Secondary Metabolites from the Marine Algal-Derived Endophytic Fungi: Chemical Diversity and Biological Activity

Authors
Peng Zhang[1,2], Xin Li[1], Bin-Gui Wang[1]

Affiliations
[1] Laboratory of Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China
[2] Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, China

Key words
- seaweed
- algal-derived endophytic fungi
- secondary metabolites
- chemical diversity
- biological activity

Abstract

Marine algal-derived endophytic fungi have attracted considerable attention in the most recent two decades due to their prolific production of structurally diverse secondary metabolites with various biological activities. This review summarizes a total of 182 natural products isolated from marine algal-derived endophytic fungi in the past two decades. The emphasis is on the unique chemical diversity of these metabolic products, together with relevant biological activities.

Introduction

Marine-derived endophytic fungi, which colonize internal tissues of their hosts harmoniously and usually without causing obvious damage to the hosts, have proven to be prolific sources of bioactive natural products with a unique structure and potent pharmaceutical activity [1,2]. Endophytic fungi are widely distributed in virtually every organism on earth and these microorganisms reside in the living tissues of the host and do so in a variety of relationships ranging from symbiotic to pathogenic. The host, such as plants or animals, can provide suitable living conditions and abundant nutrition to stimulate the growth of the endophytes, while endophytes, in return, may contribute to their hosts either by producing a range of substances that may provide protection and ultimately survival value to their hosts, or by affecting the growth and evolution process to benefit the ecological adaptability of the hosts via the signal transduction pathway [3]. Due to the reciprocal and mutually advantageous relationship between endophytes and their hosts, the endophytic fungi may activate the gene-silencing mechanism and subsequently activate the specific biosynthetic pathways to produce unique functional/bioactive metabolites. In some cases, endophytes might be the true producer of the natural products that were characterized from the hosts, or, at least, be indirectly involved in the production of the products.

Fungi have been obtained from virtually every possible marine habitat, including marine plants (algae, driftwood, and mangrove plants), marine invertebrates (sponges, corals, ascidians, and holothurians) and vertebrates (mainly fish) [4]. Among them, algae are one of the most prevalent sources of marine-derived fungi for chemical studies [4]. Algae inhabiting marine ecosystems adapt to frequent and sporadic environmental changes such as high salinity, low oxygen content, nutrient limitation, excessively high light, and drought, which may stress endophytes to produce certain bioactive secondary metabolites to participate in the defense mechanisms of the hosts. Based on this inference, algal-derived endophytic fungal natural products have been the subject of many chemical reports in the past decades, especially in the past 10 years. A number of new compounds have been isolated and identified, with a wide range of biological properties including anticancer, antibiotic, antiviral, antioxidative, and kinase inhibitory or activated activities. Several reviews have touched on the natural products of algal-derived endophytic fungi [1–5], but no review on the full aspect and in-depth view on the array of natural products from the algal-derived fungi has been published. Herein we describe the source, chemical structure, and bioactivity of the newly discovered compounds, with particular emphasis given to their potential use as drug leads. A total of 182 metabolites discovered from marine algal-derived endophytic fungi in the past decades (mainly from 2002 to mid-2015) are included. The structures were classified within a biogenetic context as polyketides (macrolides, phenols, quinones, and unsaturated lactones), terpenes, steroids, and nitrogen-containing compounds.

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Correspondence
Prof. Bin-Gui Wang
Laboratory of Marine Biology and Biotechnology
Qingdao National Laboratory for Marine Science and Technology
Key Laboratory of Experimental Marine Biology
Institute of Oceanology
Chinese Academy of Sciences
Nanhai Road 7
Qingdao 266071
China
Phone: + 86 5 32 82 89 85 53
Fax: + 86 5 32 82 89 85 53
wangbg@ms.qdio.ac.cn

Procedures to Isolate Algal-Derived Endophytic Fungi

To obtain endophytic fungal strains, fresh algal samples should be collected and processed immediately [6]. The isolation of endophytic fungi is generally carried out using an indirect isolation method. Fresh algal samples should be rinsed at least three times with sterile H_2O. After surface sterilization with 70% EtOH (v/v) or 10% sodium hypochlorite for a few seconds, the algal material should be rinsed again in sterile seawater and then aseptically cut into small pieces and placed on agar plates containing the isolation medium (15 g/L agar, 15 g/L sucrose, and chloramphenicol 200 mg/L) [6]. It should be pointed out that the sterilization time is important and it must be balanced at an appropriate level. For the microalgae, the sterilization time should be limited to 10–20 s in EtOH and/or sodium hypochlorite, whereas for the macroalgae it can be extended to 60–120 s [5]. A short time might cause the sterilization of the outer part to be incomplete, while a long time might kill the desired endophytic fungi [5]. To distinguish remaining epiphytic fungi from endophytic fungi, an imprint of the algal surface on biomalt agar has been recommended [6]. Fungi growing exclusively out of the algal tissue were separated on biomalt medium (malt extract 15 g/L, agar 15 g/L). After repeated transferring of the hyphal tips for purification of the fungal strains, the endophytes can be successfully isolated from the inner parts of the tissue.

Polyketides

Parasitenone (**1**) (**Fig. 1**), a new gabosine derivative with moderate free radical scavenging activity, was isolated from a culture of the fungus *Aspergillus parasiticus* # MFA 153 from the red alga *Carpopeltis cornea* (collected in Ulsan City, Korea). On the basis of CD data and a chemical transformation, the absolute configuration of **1** was determined to be (4*S*,5*S*,6*S*) [7]. 5-Hydroxyramulosin (**2**), originating from *Phoma tropica*, was isolated from the brown alga *Fucus spiralis* (collected around the Azorean island of Faial, Portugal) and its structure was secured by single crystal X-ray diffraction analysis [8]. A culture of the fungus *Wardomyces anomalus* OS4T3-2-1, isolated from the green alga *Enteromorpha* sp. (collected around Fehmarn island in the Baltic Sea), yielded two xanthone derivatives, anomalins A (**3**) and B (**4**). Anomalin A (**3**) possessed significant p56(lck) tyrosine kinase inhibitory and antioxidative activity [9]. Strictly speaking, the strain *W. anomalus* is an algicolous fungus. However, herein we also define it as an endophyte considering the indirect isolation method and the reciprocal advantageous relationship between this fungus and its host *Enteromorpha* sp. [9]. A new isochroman derivative named pseudodeflectusin (**5**) was isolated from a culture broth of *Aspergillus pseudodeflectus* Hiji005 (isolated from brown alga *Sargassum fusiform*, which was collected in the Miura Peninsula, Japan). This compound exhibited modest but selective cytotoxic activity against several human cancer cell lines including the stomach (NUGC-3), cervix (HeLa-S3), and peripheral blood (HL-60), with LD_{50} values of 49, 47, and 39 µM, respectively [10]. A *Cladosporium* L037 species from the brown alga *Actinotrichia fragilis* (collected off Seragaki Beach at Okinawa Island, Japan) produced two 12-membered macrolides, sporiolides A (**6**) and B (**7**). Sporiolides **6** and **7** exhibited potent cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 0.37 and 3.1 µM, respectively. In addition, sporiolide A (**6**) had moderate antifungal activity against a range of pathogenic fungi, while both sporiolides A (**6**) and B (**7**) were active against *Micrococcus luteus* [11]. The fungus *Apiospora montagnei* 581/H2 15E, isolated from the inner tissue of the North Sea alga *Polysiphonia violacea*, was the source of the polyketide apiosporic acid (**8**), monomethyl ester of 9-hydroxyhexylitaconate (**9**), and the (−)-enantiomer (**10**) of the known (+)-hexylitaconic acid [12]. The mycelium extract of an unidentified endophytic fungus (strain no. ZZF36) from the brown alga *Sargassum* sp. (obtained from the South China Sea) yielded two new 12-membered lactones, 6-oxo-de-*O*-methyllasiodiplodin (**11**) and (*E*)-9-etheno-lasiodiplodin (**12**), as well as the related known compound lasiodiplodin, and the structure of compound **11** was confirmed by single crystal X-ray diffraction analysis [13]. Lasiodiplodin and its relatives possess various antibacterial activities. However, compound **11**, with a ketone car-

