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The current status of natural products from marine fungi and their potential as anti-infective agents

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Abstract A growing number of marine fungi are the sources of novel and potentially life-saving bioactive secondary metabolites. Here, we have discussed some of these novel antibacterial, antiviral, antiprotozoal compounds isolated from marine-derived fungi and their possible roles in disease eradication. We have also discussed the future commercial exploitation of these compounds for possible drug development using metabolic engineering and post-genomics approaches.

Keywords Marine natural products · Fungi · Metabolic engineering · Marine biotechnology

Introduction

The success stories in marine biotechnology are far fewer than for other commercial biotechnology. Marine biotechnology is defined by Zilinskas et al. [168] as “the application of scientific and engineering principles to the processing of materials by marine biological agents to provide goods and services”. In 1985, Colwell [30] wrote “There are several reasons for the lack of development in the area of marine pharmaceuticals...”. The difficulties of retrieving a “sustained, reliable” harvest of a marine organism, insufficient quantities of material to allow for study completion, and difficulties culturing marine

organism in the lab were cited. These problems often still exist today, two decades later.

The marine environment is a rich source of both biological and chemical diversity, where it has been reported that oceans contain nearly 300,000 described species, representing only a small percentage of the total number of species that have to be discovered [98, 129, 165]. The oceans comprise more than 70% of the Earth’s surface, and each drop of water taken from the ocean will contain microbial species unknown to humans in a 9:1 ratio [31]. The ocean represents a rich resource for ever more novel compounds with great potential as pharmaceutical, nutritional supplements, cosmetics, agrichemicals, and enzymes, where each of these marine bioproducts has a strong potential market value [48, 49, 77].

Almost all forms of life in the marine environment e.g.—algae, sponges, corals, ascidians have been investigated for their natural products content [46, 47]. A lot of structurally and pharmacologically important substances have been isolated with novel antimicrobial, antitumor and anti-inflammatory properties [19, 43, 106, 107, 136, 141]. As interests have turned to marine microorganisms, the fungi have begun to be recognized as a likely source of potentially useful natural products, following the very little attention they received from natural products chemists. According to Fenical and Jensen [50], only 15 metabolites were discovered throughout 1992. Recently marine fungi have proved to be a rich source of bioactive natural products [1, 5, 6, 22, 23, 64, 73, 75, 82, 91, 95, 117, 139, 152, 153, 157, 158, 163]. Most of these micro-organisms grow in a unique and extreme habitat and therefore they have the capability to produce unique and unusual secondary metabolites. It is believed that the metabolites possibly act as a chemical defence as an adaptation of fungi competing for substrates [50, 51]. According to Jensen and Fenical [69], the production of these unique secondary metabolites by marine fungi are possibly because of adaptation to a very distinct set of environmental pressures.

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Fungi growing in the sea can be grouped into obligate and facultative marine fungi [61]. Kohlmeyer [76] defined obligate marine fungi as those “that grow and sporulate exclusively in a marine or estuarine habitat; facultative marine are fungi from freshwater or terrestrial areas also able to grow in the natural marine environment”.

To date, more than 272 new compounds have been isolated from the marine fungi and the number of compounds is on the increase [22]. Marine fungi have proven to be a rich and promising source of novel anticancer, antibacterial, antiplasmodial, anti-inflammatory and antiviral agents [2, 38, 39, 49, 53, 66, 79, 85, 88, 127, 137, 149, 157]. Most of these metabolites are analogues of those discovered previously from terrestrial fungi [39].

Marine fungal-derived compounds such as sargassamide, halimide and avrainvillamide have shown selective inhibition of cancer cell lines, and shown *in vivo* activity in preclinical models (P-388 lymphocytic leukaemia) (<http://www.cancer.ucsd.edu/summaries/wfenical.asp>). Two of the above potential drugs have been licensed to the pharmaceutical industry and are in preclinical development (<http://www.cancer.ucsd.edu/summaries/wfenical.asp>). In addition, two classes of metabolites have been isolated from unidentified fungi obtained from the marine sponge *Jaspis*, known as the source of the cyclic peptide jaspamide [34]. The first class comprises chlorinated sesquiterpenes, chloriolins A, B and C [25], which are related to coriolin B and dihydrocoriolin C, previously isolated metabolites of the terrestrial wood-rotting fungus *Coriolus consors* [115]. Although the chloriolins were not found to be active in the disease-oriented screen of the National Cancer Institute (NCI), coriolin B is cytotoxic against the T-47D human breast and SNB-75 central nervous system tumor cell lines [39].

In this review, we elucidate the bioactive metabolites isolated from marine fungi that have shown *in vivo* or *in vitro* activity against bacterial, viral, protozoan and fungal infections. We also discuss the future scope and prospects of commercial biotechnological production of these natural products using metabolic engineering/systems biology approaches.

Antibacterial compounds from marine fungi

There is a need for the discovery and development of new classes of antibacterial compounds, due to recent trends in antibiotic resistance among different strains of bacteria (e.g. methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*), which are causing serious problems in the containment of infectious diseases [32, 42, 74, 114, 120, 147, 164]. Therefore, there is an increasing need to develop new antibacterial compounds due to emerging antimicrobial resistance [29]. Marine fungi have been a source of diverse antibacterial compounds e.g. 14, 15-secocurvularin, hirsutanol-A etc [3, 161]. In this section, we discuss natural products from marine fungi (see Table 1) that have

shown promising antibacterial properties, and could provide further cues for clinical trials.

