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Review

Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors

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ABSTRACT

Antimicrobial resistance (AMR) is one of the serious global public health threats that require immediate action. With the emergence of new resistance mechanisms in infection-causing microorganisms such as bacteria, fungi, and viruses, AMR threatens the effective prevention and treatment of diseases caused by them. This has resulted in prolonged illness, disability, and death. It has been predicted that AMR will lead to over ten million deaths by 2050. The rapid spread of multidrug-resistant bacteria is also causing old antibiotics to become ineffective. Among the diverse factors contributing to AMR, intrinsic biofilm development has been highlighted as an essential contributing facet. Moreover, biofilm-derived antibiotic tolerance leads to serious recurrent chronic infections. Therefore, the discovery of novel bioactive molecules is a potential solution that can help combat AMR. To achieve this, sustained mining of novel antimicrobial leads from actinobacteria, particularly marine actinobacteria, can be a promising strategy. Given their vast diversity and different habitats, the extraordinary capacity of actinobacteria can be tapped to synthesize new antibiotics or bioactive molecules for biofilm inhibition. Advanced screening strategies and novel approaches in the field of modern biochemical and molecular biology can be used to detect such new compounds. In view of this, the present review focuses on understanding some of the recent strategies to inhibit biofilm formation and explores the potential role of marine actinobacteria as sources of novel antibiotics and biofilm inhibitor molecules.

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the major global health challenges of the 21st century that pose serious threats to mankind, animal, and the environment. According to the Centers for Disease Control and Prevention (CDC) 2019 AMR threats report, each year more than 2.8 million antibiotic-resistant infections occur in the United States, and more than 35,000 die as a result. The growing resistance of bacteria to antibiotics and difficulty in the discovery of new drugs have resulted in the decline of antimicrobials in the pipeline of development. The World Health Organization (WHO) highlighted AMR as one of the top ten threats to global health in 2019.

Upon frequent exposure to antibiotics, bacteria can become resistant to the antimicrobials genetically. One of the other ways by which bacteria evade antibiotic exposure is via the formation of biofilms, which are homogeneous or heterogeneous microbial communities residing in a self-produced matrix of extracellular polymeric substances. Biofilms allow its microbial cells to enter transiently into a metabolically inactive state, and as a result, antibiotics cannot work effectively, causing disease re-occurrence due to latent bacterial infection remaining inside the host [1]. Biofilm formation is the root cause of many life-threatening diseases like infections in the lung, urinary tract, wound, etc. Moreover, medical device-related infections during the time of implantation can also occur due to contamination of the surface by biofilm-forming bacteria. Biofilm control by inhibiting their formation or by dispersing the preformed biofilm, therefore, becomes important. Strategies for biofilm remediation involve the discovery of therapeutic drugs to inhibit the biofilm formation via interfering with their attachment to the surface or disturbing the communication among the biofilm residents and by disrupting the preformed biofilm. With the growing resistance of biofilms to antibiotics, research and development have focused on discovering natural products to combat biofilm formation. The need of the hour is to find potential bioactive candidates to inhibit biofilm formation or treat infections caused by them [2,3].

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Actinobacterial species are major producers of secondary metabolites. Their metabolic capability and genetic makeup holds a great promise for the source of biofilm inhibiting drugs. Their varied array of habitats, such as aquatic and terrestrial ecosystems, is reflected in their metabolic potential for producing various bioactive natural products such as antibiotics, anticancer, antivirals, anthelmintics, immunosuppressants, herbicides, and extracellular enzymes with various industrial applications [4]. Marine actinobacteria are currently being explored as an untapped source for the discovery of novel anti-biofilm drugs. In this review, we look at several mechanisms underlying AMR due to biofilm formation and the strategies aimed to tackle the issue. We capture recent developments made in this area of the last three years and highlight the potential of marine actinobacteria as a source of novel bioactive compounds to address biofilm-based AMR.

2. Biofilms

Bacteria form a biofilm to get into a habitat that provides them not only shelter from harsh environmental conditions but also promotes the accumulation of essential nutrients [5]. They are defined as homogeneous or heterogeneous microbial communities residing in a self-produced matrix of extracellular polymeric substances (EPS) that enables the exchange of various essential secondary metabolites, genetic material to withstand stress and signaling molecules. The EPS comprises about 90 % of the total biofilm, whereas microbial cells are present in less than 10 % of the dry mass. The self-organization of the EPS in the matrix is based upon the interactions among the EPS components determining the microbes' physiological activity in the biofilm. The matrix of the biofilm not only protects the microorganisms from environmental stress such as desiccation, ultraviolet radiation, metals, antibiotics, oxidizing agents, but also from host immune systems [6]. Biofilms are, therefore, communities of aggregated bacterial cells embedded in an extracellular polymeric matrix [7]. They have also been referred to as a city of microbes, metaphorically, whereas the EPS represents the house of the biofilm [8]. Biofilms may shape on an extensive sort of surfaces, along with living tissues, in dwelling clinical devices, industrial/ potable water piping, and natural aquatic systems. Both Gram-positive and Gram-negative bacteria can form a biofilm, for instance, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Neisseria gonorrheae*, *Bacillus subtilis*, *Helicobacter pylori*, *Proteus vulgaris*, *Streptococcus viridans*, and *Proteus mulbaris*, are associated with life-threatening infections [9].

