ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/7344191

The current status of natural roducts from marine fungi and their potential as anti-infective agents. J Ind Microbiol Biotechnol 33: 325–337

ARTICLE *in* JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY · JUNE 2006

Impact Factor: 2.44 · DOI: 10.1007/s10295-005-0070-3 · Source: PubMed

CITATIONS
125

READS
372

3 AUTHORS, INCLUDING:

Punyasloke Bhadury
Indian Institute of Science Education and R…
44 PUBLICATIONS **490** CITATIONS
SEE PROFILE

Phillip C Wright
The University of Sheffield
213 PUBLICATIONS **4,445** CITATIONS
SEE PROFILE

Available from: Punyasloke Bhadury
Retrieved on: 13 January 2016

J Ind Microbiol Biotechnol (2006) 33: 325–337
DOI 10.1007/s10295-005-0070-3

REVIEW

Punyasloke Bhadury · Balsam T. Mohammad
Phillip C. Wright

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

Received: 6 May 2005 / Accepted: 7 December 2005 / Published online: 21 January 2006
© Society for Industrial Microbiology 2006

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 began 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.

P. Bhadury
Plymouth Marine Laboratory, Prospect Place, The Hoe, PL1 3DH Plymouth, UK

B. T. Mohammad
Chemical Engineering Laboratory, University of A Coruna, Rua Alejandro de la Sota, no. 1, 15008-A Coruna, Spain

P. C. Wright (✉)
Biological and Environmental Systems Group, Department of Chemical and Process Engineering, University of Sheffield, S1 3JD Sheffield, UK
E-mail: p.c.wright@sheffield.ac.uk
Tel.: +44-114-2227577
Fax: +44-114-2227501

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 founds 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 integric 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	*Emericella unguis* (obtained from a mollusc)	Depside	*Staphylococcus aureus*	[121]
Lunatin (1) Cytoskyrin A (2)	*Curvularia lunata* (isolated from the sponge *Niphates olemda*)	Anthraquinone	*S. aureus* (Zones of inhibition for Lunatin and Cytoskyrin are both 8.5 and 10.0 mm in 5 and 10 μg/disk respectively)*Escherichia coli* (9.0 and 11.0 mm zone inhibition in 5 and 10 μg/disk for both compounds) *Bacillus subtilis* (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	*Emericella variecolor* (sponge derived)		*E. coli* 12.5 μg/ml*B. subtilis* 12.5 μg/ml *S. aureus* 12.5 μg/ml *Enterococcus faecalis* 50 μg/ml	[99]
Shamixanthone, Tajixanthone hydrate, Terrein (all of them)	*E. variecolor* (sponge derived)		*E. faecalis* 50 μg/ml *B. subtilis* 50 μg/ml *S. aureus* 50 μg/ml	[99]
Trichodermamide B	*Trichoderma virens*	Dipeptide	*S. aureus* 15 μg/ml *E. faecium* 15 μg/ml	[52]
Modiolides A-B	*Paraphaeosphaeria* sp N-119 (separated from a marine horse-mussel)	Macrolide	*Micrococcus luteus* 16.7 μg/ml	[156]
Sumiki's acid, acetyl Sumiki's acid	*Cladosporium herbarum* (derived from the sponge *Callyspongia aerizusa*)	Furan carboxylic acid	*B. subtilis* 5 μg/disk (7 mm zone) S. aureus 5 μg/disk (7 mm zone)	[67]
Aspergillitine	*Aspergillus versicolor* (isolated from the sponge *Xestospongia exigua*)	Chromone derivative	*B. subtilis* 5 μg/disk (7 mm zone)	[89]
Fusidic acid	*Stilbella aciculosa*	Steroid	*S. aureus* (MIC = 0.05 mg/ml)*B. subtilis* (MIC = 0.05 mg/ml)	[80]
Ascosalipyrrolidinone A	*Ascochyta salicorniae* (obligate)	Alkaloid	*Bacillus megaterium* (5 mm zone inhibition in a 50 μg/filter disk)	[123]
Phomadecalins A–D, Phomadecalin A, B, D	*Phoma sp* (isolated from the stromata of *Hypoxylon* sp)		*B. subtilis* 200 μg/disk (18, 12, 10, 9 mm zones respectively)*S. aureus* 200 μg/disk (10, 8, 8 mm zones respectively)	[24]
CJ-17665 (I)	*Aspergillus ochraceus*	Diketopiperazine & *N*-indole	*S. aureus* 12.5 μg/ml*S. pyogenes* 12.5 μg/ml *E. faecalis* 25 μg/ml	[151]
Siccayne	*Halocyphina villosa*		Inhibits gram-positive bacteria at concentrations of 10 approximately50 μg/ml	[87]
7-deacetoxyyanuthone A	*Penicillium* sp.	polyoxygenated farnesylcyclohexenones	In vitro activity against methicillin and multidrug resistant *S. aureus* 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)	*Aspergillus ostianus*	Chlorinated compounds	Compound 1 inhibited the growth of *Ruegeria atlantica* 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	*Kirschsteiniothelia maritima*	Aromatic aldehdye	Potent activity against *B. subtilis* 500 ng/ml	[22]
Enniatin B	*Fusarium* sp.	Cyclodepsipeptide	Enniatin B exhibited antibiotic activity against *S. aureus* and vancomycin resistant enterococci VRE788 with inhibition zones of 8 and 9 mm respectively (2.5 μg/ml)	[70]
Halorosellinic acid, Phenyl lactone	*Halorosellinia oceanica*	Sesterterpene, Lactone	Weak antimycobacterial acitivity against *Mycobacterium tuberculosis* H37Ra (MIC = 200 μg/ml) Active against *M. tuberculosis* (MIC = 200 μg/ml)	[26][27]
Seragikinone A	Unidentified marine-derived fungus	Anthracycline related pentacyclic compound	Modest antibacterial activity against *S. aureus* (10 μg/ml), *M. luteus* (20 μg/ml), *Corynebacterium xerosis* (20 μg/ml) and *B. subtilis* (41 μg/ml)	[145]
Neomangicol B	*Fusarium* sp.	Sesterterpenes	Antibacterial activity against *B. subtilis* (50 μg/disk)	[131]
2-(hydroxymethyl furan)	*Coniothyrium* sp (isolated from the sponge *Ectyplasia perox*)		Inhibits *B. megaterium* at 50 μg/disk	[62]

