins are a novel class of potent cytotoxic cyclodepsipeptides reported from several *Lyngbya* spp. When tested against a panel of cancer cell lines, including HT29, HeLa, and U2OS, nanomolar concentrations of apratoxins had a major anticancer effect. A total of nine compounds associated with apratoxin A has been identified to date with apratoxin A (**47**) being the most cytotoxic. Further research on apratoxin has shown that this molecule has a strong antiangiogenic activity by inhibiting the activation of retinal endothelial cells and pericytes by mediating multiple angiogenic pathways (Chen et al. 2011). Coibamide A (**48**) is a structurally novel cyclic depsipeptide with potent antiproliferative properties reported from a marine cyanobacterium of Panama. Coibamide A showed strong cytotoxicity against NCI-H460 lung cancer cells and mouse neuro-2a cells, with an LC_{50} less than 23 nM. The compound was tested in the NCI 60-cell line and it exhibited activities against MDA-MB-231, LOX IMVI, HL-60(TB), and SNB-75 with IC_{50} of 2.8 nM, 7.4 nM, 7.4 nM, and 7.6 nM, respectively. Coibamide A specifically targets the trimeric Sec61 translocon's Sec61 alpha subunit. Binding of coibamide A to Sec61 resulted in the inhibition of substrate-non-selective ER protein import and conferred strong cytotoxicity against particular cancer cell lines (Hau et al. 2013; Serrill et al. 2016; Tranter et al. 2020; Wan 2018).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17.3 Emerging Strategies for the Exploration of Marine Bioactive Compounds

17.3.1 In-Situ Isolation Technology

Thousands of microbial species remain un- or underexplored for their capacity to produce bioactive secondary metabolites as they cannot be grown in synthetic media (Nichols et al. 2010). The remarkable gap between the microbial richness in the biosphere and their often estimated as less than 1% culturability under laboratory conditions has been coined as 'The Great Plate Count Anomaly' (Staley and Konopka 1985). Accessing this missing microbial diversity almost certainly would lead to the discovery of a hitherto untapped mine of novel bioactive compounds (Epstein 2013). Recently, there has been an increase in efforts to isolate extremophiles with the use of cutting-edge equipment aiming to enhance the culturability of rare microbes. Uncovering marine rare actinomycetes has been attempted by focusing on deep-sea sediments sampled by using specialized remotely operated underwater vehicles (Bredholdt et al. 2007; Fenical et al. 1999; Pathom-Aree et al. 2006). Despite these efforts to obtain sediments and other materials, laboratory studies are still challenging as the current strategies of altering the nutritional composition and other physicochemical factors mimicking the natural habitat are painstakingly slow, emphasizing the need for radically new strategies (Berdy et al. 2017).

Kaeberlein et al. (2002) introduced the idea of moving the culturing into the natural habitat which led to the development of the in situ iChip. The use of a diffusion chamber (Fig. 17.4, 1) allowed naturally occurring growth factors to diffuse into the synthetic growth medium improving culturability (Berdy et al. 2017; Kaeberlein et al. 2002). Diffusion chambers are equipped on both sides with a membrane with 20–30 nm pore size. Using the in-situ diffusion chamber technique, marine microbes from intertidal sediment were serially diluted, mixed with warm agar made with sea salt, and the inoculated agar was placed in the diffusion chamber leaving a thin layer of air between agar and the top membrane of the diffusion chamber. Finally, the incubated diffusion chambers were transferred to an aquarium in which the natural environment was simulated by filling it with sediments collected from tidal flat and placing the diffusion chamber on the surface of the sediment and filling thin layer of air with seawater (Kaeberlein et al. 2002). The semipermeable membrane allows the exchange of nutrients and other chemicals between cells and the environment but retains the cells in their confined space. This resulted in a 300-fold improved recovery of microorganisms compared to conventional Petri dishes (Berdy et al. 2017; Kaeberlein et al. 2002). Subsequent studies have demonstrated that one to several incubations in a diffusion chamber leads to an increase in the number and diversity of environmental isolates and the ability to grow them in vitro (Bollmann et al. 2007).

A miniaturized in situ diffusion chamber has been developed by Ben-Dov et al. (2009) using a double encapsulation method for in situ culturing of microorganisms (Fig. 17.4, 2). This method cultures microorganisms in droplets of agar which are subsequently encapsulated by a polysulfonic polymeric membrane (PPM) forming a