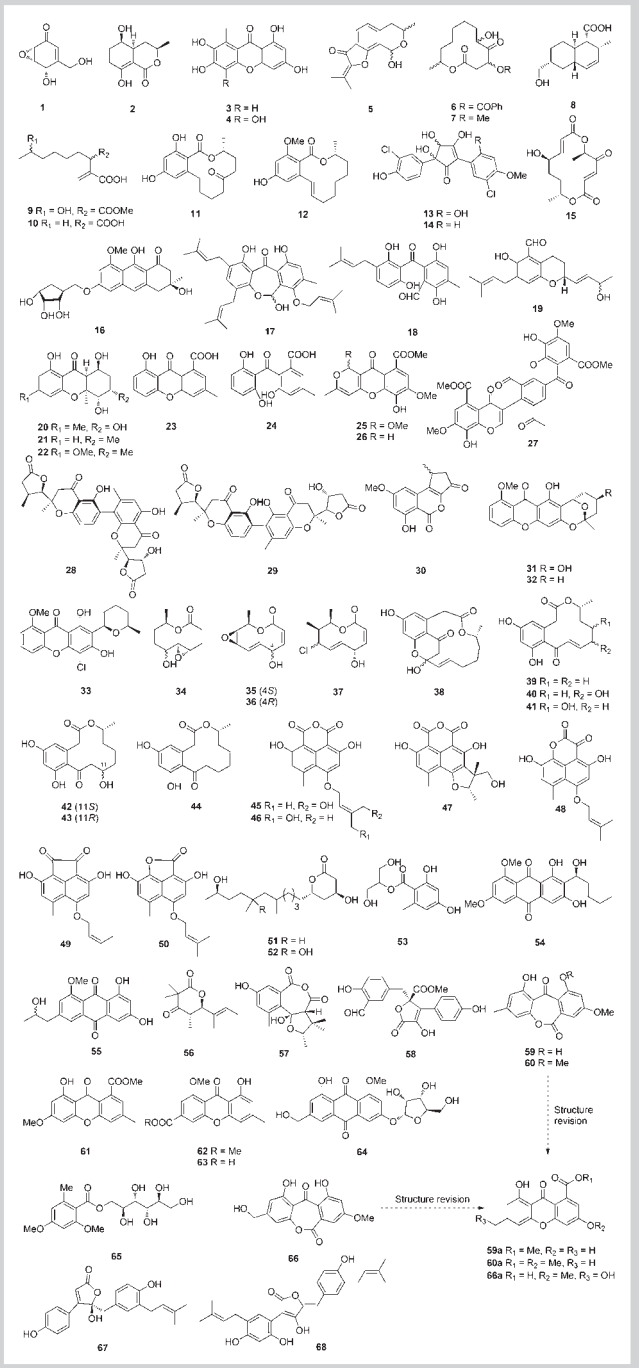

Fig. 1 Polyketides characterized from algal-derived fungi.

bonyl group, showed no activity to all of these tested aerobic reference strains [13]. The endophytic fungus *Aspergillus sydowii*, isolated from the red alga *Acanthophora spicifera* (collected from Rameswaram, south India), produced two new chlorinated 2,5-diarylcyclopentenones, sydowins A (**13**) and B (**14**). It is interesting that this kind of fungal metabolite has hitherto only been described from higher basidiomycetes, but is characterized for the first time from an ascomycete in this study [14]. 4-Ketoclonostachydiol (**15**), a known fungal metabolite possessing very strong cytotoxicity, was isolated from a marine algal-derived fungus, *Gliocladium* sp. (obtained from the New Zealand alga *Durvillaea antarctica*), by bioactivity profiling using HPLC/microtiter plate analysis. The absolute configuration of **15** was elucidated by reduction action from **15** to its derivative, clonostachydiol [15]. Asperflavin ribofuranoside (**16**), an anthracene glycoside, was characterized from the culture extract of *Microsporum* sp. MFA212-1, a fungus isolated from the marine red alga *Lomentaria catenata* (Ulsan City, Korea). Compound **16** showed moderate antibacterial activity against the methicillin-resistant and multidrug-resistant *Staphylococcus aureus* (MRSA and MDRSA) with an MIC value of 119.6 µM [16]. Cultivation of the endophytic fungus *Emericella nidulans* var. *acristata* (No. Sar 14 15E, isolated from a Mediterranean green alga) produced the prenylated polyketides arugosins G (**17**) and H (**18**). Arugosin H (**18**) was active against the fungus *Mycotypha microspora* and the green alga *Chlorella fusca* [17].