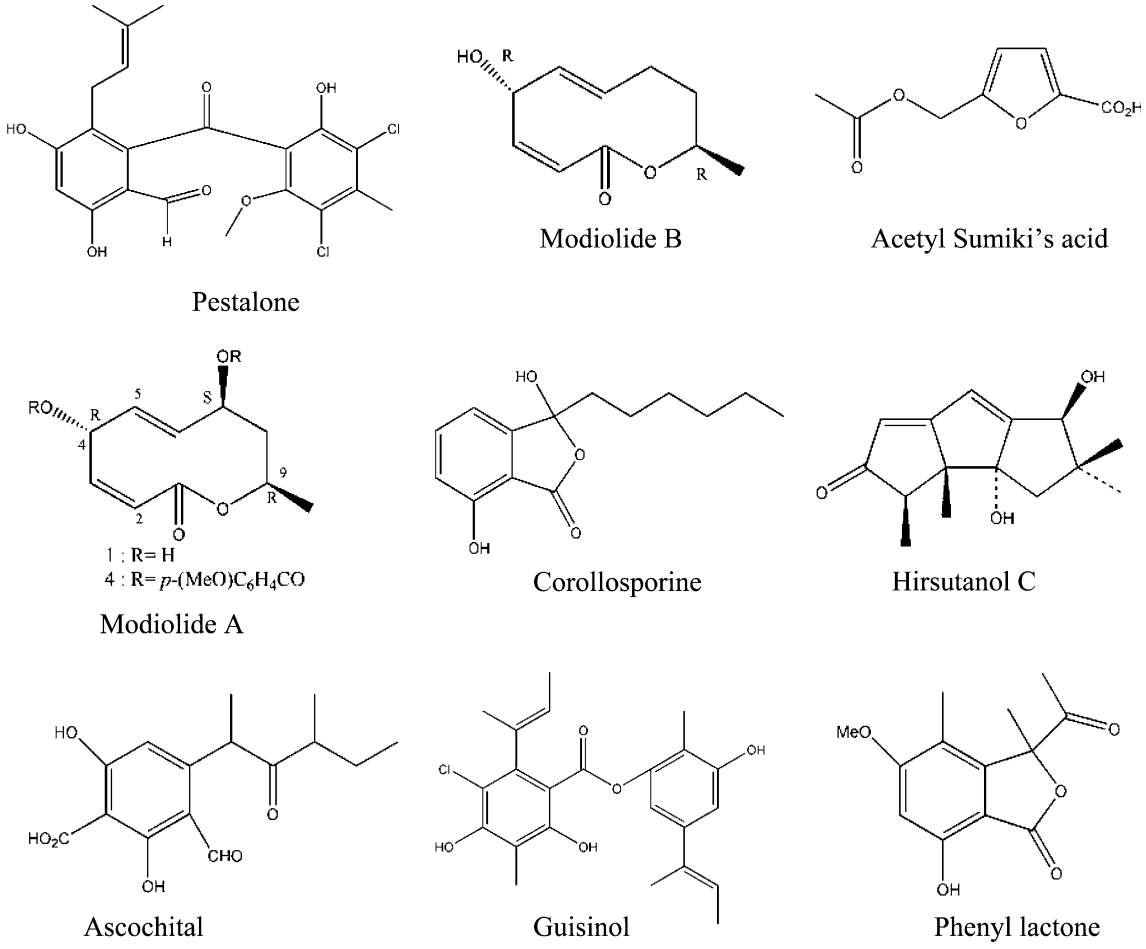

Fig. 1 Chemical structures of metabolites from marine derived fungi showing antibacterial properties

inhibition of topoisomerase catalyzed DNA relaxation, DNA-binding and covalent complex formation ($IC_{50} = 124$ μM) [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 ($IC_{50}^{S} \leq 5.1$ μg/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 μg/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_{50}d = 736 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 μg/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 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].

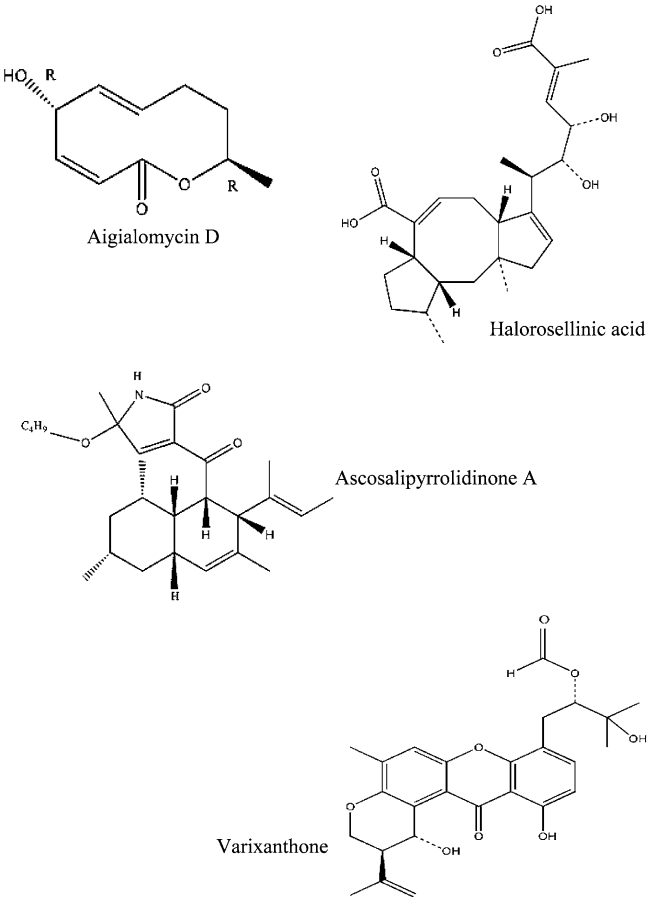

Fig. 2 Chemical structures of compounds isolated from marine fungi showing antiprotozoan activities