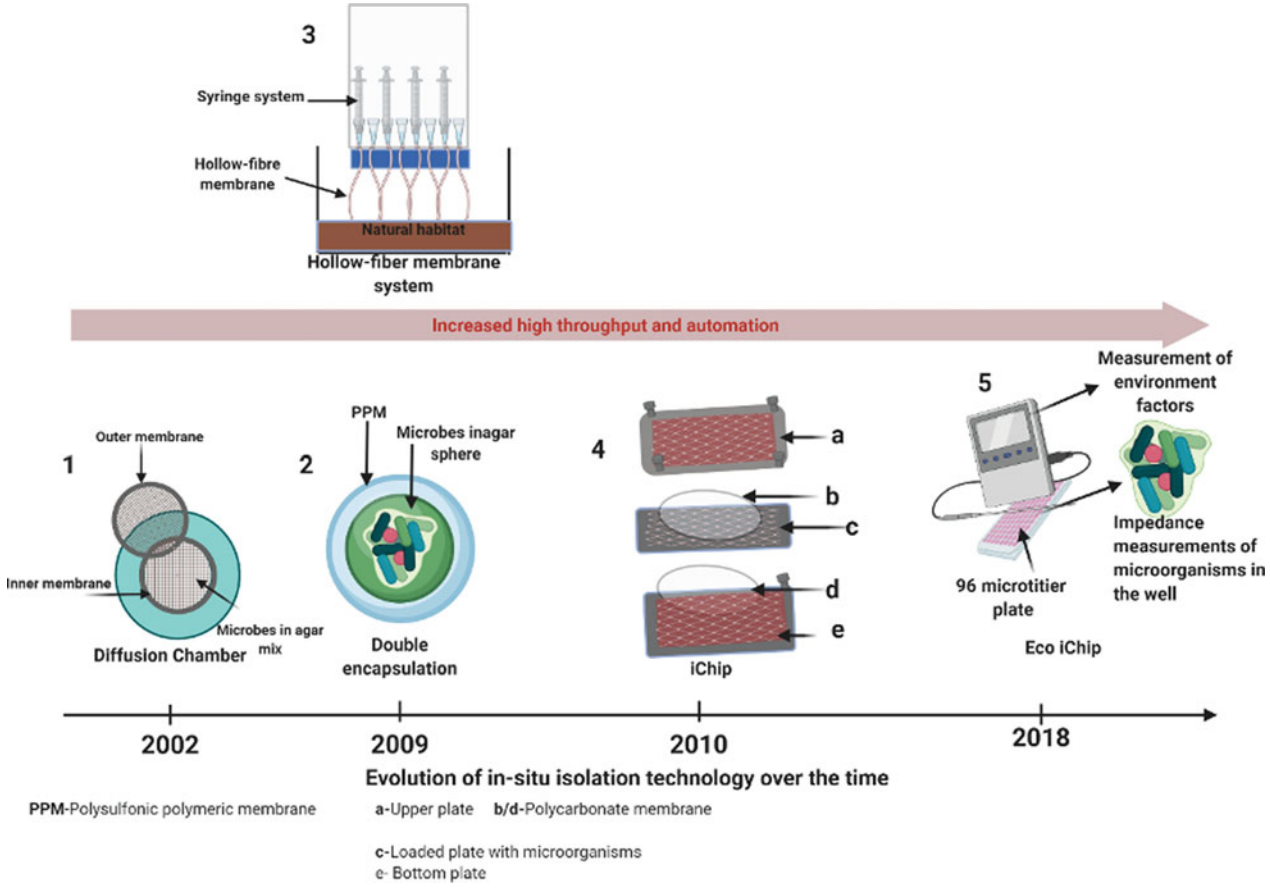


Fig. 17.4 Evolution of in situ isolation and high throughput over time (figure was produced by licensed biorender.com software)

bilayer surrounding the capsule containing the microorganism. These capsules are then incubated under simulated or in natural environments such as the mucous surface of fungia coral or sediment. After incubation until a few generations of growth of the microorganism they acquired the ability to grow in conventional laboratory setups, presumably as a result of gradual adaptation to the growth conditions (Ben-Dov et al. 2009).

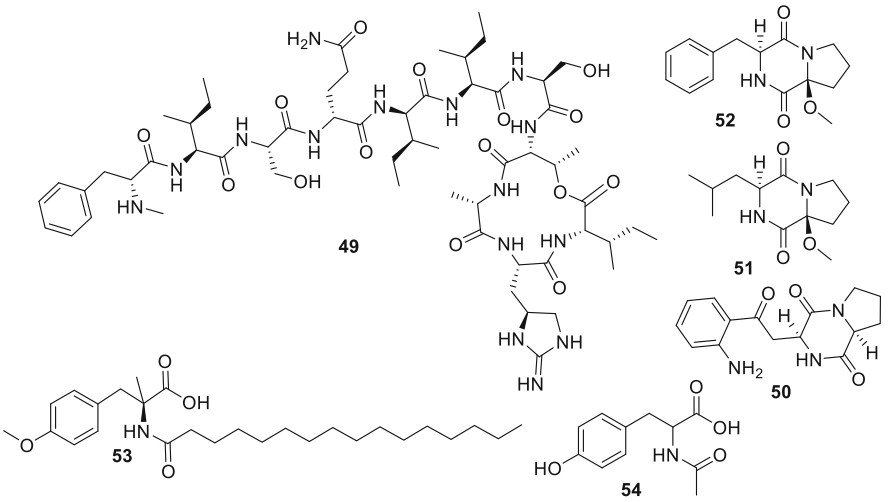
The hollow fiber membrane system is another invention that facilitates in situ isolation of microorganisms from simulated or natural environments (Aoi et al. 2009). The system consists of 48–96 hollow fiber chambers into which diluted environmental samples can be fed using syringes. One chamber unit consists of a porous hollow fiber polyvinylidene fluoride membrane (0.1 mm mean pore size, 67–70% porosity, 30 cm length, 1.2 mm outside diameter, 0.76 mm inside diameter) allowing an exchange of chemicals between microbes and the environment and also to exchange toxic by-products (Fig. 17.4, 3). The favorable feature of this system is the supply of various low concentration substrates, facilitating interspecies as well as intraspecies interactions through quorum sensing and other signaling pathways that are vital for many microorganisms for growth and survival (Aoi et al. 2009).

The above-mentioned diffusion chamber techniques for in situ isolation suffer from low throughput mainly because of the laborious isolation procedures needed for a single species. The diffusion chambers allow colonies containing different species to grow together, which limits their use for drug discovery (Berdy et al. 2017; Nichols et al. 2010). In order to improve the diffusion chamber technique, Nichols et al. (2010) invented the isolation chip or iChip (Fig. 17.4, 4). The iChip contains hundreds of miniature diffusion chambers each of which can accommodate a single microbial cell. During in situ incubation under simulated conditions or in a natural habitat, each miniature diffusion chamber allows the culturing of a single species in one step (Nichols et al. 2010). The iChip is a versatile concept that allows it to be applied in a variety of different situations such as in soil, in aquatic habitats, as well as for the human (and other) microbiomes (Berdy et al. 2017). Despite the high throughput nature of the modern in situ iChip concept, it still suffers from a few limitations which may require further modifications. Indeed, the isolation in one go of single colonies from environmental samples provides a non-laborious method for the isolation and identification of novel species. However, growing in isolation is not always ideal or even possible for some species as they may depend on symbiotic relationships or other types of association with other organisms. While the iChip seems to be an ideal device for an aquatic environment it works less well in a dry environment because the gel plaques in the micropores with microbes require moisture from its environment to prevent them from drying. Further modification may be possible when the microchambers are continuously supplied with water from the environment. Finally, long-term iChips fixed in position in an aquatic environment may run into anoxia. The thin layer of oxygen between sediment and the bottom surface of the iChip may represent an unnatural environment for the targeted microbes. It is therefore imperative to continuously aerate the water covering the sediment (Berdy et al. 2017).