Pestalone, a new chlorinated benzophenone compound isolated from the marine fungus *Pestalotia* sp. showed potent antibiotic activity against methicillin-resistant *S. aureus* (MIC = 37 ng/ml) and vancomycin-resistant *Enterococcus faecium* (MIC = 78 ng/ml), indicating that it could be evaluated further in advanced models of infectious disease [36] (Fig. 1). Interestingly, this compound was produced only when a unicellular marine bacterium strain CNJ-328 was co-cultured in a fungal fermentation, suggesting that the production of this antibiotic is initiated by bacterial competition [36].

Speradine A, a congener of cyclopiazonic acid with a 1-*N*-methyl-2-oxindole ring, isolated from the marine fungus *Aspergillus tamarii*, showed antibacterial activity against *Mycrococcus luteus* (MIC 16.7 µg/ml) [155].

Zopfiellamide A, a class of compound belonging to pyrrolidinone derivatives isolated from the facultative marine ascomycete *Zopfiella latipes*, inhibits gram-positive *Bacillus brevis*, *Bacillus subtilis*, *B. licheniformis*, *Corynebacterium insidiosum*, *Micrococcus luteus*, *Mycobacterium phlei*, *Arthrobacter citreus* and *Streptomyces* sp. and gram-negative, *Acinetobacter calcoaceticus* with minimal inhibitory concentrations ranging between 2 and 10 µg/ml [38].

Table 1 provides further information about natural products isolated from marine fungi showing antibacterial activities. Chemical structures of some of the antibacterial compounds have been elucidated in Fig. 1.

Antiviral compounds

According to the UNAIDS 2004 report (<http://www.unaids.org/bangkok2004/report.html>), HIV is causing havoc worldwide. Around 5 million people have been infected with HIV in 2003 alone, which is the greatest in a year since the start of the epidemic. Globally, the number of patients suffering from HIV has risen to 38 million in 2003. The epidemic is expanding rapidly in parts of Asia, Sub-Saharan Africa, Eastern Europe and Central Asia. Herpes simplex virus (HSV) is well known for its ability to cause lesions near the initial site of infection [135]. In the USA alone, there has been a rise of HSV2 correlated with genital infection by almost 30% since 1970. There has been a worldwide increase in HSV-1 associated genital herpes, and it is clear that it helps in the transmission of other sexual diseases like HIV [28, 134].

The search for antiviral compounds from marine fungi has yielded some promising results. Compounds like equisetin, phomasetin and integrin acid have shown significant anti-HIV activities in bioassay based experiments [157]. For example, Sansalvamide A, a cyclic depsipeptide isolated from the marine fungus *Fusarium* sp. was found to inhibit the topoisomerase of the pathogenic poxvirus *Molluscum contagiosum* (MCV) by