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

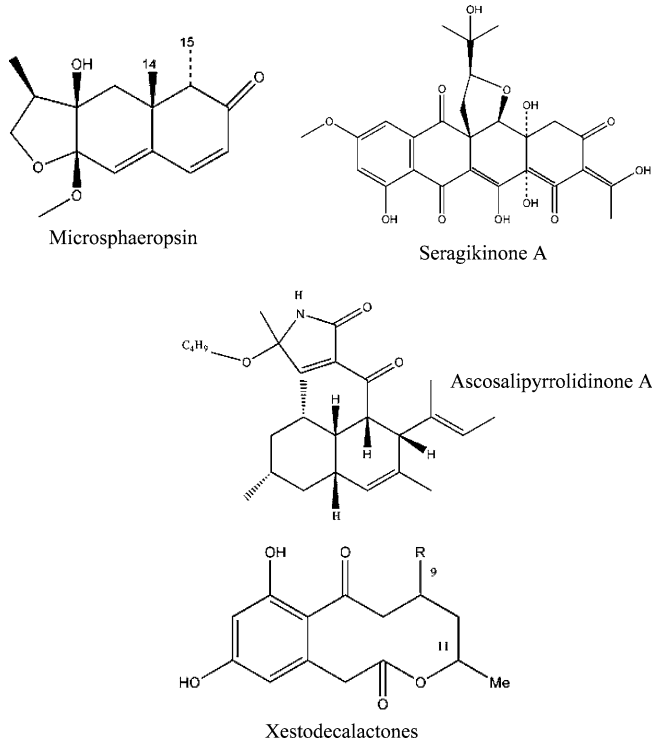

Fig. 3 Chemical structures of secondary metabolites from fungi showing promising antifungal activities

Kallichroma tethys. Schmitt et al. [140] have reported that the winged helix transcription factor CPCR1 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-16β-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 BF(3) × Et(2)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 anthelminthic 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, Cephalopsporin 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 ellipsoida* 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.

Acknowledgements Punyasloke Bhadury acknowledges the Department for International Development (DFID), Heriot Watt University (HWU) and the Association of Commonwealth Universities (ACU) for the provision of a DFID Scholarship. Phillip Wright acknowledges the EPSRC for an Advanced Research Fellowship.

References

1. Abdel-Lateff A, Fisch KM, Wright AD, Konig GM (2003a) A new antioxidant isobenzofuranone derivative from the algicolous marine fungus *Epicoccum* sp. Planta Med 69:831–834
2. Abdel-Lateff A, Klemke C, Konig GM, Wright AD (2003b) Two new xanthone derivatives from the algicolous marine fungus *Wardomyces anomalus*. J Nat Prod 66:706–708
3. Abrell LM, Borgeson B, Crews P (1996) A new polyketide, secocurvularin, from the salt water culture of a sponge derived fungus. Tetrahedron Lett 37:8983–8984
4. Aharonowitz Y, Cohen G, Martin JF (1992) Penicillin and cephalosporin biosynthetic genes: structure, organization, regulation, and evolution. Annu Rev Microbiol 46:461–495
5. Altomare C, Perrone G, Zonno MC, Evidente A, Pengue R, Fanti F, Polonelli L (2000) Biological characterization of fusapyrone and deoxyfusapyrone, two bioactive secondary metabolites of *Fusarium semitectum*. J Nat Prod 63:1131–1135
6. Amagata T, Amagata A, Tenney K, Valeriote FA, Lobkovsky E, Clardy J, Crews P (2003) Unusual C25 steroids produced by a sponge-derived *Penicillium citrinum*. Org Lett 5:4393–4396
7. Anaissie E (1992) Opportunistic mycoses in the immunocompromised host: experience at a cancer center and review. Clin Infect Dis Suppl 14:S43–S53
8. Bailey JE (1991) Towards a science of metabolic engineering. Science 252:1668–1675
9. Baker DD, Alvi KA (2004) Small-molecule natural products: new structures, new activities. Curr Opin Biotechnol 15:576–583
10. Balakrishnan K, Pandey A (1996) Production of biologically active metabolites in solid-state fermentation. J Sci Ind Res 55:365–372
11. Barredo JL, Martin JF (1991) Genes directly involved in the biosynthesis of beta-lactam antibiotics. Microbiologia 7:1–12
12. Barrett (2002) From natural products to clinically useful antifungals. Biochim Biophys Acta 1587:224–233
13. Barrett MP, Mottram JC, Coombs GH (1999) Recent advances in identifying and validating drug targets in trypanosomes and leishmanias. Trends Microbiol 7:82–88
14. Barrett MP, Burchmore RJ, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, Krishna S (2003) The trypanosomiasis. Lancet 362:1469–1480
15. Barrios-Gonzalez J, Mejía A (1996) Production of secondary metabolites by solid-state fermentation. Biotechnol Annu Rev 2:85–88
16. Barrios-Gonzalez J, Castillo TE, Mejía A (1993) Development of high penicillin-producing strains for solid state fermentation. Biotechnol Adv 11:525–537
17. Berk D, Behie LA, Jones A, Lesser BH, Gaucher M (1984) The production of the antibiotic patulin in a three phased fluidized bed reactor. II. The longevity of the biocatalyst. Can J Chem Eng 62:120–124
18. Bernal A, Ear U, Kyrpides N (2001) Genomes online database (GOLD): a monitor of genome projects world-wide. Nucleic Acids Res 29:126–127
19. Bhadury P, Wright PC (2004) Exploitation of marine algae: biogenic compounds for potential antifouling applications. Planta 219:561–578
20. Bijev A, Nankov A, Keuleyan E, Markovska R, Daneva E (2004) Synthesis and preliminary antimicrobial evaluation of new 7-(*N*-pyrrolyl) derivatives of cephalosporins. Arzneimittelforschung 54:119–124
21. Brooker S, Clarke S, Njagi JK, Polack S, Mugo B, Estambale B, Muchiri E, Magnussen P, Cox J (2004) Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. Trop Med Int Health 9:757–766
22. Bugni TS, Ireland CM (2004) Marine derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163
23. Capon RJ, Skene C, Stewart M, Ford J, O'Hair RA, Williams L, Lacey E, Gill JH, Heiland K, Friedel T (2003) Aspergillicins A–E: five novel depsipeptides from the marine-derived fungus *Aspergillus carneus*. Org Biomol Chem 1:1856–1862
24. Che Y, Gloer JB, Wicklow DT (2002) Phomadecalins A–D and Phomapentenone A: new bioactive metabolites from *Phoma* sp. NRRL 25697, a fungal colonist of *Hypoxylon stromata*. J Nat Prod 65:399–402
25. Cheng XC, Varoglu M, Abrell L, Crews P, Lobkovsky E, Clardy J (1994) Chloriolins A–C, chlorinated sesquiterpenes produced by fungal cultures separated from a *Jaspis* marine sponge. J Org Chem 59:6344–6348
26. Chinworrungsee M, Kittakoop P, Isaka M, Rungrod A, Tanticharoen M, Thebtaranonth Y (2001) Antimalarial Halorosellinic acid from the marine fungus *Halorosellinia oceanica*. Bioorg Med Chem Lett 11:1965–1969
27. Chinworrungsee M, Kittakoop P, Isaka M, Chanphen R, Tanticharoen M, Thebtaranonth Y (2002) Halorosellins A and B, unique isocoumarin glucosides from the marine fungus *Halorosellinia oceanica*. J Chem Soc Perkin Trans I 22:2473–2476
28. Christie SN, McCaughey C, McBride M, Coyle PV (1997) Herpes simplex type 1 and genital herpes in Northern Ireland. Int J STD AIDS 8:68–69
29. Coates A, Hu Y, Bax R, Page C (2002) The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov 1:895–910
30. Colwell RR (1985) Marine polysaccharides for pharmaceutical and microbiological applications. In: Colwell RR, Pariser ER, Sinskey AJ (eds) Biotechnology of marine polysaccharides. Proceedings of the 3rd annual MIT seer grant college program lecture and seminar. Hemisphere Publishing, Washington, pp 364–376
31. Colwell RR (2002) Fulfilling the promise of biotechnology. Biotechnol Adv 20:215–228
32. Cooper BS, Medley GF, Stone SP, Kibbler CC, Cookson BD, Roberts JA, Duckworth G, Lai R, Ebrahim S (2004) Methicillin-resistant *Staphylococcus aureus* in hospitals and the community: stealth dynamics and control catastrophes. Proc Natl Acad Sci USA 101:10223–10228
33. Cragg GM, Newman DJ, Snader KM (1997) Natural products in drug discovery and development. J Nat Prod 60:52–60
34. Crews P, Manes LV, Boehler M (1986) *Jasplakinolide*, a cyclodepsipeptide from the marine sponge, *Jaspis* sp. Tetrahedron Lett 27:2797–2800

