Review

Fungal Metabolites: A Promising Source for Anti-biofilm Compounds

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Review

Fungal Metabolites: A Promising Source for Anti-Biofilm Compounds

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Abstract

There is an urgent need for new alternative compounds with distinct modes of action due to the global rise in antibiotic resistance and the associated risks to public health. It is currently established that between 40 and 80% of bacterial biofilms cause antibiotic resistance. Furthermore, biofilm-forming bacteria are 1000 times more resistant to antibiotics than in their planktonic stages. Recently, the number of papers published in 2023 to find antibiofilm compounds from fungi has increased. Meanwhile, it has been proven that endophytic fungi can produce undiscovered compounds against bacterial biofilm. However, as shown in this review, there is still not enough attention given to highlight the relevance of intensifying studies amongst marine-derived fungi. This review summarizes the biologically active compounds isolated from marine-derived fungal extracts tested against bacterial biofilms published from 2015 to 2024. Moreover, this review discloses evidence on the scarcity of research on antibiofilm compounds from algal endophytic fungi. In addition, the primary approaches used in the hunt for bioactive secondary metabolites are covered in this review. Besides that, a few recent strategies have been mentioned to optimize the production of antibiofilm-active fungal metabolites by employing such techniques such as through media optimization, use of chemical elicitors, co-culture, and metabolic engineering.

Keywords: biofilm; endophytic fungi; metabolites; anti-biofilm; seaweeds; elicitors; co-culture

1. Introduction

The discovery of antibiotics accelerated after the penicillin breakthrough by Fleming. However, incorrect use and lack of monitoring by humans cause ineffectiveness or resistance to the antibiotic. The reason may also be due to the bacteria themselves, as they adapt to large doses due to their rapid reproduction, as some bacteria reproduce in less than 20 hours [1]. Recently, antibiotic resistance has emerged as a significant global health issue, and the World Health Organization (WHO) listed it as one of the top ten risks to public health worldwide in 2019 [2]. Moreover, the third disease that causes death in the world is untreated bacterial infection [1]. The global rise of antibiotic resistance will put up to 10 million people at risk yearly by 2050 [3,4]. In addition, multidrug-resistant bacterial infection is causing a burden cost amounting to more than 4.6 billion USD spent on the treatment of infectious diseases in 2017 in the United States alone, which has been only increasing since then [5]. As a result, these drive the need to identify the causes of antibiotic resistance and detect novel compounds for the antibiotic pipeline that effectively combat infections. There are various reasons behind antibiotic resistance, and the mechanisms are quite complex. In terms of molecular mechanisms, antibiotic resistance has been exhibited in the most commonly used types of antibiotics that includes: 1) decreased permeability in enterococcal bacterial resistance against low concentrations of aminoglycosides, 2) increased efflux pump in tetracycline resistance, 3) alteration of the antibiotic target as methicillin resistance, and 4) antibiotic hydrolysis by bacterial enzyme B-lactamase in penicillin and cephalosporin resistance [6–9]. Nowadays, bacterial biofilms are the cause of over 80%

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of bacterial illnesses, and about 40%-80% of bacterial biofilms lead to antibiotic resistance [10]. Bacterial biofilms have the potential to develop resistance and tolerance to antibiotics ranging from 10 to 1000 times more than planktonic bacteria [11]. Although bacterial biofilms have a positive effect as biological control agents against plant pathogens, it has been proven that bacterial biofilms had damaged human health, food safety, and the food industry [12–14]. Therefore, this mini review aims to focus on providing literature evidence from 2015 to 2024 on promising approaches and sources for the discovery of antibiofilm compounds that may help the antibiotic resistance crisis.

2. Materials and Methods

Using a certain phrase as the main search term in each year for ten years, a methodical search was carried out on Google Scholar to find relevant papers. Conference abstracts and review papers were not included in the search, which was limited to English-language publications. After initial retrieval, papers that did not contain the search keyword at all or only mentioned it in the conclusion or discussion sections were carefully eliminated manually. Furthermore, studies that did not specifically use the term "endophytic" were still included if the methodology explained techniques that were compatible with isolating endophytic or marine-derived/associated fungi (e.g., surface sterilization of plant or marine host tissues using 0.01% sodium hypochlorite to remove epiphytic microorganisms) as described in Table 1.

This search strategy was limited by several factors. The first search was conducted using only one term and no Boolean operators or alternative keywords, which might have limited the amount of material that could be found. Additionally, only Google Scholar database was searched; relevant research that was indexed elsewhere might have been missing because other databases were not included. This systematic search method was employed for literature quantification, but the compilation of fungal-derived chemicals in Table 2 depended on a more expansive and less standardized literature review, without uniform search keywords, exclusion criteria, or database limitations.

Table 1. Number of papers obtained from Google Scholar (2015–2024) using specified search phrases before and after application of exclusion criteria.

Year	Marine endophytic fungi antibiofilm compounds		Seaweed endophytic fungi antibiofilm compounds	
	Before Exclusion	After Exclusion	Before Exclusion	After Exclusion
2015	46	0	7	0
2016	53	0	12	0
2017	83	2	30	0
2018	138	1	22	0
2019	182	0	56	1
2020	249	1	73	0
2021	447	4	140	1
2022	544	3	181	1
2023	846	1	246	0
2024	764	0	233	0

Exclusion criteria: conference abstracts, review papers, non-English language articles, papers that did not mention the search keyword or mentioned it only in the conclusion/discussion sections, and studies that did not use the keyword "endophytic" and did not describe a methodology compatible with isolating endophytic fungi or not pertaining to a host-associated fungus or fungal symbiont.

3. Bacterial Biofilm

A bacterial biofilm occurs when several bacteria aggregate on a biotic/abiotic surface and excrete extracellular polymeric substances (EPS) such as sugars, proteins, extracellular DNA, and water [15]

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. The stages of bacterial biofilm formation as shown in Figure 1, begin with planktonic bacterial cells when they adhere to a biotic or abiotic surface. When the bacteria aggregate together, they become sessile bacterial cells which can move away from the adhesion on the surface and return to their reversible planktonic state. After they form sessile bacteria, EPS is secreted which completes the fixed attachment of the bacteria, contributing to their being in an irreversible state. After that, the bacteria form colonies, which in turn secrete huge amounts of EPS, which act as a barrier to protect these colonies. In their maturity stage, the bacterial colonies are shaped like mushrooms, and they form a three-dimensional biofilm structure [16]. The shape of the bacterial biofilm allows the exchange of nutrients/water and genes between bacteria of same and different species [17]. The gene expression patterns of sessile cells in a bacterial biofilm differ significantly from those found in planktonic cells as in Pseudomonas aeruginosa (P. aeruginosa) colony [12]. Finally, these mature bacterial biofilms are destroyed, releasing microorganisms that may infect other parts of the host organism or further spread on their inert surface and form new biofilms [10]. In addition, dispersion of mature bacterial biofilms can be caused by their exposure to nitric oxide (NO) [18]. Bacteria communicate to each other or to other microorganisms, whether between same or different species in the biofilm matrix by a quorum sensing system (QS). This occurs through secreting an autoinducer (AI) in response to the density of bacterial cells. QS regulates gene expression to control their pathogenicity and allows microbes to produce virulent factors [12,19,20]. QS has been linked to a variety of bacterial functions, including motility, virulence, bioluminescence, pigment disposition, biofilm formation, and polysaccharide production [21]. Besides, EPS have a major impact on the development of these aggregations, the rate of gene exchange, and antimicrobial resistance [22]. There are various proposed reasons for bacterial biofilms to become resistant to antibiotics. Since bacterial biofilm entails EPS that is negatively charged, it is expected that positively charged antibiotics will interact with it, which may either reduce or prevent their bioactivity. Another point of view is restricting the diffusion of antibiotics by EPS and therefore, decreasing the absorption of antibiotics to inhibit the growth rate of bacteria within biofilms [17,23]. This antibiotic resistance was further demonstrated by Acinetobacter baumannii (A. baumannii) and Staphylococcus biofilm where the EPS matrix was shown to limit the diffusion of antibiotics while just keeping the antibiotic near the surface [24]. Generally, penetration of beta-lactams and glycopeptides is usually less than that of other antibiotics [25]. Additionally, bacteria within biofilms rapidly adapt to external changes through altered metabolism and gene expression, which may contribute to changes in target cells or hiding target sites and thus resulting to antibiotic resistance [13,17]. Meanwhile, new resistant pathogenic strains are currently being discovered in increasing frequency, demanding the need for a new pipeline of potentially beneficial compounds [19]. As the formation of a bacterial biofilm aggravated the problem of antibiotic resistance, this encourages the discovery of new antibiofilm compounds.

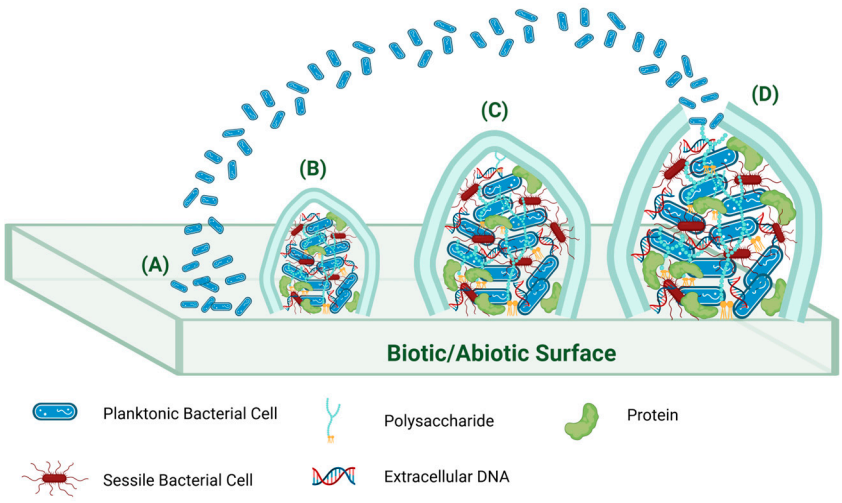

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Figure 1. Bacterial biofilm development. **A)** Reversible adherence, **B)** Micro-colony formation (irreversible attachment). **C)** Biofilm maturation. **D)** Biofilm Dispersion.