Table 1 Antibacterial compounds isolated from diverse marine fungi

Metabolite(s)	Source	Class of compound	Activity MIC (minimum inhibitory concentration)	Reference
Guisinol	<i>Emericella unguis</i> (obtained from a mollusc)	Depside	<i>Staphylococcus aureus</i>	[121]
Lunatin (1) Cytoskyrin A (2)	<i>Curvularia lunata</i> (isolated from the sponge <i>Niphates olemda</i>)	Anthraquinone	<i>S. aureus</i> (Zones of inhibition for Lunatin and Cytoskyrin are both 8.5 and 10.0 mm in 5 and 10 µg/disk respectively) <i>Escherichia coli</i> (9.0 and 11.0 mm zone inhibition in 5 and 10 µg/disk for both compounds) <i>Bacillus subtilis</i> (7.5 and 9 mm in 5 and 10 µg/disk for compound 1 whereas for compound 2 inhibition zones are 8.0 and 12.0 mm in 5 and 10 µg/disk respectively)	[68]
Varixanthone	<i>Emericella variecolor</i> (sponge derived)		<i>E. coli</i> 12.5 µg/ml <i>B. subtilis</i> 12.5 µg/ml <i>S. aureus</i> 12.5 µg/ml <i>Enterococcus faecalis</i> 50 µg/ml	[99]
Shamixanthone, Tajixanthone hydrate, Terrein (all of them)	<i>E. variecolor</i> (sponge derived)		<i>E. faecalis</i> 50 µg/ml <i>B. subtilis</i> 50 µg/ml <i>S. aureus</i> 50 µg/ml	[99]
Trichodermamide B	<i>Trichoderma virens</i>	Dipeptide	<i>S. aureus</i> 15 µg/ml <i>E. faecium</i> 15 µg/ml	[52]
Modiolides A-B	<i>Paraphaeosphaeria</i> sp N-119 (separated from a marine horse-mussel)	Macrolide	<i>Micrococcus luteus</i> 16.7 µg/ml	[156]
Sumiki's acid, acetyl Sumiki's acid	<i>Cladosporium herbarum</i> (derived from the sponge <i>Callyspongia aerizusa</i>)	Furan carboxylic acid	<i>B. subtilis</i> 5 µg/disk (7 mm zone) <i>S. aureus</i> 5 µg/disk (7 mm zone)	[67]
Aspergillitine	<i>Aspergillus versicolor</i> (isolated from the sponge <i>Xestospongia exigua</i>)	Chromone derivative	<i>B. subtilis</i> 5 µg/disk (7 mm zone)	[89]
Fusidic acid	<i>Stilbella aciculosa</i>	Steroid	<i>S. aureus</i> (MIC = 0.05 mg/ml) <i>B. subtilis</i> (MIC = 0.05 mg/ml)	[80]
Ascosalpyrrolidinone A	<i>Ascochyta salicorniae</i> (obligate)	Alkaloid	<i>Bacillus megaterium</i> (5 mm zone inhibition in a 50 µg/filter disk)	[123]
Phomadecalins A–D, Phomadecalin A, B, D	<i>Phoma</i> sp (isolated from the stromata of <i>Hypoxylon</i> sp)		<i>B. subtilis</i> 200 µg/disk (18, 12, 10, 9 mm zones respectively) <i>S. aureus</i> 200 µg/disk (10, 8, 8 mm zones respectively)	[24]
CJ-17665 (I)	<i>Aspergillus ochraceus</i>	Diketopiperazine & N-indole	<i>S. aureus</i> 12.5 µg/ml <i>S. pyogenes</i> 12.5 µg/ml <i>E. faecalis</i> 25 µg/ml	[151]
Sicayne	<i>Halocyphina villosa</i>		Inhibits gram-positive bacteria at concentrations of 10 approximately 50 µg/ml	[87]
7-deacetoxyanuthone A	<i>Penicillium</i> sp.	polyoxygenated farnesylcyclohexenones	In vitro activity against methicillin and multidrug resistant <i>S. aureus</i> 50 µg/ml	[85]
8-chloro-9-hydroxy-8, 9-deoxyasperlactone (1) 9-chloro-8-hydroxy-8, 9-deoxyasperlactone (2) 9-chloro-8-hydroxy-8, 9-deoxyaspyrone (3)	<i>Aspergillus ostianus</i>	Chlorinated compounds	Compound 1 inhibited the growth of <i>Ruegeria atlantica</i> at 5 µg/disc (Inhibition zone 12.7 mm) while 2 & 3 inhibited at 25 µg/disc (10.1 and 10.5 mm, respectively)	[118]
Ascochital	<i>Kirschsteinothelia maritima</i>	Aromatic aldehyde	Potent activity against <i>B. subtilis</i> 500 ng/ml	[22]
Enniatin B	<i>Fusarium</i> sp.	Cyclodepsipeptide	Enniatin B exhibited antibiotic activity against <i>S. aureus</i> and vancomycin resistant enterococci VRE788 with inhibition zones of 8 and 9 mm respectively (2.5 µg/ml)	[70]
Halorosellinic acid, Phenyl lactone	<i>Halorosellinia oceanica</i>	Sesterterpene, Lactone	Weak antimycobacterial activity against <i>Mycobacterium tuberculosis</i> H37Ra (MIC = 200 µg/ml) Active against <i>M. tuberculosis</i> (MIC = 200 µg/ml)	[26][27]
Seragikinone A	Unidentified marine-derived fungus	Anthracycline related pentacyclic compound	Modest antibacterial activity against <i>S. aureus</i> (10 µg/ml), <i>M. luteus</i> (20 µg/ml), <i>Corynebacterium xerosis</i> (20 µg/ml) and <i>B. subtilis</i> (41 µg/ml)	[145]
Neomangicol B	<i>Fusarium</i> sp.	Sesterterpenes	Antibacterial activity against <i>B. subtilis</i> (50 µg/disk)	[131]
2-(hydroxymethyl furan)	<i>Coniothyrium</i> sp (isolated from the sponge <i>Ectyplasia perox</i>)		Inhibits <i>B. megaterium</i> at 50 µg/disk	[62]