35. Cruz AJ, Pan T, Giordano RC, Araujo ML, Hokka CO (2004) Cephalosporin C production by immobilized *Cephalosporium acremonium* cells in a repeated batch tower bioreactor. Biotechnol Bioeng 85:96–102
36. Cueto M, Jensen PR, Kauffman C, Fenical W, Lobkovsky E, Clardy J (2001) Pestalone, a new antibiotic produced by a marine fungus in response to bacterial challenge. J Nat Prod 64:1444–1446
37. Daferner M, Mensch S, Anke T, Sterner O (1999) Hypoxysordarin, a new sordarin derivative from *Hypoxylon croceum*. Z Naturforsch C 54:474–480
38. Daferner M, Anke T, Sterner O (2002) Zopfiellamides A and B, antimicrobial pyrrolidinone derivatives from the marine fungus *Zopfiella latipes*. Tetrahedron 58:7781–7784
39. Davidson BS (1995) New dimensions in natural products research: cultured marine microorganisms. Curr Opin Biotechnol 6:284–291
40. Demain AL (2000) Small bugs, big business: the economic power of the microbe. Biotechnol Adv 18:499–514
41. Diez BS, Gutiérrez G, Barredo JL, van Solingen P, van der Voort LHM, Martin JF (1990) The cluster of penicillin biosynthetic genes. Identification and characterization of the pcbAB gene encoding the β-aminoadipyl-cysteinyl-valine synthetase and linkage to the pcbC and penDE genes. J Biol Chem 265:16358–16365
42. Dominguez TJ (2004) It's not a spider bite, it's community-acquired methicillin-resistant *Staphylococcus aureus*. J Am Board Fam Pract 17:220–226
43. Donia M, Hamann MT (2003) Marine natural products and their potential applications as anti-infective agents. Lancet Infect Dis 3:338–348
44. Du L, Sanchez C, Shen B (2001) Hybrid peptide-polyketide natural products: biosynthesis and prospects toward engineering novel molecules. Metab Eng 3:78–95
45. Edrada RA, Heubes M, Brauers G, Wray V, Berg A, Gräfe U, Wohlfarth M, Mühlbacher J, Schaumann K, Sudarsono S, Bringmann G, Proksch P (2002) Online analysis of xestodecalactones A–C, novel bioactive metabolites from the fungus *Penicillium cf. montanense* and their subsequent isolation from the sponge *Xestospongia exigua*. J Nat Prod 65:1598–1604
46. Faulkner DJ (2000a) Highlights of marine natural products chemistry (1972–1999). Nat Prod Rep 17:1–6
47. Faulkner DJ (2000b) Marine pharmacology. Antonie van Leeuwenhoek 77:135–145
48. Faulkner DJ (2001) Marine natural products. Nat Prod Rep 18:1–49
49. Fenical W (1997) New pharmaceuticals from marine organisms. Trends Biotechnol 15:339–341
50. Fenical W, Jensen PR (1993) Marine microorganisms: a new biomedical resource. In: Attaway DH, Zaborsky OR (eds) Marine biotechnology, vol 1. Plenum Press, New York, pp 419–457
51. Gallo ML, Seldes AM, Cabrera GM (2004) Antibiotic long-chain and α, β-unsaturated aldehydes from the culture of the marine fungus *Cladosporium* sp. Biochem Syst Ecol 32:545–551
52. Garo E, Starks CM, Jensen PR, Fenical W, Lobkovsky E, Clardy J (2003) Trichodermamides A and B, cytotoxic modified dipeptides from the marine derived fungus *Trichoderma virens*. J Nat Prod 66:423–426
53. Gautschi JT, Amagata T, Amagata A, Valeriote FA, Mooberry SL, Crews P (2004) Expanding the strategies in natural product studies of marine-derived fungi: a chemical investigation of *Penicillium* obtained from deep water sediment. J Nat Prod 67:362–367
54. Geng X, Danishefsky SJ (2004) Total synthesis of aigialomycin D. Org Lett 6:413–416
55. Gonzalez IJ, Varela RE, Murillo C, Ferro BE, Salas J, Giraldo LE, Zalis MG, Saravia NG (2003) Polymorphisms in cg2 and pfcrt genes and resistance to chloroquine and other antimalarials in vitro in *Plasmodium falciparum* isolates from Colombia. Trans R Soc Trop Med Hyg 97:318–324
56. Gu W, Liu SS, Silverman RB (2002) Solid-phase, Pd-catalyzed silicon-aryl carbon bond formation. Synthesis of sansalvamide A peptide. Org Lett 4:4171–4174
57. Hofmann G, McIntyre M, Nielsen J (2003) Fungal genomics beyond *Saccharomyces cerevisiae*. Curr Opin Biotechnol 14:226–231
58. Hopwood DA, Sherman DH (1990) Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu Rev Genet 24:37–66
59. Huang J, LihCJ, Pan KH, Cohen SN (2001) Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthesis pathways in *Streptomyces coelicolor* using DNA microarrays. Genes Dev 15:3183–3192
60. Hwang Y, Rowley D, Rhodes D, Gertsch J, Fenical W, Bushman F (1999) Mechanism of inhibition of a poxvirus topoisomerase by the marine natural product sansalvamide A. Mol Pharmacol 55:1049–1053
61. Höller U (1999) Isolation, biological activity and secondary metabolite investigations of marine derived fungi and selected host sponges. PhD Thesis, Universität Carolo-Wilhelmina, p 163
62. Höller U, König GM, Wright AD (1999a) Three new metabolites from marine-derived fungi of the genera *Coniothyrium* and *Microsphaeropsis*. J Nat Prod 62:114–118
63. Höller U, König GM, Wright AD (1999b) A new tyrosine kinase inhibitor from a marine isolate of *Ulocladium botrytis* and new metabolites from the marine fungus *Asteromyces cruciatus* and *Variosporina ramulosa*. Eur J Org Chem 1999:2949–2955
64. Höller U, Wright AD, Matthee GF, Konig GM, Draeger S, Aust HJ, Schulz B (2000) Fungi from marine sponges: diversity, biological activity and secondary metabolites. Mycol Res 104:1354 –1365
65. Höllker U, Höfer M, Lenz J (2004) Biotechnological advantages of laboratory-scale solid state fermentation with fungi. Appl Microbiol Biotechnol 64:175–186
66. Isaka M, Suyarnsestakorn C, Tanticharoen M, Kongsaeree P, Thebtaranonth Y (2002) Aigialomycins A–E, new resorcylic macrolides from the marine mangrove fungus *Aigialus parvus*. J Org Chem 67:1561–1566
67. Jadulco R, Proksch P, Wray V, Sudarsono, Berg A, Gräfe U (2001) New macrolides and furan carboxylic acid derivative from the sponge-derived fungus *Cladosporium herbarum*. J Nat Prod 64:527–530
68. Jadulco R, Brauers G, Edrada RA, Ebel R, Wray V, Sudarsono S, Proksch P (2002) New metabolites from sponge-derived fungi *Curvularia lunata* and *Cladosporium herbarum*. J Nat Prod 65:730–733
69. Jensen PR, Fenical W (2002) In: Hyde KD (ed) Fungi in marine environments, vol 7. Fungal diversity, Hong Kong, pp 293–315
70. Jiang Z, Barret MO, Boyd KG, Adams DR, Boyd ASF, Burgess JG (2002) JM47, a cyclic tetrapeptide HC-toxin analogue from a marine *Fusarium* species. Phytochemistry 60:33–38
71. Kim C-F, Lee SKY, Price J, Jack RW, Turner G, Kong RYC (2003) Cloning and expression analysis of the pcbAB-pcbC β-Lactam genes in the marine fungi *Kallichroma tethys*. Appl Environ Microbiol 69:1308–1314
72. Kiszewski AE, Teklehaimanot A (2004) A review of the clinical and epidemiologic burdens of epidemic malaria. Am J Trop Med Hyg 271:128–135
73. Klemke C, Kehraus S, Wright AD, Konig GM (2004) New secondary metabolites from the marine endophytic fungus *Apiospora montagnei*. J Nat Prod 67:1058–1063
74. Kmietowicz Z (2000) WHO warns of threat of superbugs. BMJ 320:1624
75. Kobayashi J, Ishibashi M (1993) Bioactive metabolites of symbiotic marine microorganisms. Chem Rev 93:1753–1769
76. Kohlmeyer J (1974) Veröff Inst. Meeresforsch. Bremerhaven Suppl 5:263–286
77. Konig GM, Wright AD, Sticher O, Angerhofer CK, Pezzuto JM (1994) Biological activities of selected marine natural products. Planta Med 60:532–537