In the context of treatment strategies against bacterial biofilm formation or disruption, as was already mentioned above, QS contributes to the development of biofilms. Since QS are not necessary for bacterial growth, QS inhibitors (QSIs) cause less evolutionary pressure on the bacteria and thus, do not cause the development of resistance compared to the more common bactericidal or bacteriostatic mechanisms of antibiotic activity [21]. It seems that one beneficial method of controlling bacterial biofilm is employing QSIs to reduce or suppress biofilm formation as a treatment strategy [10]. QSIs have shown to increase the susceptibility of bacterial biofilms to antibiotics and thus, contribute to the success of the antibiotic treatment [26]. One study showed significant synergy effects in preventing biofilm in a rat model induced with Staphylococcal vascular graft infection when combining daptomycin antibiotic with a QS inhibitor [27]. Moreover, NO can also be used to disrupt biofilms, and its use in combination to existing antibiotics will likely increase their effectiveness against bacterial biofilms [18,28]. A published article showed that exposure to sodium nitroprusside as NO donor markedly improved the ability of some antimicrobial compounds such as hydrogen peroxide, sodium dodecyl sulphate, and tobramycin to effectively eliminate P. aeruginosa biofilms [29]. Given that most biofilm-dispersing drugs, do not kill bacterial cells, it is advantageous to combine them with an antibacterial agent [22]. Therefore, combination of novel anti-biofilms agent with biofilm-dispersing drugs as NO analogue or QSIs or conventional antibiotics could eradicate bacterial biofilm to treat microbial infections and help in drug resistance crises.

4. Natural Products for the Antibiotic Pipeline

Natural products (NPs) offer an appealing reservoir of chemicals with novel anti-microbial compounds. NPs have been a strong source of antibacterial compounds since ancient times, accounting for 78% of our current drugs in the market approved between 1983 and 1994 [30]. NPs afforded structural complexity and diverse chemical composition that make them important in the search for new drugs, especially for the treatment of new emerging infectious and cancer diseases [31,32]. These variations had also lead to differences in the QS inhibitory action of these compounds [19]. In addition, NPs are less prone to develop resistance against bacteria [5]. NPs are secondary metabolites that work as defensive molecules against existing pathogens [33]. In connection with anti-bacterial activity, NPs have been found effective against many pathogenic microbes, but they are distinct from synthetic antibiotics in that they have diverse modes of action against microbes [34,35]. This feature could help against bacterial resistance to antibiotics. As some NPs, most of which are phytochemicals or plant-derived such as 27 flavonoid, 23 alkaloids and 17 terpenes have been proven effective against MRSA (methicillin-resistant *Staphylococcus aureus*) by destroying the bacterial membrane and blocking the efflux pump [36]. In addition, plant-derived compounds have successfully inhibited the formation of new biofilms for *Staphylococcus aureus* (*S. aureus*) as well as *Staphylococcus epidermidis* (*S. epidermidis*) [37]. On the other hand, because oceans covering the earth contain 500,000 living species, it is beneficial to discover novel bioactive compounds from it, which could help challenge antibiotic resistance [38].

Marine-derived fungi have also shown to play a role in inhibiting *S. aureus* biofilm. Two compounds, secalonic acids B (**70**) and D (**71**) inhibited bacterial biofilms by more than 90% at 6.25 micrograms/mL without inhibiting cell growth [39]. Therefore, there is some evidence that support metabolites derived from the marine fungi may possess anti-quorum sensing and/or antibiofilm properties. For example, the marine fungus *Penicillium chrysogenum* DXY-1 afforded cyclo(L-Pro-L-Tyr) (**68**), a cyclic dipeptide or diketopiperazine that has been shown to inhibit biofilm formation and lowers QS gene expression in *P. aeruginosa* PA01 [40]. In addition, the fungal strain *Blastobotrys parvus* PPR3, which was isolated from the woods of the mangrove plant *Avicennia marina*, exhibited anti-QS activity and antibiofilm effects against *P. aeruginosa* PAO1 [41]. Meanwhile, there are huge untapped reservoirs for the isolation of new marine microbes that have a high potential to produce bioactive

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secondary metabolites [42]. From that point on, marine fungi are full of secondary bioactive metabolites, and the microorganisms derived from seaweeds are promising sources for the discovery of antibiofilm compounds and QSIs.

Seaweeds for instance are affected by changes in season, harvest time, geographic location, and ecological variables, which increases the probability of finding novel bioactive compounds [43]. Furthermore, seaweeds have been characterized by their chemical diversity that represents an opportunity to attract compounds with distinctive properties and uses with a wide range of biological activities [44]. The first record of antimicrobial activity from seaweed extracts was published in 1944 by Pratt et al. [45]. Moreover, the three extracts of brown, green, and red seaweeds have exhibited antibacterial activity as well [46,47]. In terms of antibiofilm activity, studies have shown the occurrence of compounds derived from seaweeds to possess antibiofilm activity against pathogenic microorganisms [48]. Fucoidan (sulfated polysaccharide), derived from the brown algae, *Fucus vesiculosus*, afforded antibiofilm activity against *Streptococcus mutans* and *sobrinus* [49]. Furthermore, phlorotannins derived from another brown seaweed, *Hizikia fusiforme*, were found to inhibit biofilm formation in *P. aeruginosa* as well as inhibit QS activity of the reporter strain *Chromobacterium violaceum* [50]. Nevertheless, some of the earlier reported bioactive compounds derived from algae are now attributed to their associated microorganisms [51]. In line with this, available reports have confirmed that these seaweed-associated microorganisms consisted of bacteria, fungi, and yeast [52–54]. Some of these microbes have antimicrobial properties as well as against nosocomial pathogens [55].

5. Seaweed-Derived Microorganisms

Seaweed-associated microorganisms have drawn a lot of attention lately as a potential new supply of secure and potent bioactive. It is worth mentioning that most macroalgae have a rich diversity of epiphytic and endophytic microorganisms [56]. Epiphytic microbes are the living microbes found on the surface of seaweed, and they are important in biogeochemical cycles [57–59]. Furthermore, epiphytic bacteria are the most studied microbes in algae due to their abundance and the convenience of isolating them from thinner fronds [60]. Epiphytic fungi associated seaweeds have shown to have antibacterial activity [61]. Regarding their antibiotic activity, 38 epiphytic bacterial strains from 5 algal species were found to be exhibit antibacterial activity [62]. On the other hand, when microorganisms colonize the internal tissues of the seaweeds without causing any infection to the host, they are called endophytic microorganisms [57]. They are primarily found in natural ecosystems that invade the intercellular spaces of the host organism and could modify the primary and secondary metabolism of the host, which affects several aspects of responses by the host organism [63,64]. The effectiveness of seaweed-associated endophytic bacteria against pathogenic microbes has been reported [65]. Furthermore, multiple studies have also proven the effectiveness of algal endophytic fungi against bacterial pathogens [66–70]. Recently most of the secondary biologically active metabolites derived from microbes are of fungal origin [71]. Generally, four categories of endophytic fungi were identified: *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Oomycota* [72]. The function of endophytic fungal metabolites is to strengthen the plant immune system, combat pathogen invasions, and lessen biotic and abiotic stresses [73]. Besides that, the endophytic fungi derived from seaweeds are thought to be a talented source for the synthesis of novel secondary metabolites with antibacterial activity [74].

5.1. Antibacterial Metabolites from Seaweed Endophytes and Other Marine-Derived Fungi

Secondary metabolites of endophytic and other host-associated fungi play a role in the discovery of new compounds, especially in the search for new antibacterials. Generally, the types of bioactive compounds found in abundance in endophytic fungi from seaweeds included lactones, peptides, polyketides, alkaloids, and terpenoids [75]. As examples of polyketides metabolites that have antibacterial activity include phenalenone and diketo-lactone ring derivatives derived from endophytic fungi (*Coneutherium grains*) associated with the green algae *Enteromorpha* sp [76].

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Similarly, one of the five polyketide compounds obtained from the endophytic fungus (*Paraconiothyrium* sp.) derived from red seaweed (*Chondrus ocellatus holmes*) exhibited a moderate level of antibacterial activity [77]. The extracted an endophytic fungus derived from the green seaweed, *Ulva* sp., was capable of producing an antibacterial metabolite called ascosalipyrrolidinone, an alkaloid metabolite [78]. Likewise, the sesquiterpenoid metabolite albican-11,14-diol, isolated from the *A. versicolor* endophyte derived from *Codium fragilis,* showed antibacterial activity [79]. There are some examples of lactone metabolites that have antibacterial activity such as: 5-hydroxy-de-*O*-methyllasiodiplodin and de-*O*-methyllasiodiplodin. Subsequently, *Fusarium oxysporum*, *Bacillus subtilis* (*B. subtilis*), and *S. aureus* cannot grow in the presence of lasiodiplodin metabolites from the endophytic fungus associated with the brown algae, *Sargassum* sp. [80]. Therefore, lately there are many kinds of metabolites of algal endophytic fungi that have an antibacterial effect that makes researchers turn to them as a source of new antibiofilm compounds.