Sylvain et al. (2018) developed the initial concept of the iChip of (Nichols et al. 2010) into an automated real time in situ microbial monitoring system called the Eco

iChip (Fig. 17.4, 5) allowing the real-time measurement of growth conditions at the different growth phases of the life cycle eliminating the drawbacks associated with traditional non-automated iChips. This will be an important approach to allow these previously uncultured microorganisms to be domesticated in the laboratory by the continuous supply of their natural environmental factors.

The first successful application of the iChip in microbial drug research was the discovery of the new antibiotic teixobactin (**49)** (Hunter 2015). Teixobactin was isolated from an extract of a new species of β-proteobacteria provisionally named *Eleftheria terrae*. Another novel bacterium *Gallaecimonas mangrovi* HK-28, isolated from mangrove sediments using an in situ iChip, revealed antibacterial activity against the marine pathogen *Vibrio harveyi*. *Gallaecimonas mangrovi* HK-28 produces three new diketopiperazines gallaecimonamides A−C (**50–53**) (Ding et al. 2020; Zhang et al. 2018). A novel antibacterial *N*-acyltyrosine (**54**) was found in a new species of *Alteromonas* sp. RKMC-009 following application of an in situ iChip in the marine sponge *Xestospongia muta* (Macintyre et al. 2019).



17.3.2 Microbial Co-culture

Culturing of two or more microbes as a strategy for the induction of new bioactive molecules has been applied since the first report of the serendipitous discovery of penicillin G (Fleming 1929). Growing two microbial species together has been repeatedly proven successful as a strategy of triggering silent secondary metabolite gene clusters (Rateb et al. 2013; Thissera et al. 2020). There are several theories that explain silent genetic perturbation by microbial co-culture in a synthetic environment (Seyedsayamdost et al. 2012). Co-existence in the natural environment comes with natural stresses such as competition for food and space or natural antagonism or

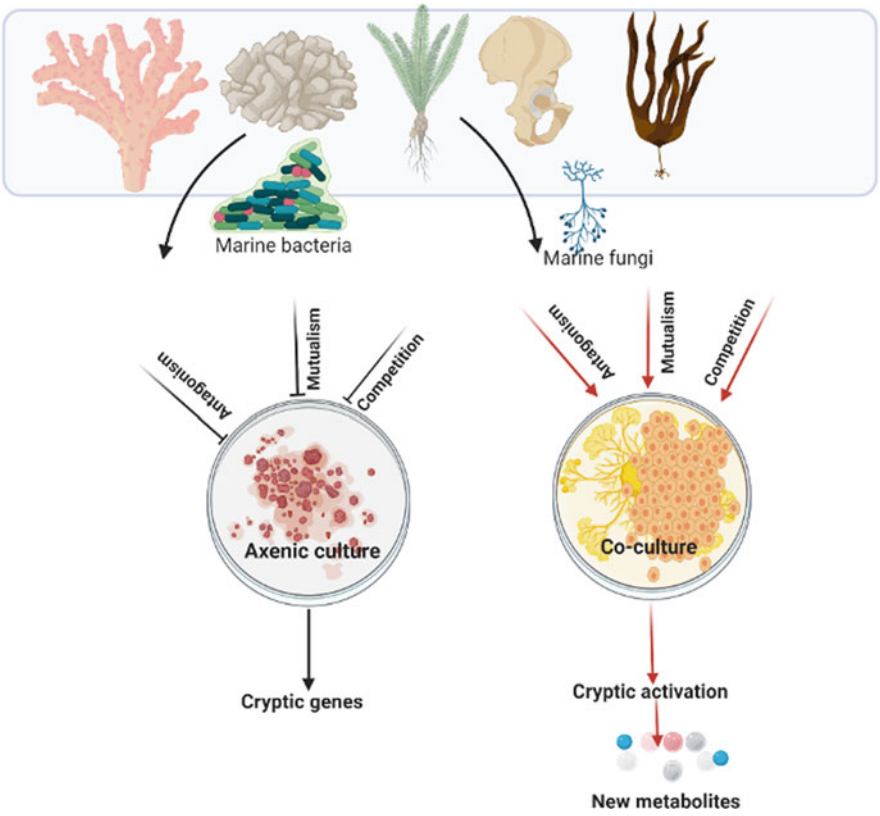


Fig. 17.5 Natural simulation of co-culture environment over the axenic environment (figure was produced by using licensed biorender.com software)

mutualism which may affect gene silencing (Fig. 17.5) (Akone et al. 2019; Reen et al. 2015; Shin et al. 2018). Triggers relieving gene silencing included tight physical intimacy of the microorganisms (Cueto et al. 2001; Schroeckh et al. 2009; Wakefield et al. 2017), exchange of signaling molecules (Shi et al. 2020), or horizontal gene transfer (Kurosawa et al. 2010). The latter is especially the case when a pathogenic species is co-cultured with a non-pathogenic organism. In this section, we will discuss the most recent developments in the use of co-culture strategies to make the drug discovery pipeline more efficient for marine natural products.