78. Kundu S, Mahapatra AC, KumarNigam V, Kundu K (2003) Continuous production of cephalosporin-C by immobilized microbial cells using symbiotic mode in a packed bed bioreactor. Artif Cells Blood Substit Immobil Biotechnol 31:313–327
79. Kupka J, Anke T, Steglich W, Zechlin L (1981) Antibiotics from basidiomycetes. XI. The biological activity of siccayne, isolated from the marine fungus *Halocyphina villosa* J. & E. Kohlmeyer. J Antibiot (Tokyo) 34:298–304
80. Kuznetsova TA, Smetanina OF, Afiyatullov SS, Pivkin MV, Denisenko VA, Elyakov GB (2001) The identification of fusidic acid, a steroidal antibiotic marine isolate of the fungus *Stilbella aciculosa*. Biochem Syst Ecol 29:873–874
81. Lee Y, Silverman RB (2000) Rapid, high-yield, solid-phase synthesis of the antitumor antibiotic sansalvamide A using a side-chain-tethered phenylalanine building block. Org Lett 2:3743–3746
82. Lee GT, Lee SY, Jeong JH, Jo BK, Li XF, Son BW (2003) PP-35 Screening of tyrosinase inhibiting activity from the marine-derived fungus. Pigment Cell Res 16:604
83. Li A, Piel J (2000) A gene cluster from a marine *Streptomyces* encoding the biosynthesis of the aromatic spiroketal polyketide griseorhodin A. Chem Biol 9:1017–1026
84. Li HY, Matsunaga S, Fusetani N (1998) Antifungal metabolites from marine sponges. Curr Org Chem 2:649–682
85. Li X, Choi HD, Kang JS, Lee CO, Son BW (2003a) New polyoxygenated farnesylcyclohexenones, deacetoxyyanuthone A and its hydro derivative from the marine-derived fungus *Penicillium* sp. J Nat Prod 66:1499–1500
86. Li X, Jeong JH, Lee KT, Rho JR, Choi HD, Kang JS, Son BW (2003b) Gamma-pyrone derivatives, kojic acid methyl ethers from a marine-derived fungus *Alternaria* [correction of *Altenaria*] sp. Arch Pharm Res 26:532–534
87. Li X, Kim MK, Lee U, Kim SK, Kang JS, Choi HD, Son BW (2005) Myrothenones A and B, cyclopentenone derivatives with tyrosinase inhibitory activity from the marine-derived fungus *Myrothecium* sp. Chem Pharm Bull (Tokyo) 53:453–455
88. Liberra K, Jansen R, Lindequist U (1998) Corollosporine, a new phthalide derivative from the marine fungus *Corollospora maritima* Werderm 1069. Pharmazie 53:578–581
89. Lin W, Brauers G, Ebel R, Wray V, Berg A, Sudarsono, Proksch P (2003) Novel chromone derivatives from the fungus *Aspergillus versicolor* isolated from the marine sponge *Xestospongia exigua*. J Nat Prod 66:57–61
90. Liu CH, Meng JC, Zou WX, Huang LL, Tang HQ, Tan RX (2002) Antifungal metabolite with a new carbon skeleton from *Keissleriella* sp YS4108, a marine filamentous fungus. Planta Med 68:363–365
91. Liu CH, Liu JY, Huang LL, Zou WX, Tan RX (2003a) Absolute configuration of keisslone, a new antimicrobial metabolite from *Keissleriella* sp. YS4108, a marine filamentous fungus. Planta Med 69:481–483
92. Liu Z, Jensen PR, Fenical W (2003b) A cyclic carbonate and related polyketides from a marine-derived fungus of the genus *Phoma*. Phytochemistry 64:571–574
93. Lorenz R, Molitoris HP (1992) High pressure cultivation of marine fungi: apparatus and method. In: Balny C, Hayashi R, Masson P (eds) High pressure and biotechnology. John Libbey & Co, London, pp 537–539
94. Lum AM, Huang J, Hutchinson CR, Kao CM (2004) Reverse engineering of industrial pharmaceutical-producing actinomycetes strains using DNA microarrays. Metab Eng 6:186–196
95. Luo J, Yang Y, Lin Y, Chen Z, Jiang G (2004) Antioxidative activities of two metabolites of cultured marine fungus, *Halorosellinia oceanicum* 323 in vitro. Zhong Yao Cai 27:188–192
96. Lépingle A, Casaregola S, Neuvéglise C, Bon E, Nguyen HV, Artiguenave F, Wincker P, Gaillardin C (2000) Genomic Exploration of the Hemiascomycetous Yeasts:14. *Debaryomyces hansenii* var. *hansenii*. FEBS Lett 487:82–86
97. MacCabe AP, Riach MBR, Unkles SE, Kinghorn JR (1990) The *Aspergillus nidulans* npeA locis consists of three contiguous genes required for penicillin biosynthesis. EMBO J 9:279–287
98. Malakoff D (1997) Extinction on the high seas. Science 277:486–488
99. Malstrøm J, Christophersen C, Barrero AF, Oltra JE, Justicia J, Rosales A (2002) Bioactive metabolites from a marine derived strain of the fungus *Emericella variecolor*. J Nat Prod 65:364–367
100. Mandwal AK, Tripathi CK, Trivedi PD, Joshi AK, Agarwal SC, Bihari V (2004) Production of L-phenylacetyl carbinol by immobilized cells of *Saccharomyces cerevisiae*. Biotechnol Lett 26:217–221
101. Mankelow DP, Neilan BA (2000) Non-ribosomal peptide antibiotics. Expert Opin Ther Patents 10:1583–1591
102. Martín JF (1998) New aspects of genes and enzymes for ß-lactam antibiotic biosynthesis. Appl Microbiol Biotechnol 50:1–15
103. Martín JF (2000) Alpha-aminoadipyl-cysteinyl-valine synthetases in beta-lactam producing organisms. From Abraham's discoveries to novel concepts of non-ribosomal peptide synthesis. J Antibiot 53:1008–1021
104. Mathison L, Soliday C, Stpean T, Aldrich T, Rambosek J (1993) Cloning and characterization, and use in strain improvement of the *Cephalosporium acremonium* gene *cefG* encoding acetyl transferase. Curr Genet 23:33–41
105. Mayer AM, Hamann MT (2002) Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 132:315–339
106. Mayer AM, Hamann MT (2004) Marine pharmacology in 2000: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral activities; affecting the cardiovascular, immune, and nervous systems and other miscellaneous mechanisms of action. Mar Biotechnol 6:37–52
107. Mehta AS, Gu B, Conyers B, Ouzounov S, Wang L, Moriarty RM, Dwek RA, Block TM (2004) alpha-Galactosylceramide and novel synthetic glycolipids directly induce the innate host defense pathway and have direct activity against hepatitis B and C viruses. Antimicrob Agents Chemother 48:2085–2090
108. Minagawa K, Kouzuki S, Yoshimoto J, Kawamura Y, Tani H, Iwata T, Terui Y, Nakai H, Yagi S, Hattori N, Fujiwara T, Kamigauchi T (2002) Stachyflin and acetylstachyflin, novel anti-influenza A virus substances, produced by *Stachybotrys* sp. RF-7260. I. Isolation, structure elucidation and biological activities. J Antibiot (Tokyo) 55(2):155–164
109. Mishra SK, Satpathy SK, Mohanty S (1999) Survey of malaria treatment and deaths. Bull World Health Organ 77:1020
110. Moore BS, Piel J (2000) Engineering biodiversity with type II polyketide synthase genes. Antonie van Leeuwenhoek 78:391–398
111. Mootz HD, Schwarzer D, Marahiel MA (2002) Ways of assembling complex natural products on modular nonribosomal peptide synthetases. Chembiochem 3:490–504
112. Munro MHG, Blunt JW, Dumdei EJ, Hickford SJH, Lill RE, Li SX, Battershill CN, Duckworth AR (1999) The discovery and development of marine compounds with pharmaceutical potential. J Biotechnol 70:15–25
113. Nagai K, Kamigiri K, Matsumoto H, Kawano Y, Yamaoka M, Shimoi H, Watanabe M, Suzuki K (2002) YM-202204, a new antifungal antibiotic produced by marine fungus *Phoma* sp. J Antibiot (Tokyo) 55:1036–1041
114. Nagaraju U, Bhat G, Kuruvila M, Pai GS, Jayalakshmi, Babu RP (2004) Methicillin-resistant *Staphylococcus aureus* in community-acquired pyoderma. Int J Dermatol 43:412–414
115. Nakamura H, Takita T, Umezawa H, Kunishima M, Nakayama Y (1974) Letter: absolute configuration of coriolin, a sesquiterpene antibiotic from *Coriolus consors*. J Antibiot (Tokyo) 27:301–302