3. Mechanism of biofilm formation

Biofilm formation is a multi-factorial and complex process. It requires the activation of a particular type of signaling known as quorum sensing and transcription of a different set of genes that differ from the planktonic form of the same species of bacteria [10]. The formation of a biofilm begins when bacteria sense environmental stress conditions that trigger the bacterial life to adhere to the surface, either biotic or abiotic. These ecological conditions vary among various microorganisms. Some bacteria, for instance, *P. aeruginosa* can form biofilm in almost any situation while growing, whereas some require specific conditions to form a biofilm such as some strains of *E. coli* form biofilm only in nutrient-limited conditions [11]. The mechanism of biofilm formation is complex among various microorganisms but generally follows four common steps: I) initial attachment to the biotic or abiotic surface, II) microcolony formation, III) maturation of the biofilm

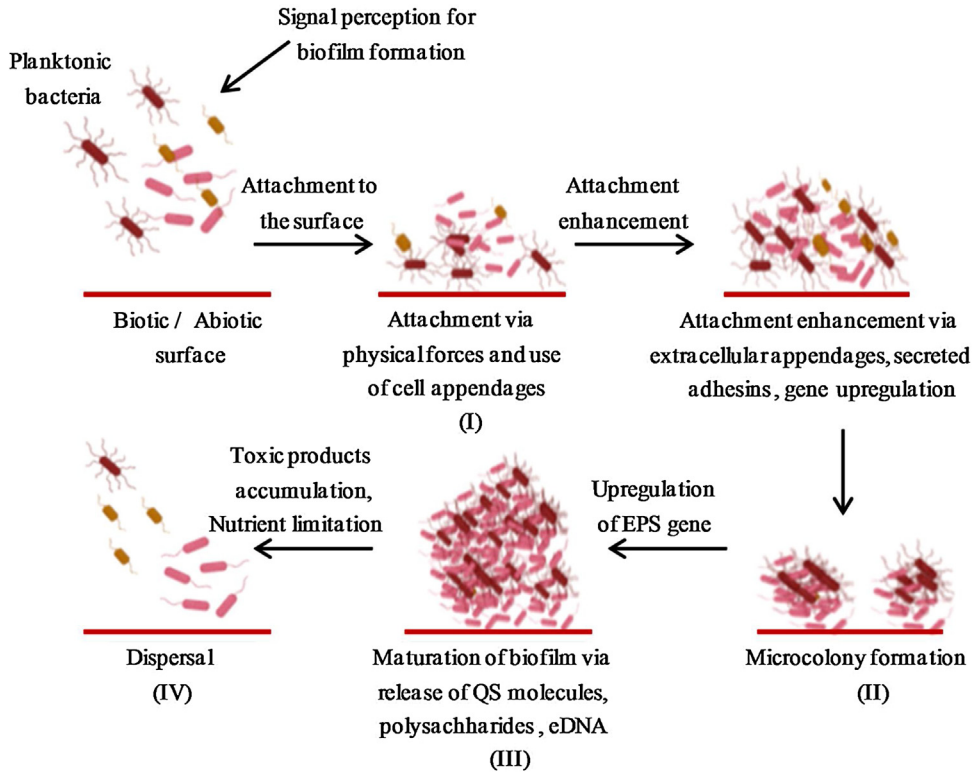

Fig. 1. Mechanism of biofilm formation. The four stages of biofilm formation: (I) Reversible attachment of free-living bacteria to the compatible surface via the use of cell appendages followed by the irreversible attachment via secreted adhesins. (II) Discrete colonies formation known as microcolonies having around 100 bacteria per cluster via upregulation of genes required to maintain attachment. (III) Biofilm maturation via upregulation of EPS genes, the release of polysaccharides, eDNA, and QS molecules. (IV) After a certain period of time, nutrients are depleted and toxic compounds are started to accumulate, this is when dispersal of bacteria residing in biofilm occurs.

and IV) dispersal of planktonic cells [10]. The steps of biofilm formation are illustrated in Fig. 1. Herein, the stages involved in biofilm formation in bacteria are discussed:

3.1. Initial attachment

The initial attachment of bacteria to a surface, termed as adhesion, is at least in a part stochastic process that is driven by gravitational forces, Brownian motion, and enhanced by hydrodynamic forces. Microbial cells get attached to the surface via physical forces and through their cell appendages like pili, fimbriae, or flagella [12,13]. The bacteria cells encounter both attractive and repelling forces within the premises depending upon the medium properties, nutrient levels, ionic strength, pH, temperature, and cell surface composition [14]. Several pathogens have motile flagella that help in overcoming repulsive forces and hydrodynamic forces. The importance of flagella is well-documented for many bacteria such as *P. aeruginosa*, *E. coli*, *Listeria monocytogenes,* and *Vibrio cholerae* [11,13].

The initial attachment is dynamic and reversible due to the weak interactions between the surface and the bacteria. During this, bacteria can detach itself and become planktonic if repulsive forces are more substantial or in response to nutrient availability. Irreversible attachment to the surface is mediated by additional extracellular adhesive appendages and secreted adhesins. For instance, *P. aeruginosa* uses type IV-pili mediated twitching motility besides flagella to maintain adherence and help move across the attachment surface [13]. Microorganisms prefer hydrophobic surfaces for attachment as they tend to reduce repulsive forces between the bacteria and the surface, thereby strengthening the attachment [10].

3.2. Microcolony formation

Attachment to the surface triggers responses that lead to changes in the gene expression and upregulation of factors that maintain adherence. Bacteria typically begin to multiply, forming small aggregates or discrete cell clusters known as microcolonies by producing small quantities of the biofilm matrix. The number of bacteria in a typical microcolony is nearly 100 [13,15]. The microcolonies that form the biofilm can have single species of bacteria or different bacteria species depending on the conditions under which the biofilm is formed. In *P. aeruginosa*, two classes of mutants were studied for microcolony formation. One class was mutant of flagella and motility, thus could not adhere to the surface, and the other was mutant of type IV pili. The type IV pili mutant of *P. aeruginosa* was unable to form microcolonies suggesting that type IV pili play an important role in microcolony formation. After microcolony formation, transcription of specific genes required for the formation of EPS matrix components of biofilm is activated [16].