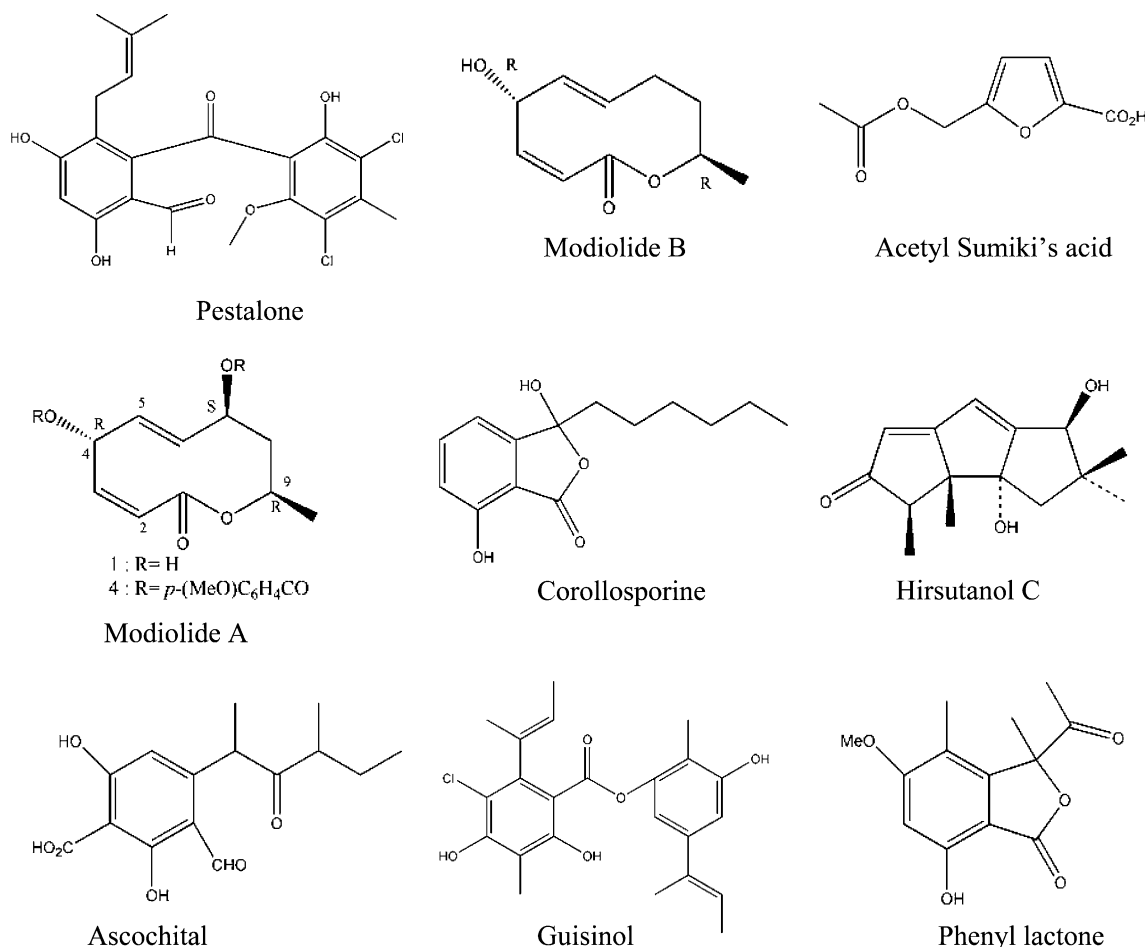


Fig. 1 Chemical structures of metabolites from marine derived fungi showing antibacterial properties

inhibition of topoisomerase catalyzed DNA relaxation, DNA-binding and covalent complex formation ($IC_{50} = 124 \mu M$) [60]. The isolation and identification of this metabolite is particularly significant, because MCV may cause severe lesions in AIDS patients [105].

A series of novel linear peptides Halovirs A–E isolated from the marine fungus *Scytidium* sp. have shown potent antiviral activity against HSVs 1 and 2. The ED_{50} values (1 h duration) for Halovirs A, B, C, D, E were 1.1, 3.5, 2.2, 2.0 and 3.1 μM respectively. In addition, halovir A was also tested for the inhibition of HSV 2. Halovir A was determined to equally inhibit replication of HSV-1 and HSV-2 with an ED_{50} value of 280 nM in a standard plaque reduction assay [137]. The mode of action is still not clear; however it is presumed that halovirs render HSV non-infectious by possible membrane destabilization [137]. An extensive biological evaluation is required to fully assess the potential of the halovirs as antiviral agents. Additional tests against viruses like HIV, human cytomegalovirus (HCMV) are required to explore the specificity of the observed activity of this particular class of compound [137].

Stachyflin, a novel terpenoid isolated from the fungus *Stachybotrys* sp. RF-7260 showed in vitro antiviral

activity against influenza A virus (H1N1) with an IC_{50} value of 0.003 μM , which is significantly better than other antivirals such as amantadine and zanamivir [108]. Yagi et al. [166] have studied the antivirals, stachyflin and its derivatives to improve their reduced in vivo activity after oral administration by chemical modification and some vehicles. Stachyflin, with a pentacyclic moiety includes a novel *cis*-fused decalin and its antiviral activity is mediated through the inhibition of fusion between the viral envelope and the host cell membrane. Such activity is thought to be unique among antiviral compounds [9].

Metabolites showing antiprotozoal activities

Parasitic diseases cause high rates of fatality worldwide [72, 132, 148]. According to the World Health Organisation (see <http://www.who.int/infectious-disease-report/pages/ch3text.html>) 100 million of people in the developing countries are affected by infectious diseases. Parasitic diseases like malaria, sleeping sickness, chagas disease are causing havoc in parts of Africa, Asia and South America. The WHO estimates that 55 million

people in 36 countries along Sub Saharan Africa are threatened by the deadly sleeping sickness, whereas in Latin America up to 18 million are infected with the Chagas disease. In this section, we focus on the natural products from marine fungi that have shown promising anti-protozoal activities against strains of *Plasmodium* sp. and *Trypanosoma* sp.

Malaria is a serious health issue in parts of the African continent and also in South-east Asia and South America [21, 109, 119]. Most of the malaria cases are caused by the parasite *Plasmodium falciparum* [109] transmitted by the Anopheles mosquito. There is an urgent need to develop drugs from natural products to stop the malaria protozoan which are increasingly becoming resistant to drugs like chloroquine, quinine, pyrimethamine, etc [55]. Although not from marine fungal sources, some headway is being made, however, in plant derived natural products for this purpose by Keasling and colleagues at The University of California Berkeley (http://www.berkeley.edu/news/media/releases/2004/12/13_gates.shtml). At this time it is unknown which will be the most effective source.

A series of compounds belonging to different classes of unusual irregular terpenoids have been isolated from the algicolous marine fungus *Drechslera dematioidea* found in the inner tissue of the marine alga *Liagora viscida*. Helminthosporol, Isocochlioquinone A, Cochlioquinone B, Drechslerine E and G have been found to inhibit the growth of malaria-causing protozoan of *P. falciparum* to a significant extent ($IC_{50} \leq 5.1 \mu\text{g/ml}$) [124].