116. Nakatani M, Nakamura M, Suzuki A, Inoue M, Katoh T (2002) A new strategy toward the total synthesis of stachyflin, a potent anti-influenza A virus agent: concise route to the tetracyclic core structure. Org Lett 4:4483–4486
117. Namikoshi M, Kobayashi H, Yoshimoto T, Meguro S, Akano K (2000) Isolation and characterization of bioactive metabolites from marine-derived filamentous fungi collected from tropical and sub-tropical coral reefs. Chem Pharm Bull (Tokyo) 48:1452–1457
118. Namikoshi M, Negishi R, Nagai H, Dmitrenok A, Kobayashi H (2003) Three new chlorine containing antibiotics from a marine-derived fungus *Aspergillus ostianus* collected in Pohnpei. J Antibiot (Tokyo) 56:755–761
119. Ndyomugyenyi R, Magnussen P (2004) Trends in malaria-attributable morbidity and mortality among young children admitted to Ugandan hospitals, for the period 1990–2001. Ann Trop Med Parasitol 98:315–327
120. Neu HC (1992) The crisis in antibiotic resistance. Science 257:1064–1073
121. Nielsen J, Nielsen PH, Frisvad JC (1999) Fungal depside, guisinol, from a marine derived strain of *Emericella unguis*. Phytochemistry 50:263–265
122. Ohzeki T, Mori K (2001) Synthesis of Corollosporine, an antibacterial metabolite of the marine fungus *Corollospora maritima*. Biosci Biotechnol Biochem 65:172–175
123. Osterhage C, Kaminsky R, König GM, Wright AD (2000) Ascosalipyrrolidinone A, an antimicrobial alkaloid, from the obligate marine fungus *Ascochyta salicorniae*. J Org Chem 65:6412–6417
124. Osterhage C, König GM, Höller U, Wright AD (2002) Rare sesquiterpenes from the algicolous fungus *Drechslera dematioidea*. J Nat Prod 65:306–313
125. Papagianni M, Mattey M, Kristiansen B (2003) Design of a tubular loop bioreactor for scale-up and scale-down of fermentation processes. Biotechnol Prog 19:1498–1504
126. Piel J, Hertweck C, Shipley PR, Hunt DM, Newman MS, Moore BS (2000) Cloning, sequencing and analysis of the enterocin biosynthesis gene cluster from the marine isolate '*Streptomyces maritimus*': evidence for the derailment of an aromatic polyketide synthase. Chem Biol 7:943–955
127. Pietra F (1997) Secondary metabolites from marine microorganisms: bacteria, protozoa, algae and fungi. Achievements and prospects. Nat Prod Rep 14:453–464
128. Pinheiro R, Belo I, Mota M (2003) Growth and beta-galactosidase activity in cultures of *Kluyveromyces marxianus* under increased air pressure. Lett Appl Microbiol 37:438–442
129. Pomponi SA (1999) The bioprocess-technological potential of the sea. J Biotech 70:5–13
130. Ramana Murthy MV, Mohan EVS, Sadhukhan AK (1999) Cyclosporin A production by *Tolypocladium inflatum* using solid state fermentation. Proc Biochem 34:269–280
131. Renner MK, Jensen PR, Fenical W (1998) Neomangicols: structures and absolute stereochemistries of unprecedented halogenated sesterterpenes from a marine fungus of the genus *Fusarium*. J Org Chem 63:8346–8354
132. Reyburn H, Mbatia R, Drakeley C, Bruce J, Carneiro I, Olomi R, Cox J, Nkya WM, Lemnge M, Greenwood BM, Riley EM (2005) Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria. JAMA 293:1461–1470
133. Robinson T, Singh D, Nigam P (2001) Solid-state fermentation: a promising microbial technology for secondary metabolite production. Appl Microbiol Biotechnol 55:284–289
134. Rodgers CA, O'Mahony C (1995) High prevalence of herpes simplex virus type 1 in female anogenital herpes simplex. Int J STD AIDS 6:144
135. Roizman B, Sears AE (1996) In: Fields B, Knipe DM, Howley PM (eds) Fundamental virology. Lippincott-Raven, Philadelphia, pp 1043–1107