Cultivation of *Chaetomium globosum*, an endophytic fungus isolated from the red alga *Polysiphonia urceolata* (Qingdao coastline, China), resulted in the isolation of chaetopyranin (**19**) (**Fig. 1**), a new benzaldehyde secondary metabolite, which was found to have moderate activity against three human tumor cell lines, with IC_{50} values of 48.7 (human microvascular endothelial cells, HMEC), 90.2 (hepatocellular carcinoma cells, SMMC-7721), and 123.7 µM (human lung epithelial cells, A549), in addition to DPPH radical-scavenging properties [6]. Four monomeric xanthones, including monodictysins A–C (**20**–**22**) and monodictyxanthone (**23**) as well as a benzophenone monodictyphenone (**24**), were identified from a culture of *Monodictys putredinis* (No. 187/195 15 I, isolated from the inner tissue of a marine green alga from Tenerife, Spain). Among them, monodictysin B (**21**) inhibited cytochrome P450 1 A activity, while monodictysin C (**22**) displayed moderate activity as inducers of NAD(P)H : quinone reductase (QR) in cultured mouse Hepa 1c1c7 cells. Besides, monodictysin C (**22**) showed weak inhibition of aromatase activity [18]. Three new polyketides, chaetocyclinones A–C (**25**–**27**), were produced by *Chaetomium* sp. Gö 100/2, which was isolated from an unidentified marine alga (source not given). Chaetocyclinone A (**25**) exhibited inhibitory activity against the selected phytopathogenic fungus *Phytophthora infestans*. By feeding ^{13}C-labelled acetate, the biosynthesis of chaetocyclinones A (**25**) and C (**27**) was revealed to corroborate a polyketide pathway and suggested an unusual condensation of two highly reactive heptaketide intermediates [19]. Investigations of the marine-derived fungus *Monodictys putredinis* 195 15 I, isolated from an unidentified green alga (collected in Tenerife, Spain), yielded two novel dimeric xanthone derivatives, monodictyochrome A (**28**) and B (**29**), as inhibitors of cytochrome P450 1 A (with IC_{50} values of 5.3 and 7.5 µM, respectively) and moderate inducers of QR activity with CD values (concentration required to double the specific activity of QR) of 22.1 and 24.8 µM, respectively [20]. Fermentation of an unidentified fungal strain (HJ33moB) derived from an unknown marine alga, which was collected on Hatijou Island, Japan, afforded a new compound, 1-deoxyrubralactone (**30**), as selective inhibitors of a selection of eukaryotic DNA polymerases [21]. Cultivation of a *Chaetomium* sp. (No. 620/GrK 1a) separated from an unidentified marine alga (originated from Kamari on the island Santorini, Greece) yielded chaetoxanthones A–C (**31**–**33**). Chaetoxanthones A (**31**) and B (**32**) are substituted with a dioxane/tetrahydropyran moiety that is rarely observed in natural products, while chaetoxanthone C (**33**) was identified as a chlorinated xanthone substituted with a tetrahydropyran ring. Chaetoxanthones B (**32**) and C (**33**) displayed selective activity against the protozoan *Plasmodium falciparum* and *Trypanosoma cruzi*, with IC_{50} values of 1.4 and 3.8 µM, respectively [22]. Fermentation of a *Curvularia* sp. (strain no. 768) obtained from the red alga *Acanthaphora spicifera* (collected in Apra Harbor, Guam) yielded four new 10-membered lactones (**34**–**37**) [23]. From the same fungus, the novel macrolide apralactone A (**38**), a 14-membered phenyl acetic acid macrolactone, as well as the antipodes of curvularin macrolides (**39**–**44**) were also isolated and were found to be cytotoxic towards human tumor cell lines [24]. The fungus *Coniothyrium cereal*, isolated from the green alga *Enteromorpha* sp. (collected from Fehmarn, Baltic Sea), produced six new phenalenone derivatives (**45**–**50**). Of the isolated metabolites, coniosclerodinol (**48**) inhibited the growth of *S. aureus* (with an MIC value of 24 µM) and proved to be the potent inhibitor of human leukocyte elastase (HLE) (with an IC_{50} value of 13.3 µM), while (*Z*)-coniosclerodinol (**45**), sclerodinol (**47**), and coniolactone (**50**) inhibited the growth of *Mycobacterium phlei* [25]. *Penicillium chrysogenum* QEN-24S, isolated from the red alga *Laurencia* sp. (collected from the Weizhou Island, south China sea), was the producer of the polyketide penicitides A (**51**) and B (**52**) and the glycerol derivative (**53**). Penicitide A (**51**) exhibited moderate cytotoxic activity against the human hepatocellular liver carcinoma cell line with an IC_{50} value of 111.9 µM [26].

From the endophytic fungus *Aspergillus versicolor* EN-7 (isolated from the brown alga *Sargassum thunbergii* that was collected along the Qingdao coastline, China), the anthraquinone compound 6,8-di-*O*-methylaverantin (**54**) was obtained with weak inhibition against *Escherichia coli* [27] (**Fig. 1**). The anthraquinone isorhodoptilometrin-1-methyl ether (**55**) was isolated from another endophytic fungus, *A. versicolor* (isolated from the Egyptian Red Sea green alga *Halimeda opuntia*), and displayed moderate antimicrobial activity against *Bacillus subtilis, Bacillus cereus*, and *S. aureus* [28]. The lactone helicascolide C (**56**) was isolated from an endophytic *Daldinia eschscholzii* from the agar-producing red alga *Gracilaria* sp. (collected in South Sulawesi, Indonesia) and was fungistatic against the phytopathogenic fungus *Cladosporium cucumerinum* [29]. The endophytic fungus *C. cereal*, isolated from the green alga *Enteromorpha* sp. (Baltic Sea), produced the structurally unprecedented polyketide cereoanhydride (**57**). Experiments with feeding ^{13}C-labeled acetate proved the polyketide nature of the major and the known *C. cereale* metabolite (−)-trypethelone, which is proposed to be the precursor of cereoanhydride (**57**) [30].

An endophytic fungus, *Aspergillus terreus*, isolated from red alga *Laurencia ceylanica* (east coast of Sri Lanka), produced a new butyrolactone (**58**) (**Fig. 1**), which was a remarkable inhibitor of the enzyme *β*-glucuronidase, with an IC_{50} value of 6.2 µM, when compared with the positive control, glucosaccharo-(1,4)-lactone (with an IC_{50} value of 48.4 µM) [31]. A new secoanthraquinone derivative, wentiquinone A (**59**), along with wentiquinone B (**60**), which was claimed as a new compound, were isolated from the culture extracts of *Aspergillus wentii* EN-48, an endophytic fungus derived from an unidentified marine brown

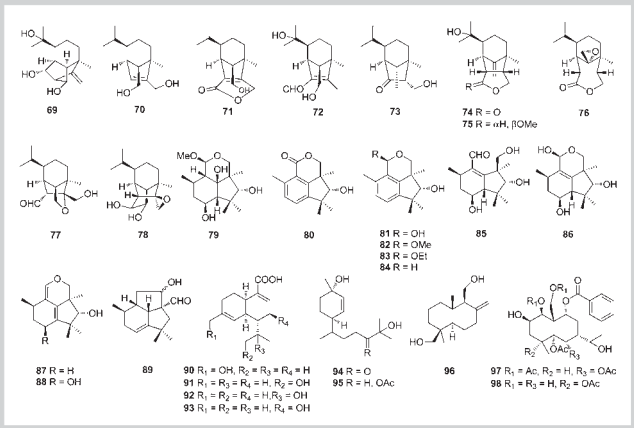

Fig. 2 Sesquiterpenes characterized from algal-derived fungi.