17.3.2.1 Marine Fungal-Bacterial Co-culture

The antibiotic agent pestalone (**55**) was reported as the first new secondary metabolite produced by a marine fungus *Pestalotia* sp., isolated from the surface of the brown alga *Rosenvingea* sp. that was collected in the Bahamas Islands and while being co-cultured with an unidentified marine bacterium (Cueto et al. 2001). Co-culturing of the marine sponge-derived actinomycete *S. rochei* MB037 with

the coral reef-derived fungus *Rhinocladiella similis* 35 induced two new antibacterial fatty acids with a rare nitrile group: borrelidins J (**56**) and K (**57**) (Yu et al. 2019). The importance of the co-culture strategy to obtain structural diversification of bioactive compounds was illustrated by the isolation of ten novel prenylated 2,5 diketopiperazines 12b-hydroxy-13a-ethoxyverruculogen TR **(58)**, 12b-hydroxy-13a-butoxyethoxyverruculogen TR-2 (**59**), hydrocycloprostatin A 9 **(60)**, hydrocycloprostatin B **(61)**, 25-hydroxyfumitremorgin B **(62)**, 12b-hydroxy-13a-butoxyethoxyfumitremorgin B **(63)**, 12b-hydroxy-13a-methoxyverruculogen **(64)**, 26a-hydroxyfumitremorgin A (**65**), 25-hydroxyfumitremorgin A (**66**), and diprostatin A (**67**) with potent BRD4 inhibitory action. These structurally diverse metabolites were produced following co-culturing of the marine fungus *Penicillium* sp. DT-F29 and the marine bacterium *Bacillus* sp. B31 (Yu et al. 2017).

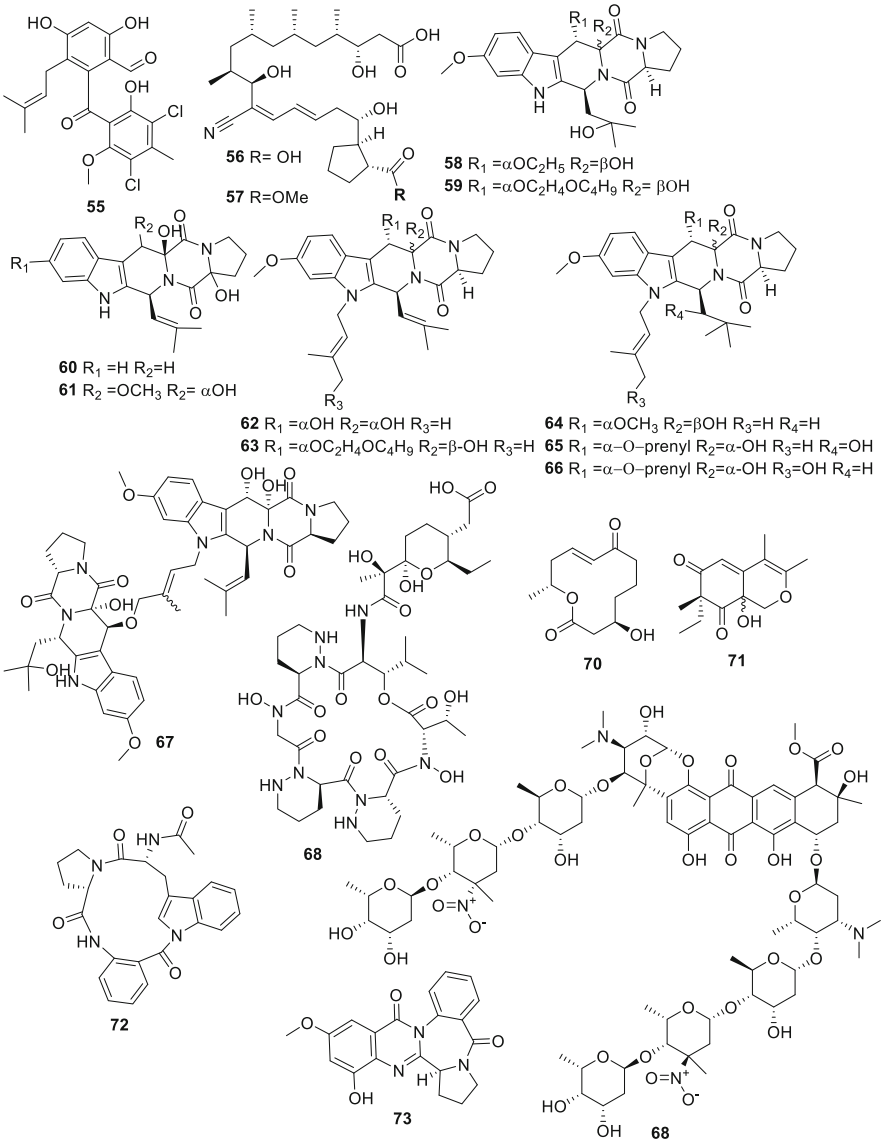
17.3.2.2 Co-culturing of Marine Bacteria

A new depsipeptide dentigerumycin E (**68**) with moderate anticancer and antimetastatic activity was produced in a co-culture of two marine bacteria, *Streptomyces* sp. and *Bacillus* sp., both isolated from an intertidal mud flat in Wando, Republic of Korea (Shin et al. 2018). The interspecies bacterial interactions of a set of marine invertebrates associated *Micromonosporaceae* were studied using an innovative co-culture platform. While these bacteria exuded secondary metabolites, they did not as axenic cultures (Adnani et al. 2015). This co-culture platform was high throughput compared to conventional Petri dish approaches and it revealed that 12 species out of 65 *Micromonosporaceae* excreted different secondary metabolites in different combinations of species. The co-culturing of a *Rhodococcus* sp. and a *Micromonospora* sp. is another noteworthy case in which silent genes were induced. This led to a new antibacterial bis-nitroglycosylated anthracycline: keyicin (**69**), that possesses selective antibacterial activity against Gram-positive bacteria including *Rhodococcus* sp. as well as *Mycobacterium* sp.