136. Rowley DC, Hansen MS, Rhodes D, Sotriffer CA, Ni H, McCammon JA, Bushman FD, Fenical W (2002) Thalassiolins A–C: new marine-derived inhibitors of HIV cDNA integrase. Bioorg Med Chem 10:3619–3625
137. Rowley DC, Kelly S, Kauffman CA, Jensen PR, Fenical W (2003) Halovirs A–E, new antiviral agents from a marine-derived fungus of the genus *Scytalidium*. Bioorg Med Chem 11:4263–4274
138. Salomon CE, Magarvey NA, Sherman DH (2004) Merging the potential of microbial genetics with biological and chemical diversity: an even brighter future for marine natural product drug discovery. Nat Prod Rep 21:105–121
139. Schiehser GA, White JD, Matsumoto G, Pezzanite JO, Clardy J (1986) The structure of leptosphaerin. Tetrahedron Lett 27:5587–5590
140. Schmitt EK, Bunse A, Janus D, Hoff B, Friedlin E, Kurnsteiner H, Kuck U (2004) Winged helix transcription factor CPCR1 is involved in regulation of beta-lactam biosynthesis in the fungus *Acremonium chrysogenum*. Eukaryot Cell 3:121–134
141. Schwartsmann G, Da Rocha AB, Mattei J, Lopes R (2003) Marine-derived anticancer agents in clinical trials. Expert Opin Investig Drugs 12:1367–1383
142. Segeth MP, Bonnefoy A, Bronstrup M, Knauf M, Schummer D, Toti L, Vertesy L, Wetzel-Raynal MC, Wink J, Seibert G (2003) Coniosetin a novel tetrameric antiobiotic from *Coniochaeta ellipsoidea* DSM 13856. J Antibiot 56:114–122
143. Selbmann L, Crognale S, Petruccioli M (2004) Beta-glucan production by *Botryosphaeria rhodina* in different bench-top bioreactors. J Appl Microbiol 96:1074–1081
144. Shibazaki M, Taniguchi M, Yokoi T, Nagai K, Watanabe M, Suzuki K, Yamamoto T (2004) YM-215343, a novel antifungal compound from *Phoma* sp. QN04621. J Antibiot (Tokyo) 57:379–382
145. Shigemori H, Komatsu K, Mikami Y, Kobayashi J (1999) Seragakinone A, a new pentacyclic metabolite from a marine derived fungus. Tetrahedron 55:14925–14930
146. Silber AM, Colli W, Ulrich H, Alves MJ, Pereira CA (2005) Amino acid metabolic routes in *Trypanosoma cruzi*: possible therapeutic targets against Chagas' disease. Curr Drug Targets Infect Disord 5:53–64
147. Silvestri L, van Saene HK, Milanese M, Fontana F, Gregori D, Oblach L, Piacente N, Blazic M (2004) Prevention of MRSA pneumonia by oral vancomycin decontamination: a randomised trial. Eur Respir J 23:921–926
148. Sinha PK, Pandey K, Bhattacharya SK (2005) Diagnosis & management of leishmania/HIV co-infection. Indian J Med Res 121:407–414
149. Son BW, Choi JS, Kim JC, Nam KW, Kim DS, Chung HY, Kang JS, Choi HD (2002) Parasitenone, a new epoxycyclohexenone related to gabosine from the marine-derived fungus *Aspergillus parasiticus*. J Nat Prod 65:794–795
150. Stachelhaus T, Marahiel MA (1995) Modular structure of genes encoding multifunctional peptide synthetases required for non-ribosomal peptide synthesis. FEMS Microbiol Lett 125:3–14
151. Sugie Y, Hirai H, Inagaki T, Ishiguro M, Kim YJ, Kojima Y, Sakakibara T, Sakemi S, Sugiura A, Suzuki Y, Brennan L, Duignan J, Huang LH, Sutcliffe J, Kojima N (2001) A new antibiotic CJ-17,665 from *Aspergillus ochraceus*. J Antibiot (Tokyo) 54:911–916
152. Tan LT, Cheng XC, Jensen PR, Fenical W (2003) Scytalidamides A and B, new cytotoxic cyclic heptapeptides from a marine fungus of the genus *Scytalidium*. J Org Chem 68:8767–8773
153. Toske SG, Jensen PR, Kauffman CA, Fenical W (1998) Aspergillamides A and B: modified cytotoxic tripeptides produced by a marine fungus of the genus *Aspergillus*. Tetrahedron 54:13459–13466
154. Toyomasu T, Nakaminami K, Toshima H, Mie T, Watanabe K, Ito H, Matsui H, Mitsuhashi W, Sassa T, Oikawa H (2004) Cloning of a gene cluster responsible for the biosynthesis of diterpene aphidicolin, a specific inhibitor of DNA polymerase α. Biosci Biotechnol Biochem 68:146–152