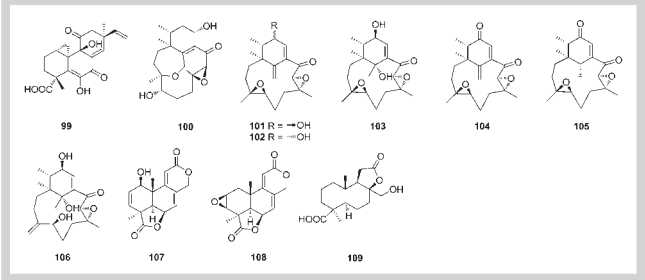

Fig. 3 Diterpenes characterized from algal-derived fungi.

algal species of the genus *Sargassum* (source not given) [32]. The structure of wentiquinone B (**60**) was found to be the same as the previously reported structure of guepinone, which was identified from *Pestalotiopsis guepinii*, an endophytic fungus obtained from the medicinal plant *Virola michelii* [33]. It should be pointed out that the structures of secoanthraquinone derivatives **59** and **60** were suggested to be revised to the xanthones **59a** and **60a**, respectively, according to the detailed analysis of the NMR data [34]. The same species of endophytic *A. wentii* pt-1, but isolated from the red alga *Gymnogongrus flabelliformis* (Pingtan Island, China), resulted in the isolation of three xanthone derivatives, yicathins A–C (**61**–**63**). Yicathin B (**62**) was active against *E. coli*, and yicathin C (**63**) could inhibit *E. coli, S. aureus*, and *C. lagenarium* [35].

Cultivation of the fungal strain *Eurotium cristatum* EN-220, an endophyte obtained from the marine brown alga *Sargassum thunbergii* (Qingdao, China), afforded a new anthraquinone glycoside, 3-*O*-(α-D-ribofuranosyl)-questinol (**64**) and a new orsellinic acid ester, cristatumside A (**65**) [36]. A new *seco*-anthraquinone wentiquinone C (**66**) was characterized from the marine algal-derived endophytic fungus *Aspergillus wentii* EN-48 (brown alga *Sargassum* sp., source not given) [37]. Detailed comparison of the NMR data between anthraquinones and xanthones suggested the revision of *seco*-anthraquinone wentiquinone C (**66**) to be a xanthone structure (**66a**) [34].

Fermentation of the marine-derived endophytic fungus *Paecilomyces variotii* EN-291 from the red alga *Grateloupia turuturu* (Qingdao, China) resulted in the isolation of two new butenolides, butyrolactone IX (**67**) and aspulvinone O (**68**) (**Fig. 1**). Aspulvinone O (**68**) showed potent DPPH radical scavenging activity with an IC_{50} value of 11.6 µM [38].

It should be pointed out that polyketides are generally produced through the acetate-malonate (AA-MA) biosynthetic pathway. However, some metabolites have provided valuable access to novel hybrid chemotypes derived from different biosynthetic routes, including mevalonic acid and shikimate mixed pathways. In view of this point, isocoumarins (e.g., compound **30**), chromones (e.g., compounds **20** and **28**), quinines (e.g., compound **54**), xanthones (e.g., compounds **31**–**33**), phenols and phenolic acids (e.g., compound **53**), lactones (e.g., compounds **6** and **67**), and miscellaneous polyketides (e.g., compounds **13** and **58**) are all classified into polyketides in this review.

Terpenoids

Sesquiterpenes: Ten new sesquiterpenoids, isosativenetriol (**69**), drechslerines A (**70**) and B (**71**), 9-hydroxyhelminthosporol (**72**), drechslerines C–G (**73**–**77**), and sativene epoxide (**78**) (**Fig. 2**), were isolated from a culture of the fungus *Drechslera dematioidea* from the inner tissue of the marine red alga *Liagora viscida* (Mediterranean Sea). Drechslerines E (**75**) and G (**77**) exhibited antiplasmodial activity against two *P. falciparum* strains (IC_{50} values ≤ 20.2 µM) [39]. The mitosporic fungus *Geniculosporum* sp. (strain no. 6580), associated with a red algal *Polysiphonia* species (Baltic Sea, Germany), was cultured to produce 11 new botryane sesquiterpenoids (**79**–**89**), some of which displayed modest herbicidal, antifungal, and antibacterial activities. Their structures were determined by X-ray single crystal analysis and found to be different from known botryanes in substitution pattern and altered sites of oxidation, alkylation, and unsaturation [40]. The fungus *Cadophora malorum* SY3-1-1MIT, isolated from the green alga *Enteromorpha* sp. (source not given), was the source of four new hydroxylated sclerosporin derivatives, 15-hydroxysclerosporin (**90**), 12-hydroxysclerosporin (**91**), 11-hydroxysclerosporin (**92**), and 8-hydroxysclerosporin (**93**). 8-Hydroxysclerosporin (**93**) showed a weak fat-accumulation inhibitory activity against 3T3-L1 murine adipocytes [41]. Chemical investigations of the fungus *Verticillium tenerum* derived from an unknown marine alga (source not given) yielded two new hydroxylated bisabolane-type sesquiterpenes, verticinols A (**94**) and B (**95**) [42]. The endophytic *A. versicolor* d129 (from green alga *Codium fragile* collected off the coast of Dalian, China) produced the sesquiterpene albican-11,14-diol (**96**), which possessed potent activity against brine shrimp, *E. coli*, and *S. aureus* [43]. Two new eudesmane sesquiterpenes, (**97**) and (**98**), were produced as stress metabolites in the cultured mycelia of *Pestalotiopsis* sp. Z233, isolated from the brown alga *Sargassum horneri* (Wenzhou island, China), in response to abiotic stress elicitation by $CuCl_2$. Both metabolites showed tyrosinase inhibitory activities with IC_{50} values of 14.8 and 22.3 µM [44].

Diterpenes: A new diterpene, myrocin A (**99**) (**Fig. 3**), was isolated from the fungus *Apiospora montagnei* 581/H2 15E, which was isolated from the inner tissue of the North Sea red alga *Polysiphonia violacea* [12]. The diterpene phomactin H (**100**), with a novel skeleton having an oxepane moiety, was obtained from an unidentified fungus isolated from a brown alga *Ishige okamurae* (Tateishi, Japan). The structure and relative stereochemistry were determined by X-ray analysis [45]. Three new diterpenes, phomactin I (**101**), 13-*epi*-phomactin I (**102**), and phomactin J (**103**), were isolated from an unidentified fungus (MPUC046) of the family Dothideales (brown alga *Ishige okamurae* collected from Kanagawa Prefecture, Japan), and their structures

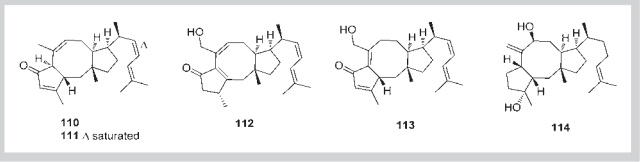

Fig. 4 Sesterterpenes characterized from algal-derived fungi.

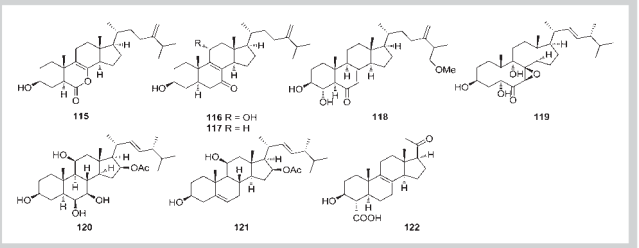

Fig. 5 Steroids characterized from algal-derived fungi.

were confirmed by X-ray crystallographic methods [46]. Subsequently, the same fungus afforded phomactins K–M (**104**–**106**) [47]. It is interesting that phomactin-type diterpenes were isolated from the fungus MPUC046, which is far from *Phoma* sp. genetically. Examination of the endophytic *Aspergillus wentii* EN-48 from an unidentified marine brown algal species of the genus *Sargassum* revealed three new tetranorlabdane diterpenoids, asperolides A–C (**107**–**109**). The structure and absolute configuration of asperolide A (**107**) were confirmed by X-ray crystallographic analysis and by application of the modified Mosher's method [48].