17.3.2.3 Co-culturing of Marine Fungi

Oppong-Danquah et al. (2020) used a systematic approach based on comparative metabolomics and bioactivity to select the best pair of fungi for co-culturing. Monoculture extracts of the marine fungi *Plenodomus influorescens*, *Penicillium bialowiezense*, *Sarocladium strictum*, *Helotiales* sp., two strains of *Pyrenochaeta* sp., and two strains of *Lentithecium* sp. were screened against a series of phytopathogens followed by ranking them through their antiphytopathogenic activities. Subsequently, species were paired as weak-weak, weak-strong, strong-strong on solid agar plates as the best visualizing modes for macro-interactions (Bertrand et al. 2013). All co-culture extracts were analyzed and compared with their monoculture extracts using metabolomics and screening for antiphytopathogenic activity in order to ascertain the deteriorated chemical profiles and enhanced bioactivities. *P. influorescens* (strong partner) with *Pyrenochaeta nobilis* (weak partner) was selected for a large-scale analysis. This resulted in the isolation of five polyketides of which dendrodolide N (**70**) and 8α-hydroxy-spiciferinone (**71**) were new (Oppong-Danquah et al. 2020). A co-culture of two fungi of the genus

Aspergillus (BM-05 and BM-05ML), isolated from a brown alga belonging to the genus *Sargassum* collected off the North Sea island Helgoland, produced a new cyclopeptide psychrophilin E (**72**), which has an antiproliferative effect against various cancer cell lines (Ebada et al. 2014). Two strains of *Aspergillus* sp., isolated from rotten fruit of the mangrove species *Avicennia marina*, produced a new antibacterial alkaloid aspergicin (**73**) during mixed fermentation (Zhu et al. 2011).



17.3.3 The OSMAC (One Strain Many Compounds) Approach

In terms of the production of bioactive compounds, the genomes of many promising marine microorganisms reveal a much larger number of biosynthetic gene clusters than are expressed under normal culture conditions, hence genetic manipulation may be required in order to have them expressed (Romano et al. 2018). Approaches such as gene knock-out (Wang et al. 2006), heterologous or homologous expression, pathway-specific activation of regulatory or promotor genes, require a full and precise knowledge of the specific functions of the genes (transcriptional/regulatory functions). In addition, much biological information about the targeted organisms is needed, requiring advanced analytical equipment and study of the data that is generated. Bioinformatics allows the prediction of tentative structures of specialized metabolites produced by silent genes although it sometimes leads to wrong conclusions (Kim et al. 2017; Rutledge and Challis 2015). An example of an incorrect prediction is that for the gene cluster responsible for the biosynthesis of the pyrrolamides, a family of secondary metabolites known as DNA minor groove binders. This gene cluster was earlier reported as the gene cluster responsible for producing congocidine (netropsin) in *Streptomyces ambofaciens*. However, later studies demonstrated that there are two distinct pyrrolamide-like gene clusters in *Streptomyces netropsis* DSM40486 working reciprocally to produce three pyrrolamides: distamycin, congocidine, and a hybridized congocidine-distamycin called disgocidine (Vingadassalon et al. 2015). Without the second study, pathway-specific activation of only the pyrrolamide gene cluster would not have revealed the production of the other two pyrrolamides, distamycin and disgocidine as they are coded in other pyrrolamide-like gene clusters (Vingadassalon et al. 2015). Such pitfalls can be avoided by the global alteration of microbial physiology (Romano et al. 2018).

Global alteration of microbial physiology which aims at the whole biosynthetic network without targeting a specific one or two biosynthetic pathways to trigger different chemical profiles from single species is termed OSMAC (One Strain Many Compounds) by Zeeck and co-workers (Bode et al. 2002). However, similar experiments in which the culture conditions were varied date back to 1975 (Okazaki et al. 1975). In OSMAC, systematical alteration of culture conditions such as media composition, aeration, salinity, culture vessels, and temperature result in altered chemical profiles leading to the discovery of new secondary metabolites. The altered chemical profile that is obtained as a result of culturing under different conditions is assumed to originate from the provision of different environmental simulations causing activation of different biosynthetic pathways without the need for genetic manipulation (Bode et al. 2002; Romano et al. 2018). Changing environmental factors influence the biosynthesis of secondary metabolites at different levels such as at the transcriptome and proteome level (Fig. 17.6).

17.3.3.1 OSMAC with Alteration of Food Source

The earliest example was reported by Okazaki et al. (1975) who used different nutrient conditions in order to trigger the production of the antibiotic SS-228 Y (**74**)

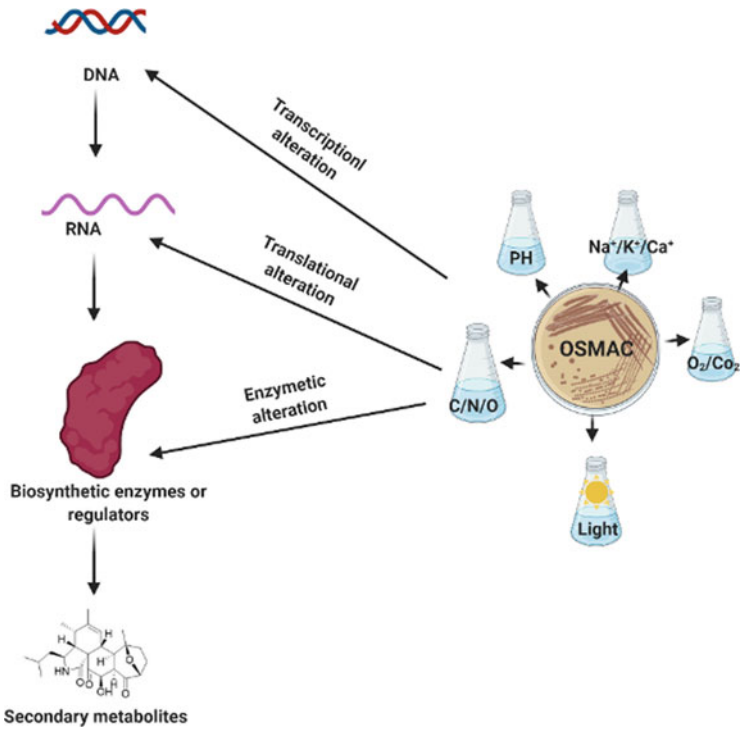


Fig. 17.6 Different levels of influences by OSMAC in the biosynthesis of secondary metabolites (figure was produced using licensed biorender.com software)

by the marine bacterium *Chainia purperogena* that was isolated from shallow sea mud. After a series of optimizations using different media components, only the medium containing Kobu-Cha (powdered Laminaria seaweed) produced the new antibiotic SS-228-Y. The marine fungus *Spicaria elegans* KLA03 produced a new polyketide eleganketal A (**75**), possessing a rare and highly oxygenated spiro [isobenzofuran-1,3′-isochroman] ring, when cultured in a modified mannitol-based medium (NH_4Cl as nitrogen source) (Luan et al. 2014). Growing the marine fungus *Scedosporium apiospermum* F41−1 in GPY medium boosted alkaloid production. Growth in GPY medium supplemented with L-tryptophan, L-phenylalanine, L-threonine, and L-methionine resulted in distinct chemical profiles and led to the isolation of 22 alkaloids, including 18 quinazoline-containing indole alkaloids, three formamides, and one isocyanide, as well as 14 new scedapins A−G (**76-82**) and scequinadolines A−G (**83-87**). Scedapin C (**88**) and scequinadolin D (**89**) have potential antiviral effects against hepatitis C (Huang et al. 2017).