Aigialomycin D, a resorcyclic macrolide and Hypothemycin isolated from the mangrove fungus *Aigialus parvus* BCC 5311 showed in vitro antimalarial activity against *P. falciparum* with IC_{50} values of 2.2 and 6.6 $\mu\text{g/ml}$ respectively [66] (Fig. 2).

Ascosalipyrrolidinone A, an unusual tetramic acid metabolite has shown a significant level of antiplasmodial activities against two strains of *P. falciparum*, namely K1 (resistant to chloroquinone and pyrimethamine) and NF54. This compound has been isolated from the obligate marine fungus *Ascochyta salicorniae* found in association with a marine green alga *Ulva* sp. [123] ($IC_{50d} = 736 \text{ ng/ml}$ for K1, 378 ng/ml for NF54) (Fig. 2).

Four metabolites isolated from the marine fungus *Halorosellinia oceanica* BCC 5149, namely Cytochalasin Q, 5-carboxymellein, halorosellinic acid (an ophoobolane sesterterpene) along with its acetonide derivative showed moderate antimalarial activity against the parasite *P. falciparum* (K1, multidrug resistant strain). The IC_{50} values were 17, 4, 13 and 19 $\mu\text{g/ml}$ respectively [26] (Fig. 2).

Trypanosoma cruzi and *Trypanosoma brucei* are the causal agents of South American Chagas disease and sleeping sickness disease respectively. Chemotherapy remains the only way to control this disease [13]. Some of the drugs are only effective during the early stages of the disease [14, 146]. There is a need to look at the

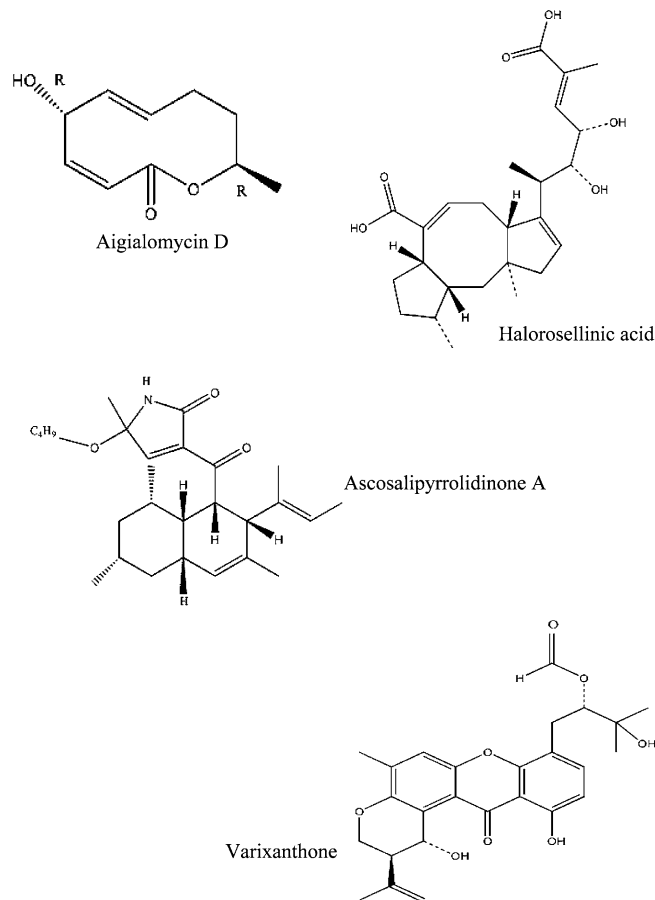


Fig. 2 Chemical structures of compounds isolated from marine fungi showing antiprotozoan activities

diverse marine natural products which can be useful for clinical trials and development of new drugs which will be different from the current drugs in terms of mechanism of action and structures [13].

Ascosalipyrrolidinone A isolated from the obligate marine fungus *A. salicorniae* has shown significant activity against the haemoflagellate *T. cruzi* and *T. brucei* subsp. *rhodesiense* with an MIC of 1.1 and 30 $\mu\text{g/ml}$ respectively [123]. The limitation for further development with ascosalipyrrolidinone is because of the level of cytotoxicity against myoblast cells [123].

Antifungal compounds

Several compounds from marine fungi have been screened for antifungal activities, and a number of compounds have been characterised with regard to their antifungal activities and chemical structures. There has been a sharp increase in fungal infections among patients suffering from HIV, receiving cancer and immuno-therapy etc [7, 12, 84]. As a result, compounds from marine fungi could be used for further clinical trials and drug development. Compounds like Hypoxysordarin, isolated from the facultative marine fungus *Hypoxylon croceum* and

1-Hydroxy-6-methyl-8-(hydroxymethyl) xanthone isolated from the *Ulocladium botrytis* have shown potent antifungal activities [28, 63]. In the following section, some of these natural products from marine fungi showing antifungal activities have been elaborated.

A new antifungal antibiotic, YM-202204, was found in the culture broth of marine fungus *Phoma* sp. Q60596 [113]. The structure was determined as a new lactone compound. The compound exhibited potent antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, and also inhibited glycosyl-phosphatidyl-inositol (GPI)-anchoring in yeast cells [113]. Also another compound, YM-215343 found in the culture extract of *Phoma* sp. QN04621 have shown antifungal activity against the pathogenic fungi, *C. albicans*, *C. neoformans* and *Aspergillus fumigatus* with MIC values of 2–16 µg/ml (approximately) [144].