On the other hand, from marine invertebrates, which are a more common source of -derived fungi, three new peptides known as asperversiamides, were isolated from the fungus *A. versicolor*, which was derived from a gorgonian soft coral. These substances demonstrated potent inhibitory action against *Mycobacterium marinum* [75]. Additionally, *Penicillium brocae*, an endophytic fungus derived from the marine mangrove plant *Avicennia marina* provided four new sulphide diketopiperazine derivatives showed antimicrobial activity [64]. Although, 47% of the peptides isolated from marine fungi have no biological activity and about 53% of them have cytotoxic effects, consistently needs intensive evaluation [81]. One of the bioactivities of the cyclic dipeptide, cis-cyclo (Leucyl-Tyrosyl) derived from a marine sponge-associated *Penicillium* sp has remarkable ability to inhibit up to 85% of the formation of biofilms from *S. epidermidis* [82].

5.2. Antibiofilm Compounds from Marine-Derived, Endophytic and Other Fungi

Figure 2 shows a paucity of studies directly pertaining to 'Seaweed endophytic fungi antibiofilm compounds', with only two identified from 1,010 articles between 2015 and 2024 [83,84]. Conversely, the more general phrase resulted in 12 articles throughout the same timeframe, suggesting insufficient focused research on this domain [41,83,85–93]. All these articles discussed in depth below. Nonetheless, this shortage indicates that although general characteristics of antibiofilm compounds from marine endophytic fungus have been investigated, the specialized area of antibiofilm compounds produced from seaweed remains insufficiently examined. Addressing this gap is crucial for advancing algal endophytic fungi, since it directly influences the discovery of novel antibiotics.

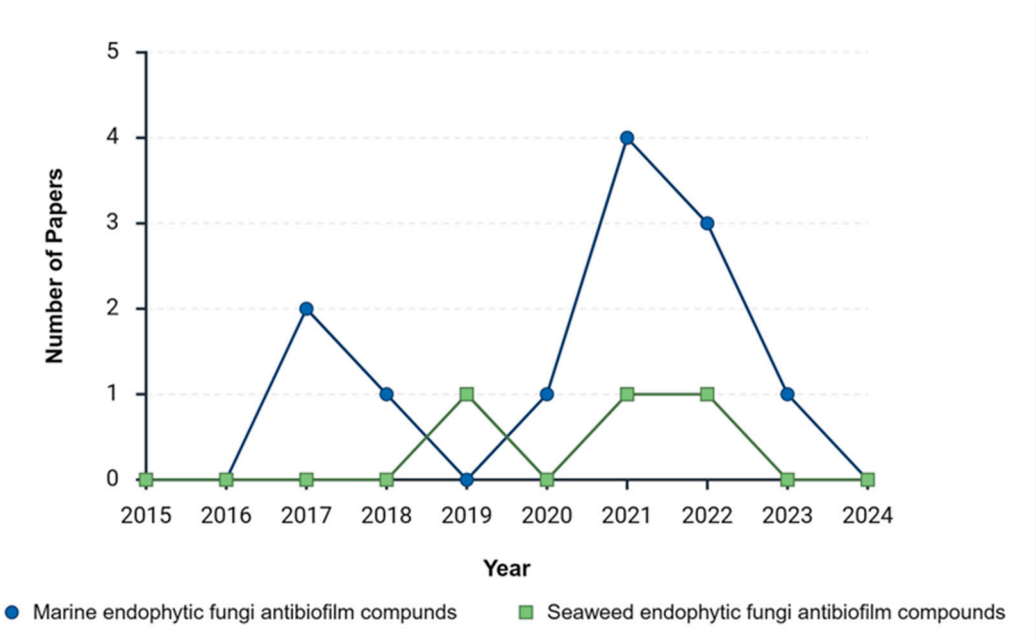

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Figure 2. Comparative data about the number of publications on antibiofilm compounds from marine endophytic fungi sources in Google Scholar from 2015 till 2024.

Fungi represent a source of antibiofilm-active secondary metabolites for a promising drug pipeline against bacterial biofilms. In this section, we are considering other sources of antibiofilm fungal metabolites because a very small percentage of these compounds have been described in comparison to the larger number of described antibacterial compounds [94]. The classification of bacterial strains identified as weak, moderate, and strong biofilm producers, include *Escherichia coli (E. coli), S. aureus*, *B. subtilis*, and *P. aeruginosa,* respectively [95]. In this review, we will focus on the formation of robust bacterial biofilm. There are two phenotypic types of *P. aeruginosa* biofilm: mucoid and non-mucoid. The mucoid variant is more challenging to eliminate compared to non-mucoid biofilms [96]. Nonetheless, the mucoid strains of *P. aeruginosa* exhibit greater sensitivity to antibiotics compared to non-mucoid isolates [97]. The non-mucoid strains of *P. aeruginosa*, including PAO1 and ATCC 27853, are commonly the focus of investigation [98]. There are numerous antibiofilm activity of the extracts of fungi against bacteria have been published.

Dendryphiella salina (*D. salina*) a fungi derived from the seaweed *Laminaria hyperborea* displayed 100 % inhibition activity against *P. aeruginosa* (ATCC 27853) biofilm at 100 µg/mL [84]. Differences in antibacterial and antibiofilm bioactivity have been described in literature depending on the type of fungus, media used for cultivation, and the extraction solvent employed. As in the investigation of non-marine fungal extracts, Attia EZ and co-workers used several media, also known as OSMAC approach (One-Strain-Many-Compounds) and got different percentages of biofilm inhibition, however, the modified potato dextrose broth (MPDB) medium gave the highest percentage of inhibition [99]. Whereas, the potato dextrose agar (PDA) media effects positively on the production of secondary metabolites [100,101]. Indeed, the biosynthesis of secondary metabolites is significantly influenced by the composition of the media [102]. Similarly, Jaber [103] also did an OSMAC approach with malt extract with and without sea salt, Wickersham media with and without sea salt, marine broth, rice media with and without sea salt, and oat with and without sea salt. For the scale-up, the malt extract broth with sea salt was also chosen to grow *D. salina* for 30 days as it provided the most potent antimicrobial activity and afforded a different chemical profile to that of *D. salina* grown on oat media with MIC of 20.0 and 17.5 µg/ml and MBEC (Minimum Biofilm Eradication Concentration) of 21.8 and 18.8 µg/ml against both *S. aureus* and *P. aeruginosa*, respectively. The optimum growth of *D. salina* with the sea salt requirement was evident that the fungus is a "true" marine algal endophyte.

Furthermore, the solvent employed for extraction will influence the activity as well. The ethyl acetate (EtOAc) extract had superior antibiofilm activity compared to both methanol and acetone extracts, achieving complete biofilm formation inhibition (100%) against non-mucoid strains of *P. aeruginosa,* whereas the acetone and methanol extracts exhibited lower activities of 40% and 50%, respectively [104–106]. Furthermore, the EtOAc extracts derived from the endophytic fungus *Neocosmospora sp.* MFLUCC 17-0253, obtained from the mangrove plant *Rhizophora apiculata,* demonstrated inhibition of biofilm formation in *Acidovorax citrulli* JT-0003 at 44 to 77%, when applied at concentrations ranging from 12.5 to 100 µg/mL [87]. Similarly, EtOAc extracts demonstrated higher potency of antibiofilm activity compared to their chloroform extracts, as evidenced by its greater percentage of biofilm inhibition against the tested *S. aureus* as demonstrated by several studies [91,107–109].

In terms of the activity of fungal crude extracts and their purified metabolites, the crude extract of *Alternaria alternata* has slightly increased its antibiofilm activity against *P. aeruginosa* than their purified metabolites [90,110]. A study likewise demonstrated that a fungal extract that afforded weak biofilm inhibition against *P. aeruginosa* at 36%, totally loss its bioactivity when the respective metabolites were purified [111]. The crude extract could have contained several metabolites that when their modes of action were combined would lead to the extract's bioactivity causing a synergistic effect as interpreted by Njateng and his colleagues [102,112]. Alternatively, another study