17.3.3.2 OSMAC with Solid and Liquid Media

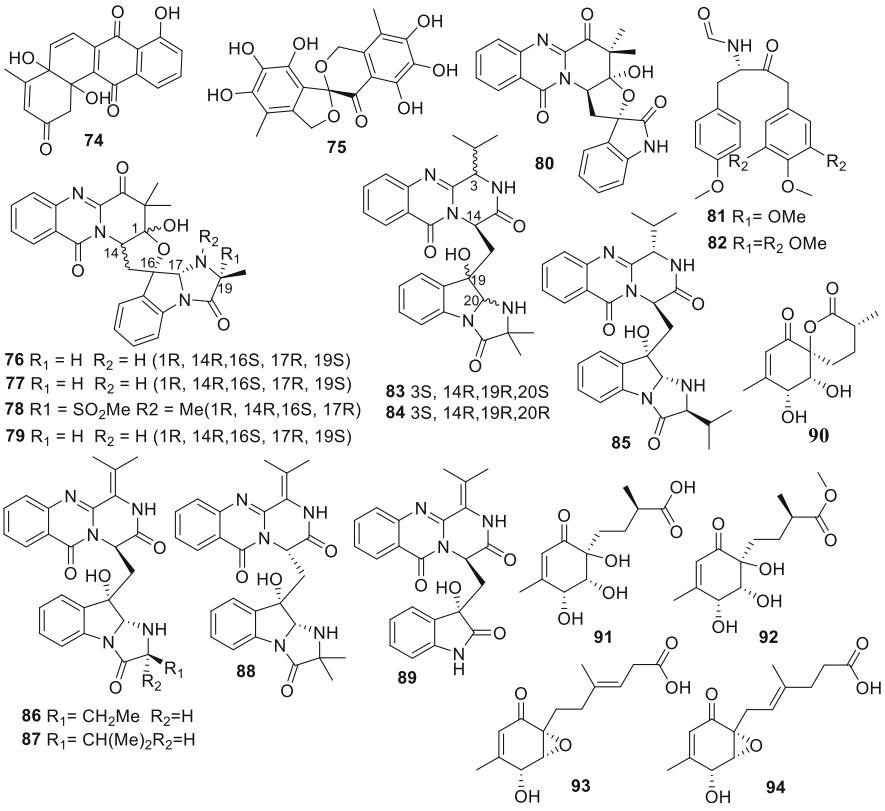
It has been shown that solidified media give rise to different metabolite profiles than their liquid version. Imanaka et al. (2010) showed that the use of a solid substrate for the fungus *Aspergillus oryzae* IAM 2706 led to a more diverse chemical profile than liquid shaking cultures with the same medium. Contrary to this work, Doran (2013) argued that the mobility of the nutrients, signaling molecules, oxygen, and foraging, are less well simulated in a solid medium compared to its liquid counterpart. However, the improved chemical profile may be understood as the lack of environmental stimulation that otherwise would produce these specialized metabolites in order to withstand environmental stresses. Another classical example of a possible chemical profile disparity and induction of new interesting metabolites when culturing was switched from liquid to solid medium is the marine *Penicillium* sp. F23-2. This fungus displayed an altered chemical profile when it was grown on a rice medium rather than in a liquid potato-based medium. Under these conditions *Penicillium* sp. F23-2 produced five new ambuic acid analogs: penicyclones A−E (**90-94**), with potential antibacterial activity against *Staphylococcus aureus* (Guo et al. 2015).

17.3.3.3 OSMAC with Changes in Physical Factors

Alteration of aeration, temperature, and pH have a strong effect on the production of microbial secondary metabolites. Aeration increases the metabolic rates and the conversion of the substrate(s) in the growth medium. This is usually achieved by shaking culture flasks or by bubbling air (Romano et al. 2018). The hypoxia-driven decrease in the production of the antibiotic napyradiomycin in the marine streptomycete strain CNQ-525 results in a spike of the intermediate 8-amino-flavioli. The conversion of 8-amino-flavioli into napyradiomycin is a redox reaction and a constant aeration is therefore important for the production and the yield of this antibiotic (Gallagher et al. 2018).

Considering the secondary metabolism of marine microbes, osmotic pressure, salinity, and pH are among the most relevant physical factors. For instance, habitats such as the deep sea, intertidal areas, shallow sea sediments, or mangroves all differ in osmotic pressure, salinity, and dissolved gases, and their microbiomes are adapted to that. Saha et al. (2005) showed the importance of mimicking the natural sea salinity conditions for the production of an antibacterial lipid from a marine-derived actinobacterium. Overy et al. (2017) published a comprehensive systematic study that confirmed the effect of osmotic pressure and salinity on the growth and production of secondary metabolites in fungi.

Hitherto, most studies involved tedious, laborious, and low throughput OSMAC applications in which one factor at time was optimized. Mathematical models are now used in order to avoid arduous and complicated optimization techniques during fermentation and improve the reproducibility of methods (Singh et al. 2017).