Sesterterpenes: Five new sesterterpenes, (6α)-21-deoxyophiobolin G (**110**), (6α)-16,17-dihydro-21-deoxyophiobolin G (**111**), and ophiobolins U–W (**112**–**114**) (**Fig. 4**), were isolated from *Aspergillus ustus* cf-42, a fungus obtained from the fresh tissue of marine green alga *Codium fragile* (Zhoushan Island, China). Their absolute configurations were predicted by quantum chemical calculations. Quantum chemical calculations of electronic circular dichroism (ECD) spectra have been proven to be reliable tools in deducing the absolute configurations of natural products [49]. To establish the absolute configuration of **110**, its ECD spectrum was determined and calculated. The energy-minimized conformer was generated by the Dreiding force field, which was subjected to the theoretical calculation of the ECD spectrum using the time-dependent density function theory (TD-DFT) method. The calculated ECD spectrum of **110** matched well with the experimental one, which suggested the absolute configuration of **110** to be the same as deduced. Ophiobolin U (**112**) moderately inhibited the growth of *E. coli* (inhibitory diameter of 15 mm) [49].

Steroids

7-Nor-ergosterolide (**115**), a rare 7-norsteroid with an unusual pentalactone B-ring system, and two related derivatives, 3β,11α-dihydroxyergosta-8,24(28)-dien-7-one (**116**) and 3β-hydroxyergosta-8,24(28)-dien-7-one (**117**) (**Fig. 5**), were characterized from the culture of *Aspergillus ochraceus* EN-31, an endophytic fungus isolated from the marine brown alga *Sargassum kjellmanianum* (Dalian coastline, China) [50]. Compounds **115** and **117**, although claimed as new, had been previously reported from a *Penicillium* sp [51]. 7-Nor-ergosterolide (**115**) displayed cytotoxicity against NCI-H460, SMMC-7721, and SW1990 cell lines with IC_{50} values of 12.1, 16.9, and 67.6 µM, respectively [50]. A new steroid (**118**) was isolated from the culture of *Aspergillus flavus* cf-5, an endophytic fungus isolated from the marine red alga *Corallina officinalis* (Yantai, China). Compound **118** exhibited low inhibition of acetylcholinesterase (AChE) [52]. The endophytic fungus *Gibberella zeae* cf-18, isolated from the green alga *Codium fragile* (Yantai, China), yielded a known steroid, which was originally incorrectly assigned as (22*E*)-5,6α-epoxy-3β,8β,14α-trihydroxy-5α-ergost-22-en-7-one, but was corrected to (22*E*,24*R*)-7β,8β-epoxy-3β,5α,9α-trihydroxyergosta-22-en-6-one (**119**) [53]. Two new polyoxygenated steroids, penicisteroids A (**120**) and B (**121**), were obtained from the culture of *Penicillium chrysogenum* QEN-24S, an endophytic fungus isolated from the red alga *Laurencia* sp (source not given). Penicisteroid A (**120**), which is a structurally unique steroid possessing tetrahydroxy and C-16-acetoxy groups, was potently antifungal and selectively cytotoxic to three HTCLs (HeLa, SW1990, and NCI-H460 with the IC_{50} values of 29.6, 61.3, and 79.1 µM, respectively) [54]. Investigation of the fungus *Phaeosphaeria spartinae*, an endophyte of the marine red alga *Ceramium* sp. (North Sea, Büsum, Germany), led to the isolation of spartopregnenolone (**122**). The unusual structure of **122** is intriguing, as it possesses features of triterpenes and steroids [55].

Nitrogenated compounds

Amines and amides: A novel isocoumarin (**123**) (**Fig. 6**), which possesses an unusual seven-membered ring in its side chain, was isolated from the culture broth of *Alternaria tenuis* Sg17-1, from an unidentified alga (Zhoushan Island, China), showed cytotoxicities *in vitro* against human malignant A375-S2 and Hela cell lines with IC_{50} values of 0.3 and 0.05 mM, respectively [56]. Fermentation of a *Penicillium* sp. (CANU MCPT14-1–5), isolated from a surface-sterilized thallus segment of the brown alga *Xiphophora gladiata* (Macrocarpa Point, New Zealand), led to the isolation and characterization of two new 2-pyridone alkaloids, (**124**) and (**125**) [57], while a new phenethyl-α-pyrone derivative, isopyrophen (**126**), was characterized from the culture of *Aspergillus niger* EN-13, an endophytic fungus isolated from the inner tissue of the marine brown alga *Colpomenia sinuosa* (Qingdao coastline, China) [58]. *Aspergillus flavus* MFA500 from the green alga *Codium fragile* (GeoMun Island, Korea) provided the cerebroside derivatives flavusides A (**127**) and B (**128**), which exhibited mild antibacterial activity against *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus* [59]. From the endophytic *Gibberella zeae* cf-18 mentioned above, a new pyrrolidine (**129**), an inhibitor of A549 and BEL-7402 cell lines, was obtained [53], while a new benzamide derivative [methyl 4-(3,4-dihydroxybenzamido)butanoate (**130**)], was characterized from the marine algal-derived endophytic fungus *Aspergillus wentii* EN-48 with significant DPPH radical scavenging activity and an IC_{50} value of 23.1 µM [37].

Quinolines and quinazoline derivatives: A novel benzonaphthyridinedione derivative, chaetominedione (**131**) (**Fig. 6**), proved to be a potent tyrosine kinase inhibitor and was isolated from culture of *Chaetomium* sp. Az 3-10 separated from the alga *Valonia utricularis* (Azores, Atlantic Ocean) [60]. A new fungus-derived benzodiazepine analogue, 2-hydroxycircumdatin C (**132**), was isolated from *Aspergillus ochraceus* EN-31, an endophytic fungus derived from the marine brown alga *Sargassum kjellmanianum* (Dalian coastline, China). 2-Hydroxycircumdatin C (**132**)