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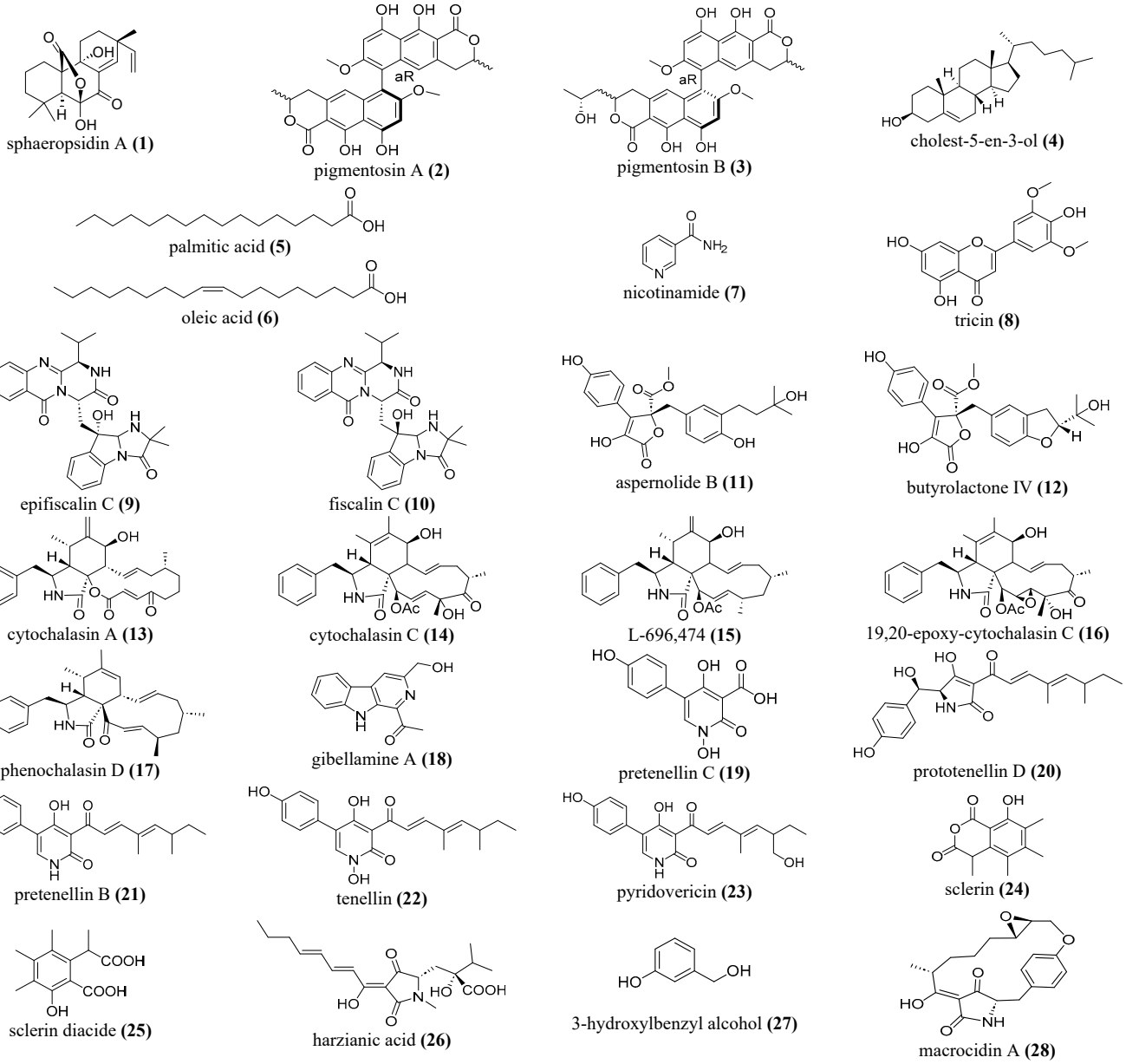
showed that the crude extract of a marine-derived *Penicillium* sp. inhibited biofilm formation in *S. aureus* at only 19% while their purified metabolites, particularly β-sitosterol (**77**), exhibited antibiofilm activity at 64% [113]. In this case, bioactivity required specificity and concentration dependent on a single metabolite. It is also worth mentioning that the bioactivity of the host organisms is totally independent from that of the endophyte. The plant extract of *Phragmites australis* lacks antibacterial properties, while the metabolites of its endophytic fungi associated with the plant showed moderate bioactivity against gram-positive strains, with some achieving 80% biofilm inhibition of *S. aureus* [90,114] .

Table 2 lists the various studies on the antibiofilm activities of fungal metabolites against several bacterial test strains. The fungal metabolites were categorized under three groups of sources: 1) non-endophytic-terrestrial, 2) terrestrial endophytes, and 3) marine symbionts. The chemical structures of these metabolites are shown in Figure 3. Polyketides are the most common type of bioactive metabolites found in literature and have a major role in the inhibition of biofilm activities. Steroids and terpenoids as secondary metabolites have been reported from both marine- and soil-derived fungi against various harmful bacteria.



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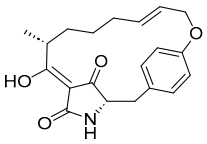
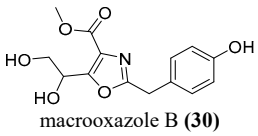
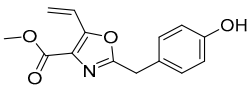
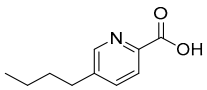
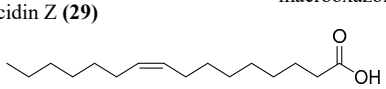
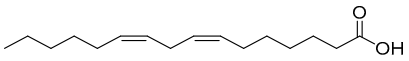

macrocidin Z **(29)**

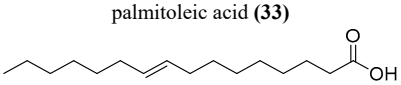

macrooxazole B **(30)**

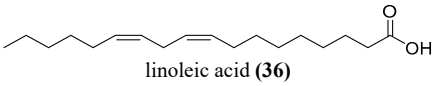

macrooxazole C **(31)**

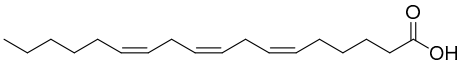

5-butyl-2-pyridine carboxylic acid **(32)**

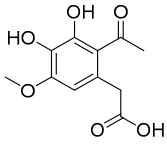

palmitoleic acid **(33)**

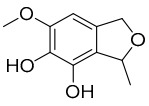

7Z,10*Z*-hexadecadienoic acid **(35)**

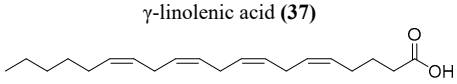

palmitelaidic acid **(34)**

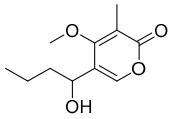

linoleic acid **(36)**

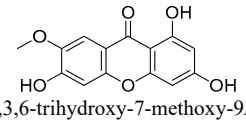

γ-linolenic acid **(37)**

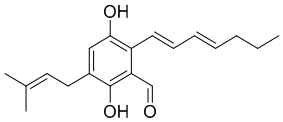

vulculic acid **(39)**

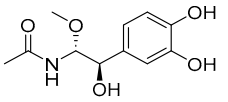

curvulol **(40)**

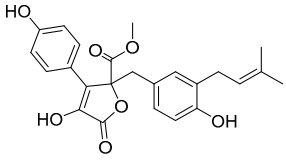

arachidonic acid **(38)**

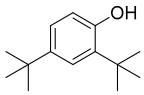

5-(1-hydroxybutyl)-4-methoxy-3-methyl-2*H*-pyran-2-one **(41)**

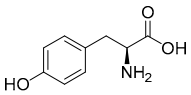

1,3,6-trihydroxy-7-methoxy-9*H*-xanthen-9-one **(42)**

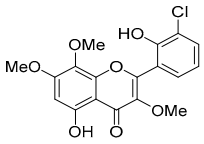

dihydroauroglaucin **(43)**

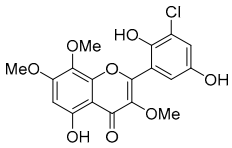

(±)-periplanamide B **(44)**

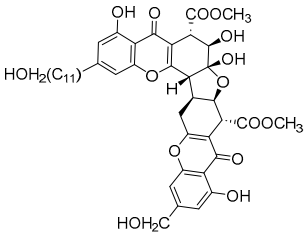

butyrolactone I **(45)**


2,4-di-tert-butylphenol **(46)**


L-tyrosine **(47)**


chlorflavonin **(48)**


chlorflavonin A **(49)**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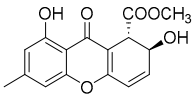
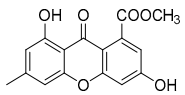
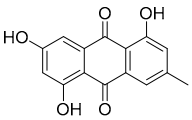
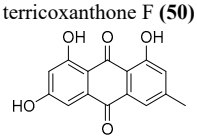
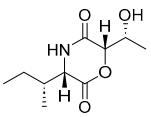

aspergillusone A **(51)**


4-hydroxyvertixanthone **(52)**


alatinone **(53)**


emodin **(54)**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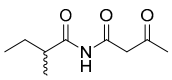
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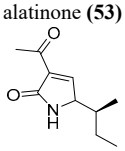
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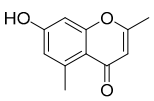
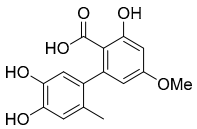
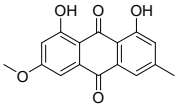
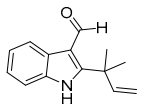
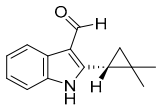
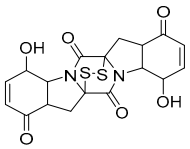