3.3. Maturation of biofilm

In this phase of biofilm formation, cells contact each other via the production of autoinducer molecules, which in turn leads to activation of various genes required for the formation of the biofilm matrix [9]. The autoinducer signaling molecules facilitate quorum sensing which helps in determining the surrounding cell density. Three polysaccharides (alginate, pel, and psl) are released at this stage of biofilm formation in *P. aeruginosa* that provides stability to the biofilm. The regulation of these three polysaccharides is governed by the level of signal 3,5-cyclic diguanylic acid (c-di-GMP) [13]. Also, several studies have elucidated that extracellular DNA (eDNA) has a role in establishing cell to cell connection and stabilizing the biofilm matrix. Overall, there are two stages in biofilm maturation; the first stage involves cell to cell communication, and the production of autoinducer molecules such as N- acylated homoserine lactone (AHL), and the other is the differentiation of microcolonies to macrocolonies by activating all genes required for EPS matrix formation [9].

3.4. Dispersal

To colonize the new area, bacteria from the sessile state undergo to mobile state via dispersal from the biofilm resulting in the start of the new lifecycle of biofilm formation [16]. Besides passive dispersal which results from shear stresses, bacteria have evolved its ways to decide whether to continue in the present biofilm or to start the new biofilm formation [13]. As the biofilm matures, there is an accumulation of toxic products, oxygen fluctuations, and also nutrients become limited [9]. There are several sensory systems involved that monitor the number of small molecules in the environment and induce activation of specific genes, thereby facilitating dispersal upon any stress. For instance, in *P. aeruginosa*, dispersal occurs in response to an increasing amount of nitrogen and carbon sources. Also, an increase in c-di-GMP molecules is an indication of microcolony formation, whereas reduction in level leads to the upregulation of motility. Enzymes are also reported to aid biofilm dispersal, such as alginate lyase in *P. aeruginosa*. An increase in surfactant molecules such as rhamnolipid reduces the interaction of bacteria to the surface resulting in biofilm dispersal [13]. When bacteria disperse as a result of shear stress by sloughing off aggregates, they might retain their biofilm characteristics such as antibiotic resistance, whereas bacteria cells that are removed as a result of active dispersal mechanism may revert to their original planktonic phenotype [14].

Microorganism sovereignty to make biofilm on both biotic and abiotic surfaces has posed serious threats to human health. The CDC, in 2007, stated that about 1.7 million hospital-acquired infections were due to biofilm formation, which led to more than a million deaths annually [17]. The National Institute of Health (NIH) states that nearly 80 % of total microbial infections in human beings are due to the formation of biofilm [9]. Biofilm formation on medical devices such as breast implants, catheters, pacemakers, contact lenses, prosthetic heart valves, and cerebrospinal fluid shunts are responsible for life-threatening infections. *P. aeruginosa*, *S. aureus,* and *S. epidermis* are some of the most notable bacteria which can form biofilm on medical devices. Biofilm-associated infections such as chronic wounds, cystic fibrosis, infective endocarditis, osteomyelitis, and periodontitis are difficult to treat because of compromised efficiency of antibiotics to act on bacteria residing in biofilm [18,9]. The NIH has estimated that biofilm-forming microorganisms account for 65 % of microbial diseases [10].

4. Biofilm-imparting resistance towards antibiotics

Several mechanisms of antimicrobial resistance have been reported, out of which one of the major factors is biofilm formation [23]. Herein we discuss the mechanisms which allow biofilms to tolerate or resist the action of antibiotics.

4.1. Poor antibiotic penetration

The EPS matrix of biofilm plays a key role in the structural stability of biofilm. Besides this, it has also been suggested that this also acts as a barrier to many of the antibiotics [19]. Tolerance arises through inactivation or entrapment of antimicrobials by the biofilm matrix. However, antimicrobials that do not interact with EPS components can diffuse through biofilms as easily as through water suggesting the diffusion barrier is not the only reason for the reduced susceptibility of biofilms to antibiotics [20]. Then the question

arises if not by inhibition due to diffusion, then what results in the quenching of the antibiotics? EPS matrix components can chelate antibiotics by forming complexes. Additionally, antibiotics can also be degraded by the enzymes present in the biofilm matrix. As a result, the antibiotics do not reach the target at an adequate concentration to kill the bacteria ensuing in the survival of bacteria even after exposure [5]. For instance, *Erwinia amylovora* can tolerate toxic metals stress, such as copper by making a complex with the polysaccharides of the EPS matrix [21]. For instance, many of the antibiotics belonging to the class of aminoglycosides are positively charged. They interact with the negative component of the biofilm matrix, for example, eDNA and phage particles; this eventually reduces the penetration of the antibiotic [5,22].

4.2. Reduced growth rate

When bacterial culture starves for a particular nutrient, it slows its growth. Bacteria transition from the log phase to the stationary phase is generally linked to the reduced susceptibility to many of the antibiotics [23]. Indeed, at least 1 % of the bacteria that are in the stationary phase become tolerant of antimicrobials in the biofilm [5]. The growth study of planktonic cells and biofilm residing cells when growing at a slower rate was evaluated upon exposure to tobramycin and ciprofloxacin. Studies have revealed that the effect of the antibiotics was the same for *P. aeruginosa*, *E. coli*, and *S. epidermidis* when growing either in free form or residing in the biofilm. However, as the growth rate increased, planktonic cells become more susceptible to the antibiotics than the bacteria residing in the biofilm, concluding the involvement of other factors along with the slow growth of bacteria in the biofilm. Moreover, bacteria residing deep in the biofilm experience low or complete absence of oxygen as compared to bacteria present at the upper layer in the biofilm. This oxygen gradient within the biofilm stops bacterial growth, thereby making antibiotics ineffective as many of the antibiotics target bacterial macromolecules synthesis pathways [19,23,24].