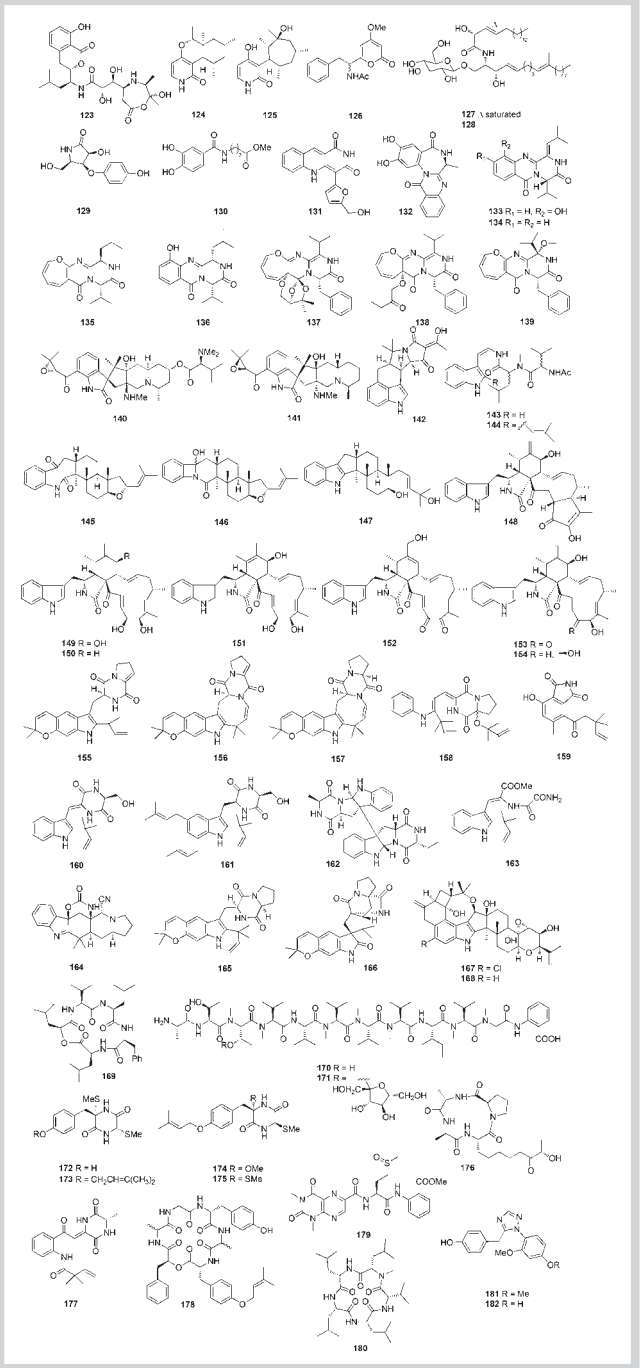

Fig. 6 Nitrogenated compounds characterized from algal-derived fungi.

exhibited significant DPPH radical scavenging activity, with an IC_{50} value of 9.9 µM [61]. A chemical investigation of the endophytic fungus *Aspergillus carneus* KMM 4638 from the brown alga *Laminaria sachalinensis* (Kunachir Island) yielded two new quinazolinone derivatives, carnequinazolines B (**133**) and C (**134**), with antimicrobial and cytotoxic activities [62], while an oxepin-containing alkaloid (**135**) and a quinazolinone-containing alkaloid (**136**) were obtained from the same fungus [63]. A marine-derived endophytic fungus, *Paecilomyces variotii* EN-291 from the marine red alga *Grateloupia turuturu* (Qingdao, China), yielded varioxepine A (**137**) with inhibitory activity against the plant pathogenic fungus *Fusarium graminearum*, with an MIC value of 8.6 µM. Varioxepine A is a 3 *H*-oxepine-containing alkaloid containing a condensed 3,6,8-trioxabicyclo[3.2.1]octane unit, which has not been reported yet in natural products [64]. Further investigation of the same fungal strain led to the identification of two new oxepine-containing diketopiperazine-type alkaloids, varioloids A (**138**) and B (**139**). The identification of varioloid A as a metabolite of *P. variotii* EN-291 supported its role as a biosynthetic precursor to varioxepine A (**137**). Meanwhile, varioloids A and B also exhibited potent activity against the plant pathogenic fungus *F. graminearum*, with MIC values of 17.3 and 10.2 µM, respectively [65].

Indole derivatives: Penicillium citrinum N-059, isolated from the red alga *Actinotrichia fragilis* (Hedo Cape, Okinawa Island), was the source of a new pentacyclic spiroindolinone alkaloid, citrinadin A (**140**) (**Fig. 6**), that displayed modest cytotoxicity against L1210 and KB cell lines (with IC_{50} values of 9.9 and 16.0 µM, respectively) [66], while the same fungus also yielded a new congener, citrinadin B (**141**), with modest cytotoxicity against L1210 cells (with an IC_{50} value of 20.8 µM) [67]. The absolute stereochemistry of citrinadins A and B was elucidated on the basis of ECD and vibrational circular dichroism (VCD) data. *iso*-α-Cyclopiazonic acid (**142**), isolated from *Aspergillus flavus* C-F-3 (green alga *Enteromorpha tubulosa*, Putian Pinghai, China), was modestly cytotoxic to several human tumor cell lines (HL-60, MOLT-4, A-549, and BEL-7402 cell lines) [68]. Two new terpeptin analogues, designated as JBIR-81 (**143**) and JBIR-82 (**144**), were obtained from an *Aspergillus* sp. SpD081030G1f1 (isolated from the brown alga *Sargassum* sp., collected from the sea shore of Ishigaki Island, Okinawa Prefecture, Japan). Both metabolites were strong radical scavengers due to theie protective effect against L-glutamate toxicity (with EC_{50} values of 0.7 and 1.5 µM) [69]. Two new indoloditerpene derivatives, asporyzins A (**145**) and B (**146**), and one new congener, asporyzin C (**147**), were obtained from the endophytic fungus *Aspergillus oryzae* cf-2, which was isolated from the marine red alga *Heterosiphonia japonica* (Yantai, China). To determine the chemical defensive function of this endophytic fungus for the host alga, all the isolates were evaluated for insecticidal and antimicrobial activities. Asporyzins A (**145**) and B (**146**) showed insecticidal activity against brine shrimp (*Artemia salina*), and asporyzin C (**147**) possessed potent activity against *E. coli*. These preliminary results implied that endophytic *A. oryzae* plays an important role in the defense against marine herbivores and bacteria from the host alga *H. japonica* [70]. Cytoglobosins A–G (**148**–**154**), seven new cytochalasan derivatives, were isolated and identified from the culture of *Chaetomium globosum* QEN-14, an endophytic fungus derived from the marine green alga *Ulva pertusa* (Qingdao coastline, China). Cytoglobosins C (**150**) and D (**151**) displayed cytotoxic activity against the A-549 tumor cell line (with IC_{50} values of 2.26 and 2.55 µM, respectively) [71]. Apart from the quinazoline derivatives (**133**–**136**), the fungus *Aspergillus carneus* KMM 4638 isolated from a brown alga also produced three prenylated indole alkaloids, carneamides A–C (**155**–**157**) [62]. A new indole alkaloid, 9ξ-*O*-2(2,3-dimethylbut-3-enyl)brevianamide Q (**158**), was obtained from the culture of *Aspergillus versicolor* pt20, an endophytic fungus isolated from the marine brown alga *Sargassum thunbergii* (Pingtan Island, China) [72]. The endophytic *Coniothyrium cereale* from the green alga *Enteromorpha* sp. produced the isoindole pseudoalkaloid conioimide (**159**) with selective inhibition of human leukocyte elastase [30].