17.3.4 Chemical Elicitation

Additions to culture media were made in order to divert existing or induce new biochemical pathways in microorganisms. Alginate added to a culture of bifidobacteria (Akiyama et al. 1992) and chitosan to a culture of *Fusarium oxysporum* (El Ghaouth et al. 1994) enhanced growth and caused morphological and structural changes in the microorganisms. These studies indicated that small molecules reported as elicitors mainly served as signaling molecules (Eberhard et al. 1981; Nealson et al. 1970) or as epigenetic modulators (Shwab et al. 2007).

17.3.4.1 Quorum Sensing Elicitors

The chemical signaling network in Gram-negative bacteria is mainly mediated by AHLs (Acylated Homoserine Lactones) the release of which exhibits a population density-dependent regulation. The first report of an AHL involved in cell signaling was from the symbiotic marine bacterium *Vibrio fischeri* in which it induced

luciferase synthesis (Eberhard et al. 1981). Four out of 43 marine snow-derived bacteria including *α-Proteobacteria* and *Roseobacter* spp. produced AHLs that induced phenotypic traits such as biofilm formation, co-enzyme synthesis, and antibiotic production (Gram et al. 2002). The use of quorum sensing (QS) molecules produced by distantly related species to introduce antagonism by disrupting the native QS system by altering the gene transcription is an intriguing strategy for the induction of QS-controlled antagonistic secondary metabolites. In Gram-negative bacteria, native AHL receptors are activated by native AHL as well as non-native AHL molecules that possess slightly changed structures. Thus, the exogeneous addition of non-native AHLs might initiate new biosynthetic pathways by interfering with native QS networks. This phenomenon is demonstrated by the inhibition of bioluminescence of the marine bacterium *Vibrio harveyi* by two phenethylamine metabolites produced by the marine bacterium *Halobacillus salinus* by competing with 3-oxo-hexanoyl-homoserine lactone (OHHL) which accounts for bioluminescence in *Vibrio harveyi* (Teasdale et al. 2009). *N*-acetyl-D-glucosamine (GlcNAc), a component of peptidoglycan in the bacterial cell wall and of chitin in fungal cell wall, was released into the environment during cell repairing and may play a role as a signaling molecule (Dashti et al. 2017; Konopka 2012). Incorporation of GlcNAc in the culture media of the sponge-derived actinobacteria *Rhodococcus* sp. RV157 and *Actinokineospora* sp. EG49 demonstrated the chemical elicitation of new secondary metabolites. These include the induction of the new siderophore bacillibactin (**95**) and the surfactin antibiotic (**96**) from *Rhodococcus* sp. RV157, and the amplification of the new metabolites actinosporins E−H (**97–100**) from *Actinokineospora* sp. EG49 (Dashti et al. 2017).

17.3.4.2 Epigenetic Elicitation

Epigenetic elicitation or genetics-free manipulation allows upregulation of secondary metabolite pathways (Ganesan et al. 2019). Achieving epigenetic elicitation by means of small-molecule epigenetic modifiers is an efficient alternative for invasive genetic manipulations such as gene knockout, heterologous expression, homologous expression, and ribosome engineering. Epigenetics is the study of heritable phenotypes including secondary metabolites without the need to make changes to the DNA (Pfannenstiel and Keller 2019). In some instances, the biosynthetic gene cluster is not transcribed (so called "gene silencing") in eukaryotes, including fungi, because of the compact arrangement of the DNA strands around histones that form tightly packed nucleosomes. This is a reversible enzymatic process. The three main regulatory enzymes taking part in this process are histone acetyltransferases (HAT), histone deacetylases (HDAC), and DNA methyltransferase (DMT). These enzymes alter the nature of the packaging of DNA around histones by adding $–CH_3$ or –C (=O)–CH_3 (Ac) into DNA or histone tails. Incorporation of acyl moieties into histone tails driven by HAT, forming loosely packed chromatin (so called euchromatin regions), makes the conserved biosynthetic gene clusters (BGCs) available for

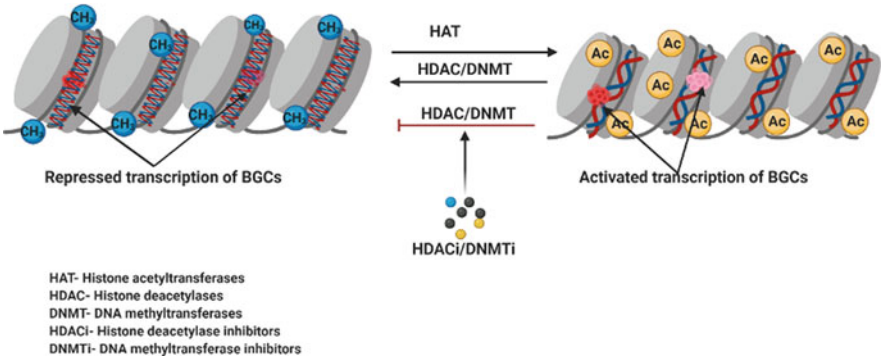


Fig. 17.7 Cryptic gene induction by epigenetic elicitors (figure was produced using licensed biorender.com software)