4.3. Horizontal gene transfer

One of the mechanisms of resistance towards antimicrobial arises due to the genes acquired through horizontal gene transfer (HGT). Inside the biofilm, the accumulation of genetic elements occurs through the cell lysis of heterogeneous species. This provides the ideal environment for the uptake of resistance genes [25]. It was revealed that plasmids that confer resistance to antibiotics were transferred via conjugation in dual-species biofilms of *P. putida* and *E. coli* [26]. Additionally, conjugation is 700-fold more effective in biofilms as compared to planktonic bacterial cells [27]. The eDNA in the matrix can also be the source of HGT [28]. In *V. cholerae* biofilms, HGT occurs via type VI secretion system, which requires cell-to-cell contact [29]. In vitro studies using transcriptome analysis has revealed that vancomycin-treated *E. faecalis* biofilm has 101 differentially regulated genes as compared with planktonic bacterial cells. Genes encoding penicillin-binding 1A family proteins and ATP-binding cassette (ABC) transporters were the most highly upregulated, resulting in an increased level of antibiotic efflux pump and resistance to β-lactam antibiotics. Moreover, it was observed that *E. faecalis* vancomycin-resistant gene, vanA, was transmitted to *S. aureus* in a polymicrobial biofilm [30].

5. Current strategies to inhibit and eradicate the preformed biofilm formation

Biofilm formation is the root cause of a range of life-threatening diseases like cystic fibrosis-associated lung infection, chronic wound infection, recurrent urinary tract infection, and chronic osteomyelitis. Medical device-related infections occur due to surface contamination by biofilm-forming bacteria during the time of implantation. Further, their ability to resist or tolerate antibiotic action adversely impacts human health. Therefore, there is a need for biofilm control either by inhibiting the biofilm formation or by dispersing the preformed biofilm. Biofilm formation on medical devices could be prevented by modifying the surface properties so that bacteria are not able to attach on the devices. Recently employed strategies to inhibit and disperse the preformed biofilm are discussed below.

5.1. Plant-derived antimicrobial compounds

Many medicinal plants are used for a long time to treat various diseases. Plant-derived compounds are safe, with no side effects and are cost-effective. They are mostly secondary metabolites with diverse functional groups such as alkaloids, terpins, polyphenolics, flavonoids, resins, phenolics, essential oils, etc., and have shown antibacterial and anti-biofilm activity [31]. Recently, an oral spray containing plant-derived compounds: α-mangostin (α-MG) and/or lawsone methyl ether (2-methoxy-1,4-naphthoquinone) (LME) has been developed. This spray has proven to be effective against common oral pathogens that form a biofilm. When used in combination (α-MG + LME), >90 % of biofilm was inhibited with no significant cytotoxicity [32]. Studies have also shown that one of the analogs of oleanolic acid, Compound 9 inhibited biofilm formation of *E. coli*, *P. aeruginosa*, *Salmonella enterica*, and *Burkholderia cepacia* by altering the gene expression of type IV pili in *P. aeruginosa* [33].

5.2. Polymers

Biofilm formation can be inhibited by coating the abiotic surfaces with substances having antifouling properties. The hydrophilic coating of polyethylene glycol (PEG) on the surfaces does not allow adhesion of the cells making the surface anti-adhesive. The high molecular weight PEG showed greater repulsion between the surface and cell appendages as compared to low molecular weight PEG. However, the use of PEG is restricted due to its auto-oxidation in aerobic conditions, eventually decreasing the efficiency of PEG [31]. Recently, the dual function of silver-polymer nanocomposites was reported aiding not only the rapid killing of microbes but also inhibited biofilm formation. These nanocomposites suppressed bacterial motility and also reduced the production of many virulence factors. The nanocomposite was developed using polymer N, N-dimethyl-*N*-hexadecyl ammonium chitin tosylate, and silver para-toluene sulfonate having intrinsically biodegradable and antimicrobial properties [34]. Polylactide (PLA)-based polymers also received considerable attention as antimicrobial materials [35]. The efficacy of the nanocomposite was tested on drug-resistant pathogens such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and beta-lactam-resistant *K. pneumoniae* [36]. It inactivated various drug-resistant pathogens.

5.3. Enzymes

Enzymes are being used to disperse the preformed biofilm. In a recent study, a combination of self-immobilized dopamine with alpha-amylase and silver nitrate has significantly eradicated the biofilm of *S. aureus*. Furthermore, polydopamine assisted treatment was found to immobilize silver on the surface thereby inhibiting the bacterial interaction with the surface and further recolonization [37]. Xylanase, a cell wall degrading enzyme has inhibited biofilm formation by 70 % and dispersed the biofilm of

P. aeruginosa PAO1 without affecting the planktonic bacteria. This enzyme has also shown anti-biofilm activity against methicillin-resistance *S. aureus* and two *E. coli* strains [38].

5.4. Polysaccharides

Recent studies have shown that polysaccharides can be used to inhibit biofilm formation. Sulfated polysaccharides purified from *Chlamydomonas reinhardtii* inhibited biofilm formation of *Salmonella enterica* and *Vibrio harveyi* and also eradicated biofilm formed by these bacteria. The sulfated polysaccharides degraded eDNA of the EPS matrix, further resulting in the disruption of biofilm [39]. However, more research is needed to validate its application in the biomedicine field.

5.5. Biosurfactants

Recently, the activity of lipopeptide biosurfactants (LPBs) produced by *Acinetobacter junii* was evaluated. LPBs are amphiphilic compounds that inhibited the biofilm of *Proteus mirabilis*, *S. aureus*, and *P. aeruginosa* [40]. LPBs extracted from *Bacillus tequilensis* strain SDS21 eradicated more than 99 % of the *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Salmonella typhi*, and *Salmonella typhimurium* biofilm on polystyrene, stainless steel, and glass surface. The biosurfactant has retained its biofilm eradicating activity even after exposure to high temperature and pH, suggesting it can be used in disinfectant like formulations [41]. Mannosyl erythritol lipids (MELs) are novel biosurfactants produced by *Pseudozyma aphidis* DSM 70725. It has demonstrated antibacterial activity against *S. aureus* by damaging the cell membrane of the bacteria. Furthermore, MELs inhibited biofilm formation of *S. aureus* by disrupting the bacterial adhesion to the surface. However, their large-scale production is expensive and limits their use in many areas [42]. In addition to the above, novel sources of biofilm inhibitors are being explored recently.