Cultivation of the fungal strain *Eurotium cristatum* EN-220 [36] led to the isolation of four new indole alkaloids, cristatumins A–

D (**160**–**163**) (**Fig. 6**), of which cristatumin A (**160**) was active against *E. coli* with an MIC value of 188.8 µM, while cristatumin B (**161**) was moderately toxic to brine shrimp with an LD_{50} value of 155.9 µM [73]. Aspeverin (**164**), a novel carbamate- and cyano-containing alkaloid isolated from an algicolous *Aspergillus versicolor* dl-29 (green alga *Codium fragile*, Dalian, China), was a moderate growth inhibitor of the phytoplankton *Heterosima akashiwo* (with EC_{50} values of 16.7 and 9.0 µM for 24 and 96 h, respectively) [74]. Further work on the remaining fractions of the fungus *Paecilomyces variotii* EN-291 that produced oxepine-containing diketopiperazine-type alkaloids (**137**–**139**) [64,65] resulted in the isolation of two new prenylated indole alkaloids, dihydrocarneamide A (**165**) and carneamide D (**166**). This is the first report of these alkaloids from a genus other than *Penicillium* or *Aspergillus* [75]. Two new indole diterpenoids, 19-hydroxypenitrem A (**167**) and 19-hydroxypenitrem E (**168**), were isolated and identified from the culture of *Aspergillus nidulans* EN-330, an endophytic fungus obtained from the marine red alga *Polysiphonia scopulorum* var. *villum* (Yantai coastline, China). Both compounds exhibited inhibitory activity against brine shrimp (with LD_{50} values of 3.2 and 4.6 µM) and the chlorinated 19-hydroxypenitrem A (**167**) also showed antimicrobial activity against several human (*E. coli* and *S. aureus*) and aqua pathogens (*Edwardsiella tarda* and *Vibrio anguillarum*), with MIC values ranging from 24.6 to 49.2 µM [76].

Peptides: A *Fusarium* sp. CNL-619, which was isolated from the green alga *Avrainvillea* sp. (Bovoni Cay, United States Virgin Islands), produced *N*-methylsansalvamide (**169**), a new cyclic depsipeptide having weak *in vitro* cytotoxicity in the NCI human tumor cell line screen with an GI_{50} value of 8.3 µM [77]. Investigations of an unidentified fungus isolated from the red alga *Ceradictyon spongiosum* (Seragaki Beach, Okinawa) resulted in the isolation of two linear dodecapeptides, dictyonamides A (**170**) and B (**171**). The characteristic features of **170** and **171** are the presence of several *N*-methylamino acids and an anthranilic acid (Abz) at the C-terminus. LC-ESIMS and chiral HPLC analyses of the acid hydrolysate of **170** using Marfey's procedure determined the absolute configuration of each amino acid residue. Dictyonamide A (**170**) inhibited cyclin-dependent kinase 4 (with an IC_{50} value of 13.0 µM), while dictyonamide B was inactive (IC_{50} value > 30 µM) [78].

Cultured *Fusarium chlamydosporum* OUPS-N124, isolated from the marine red alga *Carpopeltis affinis* (Toyooka city, Japan), was the source of two new sulfur-containing diketopiperazine derivatives, fusaperazines A (**172**) and B (**174**) (**Fig. 6**) [79], and two known compounds, (**173**) and (**175**), which had been originally isolated from the culture of the fungus *Tolypocladium* sp. [80]. JM47 (**176**), a cyclic tetrapeptide, was isolated from a marine *Fusarium* species (MOBCOF-1) isolated from the green alga *Codium fragile* subsp. *Atlanticum* (collected off the east coast of Scotland) and the structure was determined to be cyclo(Ala-Ala-Aoh-Pro), where Aoh is (2*S*,9*S*)-2-amino-8-oxo-9-hydroxydecanoic acid [81]. Golmaenone (**177**), a diketopiperazine alkaloid, was obtained from an *Aspergillus* species (# MFA 212) isolated from a red alga *Lomentaria catenata* (Golmae Village, Ulsan City, Korea) with significant radical scavenging (with an IC_{50} value of 20 µM) and UV-A protecting properties (with an ED_{50} value of 90 µM) [82]. The marine-derived fungus *Gliocladium* sp., obtained from the alga *Durvillaea antarctica*, produced gliotide (**178**), a new cyclodepsipeptide containing several rare D-amino acids [15], and a marine-derived fungal strain, identified as *Penicillium* sp. CNL-338 (isolated from the red alga *Laurencia* sp. that was collected in the Bahamas islands), yielded a novel lumazine peptide, penilumamide (**179**) [83]. Application of OSMAC (one strain-many compounds) methodology to *Asteromyces cruciatus* 763, isolated from an unidentified decaying green alga (La Jolla shore, San Diego), resulted in the isolation of the pentapeptide lajollamide A (**180**) and the absolute configuration was solved by total synthesis [84]. The OSMAC approach was based on cultures of the fungus in media derived from the basic components of the Czapek-Dox broth, with glucose as the carbon source, which was either supplemented with cofactors or contained solely the amino acids Arg, Asn, and Glu as a nitrogen source instead of $NaNO_3$ to afford a good variety of known metabolites and previously unknown compound **180**.

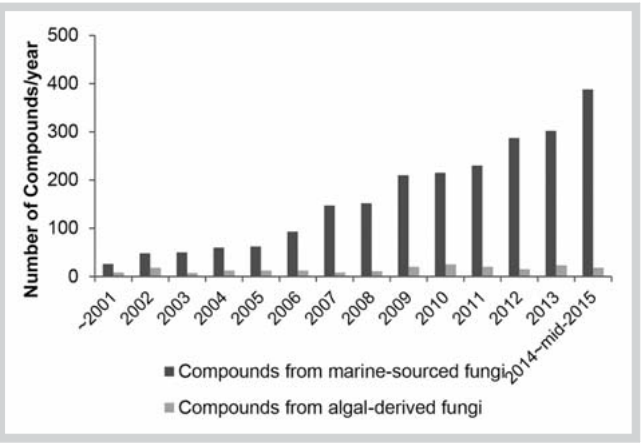

Fig. 7 Annual output of algal-associated fungal metabolites from 1990 to mid-2015.

Miscellaneous nitrogenated derivatives: Two new triazoles, chrysotriazoles A (**181**) and B (**182**) (**Fig. 6**), were isolated and identified from the culture of *Penicillium chrysogenum* EN-118, an endophytic fungus obtained from the marine brown alga *Sargassum pallidium* (Fujian Province, China). The structure of chrysotriazole A (**181**) was confirmed by X-ray crystallographic analysis of its *p*-bromobenzoate derivative [85].

Concluding Remarks

Marine-derived fungi including those derived from marine algae, which have emerged as a new frontier for finding novel pharmaceutical candidates, continue to be a rich source of structurally unique and biologically active natural products. **Fig. 7** summarizes new compounds isolated from marine-sourced fungi and from the endophytic fungi derived from marine algae. Analyzing the data presented, we can infer that the rate at which these fungal metabolites have been discovered within the past 10 years has increased sharply. This increase is largely due to improvements in the isolation/purification techniques, structural analyses, and biological activity evaluation [86]. The search for new metabolites from algal-derived endophytic fungi has developed quite recently. There are approximately 75 papers dealing with chemical investigations of endophytic fungi from marine alga, more than 50% of which appeared in the literature after the year 2008.