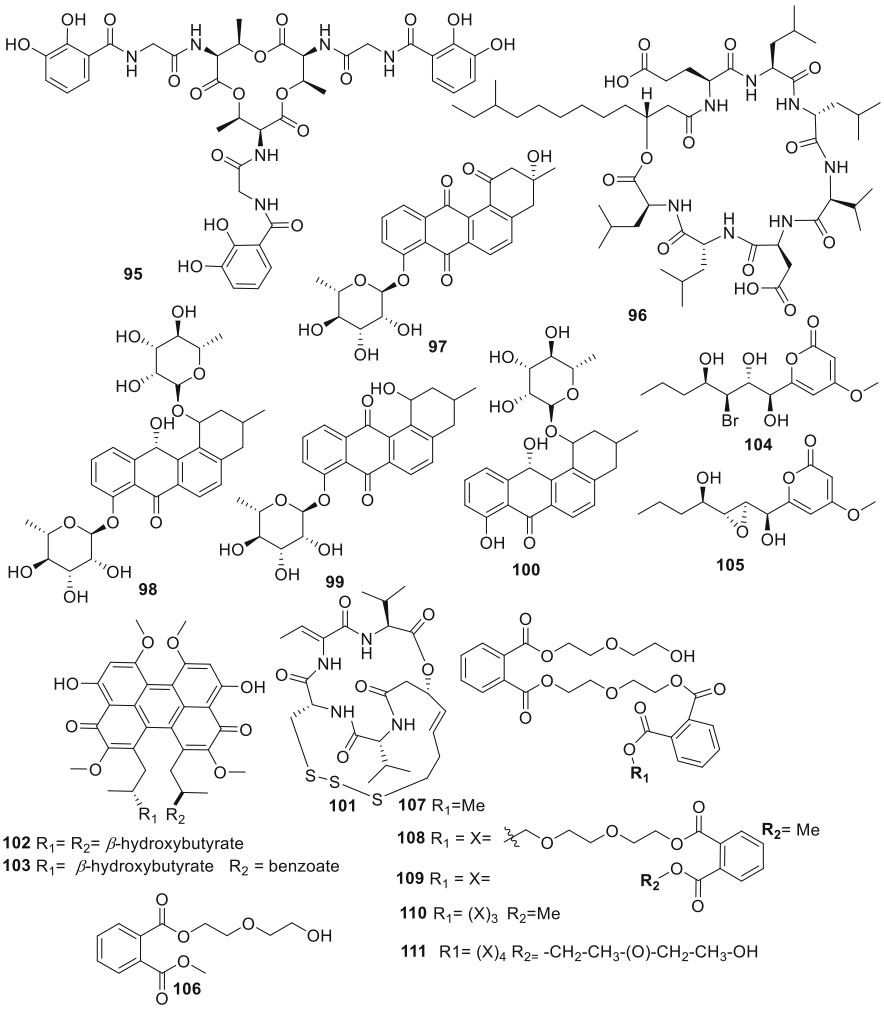
transcription. While the incorporation of $-CH_3$ into DNA or histone tails driven by respectively DNMT and HDAC does the opposite action of acyl moieties making the chromatin packaging denser and tighter, forming so-called heterochromatins leaving them more conserved and transcriptionally inactive (Ganesan et al. 2019; Jin et al. 2011; Pfannenstiel and Keller 2019) (Fig. 17.7). The reverse action of HAT is driven by HDAC re-producing heterochomatins showing the importance of HDAC inhibitors (HDACi) thereby triggering cryptic gene activation. The use of DNMT inhibitors (DNMTi) discourages the formation of heterochromatin (Mao et al. 2018; Pfannenstiel and Keller 2019; Ramadan et al. 2015).

HDACi are small molecules and beneficial for use in oncology as promising cancer therapies. Their ability to revert abnormal epigenetic features associated with many types of cancers made them popular as anticancer medication (Hull et al. 2016; Sanaei and Kavoosi 2019). In eukaryotes, HDACi are known to regulate gene expression at different levels such as transcription factor activity, miRNA expression, and signal transduction pathways (Alao 2004; Fournel et al. 2008; Hull et al. 2016; Romano et al. 2018). These findings encouraged researchers to use HDACi for the induction of cryptic pathways in fungi to discover the hidden genome and hence the discovery of new secondary metabolites. The first application of HDACi was an induction of new metabolites from *Alternaria alternata* and *Penicillium expansum* with HDACi and the antifungal antibiotic trichostatin A (Shwab et al. 2007). This introduced the use of HDACi inhibitors into microbial natural product chemistry. The main mechanism of cryptic induction by HDACi is by inhibiting the histone deacetylases leaving the euchromatins accessible for gene transcription and initiating new cryptic biosynthetic pathways (Janssens et al. 2019; Pfannenstiel and Keller 2019). The marine microbiome has been reported to be a profound resource for producing HDAC inhibitors which are currently being used as promising anticancer therapies such as chromopeptide A (**101**), a depsipeptide isolated from the marine

sediment-derived bacterium *Chromobacterium* sp. (Sun et al. 2017). Varghese et al. (2015) demonstrated the importance of marine actinomycetes for the production of HDACi. A study involving a variety of fungi, including several marine species, was conducted in order to assess the broad-spectrum elicitation effect on their chemical profiles by both HDACi and DNMTi (Williams et al. 2008). This demonstrated the importance of these molecules for cryptic induction. The study of Williams et al. (2008) showed that the marine fungus *Cladosporium cladosporioides*, treated with HDACi suberoylanilide hydroxamic acid, produced a complex series of perylenequinones, including the new metabolites cladochromes F (**102**) and G (**103**) along with four known cladochromes.

Basically, the mode of cryptic induction by DNMTi is by reversing the action of DNMT, which catalyzes the amalgamation of methyl groups from S-adenyl methionine to the fifth carbon of a cytosine residue to form 5-methylcytosine. Methylated DNA strands enhance the attractive forces with histones wrapping tightly around them making DNA transcriptionally quiescent (Moore et al. 2013). The marine fungus *Cochliobolus lunatus* treated with well-known DNMTi 5-azacytidine induced two new biologically active α-pyrones: cochliobopyrones A (**104**) and B (**105**) (Wu et al. 2019). In another study with the same species (strain TA26–46), the organism was cultured with exogenously incorporated 5-azacytidine. This study exemplifies the potential cryptic induction by producing seven new diethylene glycol phthalate esters: cochphthesters A −G (**106–111**) (Chen et al. 2016b).

The application of epigenetic elicitors in the cryptic induction of bacterial species was not encouraging due to the absence of highly organized DNA and histone complexes forming nucleosomes. This explains the failure of the epigenetic elicitation by HDACi and DNMTi. However, several studies have reported the possible epigenetic elicitation in bacterial cells by HDACi and DNMTi (Moore et al. 2012; Okada and Seyedsayamdost 2017). The identification of natural and synthetic elicitors at the microscale level, for instance, the ground-breaking invention of high throughput elicitor screening, (HiTES) (Seyedsayamdost 2014) and its recent advancement HiTES-IMS (Seyedsayamdost 2019) will be intriguing for future applications in the area of chemical elicitation.



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