6. Actinobacteria: A source of biofilm inhibitors

Actinobacteria constitute one of the most diverse and ubiquitous microorganisms in nature. They range from anaerobic to aerobic, motile to non-motile, and spore to non-spore-forming bacteria. They are Gram-positive in nature, with high guanine-cytosine content. Traditionally, they were considered as soil bacteria but are known to be present in virtually all ecosystems as depicted in Fig. 2 [43]. Actinobacterial species are known producers of secondary metabolites such as antimicrobial, antiviral, anticancer agents. Currently, about 70 % of the natural bioactive compounds are in clinical use. Genome sequencing studies have revealed the presence of >50 biosynthetic gene clusters (BGC) in the *Streptomyces* sp. depicting the potential nature of producing anticipated antibiotics in the future. While *Streptomyces* has been known for producing many of the known antibiotics, the potential of other actinobacterial species cannot be undermined. Some of the recent reports of novel biofilm control molecules from actinobacteria are listed in Table 1. The bioactives have demonstrated biofilm control on a range of bacterial strains. *Frankia casuarinae* is a mycelium forming actinobacteria which is isolated from root nodules of *Casuarina* spp., Tamil Nadu, India. The secondary metabolites extracted from *F. casuarinae* has inhibited *Candida* sp. biofilm formation by 81 % at 62.5 μg/mL and by 80 % in *Pseudomonas* at 125 μg/mL concentration. The novel findings concluded that *F. casuarinae* are anti-biofilm agents' producers against *Pseudomonas* and *Candida* sp. biofilm [44]. Moreover, secondary metabolites extracted from *Streptomyces californicus* ADR1 has inhibited 90 % biofilm formation of *S. aureus* ATCC 29213 and MRSA ATCC 43300 at concentration 1.80 μg/mL and 4.92 μg/mL, respectively [45], highlighting the importance of actinobacteria as a promising strategy for the discovery of novel anti-biofilm agents.

7. *Streptomyces*: A hub for varied array of bioactive molecules

Actinomycetes are known to produce almost two-thirds of the antibiotics, about 80 % of which are produced by *Streptomyces* genus. With more than 7000 compounds produced by *Streptomyces* sp., the majority of them account for the secondary metabolites that are potent antibiotics, making *Streptomycetes* the most exploited primary antibiotic-producing source [46]. Genomic analysis has shown that a single strain of *Streptomyces* can produce about tens of such metabolites. Additionally, the metagenomic profile revealed a large number of biosynthetic gene clusters in the *Streptomyces* genus. Therefore, *Streptomyces* are being studied

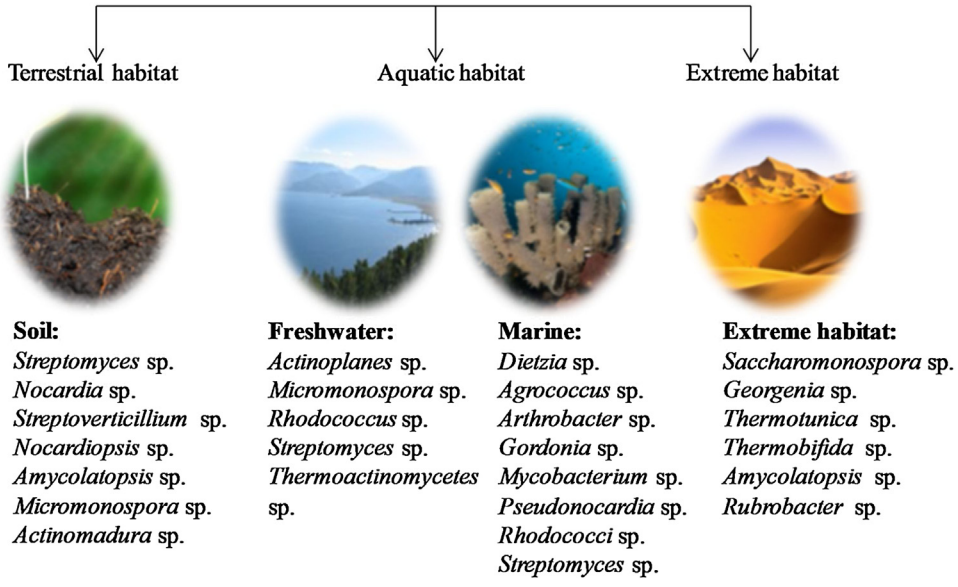

Fig. 2. Ecological distribution of Actinobacteria.

Table 1
Recent biofilm inhibitors reported from Actinobacteria.