altechromone **(58)**

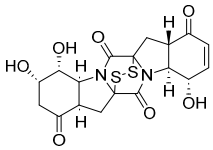

altenusin **(59)**

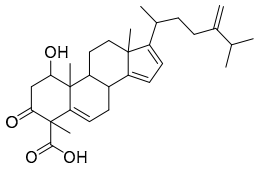

physcion **(60)**

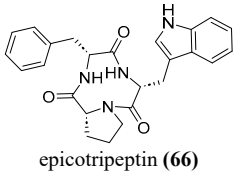

prenylated indole carbaldehydes **(61)**

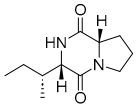

prenylated indole carbaldehydes **(62)**

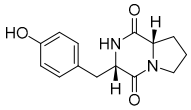

epicorazine A **(63)**


epicorazine C **(64)**


terpenoid **(65)**


epicotripeptin **(66)**


cyclo(L-Pro-L-Ile) **(67)**


cyclo(L-Pro-L-Tyr) **(68)**

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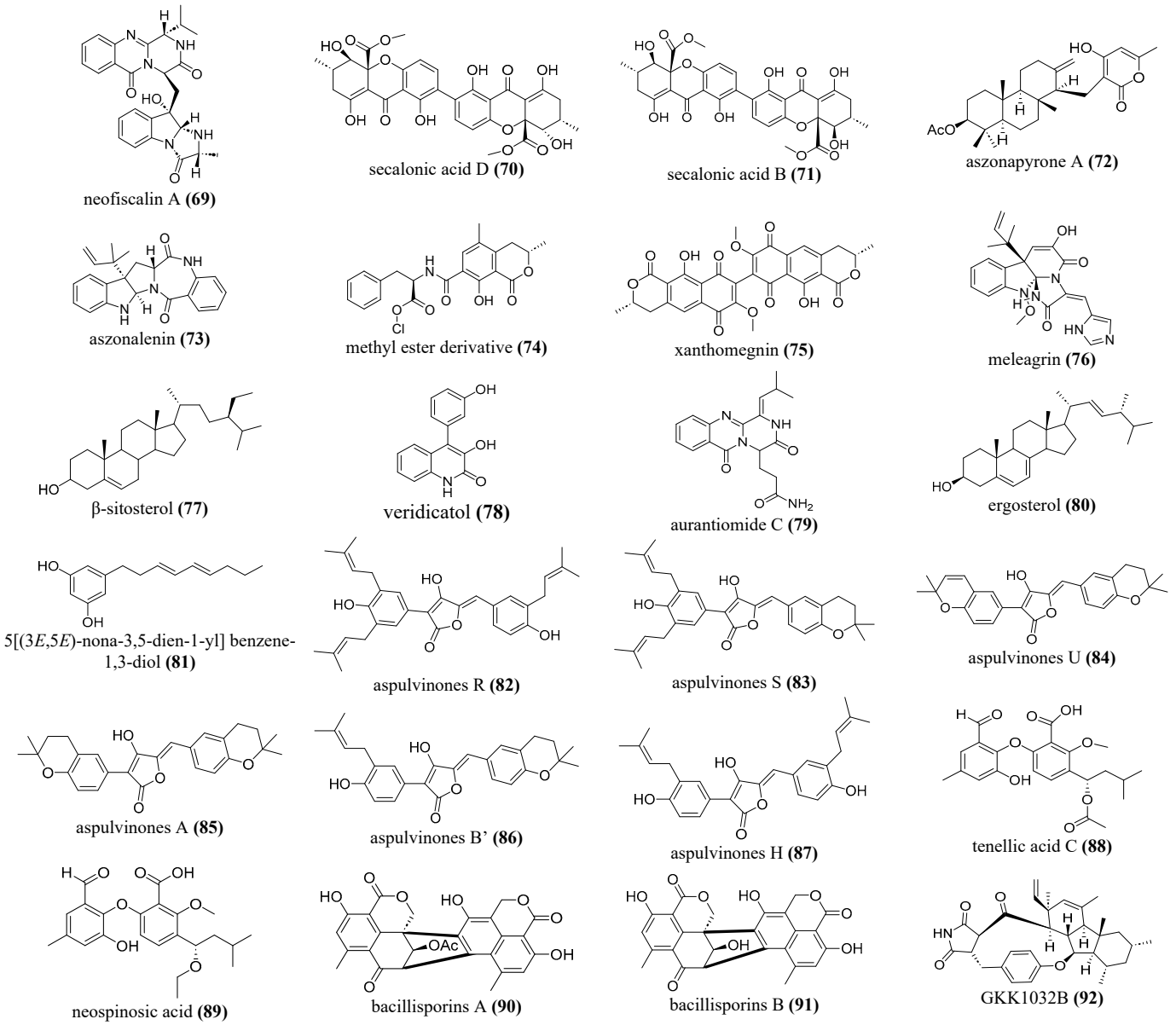

Figure 3. Chemical structures of antibiofilm-active fungal secondary metabolites.

5.2.1. Terpenoids and Steroids

Soil-derived fungi demonstrated superior efficacy, with cholest-5-en-3-ol (**4**), exhibiting 78% biofilm inhibition against *B. subtilis*, 59% against *S. aureus*, and 59% against *E. coli* [111]. In contrast, two steroidal compounds from a marine-derived *Penicillium sp.* revealed that β-sitosterol (**77**) achieved 28% inhibition of biofilm formation in *B. subtilis* and 64% in *S. aureus*, while ergosterol (**80**) displayed 40-55% antibiofilm activity solely against *E. coli* [113]. Diterpenes such as aszonapyrone A (**72**) from marine-derived fungus *Neosartorya siamensis* isolated from a sea fan exhibited 72% efficacy at 9 µg/mL and 94% efficacy at 6.25 µg/mL against *S. aureus* ATCC 29213 and *S. aureus* 272123, respectively [115]. However, the *Diplodia corticola* fungal phytotoxin inhibited biofilm formation at 53% at 1/4 MIC of 1.56 µg/mL against a clinical strain of *MRSA* and 62% at a 1/4 MIC of 3.12 µg/mL against a clinical strain of *P. aeruginosa* [116]. A recently described terpenoid from *Epicoccum nigrum* of the *Phaeurus antarcticus* seaweed showed significant activity against MRSA with a MBEC of 25 µg/mL and ruptured formed biofilm at 100 µg/mL [83]. *Penicillium erubescens* KUFA0220, isolated from the marine sponge *Neopetrosia sp*, showed significant biofilm formation inhibition against *Enterococcus faecalis* ATCC29212 at 8 µg/mL and 16 µg/mL [89]. This revealed that the terpenoid compounds derived from marine fungi exhibited greater activity against gram-positive bacteria,

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aligning with a recent study on marine-derived terpenoids showcasing their antibacterial properties [117].

5.2.2. Alkaloids and Peptides

Table 2 reveals that most active alkaloids extracted from fungi are the indole type that includes epifiscalin C (**9**), fiscalin C (**10**), gibellamine A (**18**), two prenylated indole carbaldehydes (**62**) and (**63**), neofiscalin A (**69**), aszonalenin (**73**), and meleagrin (**76**). Marine-derived indole alkaloids exhibit significant antibacterial activity against various pathogens, as outlined in the preceding review [118]. In addition, synthetic indole derivatives exhibited antibiofilm activity against *Serratia marcescens* and interfere with QS [119]. While a non-marine fungal source derived from the Canadian thistle *Circium arvense* afforded alkaloids, macrocidin A (**28**), macrocidin Z (**29**), macrooxazole B (**30**), macrooxazole C (**31**) exhibiting no more than 80% inhibition of biofilm formation in *S. aureus* DSM 1104 [92,120] .

Eight non-ribosomal peptide compounds exhibited significant biofilm inhibition, with seven isolated from marine sources such as phragamide A (**55**), tenuazonic acid (**57**), epicorazines A-**C** (**63**) and (**64**), epicotripeptin (**66**), cyclo(L-Pro-L-Ile) (**67**), cyclo(L-Pro-L-Tyr) (**68**) in figure 3. The three secondary metabolites, epicotripeptin (**66**), cyclo(L-Pro-L-Ile) (**67**), cyclo(L-Pro-L-Tyr) (**68**)) were isolated from the endophytic fungus *Epicoccum nigrum* M13, derived from seagrass and displayed moderate bioactivity against positive bacterial strains [90].

5.2.3. Flavonoids, Phenolics and Polyketide Compounds

Two studies identified fungal flavonoid compounds that exhibited antibiofilm activity. Tricin (**8**) was isolated from the soil fungus *Sarocladium kiliense* SDA20, and exhibited weak inhibition of biofilm formation in *E. coli* and *S. aureus* [111]. Flavonoids isolated from the endophytic fungus *Aspergillus candidus* T1219W1, derived from *Pittosporum mannii* Hook f. exhibited significant biofilm inhibition (exceeding 60%) in *E. coli* and *S. aureus* [121]. Other phenolic metabolites are also listed in Table 2. This includes 5[3*E*,5*E*)-nona-3,5-dien-1-yl]benzene-1,3-diol (**81**) isolated from the marine sponge-derived *Aspergillus stellatus* KUFA 2017 exhibited 100% inhibition of biofilm formation in *S. aureus* and *E. faecalis*. In terms of low MWs phenolic compound derived from fungi, one example is 3'-hydroxylbenzyl alcohol (**27**), from *Aspergillus nidulans* isolated from a forest soil, could inhibit a broader range of bacterial biofilms that included *S. aureus*, *B. subtilis*, *Micrococcus luteus* (*M. luteus*), *Actinomyces viscosus* (*Act. viscosus*), *C. violaceum*, *E. coli*, *Klebsiella planticola* (*K. planticola*), and *P. aeruginosa*. While the compound isolated from the endopyte *Daldinia eschscholtzii* showed 49% inhibition against *P. aeruginosa* [122,123] . Three studies found antibiofilm active metabolites against *S. aureus* DSM 1104 such as the cytochalasin polyketides (**13** to **17**), phenolic sclerin (**24**) and its diacide (**25**), along with unsaturated fatty acids (**33** to **38**) isolated from *Hypoxylon fragiforme* fungus derived from the Harz Mountains [124–126].

Antibiofilm phenolic compounds biosynthesized from the polyketide pathway have been afforded by marine sponge-derived fungi. These include aspulvinones (**82** to **87**) isolated from *Aspergillus flavipes* KUFA1152 [85], tenellic acid C (**88**) and neospinosic acid (**89**) from *Neosartorya spinosa* KUFA 1047[86], and bacillisporins (**90** and **91**) from *Talaromyces pinophilus* KUFA 1767 [93].