Keisslone, a metabolite isolated from the marine filamentous fungus *Keissleriella* sp. has shown inhibitory activities against the human pathogenic fungi *C. albicans*, *Trichophyton rubrum* and *Aspergillus niger* with MICs of 50, 70, 40 µg/ml respectively [92].

Trichodermamide B, a dipeptide based compound isolated from the marine fungus *Trichoderma virens* have shown antifungal activity towards amphotericin resistant *C. albicans* with MIC value of 15 µg/ml [52].

Some further compounds with antifungal activities and chemical structures have been also elaborated in Table 2 and Fig. 3 respectively.

Discussion

Some of these metabolites with potential clinical importance could be produced in bulk by total or semi-synthetic pathways, through implementation of fermentation technologies and using (post) genomic technologies in which biosynthetic gene clusters are cloned and expressed in vector systems [138]. Salomon et al. [138] have suggested that most of the secondary metabolites originate from polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) pathways. Little is known about the biosynthetic gene clusters that are involved in the production of these secondary metabolites (as described above) in marine fungi. Authors like Martin [102, 103] have mentioned that NRPSs, a group of giant multidomain enzymes are responsible for the biosynthesis of important β-lactam-containing peptide antibiotics in terrestrial fungi. Antibiotics like the Penicillins, Vancomycin, Cephalosporins and Cephamycins produced by terrestrial fungi have been found to be synthesised by the NRPSs domain [4, 11, 41, 97, 101, 104]. Compounds produced by the NRPSs pathway are generally non-proteinogenic, branched, contain D-amino acids and are usually cyclic in structure [138]. Generally peptide metabolites produced by NRPS pathways display a variety of activities and are extremely important as pharmaceuticals [111, 150, 159]. Kim et al. [71] have identified genes involved in β-lactam biosynthesis in the marine fungus

Table 2 Antifungal compounds from marine fungi

Metabolite	Source	Class of compound	Activity MIC	Reference
Xestodecalactone B	<i>Penicillium cf. montanense</i> (derived from the sponge <i>X. exigua</i>)		Xestodecalactone B showed 25, 12, and 7 mm zones of inhibition against <i>Candida albicans</i> at 100, 50 and 20 µmol respectively	[45]
Seragikinone A	Unidentified marine fungus (derived from the rhodophyte <i>Ceratodictyon</i> <i>spongiosum</i>)	Anthracycline related pentacyclic compound	Weak antifungal activity against <i>C. albicans</i> (MIC = 83 µl)	[145]
Modiolides A-B	<i>Paraphaeosphaeria</i> sp	Macrolide	<i>Neurospora crassa</i> 33.3 µg/ml	[156]
Ascosalipyrrolidinone A	<i>Ascochyta salicorniae</i>	Alkaloid	<i>Mycotypha microsporum</i> (4 mm zone inhibition at a concentration of 50 µg/disk) <i>Microbotryum</i> <i>violaceum</i> (2 mm zone inhibition at a concentration of 50 µg/disk) <i>M. violaceum</i> (1 mm zone inhibition, 50 µg/disk) <i>Eurotium repens</i> (2 mm zone inhibition)	[123]
2,3-Dihydro-2-hydroxy-2, 4-dimethyl-5-trans- propenyfuran-3-one				
3,6,8-trihydroxy-3-[3, 5-dimethyl-2-oxo-3 (E)-heptenyl]-2, 3-dihydronaphthalen- 1(4H)-one	<i>Keissleriella</i> sp. YS4108		Inhibits <i>C. albicans</i> , <i>T. rubrum</i> and <i>A. niger</i> with MIC values of 40, 20, 80 µg/ml, respectively	[90]
Zopfiellamides A and B	<i>Zopfiella latipes</i>	Pyrrolidinone derivative	Inhibits <i>Nematospora coryli</i> and <i>Saccharomyces cerevisiae</i> with MIC values starting at 2 µg/ml	[38]
Microsphaeropsin	<i>Microsphaeropsis</i> sp (derived from the sponge <i>Myxilla incrustans</i>)	Eremophilane derivative	Antifungal activity against <i>Ustilago</i> <i>violacea</i> and <i>Mycotypha microspora</i> at 50 µg level	[61]

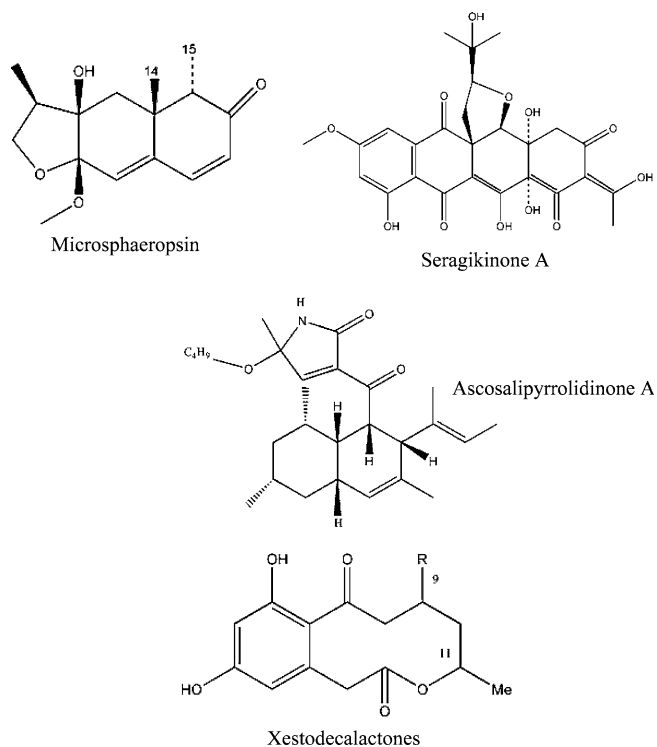


Fig. 3 Chemical structures of secondary metabolites from fungi showing promising antifungal activities

Kallichroma tethys. Schmitt et al. [140] have reported that the winged helix transcription factor CPC1 is involved in regulation of β -lactam biosynthesis (Cephalosporin-C biosynthesis) in the filamentous fungus *Acremonium chrysogenum*.