Actinobacteria	Anti-biofilm effect	Activity reported	Reference (s)
Bioactive metabolites of *Frankia casuarinae* DDNSF-02	Inhibited biofilm of *Pseudomonas* sp. and *Candida* sp.	Dose dependent percent biofilm inhibition of *Candida* sp. from 59 % to 81 % and *Pseudomonas* sp. from 65 % to 80 %	[44]
Secondary metabolites of *Streptomyces californicus* strain ADR1	Inhibited biofilm of *S. aureus* and methicillin resistant *S. aureus*	90 % biofilm inhibition at a significantly lower concentration of metabolites (0.74 ± 0.08 μg/mL for *S. aureus* and 4.59 ± 0.71 μg/mL for methicillin-resistant *S. aureus*)	[45]
Melanin pigments from *Nocardiopsis dassonvillei* strain JN1 and *Nocardiopsis* sp. JN2	Inhibited biofilm of *Staphylococcus* sp.	Melanin extracted from strain JN1 showed 64.20 % and JN2 65.99 % biofilm inhibition of *Staphylococcus* sp.	[65]
Organic solvent extracts of *Streptomycetes*	Inhibited biofilm and dispersed preformed biofilm of *Candida albicans*	Minimum biofilm inhibition concentration (MBIC) of *Streptomycetes* BV283 extracts was 31 μg/mL	[66]
Carotenoid pigment extracted from *Streptomyces parvulus*	Inhibited biofilm of *C. albicans*	>50 % biofilm reduction and MBIC value reported was 50 μg/mL	[67]
1-hydroxy-1-norresistomycin (HNM) extracted from *Streptomyces variabilis*	Inhibited the biofilm of human clinical pathogens *V. cholerae*, *E. coli*, and *S. aureus*	HNM (at 200 μg/mL) inhibited biofilm formation of *V. cholerae*, *E. coli*, and *S. aureus* with an efficiency of 92, 96, and 93 % respectively	[68]
Extract of *Glycomyces sediminimaris* UTMC 2460	Inhibited the biofilm formation of *Kocuria* sp. and *Mesorhizobium* sp.	Inhibited 93.2 % biofilm of *Kocuria* sp. and 71.4 % of *Mesorhizobium* sp.	[69]
Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) extracted from endophytic actinomycetes *Nocardiopsis* sp. GRG 1 (KT235640)	Inhibits biofilm formation and also reduces the viability of preformed biofilms of *P. mirabilis* and *E. coli*	82 % biofilm inhibition of *P. mirabilis* and 77 % of *E. coli*	[70]
Actinomycin D from *Streptomyces parvulus*	Anti-quorum sensing and biofilm inhibition activity	Percent biofilm inhibition of *P. aeruginosa*, *S. aureus*, *Micrococcus luteus*, and *Ruegeria* sp. were reported to be 37.12, 53.72, 22.20, and 45.98 %, respectively	[71]

more intensively for the production of novel antibiotics to combat AMR [47]. The life cycle of *Streptomyces* starts when a free spore undergoes germination forming vegetative mycelium, also known as substrate mycelium. Under nutrient depletion condition, the substrate mycelium is degraded by programmed cell death (PCD) mechanism to acquire nutrients required to form aerial mycelium. During this period, antibiotics production occurs to compete with other microorganisms attracted to nutrients after PCD [43]. *S. coelicolor*A3(2), as a model organism, has been studied for about 60 years. Following the discovery of streptomycin in 1943, many bioactives including anticancer, antifungal, antiparasitic, antioxidant agents have been discovered from *Streptomyces*. The recently discovered bioactive from *Streptomyces* are listed in Table 2. With the rising concerns of multidrug resistance (MDR) in cancer cells, it has led to compromise efficacy of current therapeutics applied in chemotherapy. MDR makes cancer cells resistant to several drugs with completely different structures and mechanisms of action. Reversing the action of resistance in cancer cells has an important clinical value. Cisplatin (DDP) is most widely used as a first-line anticancer drug. However, drug resistance has limited its usage. A new milbemycin compound VM48130, which is a product of type I polyketide synthase isolated from *Streptomyces* sp. FJS31−2 has significantly restored the sensitivity of multidrug-resistant cisplatin-resistant human lung adenocarcinoma (A549/DDP) cells to DDP [48], highlighting the importance of *Streptomyces* not only in antibiotics production but for many valuable clinical drugs.

8. Tapping the potential of marine actinobacteria for novel biofilm inhibitors

With the significant contribution of actinobacteria to the reservoir of clinical drugs and biofilm control molecules, the less-explored actinomycetes provide even more scope of being a source of a large number of highly inhibitory antimicrobials. Recent studies are, therefore, focusing on non-*Streptomyces,* or so-called rare actinobacteria to isolate novel bioactive molecules [49,50]. Marine actinobacteria is one such source which have not yet been fully explored, but published literature has shown their potential to produce novel antimicrobial compounds [110]. Their role in inhibiting the biofilm formation and to eradicate the preformed biofilm has gained much interest.

The marine environment comprises about 70 % of the earth's surface and is the largest ecosystem of earth's aquatic system representing an untapped source for novel drug discovery [51,52]. Marine microorganisms are known to produce over 23,000 compounds to date, of which 70 % are from actinobacteria, 20 % by fungi, and remaining from other microbes [53,54]. To survive in extreme conditions such as limited light, elevated temperature, pressures along with nutrients limitation, low pH, and high salt concentrations, marine actinobacteria produces a diverse array of secondary metabolites. They thrive in either free form or get associated with other lives [55]. Some of the recently isolated compounds from marine actinobacteria are listed in Table 3. Khodamoradi et al. recently isolated *Streptomonospora* sp. M2 from a soil sample collected at the Wadden Sea beach in order to isolate rare actinobacteria that were eventually aiming the discovery of new antibiotics. The two new thiopeptide antibiotics named as litoralimycin A (1) and B (2) were discovered. The biological activity reported for the two compounds was uncommon for thiopeptide antibiotics, as they showed only negligible antibacterial activity, but litoralimycin A (1) showed strong cytotoxic activity [99].