Primary metabolites such as fatty acids (**5, 6, 33** to **38**) do play a role as well in biofilm inhibition [111,126] , particularly in their absorbance or crossing through the EPS matrix to disrupt the biofilm. Amino acids from endophytic fungi, such as *Rhizopus oryzae* and *Aspergillus tubingensis*, have significant biofilm inhibition activity as well [127,128] . Primary metabolites are crucial for microbial growth and exhibit similarities among microbial species; consequently, researchers often investigate secondary metabolites to discover novel antibiofilm compounds that have not been thoroughly examined. Furthermore, climate change and salinity alteration in cases of marine resources affect the production of these secondary metabolites, suggesting fungi or other microorganisms could be good reservoirs of various active substances [129]. However, as shown in Table 2, only two investigation that has extracted antibiofilm compounds from endophytic fungi from seaweeds [84]. Maybe because

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working with endophytic fungi of seaweeds can be challenging requiring specific sterilization methods to avoid epiphytic contaminants [130]. Then again, there is a trend for researchers to find secondary metabolites from endophytic fungi that have antibiofilm activity, but the challenge arises in increasing the production of these metabolites.

Table 2. Anti-biofilm activity of reported fungal metabolites.

Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
1) Non marine and non-endophytic fungal source					
sphaeropsidin A **(1)**	*Diplodia. corticola*	fungal phytotoxin	Biofilm formation inhibition: Reference MRSA strains: 60% at 3. 12 µg/mL. MRSA clinical: 53% at 1/4 MIC (1.56 µg/mL) Preformed biofilms: (adhesion) Reference *P. aeruginosa* strains: 50% at a 1/4 MIC: 3.12 µg/mL. clinical *P. aeruginosa:* 62% at a 1/4 MIC: 3.12 µg/mL.	MRSA ATCC 43300 reference strains MRSA 1118-116 *P. aeruginosa* PAO1 as reference strains extended-spectrum beta-lactamase (ESBL) producing *P. aeruginosa* 0418-925 *P. aeruginosa* 0418-925.	[116]
pigmentosin A **(2)** and pigmentosin B **(3)**	*Gibellula sp.*	spider	Biofilm formation inhibition: MIC values: 1.9 and 15.6 µg/mL	*S. aureus* DSM1104	[131]
astucin composed of 92 amino acid residues.	*Aspergillus tubingensis*	soil	Biofilm formation inhibition: 99.9%: MBIC: 2 µg/mL against *S. aureus* MBIC: 8 µg/mL against MRSA	*S. aureus* & MRSA	[128]
cholest-5-en-3-ol **(4)**, palmitic acid **(5)**, oleic acid **(6)**, nicotinamide **(7)**, tricin **(8)**	*Sarocladium kiliense* SDA20	soil	Biofilm formation inhibition: *B. subtilis:* Compound **4**: 78 %, Compound **5**: 71 %, Compound **6**: 52% *S. aureus:* Compounds **4** and **5**: 59 %, Compound **8**: low inhibition activity around 35%. *E coli*: Compound **4**: 59% Compound **6**: 53% Compound **7**: 40% Compound **8**: ca. 42% *P. aeruginosa:* No activity	*S. aureus* *B. subtilis* *P. aeruginosa* *E. coli*	[111]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
epifiscalin C **(9)**, fiscalin C **(10)**, aspernolide B **(11)**, and butyrolactone IV **(12)**	fungal extract	NA	Biofilm formation inhibition: Compounds **9**, **10**, and **11**: Above 60%: 16 µg/mL. Compound **12**: Above 50%: 64 µg/mL	MRSA (ATCC 43300)	[132]
cytochalasin A **(13)**, cytochalasin C **(14)**, L-696,474 **(15)**, 19,20-epoxy-cytochalasin C **(16)**, and phenochalasin D **(17)**	*Hypoxylon fragiforme*	Harz mountains	Biofilm formation inhibition: Compound **13**: 91%: 16 µg/mL, Compound **14**: 42%: 256 µg/mL, Compound **15**: 44%: 16 µg/mL Compounds **16** and **17**: 20 to 40%.	*S. aureus* DSM 1104	[124]
gibellamine A **(18)**	*Gibellula gamsii* strain BCC47868	spider	Biofilm formation inhibition: MIC value of 62.5 µg/mL It significantly inhibited biofilm formation.	*S. aureus* DSM1104 (ATCC 25923)	[133]
pretenellin C **(19)** prototenellin D **(20)**, pretenellin B **(21)**, tenellin **(22)** and pyridovericin **(23)**	*Entomopatho-genic fungus Beauveria neobassiana*	Coleoptera	Biofilm formation inhibition: Compound **19**: 46 ± 9% at 31.3 µg/mL Compound **20**: 53 ± 7% at 7.8 µg/mL Compound **21**: 37 ± 7% at 7.8 µg/mL Compound **22**: 36 ± 13% at 7.8 µg/mL. Compound **23**: 48 ± 5 % at 62.5 µg/mL.	*S. aureus* DSM 346	[134]
sclerin **(24)** and its diacid **(25)**	*Hypoxylon fragiforme*	Harz mountains	Biofilm formation inhibition: Compound **24** 86% at 256 µg/mL Compound **25** 80% at 256 µg/mL	*S. aureus* DSM 1104	[125]
harzianic acid **(26)**	*Trichoderma harzianum*, two strains E45 and ET45	soil	Biofilm formation inhibition: at 16 µg/mL: Significant inhibition of biofilm formation in both MRSP and MSSP Pre-formed biofilms: at 32 and 64 µg/mL: Statistically significant disaggregation of pre-formed biofilm produced by MSSP.	*Staphylo-coccus pseudinter-medius (S. pseudinter-medius)* methicillin-resistant (MRSP) methicillin-susceptible (MSSP) strains.	[135]
Exopolysaccharide (EPS)	*Fomitopsis meliae* AGDP-2	mushrooms	Biofilm formation inhibition: at 10,000 µg/mL: *P. aeruginosa:* 86.01% *S. typhi*: 17.64%	*P. aeruginosa Salmonella typhi*	[136]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
				(S. typhi) ATCC 6539	
3-hydroxyl-benzyl alcohol **(27)**	*Aspergillus nidulans strain* KZR-132	forest soil	Biofilm formation inhibition: at 18.75 µg/mL: *S. aureus* MTCC 96: 89.2% *S. aureus* MLS16 MTCC 2940: 88.2 % *S. aureus* ATCC 6538P: 85.6% *B. subtilis* MTCC 121: 80% *M. luteus* MTCC 2470: 90.2% *Act. viscosus* ATCC 15987: 80.6% *C. violaceum*: 88% at 37.5 µg/mL: *E. coli:* 75% *K. planticola:* 70.9% *P. aeruginosa*: 65.5%	*S. aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940, *S. aureus* ATCC 6538P, *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *Act. viscosus* ATCC 15987 *C. violaceum* *E. coli,* *K. planticola* *P. aeruginosa*	[122]
macrocidin A **(28)**, macrocidin Z **(29)**, macrooxazole B **(30)**, macrooxazole C **(31)**	*Phoma macrostoma* DAOMC 175,940	*Circium arvense*	Biofilm formation inhibition: at 250 µg/mL: Compound **30**: 65%, Compound **31**: 75% Compound **28**: 79% Compound **29**: 76% Pre-formed biofilms: at 250 µg/mL: Compound **28**: 75% Compound **29**: 73%	*S. aureus* DSM 1104	[120]
5-butyl-2-pyridine carboxylic acid **(32)**	*Aspergillus fumigatus* nHF-01	NA	Biofilm formation inhibition: 22.30%: 4 µg/mL against *B. cereus* 129 µg/mL against *E. coli*	*Bacillus cereus (B. cereus)* MTCC 1272, *E. coli* MTCC 723, *E. coli* ATCC DH5α,	[137]
palmitoleic acid **(33)**, palmitelaidic acid **(34)**, 7Z,10Z-hexadecadienoic acid **(35)**, linoleic acid **(36)**, γ-linolenic acid **(37),** arachidonic acid **(38)**	*Hypoxylon fragiforme*	Harz mountains	Biofilm formation inhibition: Compound **33**: Sub-MIC: at 64 µg/mL: 54 ± 2% against *S. aureus* at 120 µg/mL: 49 ± 2% against *S. aureus* Compound **34**: Sub-MIC: at 256 µg/mL: 25 ± 4% against *E. coli* at 16 µg/mL: 21± 4 % against *S. aureus* Compound **35**: Sub-MIC: at 128 µg/mL:	*B. cereus DSM 626,* *E. coli MT102,* *P. aeruginosa PA14,* *S. aureus DSM 1104,* *S. epidermidis ATCC 35984,* *S. mutans UA59*	[126]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
			62 ± 8% against *E. coli* at 8 µg/mL: 60± 9 % against *S. aureus* at 8 µg/mL: 22± 14 % against *S. epidermidis* at 128 µg/mL: 38 ± 12 % against *S. mutans* Compound **36**: Sub-MIC: at 16 µg/mL: 35 ± 15 % against *B. cereus* at 8 µg/mL: 54 ± 4 % against *S. aureus* Compound **37**: at 4 µg/mL: 58 ± 1 % against *S. aureus* at 128 µg/mL: 44 ± 19 % against *S. epidermidis* Compound **38**: Sub-MIC: at 64 µg/mL: 36 ± 2 % against *B. cereus* 35 ± 11 % against *S. aureus*		
extracellular protein	*Aspergillus oryzae*	NA	Biofilm formation inhibition: Significantly inhibited at 1/4 MIC (75 µg/mL) and 1/2 MIC (150 µg/mL).	*Klebsiella pneumoniae (K. pneumoniae)* ESBL	[138]
2) Endophytic Fungal Metabolites					
vulculic acid **(39)**, curvulol **(40)**	*Chaetosphaeron ema achilleae*	*Taxus baccata*	Biofilm formation inhibition: Both compounds: 100% at 256 µg/mL.	*S. aureus*	[139]