Toyomasu et al. [154] have cloned the gene cluster in *Phoma betae* which are responsible for the synthesis of diterpene Aphidicolin, a specific inhibitor of DNA polymerase α . They identified six ORFs namely PbGGS, ACS (Aphidicolan-16 β -ol synthase), PbP450-1, PbP450-2, PbTP and PbTF using genome walking. The identification of the biosynthetic gene cluster in fungal terpenoids is thought to be rare like the polyketides [154].

Polyketides are a diverse group of metabolites that are produced by modular PKSs system through the sequential condensation of small carboxylic acids in eukaryotic organisms including fungi [58]. In fungi, PKSs consist of a single, giant protein that uses the same domain to build up the polyketides [138]. Researchers have shown the presence of 16 modular PKS/NRPS clusters in the genome of the marine cyanobacterium *Nostoc punctiforme* [138]. Marine actinomycetes are another group of organism where the PKS and NRPS biosynthetic gene clusters are responsible for the production of unique metabolites [83, 126].

As mentioned earlier, the other possible route for the production of these metabolites could be via total or semi-synthetic pathways. There are numerous examples for the production of these secondary metabolites as discussed above using chemical synthetic routes. Corollosporine, a phthalide derivative and an

antibacterial metabolite isolated from the marine fungus *Corollospora maritima* [88] have been synthesised chemically using 3-hydroxyphthalic or 2-methoxybenzoic acid as the starting material [122]. Cephalosporin derivatives synthesised chemically by *N*-acylation of 7-aminocephalosporanic acid with substituted *N*-pyrrolylcarboxylic acids via mixed anhydrides have shown potent antibacterial activities against gram positive micro-organisms [20]. Nakatani et al. [116] have synthesised Stachyflin, a potent anti-viral agent through enantioselective synthesis of the tetracyclic core structure. The synthetic method involves a $\text{BF}_3 \times \text{Et}_2\text{O}$ -induced domino epoxide-opening/rearrangement/cyclization reaction step. Gu et al. [56] have reported the solid phase synthesis of the antiviral peptide Sansalvamide A using phenylalanine silane resin. Lee and Silverman [81] have synthesised Sansalvamide using a side-chain-tethered phenylalanine building block. Geng and Danishefsky [54] have reported the synthesis of the antiplasmodial antimacrolide Aigialomycin D, using a disiloxydiene and a 14-membered "ynolide" by ring-forming olefin metathesis.

The future

There has been an explosion of interest in recent years into post-genomics technologies after the sequencing of the genomes of many organisms. Currently (April 2005) according to the genomes online database (GOLD) (<http://www.genomesonline.org/> and Bernal et al. [18]) there are 1,421 genome projects, broken down into 261 published complete genomes, 669 on-going prokaryotic genome sequencing projects, and 489 (including 12 chromosomes) ongoing eukaryotic genome projects. Of the completed genome projects, 33 are of eukaryotes, and of these 11 are fungal (representing 33.3% of published genomes). Interestingly, of these 11 published genomes, only one appears to be from fungal organisms of marine origin [*Debaryomyces hansenii*, sequenced by INRA/Genoscope (in draft, see also below)]. With regards to on-going genome projects, 73 of these are fungal genome projects (representing 8% of the total on-going publicly advertised genome projects) thus showing a strengthening interest from the genomics community. However, at least from the natural products producers discussed in this paper, no genomes have been sequenced as mentioned above, nor are any appearing to be (on GOLD). However as a partial genomics lead, some examples from the same genus are being sequenced: *Trichoderma* (At North Carolina State), *Penicillium* (at the Beijing Genomics Institute) and *Fusarium* (at NCBI/Broad Institute, USDA Cereal Disease Laboratory and NCBI/University of Oklahoma). Lépingle et al. [96] have also explored the genome of the marine, osmo- and halo-tolerant yeast *D. hansenii* var. *hansenii* by analyzing 2,830 random sequence tags (RSTs).

With regard to post-genomic work on fungi, obviously the most well studied organism is *Saccharomyces*

cerevisiae. *S. cerevisiae* is an important organism, as it has been used for centuries as a tool for production of, for example, foods (bread) and beverages (wine, beer etc). One of the perceived fields of massive future exploitation of genetic engineering and bioinformatics in the fine chemical and pharmaceuticals industry is the optimisation of bioreactor production of target metabolites (products). *S. cerevisiae* is likely to be an important organism for this additional exploitation, due to the wealth of knowledge and demonstrable industrial exploitation, and it is through metabolic engineering that this exploitation will occur. Metabolic engineering can be defined as “application of recombinant DNA methods to restructure metabolic networks which can improve production of metabolite and protein products by altering pathway distributions and rates” [2]. This is an important field, and the strategies/techniques have been already applied widely (and continue to grow) in *S. cerevisiae* for changing yields, product specificity and for heterologous protein production etc [3]. It seems likely that due to this systems biological level understanding of *S. cerevisiae*, then it may be an ideal choice for incorporation of interesting genes from marine fungi for products generation [57].