The frequency of isolation of rare actinobacteria is low as compared to the commonly found actinobacteria such as the *Streptomyces* [49]. Marine actinobacteria are also difficult to culture in laboratory conditions due to insufficient knowledge about their growth requirements. Recently, laboratory conditions that can mimic the natural conditions for the growth of actinobacteria are being employed. For instance, using an isolation chip (ichip), many actinobacteria have been isolated that were previously uncultivable in laboratories. Moreover, strenuous microbiology techniques are being replaced with techniques such as genomics, proteomics, metagenomics, and transcriptomics for identification and characterization of marine microbial diversity with unique characteristics using bioinformatics tools [56,57]. Persiamycin A and 1-hydroxy-4-methoxy-2-naphthoic acid, new bioactive aromatic polyketide compounds are discovered using a genotyping-guided technique from sponge-associated halophilic *Streptomonospora* sp. PA3 isolated from the Persian Gulf. Genome sequencing of isolated *Streptomonospora* sp. PA3 led to the identification of seven biosynthetic gene clusters (BGCs) involved in secondary metabolism. The newly isolated compound

Table 2
Bioactive compounds isolated from *Streptomyces*

Streptomyces sp.	Bioactive compounds	Activity	Reference (s)
Streptomyces sp. CPCC 203577	Lavanducyanin Naphthomevalin	Activity against Gram-positive bacteria	[72]
Streptomyces sp. FJS31-2	Milbemycin	Anticancer	[48]
Streptomyces sp. ZZ446	Maculosin and Maculosin-O-α-L-rhamnopyranoside	Antimicrobial activity against methicillin-resistant *S. aureus*, *E. coli*, and *C.albicans*	[73]
Streptomyces sp. SD53	Piceamycin and Bombyxamycin C	Antimicrobial activity against *Bacillus thuringiensis*, *Salmonella enterica* and *Proteus hauseri*, Anticancer	[74]
Streptomyces blancoensis	Adipostatins E-J	Inhibitors of Coenzyme-A Biosynthesis	[75]
Streptomyces sp. TBRC7642	Abyssomycins Y–Z, methyl aeruginoate, desferri-ferrithocin-4-hydroxyphenethylester, streptomethiocins A–B, furaquinocin I, and streptolactone	Evaluated for antimalarial, antitubercular, antibacterial (both Gram-positive and Gram-negative bacteria), as well as for cytotoxicity	[76]
Streptomyces sp. ICBG1318	Meliponamycin A and Meliponamycin B	Activity against *Paenibacillus larvae*, *S. aureus*, and *Leishmania infantum*	[77]
Streptomyces sp. A1013Y	4,813-trihydroxy-611-dione-trihydrogranaticins A (TDTA)	Radical scavenging property	[78]
Streptomyces sp. shell-016	Shellmycin A–D	Anticancer	[79]
Streptomyces sp. VN1	Cinnamamide, Lobophorin A, diketopiperazines cyclo-L-proline-L-tyrosine, and a unique furan-type compound	Anticancer	[80]
Streptomyces morookaense SC1169	Streptovertimycins A-H	Activity against MRSA and vancomycin-resistant *Enterococcus faecium* (VRE)	[81]
Streptomyces sp. MN41	Pyrrole-derivative	Activity against MRSA	[82]
Streptomyces sp. IOPU	Hexahydro-menaquinone MK-9, borrelidin, ferulic acid, N-acetylanthranilic acid, uracil, uridine, thymidine, sitosteryl-3β-D-glucoside, linoleic acid and methyl linoleate	Antioxidant, antibacterial and anticancer	[83]
Streptomyces sp. RKND004	Terrosamycins A and B	Activity against Gram-positive bacteria and anticancer	[84]
Streptomyces albolongus	Compounds K-252-C-Aglycone, indolocarbazole alkaloid, decoyinine, cycloheximide	Activity against *Bacillus cereus* and *S. aureus*	[85]
Streptomyces enissocaesilis	Daunorubicin, hygromycin B, agecorynin F, indinavir-N-glucuronide and minocycline	Activity against *Bacillus cereus* and *S. aureus*	[85]
Streptomyces sp. ZZ741	Streptoglutarimides A–J (1−10) and streptovitacin A (11)	Antiproliferative, activity against MRSA, *E. coli*, and *C. albicans*	[86]
Streptomyces sp. Strain CA-271078	Napyradiomycins D1	Activity against MRSA, *M. tuberculosis* H37Ra and HepG2	[87]
Streptomyces sp. NB-A13	Staurosporine derivatives	Cytotoxic activity against SW-620 cell lines	[88]
Streptomyces bingchenggensis ULS14	ULDF4 and ULDF5 structurally similar to staurosporine and kigamicin	Cytotoxic activity against K562, HeLa, AGS, MCF-7, and HL-60 cell lines	[89]
Streptomyces species Call-36	Actinozine A (1), cyclo(2-OH-D-Pro-L-Leu) (2), two nucleosides: thymidine-3-mercaptocarbamic acid (3) and thymidine-3-thioamine (4). Cyclo(d-Pro-L-Phe) (5) and cyclo(l-Pro-L-Phe) (6)	Cytotoxic and antimicrobial activities	[90]
Streptomyces strain 4205	10 albocycline-type macrolides	Compounds 5−7 displayed antimicrobial activity against C. albicans ATCC 90,028	[91]
Streptomyces strain M7	Actinomycins V, X_2, and D	Activity against *B. subtilis*,*K. pneumoniae* sub sp. *pneumoniae*, *S. aureus*, *S. epidermidis*, *S. typhi*, *E. coli*, MRSA and VRE	[92]
Streptomyces albus JSY-2	Salinomycin (SAL)	Antiparasitic efficacy against theronts of Ichthyophthirius multifiliis	[93]
Streptomyces caniferus CA-271066	Caniferolides A–D	Antifungal activity against *C. albicans* and *Aspergillus fumigatus* Cytotoxic activity against tumor cell lines	[94]
Streptomyces coeruleorubidus GRG 4 (KY457708)	Bis (2-ethylhexyl) Phthalate	Inhibited Colistin resistant *P. aeruginosa* and *K. pneumoniae* and anticancer activity against A549 lung cancer cells	[95]
Streptomyces sp. DA3−7	Pyridine-2,5-diacetamide	Activity against *E. coli* and *Cryptococcus neoformans*	[96]
Streptomyces puniceus strain AS13	Dinactin and 1-(2,4-dihydroxy-6-methylphenyl)-ethanone	Antimicrobial and antitumor activity	[97]

Persiamycin A has shown moderate antibacterial activity against *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* [62].