5-(1-hydroxybutyl)-4-methoxy-3-methyl-2*H*-pyran-2-one **(41)**	*Colletotrichum acutatum*	*Angelica sinensis*	NA	*S. aureus* *K. pneumonia*	[140]
fractions (AF1 and AF2)	*Alternaria destruens* (AKL-3)	*Calotropis gigantea*	Biofilm formation inhibition: AF1: 22.5 µg/mL: *P. aeruginosa:* (58.15%) *E. coli* (around 49%) *S. enterica* (around 42%). AF2: 22.5 µg/mL: *S. enterica* (23.2%). Pre-formed biofilms inhibition: AF1: 22.5 µg/mL: *P. aeruginosa* (35.58%) *E. coli* (21.84%) AF2: 22.5 µg/mL: *P. aeruginosa* 19.2%	*P. aeruginosa* *E. coli* *Salmonella enterica* *(S. enterica)*	[141]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
1,3,6-trihydroxy-7-methoxy-9*H*-xanthen -9-one **(42)**	*Penicillium citrinum-314*	*Halocnemum strobilaceum*	Biofilm formation inhibition: 100 %: MBIC value of 62.5 µg/mL.	*P. aeruginosa*	[142]
dihydroauroglaucin **(43)**	*Aspergillus amstelodami* (MK215708)	*Ammi majus* L. Fruits	Biofilm formation inhibition: *S. aureus* and *E. coli:* MBIC = 7.81 µg/mL *S.mutans*: MBIC = 15.63 µg/mL *P. aeruginosa*: MBIC = 31.25 µg/mL	*S. aureus* *E. coli* *S. mutans* *P. aeruginosa*	[143]
(±)-periplanamide B **(44)**, butyrolactone I **(45)**	*Aspergillus terreus* AH1	*Ipomoea carnea*	Biofilm formation inhibition: Compound **44**: *S. aureus* 90.65%: *B. subtilis* 85.09% *E. coli* 63.144% Compound **45**: *P. aeruginosa*. 71.81%	*P. aeruginosa* (ATCC 27853) *S. aureus* (ATCC 6538-P), *E. coli* (ATCC 25955) *B. subtilis* (ATCC 6633)	[144]
2,4-di-tert-butylphenol **(46)**	*Daldinia eschscholtzii* (TP2-6)	*Tridax procumbens*	Biofilm formation inhibition: 49% at 80 µg/mL	*P. aeruginosa* PAO1	[123]
L-tyrosine **(47)**	*Rhizopus oryzae* AUMC14899	*Opuntia ficus-indica* (L.)	Biofilm formation inhibition: PA-02: 83% (3 µg/mL) SA-04: 87% (7.5 µg/mL)	*P. aeruginosa* PA-02, *S. aureus* SA-04	[127]
chlorflavonin **(48)** and chlorflavonin A **(49)**	*Aspergillus candidus* T12 19W1	*Pittosporum mannii* Hook f.	Biofilm formation inhibition: *S. aureus:* MBEC50: 256 µg/mL Compound **48**: 72.15% Compound **49**: 75.32% *E. coli* MBEC50: 128 µg/mL Compound **48**: 80.12% Compound **49**: 81.22%	*S. aureus* *E. coli*	[121]
terricoxanthone F **(50)**, aspergillusone A **(51)**, 4-hydroxy vertixanthone **(52)** alatinone **(53)** emodin **(54)**	*Neurospora terricola* HDF-Br-2	vulnerable conifer *Pseudotsuga gaussenii*	Biofilm formation inhibition: (MBIC) Compounds **50** and **51**: 128 µg/mL Compounds **52** and **53**: 32 µg/mL Compound **54**: 16 µg/mL Pre-formed biofilms: (MBIC) Compound **50**: 256 µg/mL. Compounds **51**, **52** and **53**: 128 µg/mL	*S. aureus*	[145]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
			Compound **54**: 32 μg/mL		
Mixture of fatty acids	*Arthrographis kalrae*	*Coriandrum sativum*	Biofilm formation inhibition: Minimal biofilm inhibitory concentration (MBIC) of 31.3 μg/mL completely inhibited *S. mutans* biofilm	*S. mutans* ATCC 25175	[146]
3) Marine fungal metabolites					
phragamides A **(55)** and B **(56)**, tenuazonic acid **(57)** and altechromone **(58)** altenusin **(59)**	*A. alternata* 13A	*Phragmites australis*	Biofilm formation inhibition: Gram-positive strains: 70 to 80% Gram-negative strains: 40 to 60%. Compound **59** exhibited moderate biofilm formation inhibition only against *B. subtilis.*	*S. aureus* *B. subtilis* *E. coli* *P. areuginosa*	[90]
emodin **(54)** and physcion **(60)**, and two prenylated indole carbaldehydes **(61)** and **(62)**	*Eurotium chevalieri* KUFA 0006	*Rhizophora mucronata*	Biofilm formation inhibition: Compounds **54**, **60**, **61** and **62** showed inhibition of biofilm production in *S. aureus* ATCC 25923 significantly (*p<0.05). Compound 61: at 64 μg/mL. nearly 80% reduction of *S. aureus.*	*S. aureus* ATCC 25923 *E. coli* ATCC 25922	[88]
epicorazines A **(63)** and C **(64)** as well as a new terpenoid **(65)**	*Epicoccum nigrum*	*Phaeurus antarcticus* (seaweed)	Biofilm formation inhibition: MBEC: Compound **63**: 50 μg/mL Compound **64**: 25 μg/mL Compound **65**: 25 μg/mL Post- biofilms Inhibition: Compound **65:** 100 μg/mL.	MRSA	[83]
epicotripeptin **(66)** cyclo(L-Pro-L-Ile) **(67),** cyclo(L-Pro-L-Tyr) **(68)**	*Epicoccum nigrum* M13 (marine endophytes)	*Thalassia hemprichii* leaves seagrass	Biofilm formation inhibition: Compound **66**: Gram-positive strains (55 to 70% inhibition) Gram-negative strains (20 to 30% inhibition) Compounds **67** and **68**: moderate inhibition of biofilm formation in both Gram-positive strains but were not active against the tested Gram-negative strains.	*S. aureus* *B. subtilis* *E. coli* *P. areuginosa*	[90]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
neofiscalin A **(69)**	*Neosartorya siamensis* (KUFA 0017)	marine sponge	Biofilm formation inhibition: Compound **69** against: MRSA: 96 μg/mL VRE: 80 μg/mL at a concentration of 200 μg/mL, it was able to reduce the metabolic activity of the biofilms by 50%.	*MRSA* Vancomycin -resistant *E. faecalis (VRE)*	[147]
secalonic acid B **(70)** and D **(71)**	*Penicillium sp.* SCSGAF 0023 CCTCCM 2012507	marine	Biofilm formation inhibition: Both Inhibited by >90% at 6.25 μg/mL	*S. aureus*	[39]
aszonapyrone A **(72)**, aszonalenin **(73)**, methyl ester derivative (**74**), xanthomegnin **(75)**	*Neosartory siamensis Neosartorya takakii Aspergillus elegans*	marine	Biofilm formation inhibition: Compound **72**: *S. aureus* ATCC 29213 at 9 μg/mL: 72% *S. aureus* 272123 6.25 μg/mL: 94% Compound **73**: *S. aureus* ATCC 29213 at 100 μg/mL: 63% *S. aureus* 272123 at 6.25 μg/mL: 93% Compound **74**: *S. aureus* ATCC 29213 at 10 μg/mL: 88% *S. aureus* 272123 at 25 μg/mL: 98% Compound **75**: *S. aureus* ATCC 29213 at 100 μg/mL: 96% *S. aureus* 272123 at 50 μg/mL: (84%)	*S.aureus* ATCC 29213 *S. aureus* 272123	[115]
meleagrin **(76)**	*Emericella dentata* Nq45	marine	Biofilm formation inhibition: 250 μg/mL: 87.1%	*S. aureus ATCC 29213*	[148]
β-sitosterol **(77)**, veridicatol **(78),** aurantiomide C **(79)**, ergosterol **(80)**	*Penicillium* sp. MMA	marine	Biofilm formation inhibition: Compound **77**: *B. subtilis* 28% *S. aureus* 64% Compound **78**: *B. subtilis* 35% Compounds **78**, **79, 80**: *E. coli* from 40 -55%	*S. aureus E. coli B. subtilis*	[113]
5[(3*E*,5*E*)-nona-3,5-dien-1-yl]benzene-1,3-diol **(81)**	*Aspergillus stellatus* KUFA 2017	marine sponge *Mycale sp.*	Biofilm formation inhibition: 100 % at *E. faecalis*:	*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212	[92]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
			MIC (16 µg/mL) *S. aureus:* 2xMIC (32 µg/mL).		
Fraction AW1011	*Aspergillus welwitschiae* FMPV 28	marine sponge *Taedania sp.*	Biofilm formation inhibition: remarkable decrease in biofilm formation, in a dose-dependent antibiofilm activity.	*S. aureus* ATCC 25904	[149]
Extracellular thermostable antibacterial peptide designated as MFAP9	*Aspergillus fumigatus* BTMF9	marine	Biofilm formation inhibition: - > 85% against all test bacteria.	*B. cereus* (NCIM 2155), *Bacillus circulans* (*B. circulans*) (NCIM 2107), *Bacillus coagulans* (NCIM 2030), *Bacillus pumilus* (NCIM 2189) *S. aureus* (NCIM 2127)	[150]
aspulvinones R **(82)**, S **(83)**, and U **(84)** aspulvinones A **(85)**, B′ **(86)**, H **(87)**	*Aspergillus flavipes* KUFA1152	marine sponge *Mycale sp*	Biofilm formation inhibition: Compound **87**: at MIC (32µg/ mL) and 2xMIC for both strains Compound **86** at ½ MIC (16 µg/ mL). Compounds **82** and **83**, all concentrations tested 2xMIC (16µg/ mL), MIC (8 µg/ mL), ½ MIC (4 µg/ mL), including ¼ MIC (2µg/ mL) Mixture of **84** and **85** *E. faecalis* at MIC (32µg/ mL) and 2xMIC (64 µg/ mL).	*E. faecalis* ATCC 29212 *S. aureus* ATCC 29213	[85]
tenellic acid C **(88)**, neospinosic acid **(89)**	*Neosartorya spinosa KUFA 1047*	marine sponge	Biofilm formation inhibition: Compound **88**: at 64 µg/ mL: *E. coli* (11.61± 0.09%) *E. faecalis* (24.11 ± 0.1%) *S. aureus* (15.54 ± 0.1%) Compound **89**: at 64 µg/ mL:	*E. coli* ATCC 25922 *E. faecalis* ATCC 29212 *S. aureus* ATCC 29213	[86]