In this review, we summarized the new findings regarding the chemistry and bioactivity of natural products found in marine

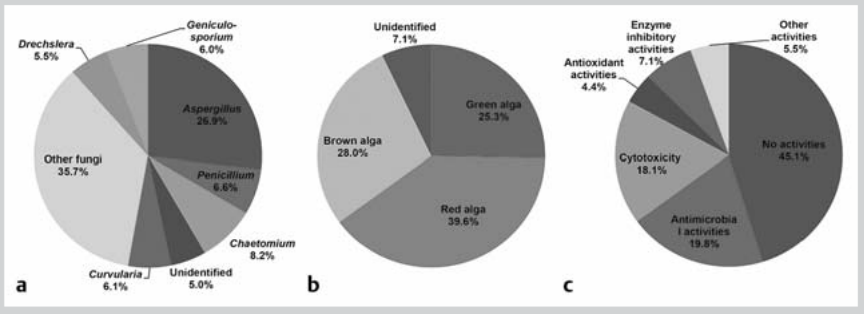

Fig. 8 **a** Fungal source categories of the reported metabolites. **b** Algal source classification of the endophytic fungi. **c** Bioactivity categories of the reported metabolites.

algal-derived endophytic fungi during the past two decades. These include 182 naturally occurring natural products, and most of them showed a variety of bioactivities. It is noteworthy that *Aspergillus, Chaetomium*, and *Penicillium* are predominant genera as producers of these metabolites (**Fig. 8a**). They constitute 41.7% of the compounds reported, and if three other prolific genera, *Curvularia, Geniculosporium*, and *Drechslera*, are taken into account, they include nearly 59.3% of all of the metabolites. Around 35.7% of the compounds are scattered across another 20 genera, while 5.0% of the metabolites are from unidentified fungi. As summarized in **Fig. 8b**, 25.3%, 39.6%, and 28.0% of the 182 compounds come from green, red, and brown algae, respectively. It appears that the red algal genera are better sources of endophytic fungi than others. However, more examples and investigations are needed to support this deduction and to explain this phenomenon. Related to the source of endophytic fungi, it is clear that the macroalgal phylum, such as the green algae of the genera *Enteromorpha, Ulva*, and *Codium*, the brown algae of the genus *Sargassum*, and the red algal genera *Laurencia, Polysiphonia*, and *Grateloupia* are the most representative sources of endophytes. The majority of these metabolites not only have intriguing structures but also possess a variety of biological activities including cytotoxic, antimicrobial, enzyme inhibitory, and radical scavenging effects (**Fig. 8c**), as well as potential ecologically relevant functions, such as antifeedant, insecticidal, antiprotozoal, and herbicidal activity. An extraordinarily high proportion (54.9%) of the isolated metabolites showed a wide range of biological activities, while the inactive metabolites discovered in previous studies were probably due to bias in the screening experiments and limitations in analytical technology, and further evaluation of these compounds with a more wide spectrum of bio-screening systems are recommended, which might leading to the discovery of their interesting bioactivities.

Among the 182 bioactive components presented in this review, some compounds intrigue the natural product researchers because of their unusual structures, such as the diterpene phomactin H (**100**) with a novel oxepane moiety [45], the novel isocoumarin (**123**) with an unusual seven-membered ring [56], and varioxepine A (**137**) with a condensed 3,6,8-trioxabicyclo[3.2.1]octane unit [64]. Most importantly, some of these compounds showed fascinating bioactivities comparable to those of modern pharmacological products, which suggests that they might be potential substitutes of traditional drugs. These include the antitumor agents sporiolides A (**6**) and B (**7**), 4-ketoclonostachydiol (**15**), and cytoglobosins C (**150**) and D (**151**). Sporiolides A (**6**) and B (**7**) from *Cladosporium* sp. exhibited potent cytotoxicity against murine lymphoma L1210 cells, with IC_{50} values of 0.37 and 3.1 µM, respectively, while cytoglobosins C (**150**) and D (**151**) from *Chaetomium globosum* QEN-14 displayed cytotoxic activity against the A-549 tumor cell line, with IC_{50} values of 2.26 and 2.55 µM, respectively. Antiplasmodial agents such as chaetoxanthones B (**32**) and C (**33**) from *Chaetomium* sp. displayed selective activity against the protozoan *P. falciparum* and *T. cruzi*, with IC_{50} values of 1.4 and 3.8 µM, respectively. Antienzyme agents such as the dimeric xanthone derivatives monodictyochrome A (**28**) and B (**29**) from *Monodictys putredinis* 195 15 I are inhibitors of cytochrome P450 1 A, with IC_{50} values of 5.3 and 7.5 µM, respectively. A butyrolactone (**58**) from *Aspergillus terreus* was considered a remarkable inhibitor of the enzyme β-glucuronidase, with an IC_{50} value of 6.2 µM, which was almost eight times stronger than that of the positive control glucosaccharo-(1,4)-lactone. These impressive activities make many of these compounds suitable candidates for drug discovery programs and might trigger synthesis studies in future research.

Although many metabolites of fungal endophytes displayed fascinating bioactivities, some of them were inactive or showed weak activities in the current reports. This was probably due to the bias in the screening programs. Further biological evaluation in other screening systems may uncover their specific biological activities. In addition, more sensitive and effective mass screening models in searching for active metabolites are strongly suggested. For example, computerized virtual screening, which has shown great promise in drug discovery, may play an important role in exploring lead compounds from natural products [87].

In conclusion, an ever-increasing number of compounds are being reported from algal-derived endophytic fungi. The endophytic fungi from marine algae are promising subjects for extensive investigations to find novel natural products, which make them a potentially rich and innovative source for new drug candidates or drug leads. As for the future of the research on the marine natural products of the algal-derived endophytes, the following aspects should be considered: (1) Although numerous secondary metabolites from algal-derived endophytic fungi with various biological activities and ecological functions were studied to some degree, little is known regarding the chemical defensive function of the endophytic fungi for their host algae. A deeper insight into mutualistic symbiosis involving the metabolites of the endophytic fungi and their host algae is therefore a great, interesting subject for future study, which would probably result in the isolation of unusual or rarely studied fungi that could produce structurally interesting and biologically active molecules with potential use in medicinal and agricultural applications; (2) The elucidation of fungal secondary metabolites for the authentic functions in their host algae habitats and the identification of the physiological and ecological conditions that have led to the activation of secondary metabolism gene clusters might be useful to understand the interactions between the endophytes and their host algae; and (3) Optimum fermentation

conditions are necessary to adapt the special physiology and genetic background of algal endophytes and to activate their "silent" gene clusters to explore more "silent" secondary metabolites that could not be produced under normal laboratory culture conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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