The diversity of the natural products from marine fungi clearly demonstrates that there are potentials for transferring some of these compounds into clinical trials for future development of anti-infective drugs. One of the challenges in future will be the large scale production of these compounds to meet the demand for clinical trials and drug development. Many researchers believe that some form of combinatorial genetic and metabolic engineering will be the future solution for commercial production of these compounds (see Bailey [8]). Integration between combinatorial biochemistry and computer-based molecular modelling designs [33] along with postgenomic technologies could be used for sustainable production of these metabolites. Already some of the marine metabolites being tested clinically are being produced either through aquaculture (e.g. compounds like Bryostatin, ET-743), chemical synthesis (compounds like Dolastatin, Ziconotide, Halichondrin B derivative, etc) or by fermentation process (Thiocoraline) [112]. One successful example is the chemical synthesis of Corollosporine, an antibacterial metabolite from the marine fungus *C. maritima* [122]. However chemical synthesis may be a solution for some compounds but it could economically non viable for other compounds.

Metabolic engineering has the potential to be used for large scale production of these compounds using rationale biochemical designs. There are reports of implementation of metabolic engineering for production of novel sesterterpenoid from the marine fungi *Fusarium heterosporum* and *Aspergillus versicolor* ([162], see also <http://www.hnei.hawaii.edu/template.asp?userID=61>). Yanai et al. [167] have engineered the metabolic biosynthetic pathway of PF1022A, a cyclooctadepsipeptide possessing strong anthelmintic properties in the

filamentous fungus *Rosellinia* sp. PF1022 for the synthesis of compounds with improved anthelmintic activities. Wang and Keasling [160] have expressed the 1658-bp region of the HMG1 gene encoding the catalytic domain (cHMG1) of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase of *S. cerevisiae* in the filamentous fungus *Neurospora crassa* and found an increase in the production of carotenoids, Lycopene and Neurosporaxanthin. Du et al. [44] have proposed the idea of engineering hybrid peptide-polyketide biosynthetic pathways for making novel “unnatural” natural products because of the structural and catalytic similarities between modular NRPS and PKS.

Large scale cultivation of marine fungi using bioreactor technology or by other means will be also essential for steady supply of natural products in the marine based drug market. Lorenz and Molitoris [93] have described the use of high pressure for cultivation of marine fungi. Scientists at the University of Kaiserslautern have used 20–100 l scale up for cultivation of obligate marine fungi (see <http://www.uni-kl.de/biotech>). Selbmann et al. [143] have conducted a study on the production of β -glucan production by the fungus *Botryosphaeria rhodina* DABAC-P82 in different bench top bioreactors, and have found that production of β -glucan is technically feasible. Cruz et al. [35] have reported higher production of the antibiotic, Cephalosporin C by using immobilised cells of *Cephalosporium acremonium* ATCC 48272 in a repeated batch tower bioreactor. Pinheiro et al. [128] have used air pressure as an optimization parameter of β -galactosidase production in high-density cell cultures of *Kluyveromyces marxianus* CBS 7894 strains. Mandwal et al. [100] have reported high yields of L-phenylacetyl carbinol by immobilizing cells of *S. cerevisiae* in a stirred tank reactor. There is also a report of higher production of an antibiotic, Patulin (increase of up to 35%) using immobilised *Penicillium urticae* in a 3-phase fluidised bed reactor [17]. Papagianni et al. [125] have compared the citric acid fermentation process by *A. niger* in tubular loop bioreactor and stirred tank bioreactor (10 and 200 l capacity). It appears that the loop reactor simulates the corresponding stirred tank representing a valuable tool in scaling up and scaling down of fermentation process. Kundu et al. [78] have reported co-immobilization of whole cell fungus, *C. acremonium* and alga, *Chlorella pyrenoidosa* to increase the oxygen transfer rate in a packed bed bioreactor for continuous production of Cephalosporin-C.

Fermentation processes have gained considerable importance in the last few years for commercial production of these metabolites [40]. Solid state fermentation (SSF) has been used widely for the production of biologically active secondary metabolites from fungi [10, 15, 65, 133]. Barrios-Gonzalez et al. [16] have shown that SSF technology could be applied to produce high quantity of β -lactam antibiotics like penicillin in a short time period. Ramana Murthy et al. [130] have also produced Cyclosporin A, an antifungal peptide under SSF conditions, using a high yielding mutant of the

fungus *Tolypocladium inflatum*. Coniosetin, a tetrameric acid antibiotic was found to be produced by the fungus *Coniochaeta ellipsoidea* only through SSF [142].

Combinatorial biosynthesis involving introduction of novel biosynthesis genes into micro-organisms will result in the synthesis of the novel metabolites due to the effect of new enzymes on the metabolic pathways [110]. Some authors have even suggested reverse engineering of biological strains for enhanced production of pharmaceutically important compounds [59, 94].

It is clear that the marine environment will play a vital role in the future development and trials of anti-infective drugs. Efforts are still needed in terms of large scale production and exploitation through metabolic engineering and postgenomic technologies for future supply of these natural products from the marine environment.

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