Metagenomics has also gained attention to explore marine actinobacteria's biosynthetic potential for the production of a diverse array of metabolites. Analysing the DNA sequence obtained using bioinformatics tools helps in screening the various BGCs responsible for many antibiotic productions such as Polyketide Synthases (PKSs), Non-ribosomal Peptide Synthetase (NRPS), and Post-Translationally Modified Peptides (RiPPs) and other metabolites. Metagenomic approaches thus provide a breakthrough to integrate previously unknown synthetic genes, obtained from environmental DNA samples using bioinformatics tools, into an appropriate host [61,63]. Weiland-Bräuer et al. identified two novel QQ proteins, QQ-5 and QQ-7 that are able to inhibit *S. epidermidis* and *C. albicans* biofilm formation derived from a metagenomic library. The two QQ proteins interfered with different QS signaling pathways responsible for biofilm formation in *S. epidermidis* and *C. albicans*. In the case of *C. albicans*, QQ-5 and QQ-7 inhibited the yeast to hyphae transition leading to impaired biofilm formation. QQ-7 has inhibited biofilm formation in *S. epidermidis* by inducing icaR gene expression that encodes repressor for polysaccharide intercellular adhesin (PIA) synthesis, the main determinant responsible for biofilm formation in *S. epidermidis* [64].

Genomic editing using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas was employed in few cases of

Table 3
Compounds isolated from marine actinobacteria with various biological activities in recent years.

Marine actinobacteria	Isolation source	Compound name	Biological activities	Reference (s)
Streptomonospora sp. PA3	Marine sponges isolated from Persian Gulf	Persiamycin A and 1-hydroxy-4-methoxy-2-naphthoic acid	Antibacterial	[62]
Streptomyces griseorubens DSD069	Marine-sediment collected from Alad and Lugbong islands	Bisanhydroaklavinone and 1-Hydroxybisanhydroaklavinone	Antibacterial	[98]
Streptomonospora sp. M2	Soil sample collected from the Wadden Sea beach	Litoralimycins A and B	Anticancer	[99]
Micrococcus luteus	Sample collected from hull of a ship Cochin Port, Kerala	Pigments	Antibacterial, Antifungal, Anticancer, Antioxidant	[100]
Streptomyces sp. ZS-A45	Muddy sea sediments collected from the Zhoushan	New medermycin analog	Anticancer	[101]
Streptomyces bingchenggensis ULS14	Sediment samples collected from 12 different locations in Lagos Lagoon	ULDF4 and ULDF5	Anticancer	[89]
Nocardiopsis sp. SCA21	Marine sediment of Havelock Island, Andaman and Nicobar Islands, India	4-bromophenol and Bis (2-ethylhexyl) phthalate	Antibacterial, Antioxidant, Enzyme Inhibitory	[102]
Streptomyces sp. strain AMA49	Marine samples collected from Nakhon Si Thammarat, Trang, Satun, Songkhla and Phuket provinces in southern Thailand	Oligomycin A and diketopiperazines	Antifungal	[103]
Streptomyces sp. SCA29	Marine sediment of Havelock Island, Andaman and Nicobar Islands, India	4-methoxyacetanilide	Antibacterial, Anticancer, and Enzyme inhibitory	[104]
Streptomyces djakartensis, *Streptomyces olivaceus* and *Nocardiopsis dassonvillei*	Marine sediment samples collected from various locations of the Oman Sea in Hormozgan Province, Iran	Solvent extracts	Antioxidant, and Anticancer	[105]
Streptomyces carpaticus	Marine sediment at the Shark's Bay Beach, Egypt	Viscosine	Antibacterial, Antifungal, and Anticancer	[106]
Nocardia alba KC710971	Marine organisms' samples collected from Andhra Pradesh, India	(Z)-1-((1-hydroxypenta-2,4-dien-1-yl)oxy)anthracene-910-dione	Antiviral, and Antilarval	[107]
Streptomyces sp. DUT11	Marine sediment collected from Xinghai Bay Dalian, China	Tunicamycin	Anticomplement	[108]
Streptomyces cyaneofuscatus M-157	Marine samples collected from submarine Avilés Canyon, Cantabrian Sea	3-hydroxyquinaldic acid derivatives	Anticancer	[109]

actinobacteria [58,59]. The studies of Huang et al. established this for site-specific recombination strategy for gene replacement via Cas9 protein, pKCcas9dO, sgRNA, and homologous arms. The genome-editing efficiency was varied from 60 to 100 % in *S. coelicolor* M145. Furthermore, the time required to edit the genome has reduced by one third compared to the conventional methods [60]. This indicated that this system can be of substantial interest for genome editing and modified secondary metabolite production in other actinobacteria and can be further explored for marine actinobacteria.

9. Conclusions and future perspectives

The emergence and spread of catastrophic AMR can only be dealt with the current understanding of the underlying mechanisms of biofilm recalcitrance toward antibiotics. The review describes how recent progress has improved our capacity to design original and efficient strategies to prevent or eradicate biofilm-related infections. For most of the difficulties encountered in the treatment of biofilm-related infections, the biofilm recalcitrance toward antibiotics is the leading cause. Even after advances being made in the characterization of factors associated with this problematic biofilm property, there are several potential anti-biofilm treatments yet to be validated. With the high risk of treatment failure and infection recurrence associated with the biofilm lifestyle, there is dire need to look for novel bioactives from the novel sources, such as marine actinobacteria. Clinical trials of new bioactives from such sources will likely require renewed interactions between fundamental research and clinical practice before these novel approaches can be included in future therapeutic arsenals for use against difficult-to-treat infections.

Ethical approval

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Nikky Goel-Writing original draft; **Syeda Warisul Fatima-**Review and writing MS; **Sumit Kumar-**Review; **Rajeshwari Sinha-**Review; **Sunil K. Khare-**Supervision, Review MS.

Declaration of Competing Interest

The authors declare no conflict of interest.

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