155. Tsuda M, Mugishima T, Komatsu K, Sone T, Tanaka M, Mikami Y, Shiro M, Hirai M, Ohizumi Y, Kobayashi J (2003a) Speradine A, a new pentacyclic oxindole alkaloid from a marine derived fungus *Aspergillus tamarii*. Tetrahedron 59:3227–3230
156. Tsuda M, Mugishima T, Komatsu K, Sone T, Tanaka M, Mikami Y, Kobayashi J (2003b) Modiolides A and B, two new 10-membered macrolides from a marine-derived fungus. J Nat Prod 66:412–415
157. Tziveleka LA, Vagias C, Roussis V (2003) Natural products with anti-HIV activity from marine organisms. Curr Top Med Chem 3:1512–1535
158. Vongvilai P, Isaka M, Kittakoop P, Srikitikulchai P, Kongsaeree P, Thebtaranonth Y (2004) Ketene acetal and spiroacetal constituents of the marine fungus *Aigialus parvus* BCC 5311. J Nat Prod 67:457–460
159. Walsh CT, Chen H, Keating TA, Hubbard BK, Losey HC, Luo L, Marshall CG, Miller DA, Patel HM (2001) Tailoring enzymes that modify nonribosomal peptides during and after chain elongation on NRPS assembly lines. Curr Opin Chem Biol 5:525–534
160. Wang GY, Keasling JD (2002) Amplification of HMG-CoA reductase production enhances carotenoid accumulation in *Neurospora crassa*. Metab Eng 4:193–201
161. Wang GYS, Abrell LM, Avelar A, Borgeson BM (1998) New hirsutane based sesquiterpenes from salt water cultures of a marine sponge-derived fungus and the terrestrial fungus *Coriolus consors*. Tetrahedron 54:7335
162. Wang GY, Laidlaw RD, Marshall J, Keasling JD (2003) Metabolic engineering of fungal secondary metabolic pathways. In: An ZQ (ed) Handbook of industrial mycology. Marcel Dekker, New York, p 10016
163. Wegner C, Schwibbe M, König GM, Wright AD (2000) HPLC-DAD and HPLC-MS investigations of marine and terrestrial *Phoma* species. Phytochem Anal 11:288–294
164. Williams RJ, Heymann DL (1998) Containment of antibiotic resistance. Science 279:115–154
165. Winston JE (1988) The systematists' perspective. In: Fautin DG (ed) Biomedical importance of marine organisms. California Academy of Science, San Francisco, pp 1–6
166. Yagi S, Ono J, Yoshimoto J, Sugita K, Hattori N, Fujioka T, Fujiwara T, Sugimoto H, Hirano K, Hashimoto N (1999) Development of anti-influenza virus drugs I: improvement of oral absorption and in vivo anti-influenza activity of Stachyflin and its derivatives. Pharm Res 16:1041–1046
167. Yanai K, Sumida N, Okakura K, Moriya T, Watanabe M, Murakami T (2004) Para-position derivatives of fungal anthelmintic cyclodepsipeptides engineered with *Streptomyces venezuelae* antibiotic biosynthetic genes. Nat Biotechnol 22:848–855
168. Zilinskas RA, Colwell RR, Lipton DW, Hill RT (1995) The global challenge of marine biotechnology: a status report on the United States, Japan, Australia and Norway College Park: Maryland Sea Grant, p 372