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Bioactive compounds	Fungal species	Fungal source	Antibiofilm activity	Test Bacteria used	Reference
			E. coli (16.11 ± 0.19%) *S. aureus* (44 ± 0.06 %)		
bacillisporins A **(90)** and B **(91)**	*Talaromyces pinophilus KUFA 1767*	marine sponge	Biofilm formation inhibition: Compound 90: at 8 μg/ mL (2xMIC): 99.92 ± 0.03% 4 μg/ mL (MIC): 99.81 ± 0.17% Compound **91**: at 16 μg/ mL (2xMIC): 99.87 ± 0.05% 8 μg/ mL (MIC): 99.71 ± 0.13%	*S. aureus* ATCC 29213	[93]
GKK1032B **(92)**	*Penicillium erubescens KUFA0220*	marine sponge *Neopetrosia sp*	Biofilm formation inhibition: at: 8 μg/ mL (MIC) and 16 μg/ mL (2xMIC) displayed significant activities.	*E. faecalis* ATCC29212	[89]

6. Future Directions

6.1. Inducing the Production of Fungal Metabolites

Strategies aimed at increasing the production of fungal metabolites have been currently being developed and are continuously progressing for the purpose of biotechnological scale up. Primarily, the metabolism of fungal strains are being improved to enhance metabolite production by identifying genes involved in the biosynthesis of specific secondary or specialized metabolites while metabolic engineering techniques are being employed to understand these pathways involved in their biosynthesis [151]. The biosynthetic gene cluster (BGC) in the filamentous fungus *Curvularia clavata*, that is engaged in the synthesis of a potent antifungal cyclic peptide KK-1 have been discovered [151]. Cyclic peptide KK-1 consists of 10-amino acids particularly against the plant pathogen *Botrytis cinerea* that causes gray mold affecting more than 200 dicotyledonous plant species including essential agricultural crops. Another common practice is employing chemicals, such as 5-azacytidine and suberoylanilide hydroxamic acid, to increase fungal secondary metabolite production [152,153].

As mentioned above, the employment of various media affects the biosynthesis of respective secondary metabolites, thus media optimization must be used for enhancing the production of target bioactive secondary metabolites in fungi as well. Changing the culture conditions by utilizing various media and incubation periods can have an impact on the variety and number of metabolites [154]. Similarly, some fermentation environmental factors such as temperature, aeration, and media composition as high salt stress could either regress or improve the metabolites production [155,156]. Finally, using a variety of combinations of microorganisms as co-cultures is an effective method for inducing the production of secondary metabolites [157,158]. A study demonstrated that interactions with neighboring organisms in the same media can significantly affect the production of fungal secondary metabolites [159].

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6.2. Metabolomic Approach

Overall, metabolomics makes it possible to comprehend the metabolic reactions of fungi in detail. The metabolomics approach has brought about a significant expansion in the field of metabolite fingerprinting and profiling, along with the identification and selection of marker metabolites [160]. The analysis of small metabolites (Mr ≤ 1 kDa) produced in cells and organisms in a sample is known as metabolomics. The metabolomics approach is a method that reflects a biological process at a systemic level using statistical techniques and equipment's [161]. Either targeted or untargeted metabolomics is chosen depending on the aim of the study. As for a non-targeted approach, it is holistic method to profile the metabolites in the sample and detect the presence of new biologically active compounds based on high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR) coupled to a database for dereplication purposes [162]. Whereas, the targeted metabolomics are quantitative analysis by using mass spectrometry to quantify a specific class of metabolites [163]. Sometimes targeted metabolomics are used after non-targeted metabolomics to verify the validity of results and perform quantitative analysis [161]. Metabolomics studies typically begin with screening metabolites from the target source and isolating the target features that could discriminate respective variables under certain experimental conditions, by acquiring spectral datasets using analytical tools such as HRMS coupled with liquid or gas chromatography (LC or GC) and NMR, followed by processing/analyzing these data, and finally identifying these metabolites using databases [164–169]. The potential for future pharmaceutical applications of secondary metabolites is then determined through biological assays [71]. Coupling the metabolomics profile of the spectral dataset and the biological assay results through multivariate analysis helps visualizes the distribution of metabolites between two or more experimental conditions (i.e active versus inactive or between various fermentation conditions) [168–170]. Moreover, it will clarify the relationship between metabolites and their behavior in biological processes and displays the metabolites with the assistance of simpler visual plots (i.e. scatter and S-plots) [164,171]. The most common multivariate analyses used along with metabolomics studies are Principal Component Analysis (PCA), Partial Least Square-Discriminant Analysis (PLS-DA), and Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) [170].

6.3. Detecting Antibiofim Compounds

In terms of biological assays, the bioassay screens the activity of respective metabolites against the biofilm of specific bacteria. That is done by testing the viability of biofilm and inhibit the bacterial growth through the Alamar blue assay and planktonic assay along with the Minimum inhibitory concentration (MIC) and Minimum biofilm eradication bacteria (MBEC) assay, respectively, as the two metrics used to assess the bioactive antibiofilm compounds [96,169,172–174] . QS is another mechanism involved in the development of thin microbial biofilms and regulates bacterial motility and enzymes, inhibiting QS would decrease EPS production. Therefore, the anti-QS activity can also be detected by measuring the motility inhibition activity (i.e. swimming and swarming) of the test bacteria. In addition, it is commended to focus on the virulence factors associated with biofilm-forming bacteria and monitor QS regulation through various mechanisms such as pyocyanin activity, chitinase activity, LasA protease activity, LasA staphylolytic activity, LasB elastase activity, and HCN production [175].

7. Summary and Conclusions

In conclusion, fungal natural products have been successfully offering bioactive antibiofilm compounds which might help in antibiotic resistance crisis. From cited papers, it was shown that fungal-derived natural products can produce secondary metabolites with antibiofilm activity albeit less investigated in comparison to their antimicrobial capability. Moreover, we demonstrated that seaweed endophytes have not been comprehensively explored for their bioactive secondary metabolites. Therefore, further exploring antibiofilm compounds from endophytic fungi associated

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with seaweed could be found effective in combination with existing antibiotics and prevent multi-resistance. In addition, the synergistic effects of the discovered compounds against bacterial biofilms can be increased by using proven elicitors such as NO-donors and QSIs. So currently, there is an urgent need to turn to this area of research, and we are looking to discover new antibiofilm compounds from seaweed endophytes. At the same time, there is a compulsion for vital effort to accelerate the production of these metabolites and expand our current antibiotic pipeline. Since seaweed metabolites are influenced by geographical location and variable climate changes, the occurrence of endophytic fungal metabolites is expected to fluctuate and vary as well, which further encourages the study of seaweed endophytic fungal metabolites as diverse but sustainable sources to increase the chance of discovering new anti-biofilm compounds. Furthermore, the development of metabolomics approaches employing high resolution instrumentation to afford more reliable spectral datasets that can be coupled with the biological assay results improves the efficiency of detecting and isolating novel antibiofilm compounds.

The challenge of bacterial resistance remains a critical concern that necessitates collaborative efforts across various fields and innovative technological developments. The investigation of marine endophytic fungal strains exhibiting distinctive metabolic adaptations, especially in extreme marine environments, offers an exciting opportunity in drug discovery. Targeting underexplored genera of seaweed endophytic fungi and their metabolites may provide a partial solution to bacterial resistance. Recent technological advancements have facilitated the isolation of new compounds via metabolomics methodologies. Utilizing bioactivity-guided metabolomics alongside LC-MS/MS-based untargeted metabolomics facilitates rapid identification of these compounds. The metabolomics approach supported by multivariate analysis enables the tracking of anti-biofilm compounds and the examination of responses to environmental stressors. Challenges include low product yield and difficulties in compound purification. To address the increasing demand, emphasis must be placed on improving the production and yield of bioactive molecules. Metabolomics-transcriptomics pipelines and synthetic biology tools should establish connections between compound production and BGC expression.

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Abbreviations

The following abbreviations are used in this manuscript:

WHO	World Health Organization
EPS	Extracellular polymeric substances
NO	Nitric oxide
QS	Quorum sensing system
AI	Auto inducer
SNP	Sodium nitroprusside
NPs	Natural products
EtOAc	Ethyl acetate
sp	species
BGC	Biosynthetic gene cluster
Mr	Molecular weight
KDa	KiloDaltons
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
LC	Liquid chromatography
MIC	Minimum inhibitory concentration

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MBEC	Minimum biofilm eradication bacteria
MBIC	Minimum biofilm inhibitory concentration
PCA	Principal Component Analysis
PLS-DA	Partial Least Square-Discriminant Analysis
OPLS-DA	Orthogonal Partial Least Square-Discriminant Analysis

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