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Microbial anti-biofilms: types and mechanism of action

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ABSTRACT

Biofilms have been recognized as a serious threat to public health as it protects microbes from antimicrobials, immune defence mechanisms, chemical treatments and nutritional stress. Biofilms are also a source of concern in industries and water treatment because their presence compromises the integrity of equipment. To overcome these problems, it is necessary to identify novel anti-biofilm compounds. Products of microorganisms have been identified as promising broad-spectrum anti-biofilm agents. These natural products include biosurfactants, antimicrobial peptides, enzymes and bioactive compounds. Anti-biofilm products of microbial origin are chemically diverse and possess a broad spectrum of activities against biofilms. The objective of this review is to give an overview of the different types of microbial anti-biofilm products and their mechanisms of action.
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1. Introduction

Biofilm is an organized complex structure of microbial communities that has been a source of concern to public health. Biofilm production is one of the main mechanisms of antimicrobial resistance in bacteria and fungi [1]. Biofilm formation by microorganisms can result in infection persistence, difficulty in infection eradication, increased morbidity and mortality rates as well as increased treatment cost and treatment failure [2,3]. Additionally, it can lead to serious economic losses in the industrial sectors as a result of a compromise of the integrity of machines and equipment [4,5].

Biofilms play a vital role in infections especially infections associated with in-dwelling medical devices. They are found on biotic surfaces such as human teeth, tissues, wounds and lungs as well as surfaces of medical devices such as catheters, cardiac valves, contact lenses and other medical implant devices [6]. The most frequent complications associated with the use of medical devices are infections caused by microorganisms that dissociate from the biofilms formed on the devices. Examples of these complications include catheter-associated urinary tract infections and ventilator-associated pneumonia. Biofilms associated with medical devices are the most important cause of nosocomial infections [7].

Biofilms protect bacteria from antimicrobials and the host's immune system by acting as a physical barrier. This results in resistance to treatment with antimicrobials and relapse after treatment, thereby making infection eradication difficult. Infections caused by biofilm-producing pathogens are associated with severe symptoms as well as high rates of morbidity and mortality [8]. Biofilm-associated infections are usually difficult to treat due to the poor biofilm penetration ability of antimicrobials and the high level of antimicrobial resistance among microorganisms within the biofilm [6]. Resistance of biofilm-associated microorganisms to antimicrobials is also linked to their slow growth rate and dormancy [9].

Biofilms account for approximately 60% of foodborne outbreaks [10], hence their presence in food processing facilities indicates a significant risk to food safety. Biofilm-producing strains of *Bacillus cereus* are a common source of concern in industries, especially dairy industries. The biofilms they produce at the interface between air and liquid as well as on submerged surfaces serve as sources of food contamination, which can lead to food infection and

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food intoxication [11]. Biofilms of *Listeria monocytogenes* are found on various types of surfaces in industries and are dispersed to foods from these surfaces. This pathogen is capable of replication at refrigeration temperature and its biofilm formation ability makes it resistant to procedures used for cleaning in food factories [12,13]. *L. monocytogenes*, when consumed, can result in listeriosis in young children, the elderly, and people with weakened immune systems, as well as spontaneous abortion in pregnant women [14,15].

Researches on alternative and effective anti-biofilm agents are on the increase owing to the resistance and tolerance of biofilm-producing microorganisms to current antimicrobials [16]. Diverse natural products sourced from microorganisms are among the most effective broad-spectrum anti-biofilm agents. They include bioactive compounds, biosurfactants, antimicrobial peptides and microbial enzymes. This review aims to provide an overview of biofilms, stages of biofilm production, biofilm-producing microorganisms, types of anti-biofilm agents of microbial origin and the mechanisms of action of microbial anti-biofilm agents.

2. Biofilms

Biofilm is a highly developed structure composed of a complex association of bacteria and fungi found enclosed in a complex self-produced extracellular polymeric substances (EPS) matrix, attached to surfaces (biotic or abiotic) [4,17]. It allows bacteria and fungi to survive harsh environmental conditions, evades the immune system of the host and resists most antimicrobials [2]. Biofilm also protects microorganisms from the activity of antimicrobials, UV radiation, desiccation, starvation, metal toxicity, acids and adverse pH [9,18,19]. Nutrients conveyed into biofilm through channels in the matrix are usually trapped by the EPS, thereby creating a nutrient-rich microenvironment [20]. Hence, microorganisms within the biofilm have greater access to nutrients [21]. Transfer of resistance genes among bacteria occurs at higher frequencies within the biofilms because biofilms provide favourable conditions for horizontal transfer of resistance genes including high microbial density and accumulated genetic materials [22,23]. Transfer of resistance genes among microorganisms within the biofilm occurs through conjugation (cell-to-cell direct contact), transformation (acquisition of extracellular genetic elements by competent bacteria) or transduction (transfer of gene mediated by bacteriophages) [24].

Biofilm formation by microorganisms occurs in stages (Fig. 1) and the stages are categorized into four [25]. The first stage involves the initial adhesion of primary colonizers (i.e. the planktonic cells) to surfaces and the formation of conditioning film, which will facilitate adhesion. The second stage is characterized by the formation of an extracellular polymeric matrix leading to the irreversible adhesion of microorganisms and subsequent multiplication of adhered microbes to form microcolonies (groups of microbial cells) with extracellular polysaccharides serving as a glue. The third stage is where biofilm maturation takes place. Intense microbial cell multiplication and formation of water channels to allow the inflow of oxygen, protein, as well as other substrates occur during this stage. The fourth stage involves the release of persister microbial cells from the biofilm [9]. These cells are homogeneous to other cell populations genetically but heterogeneous phenotypically; they divide slowly and are less susceptible to antibiotics [19]. Persister cells adhere to new surfaces and initiate the formation of new biofilm [3,25]. Cell-to-cell communication within the biofilm is through a communication mechanism called quorum sensing (QS). This communication mechanism plays an important role in biofilm and EPS production [26]. It is also important in controlling secondary metabolite production and expression of virulence factors [27].

3. Structural composition of biofilm

The extracellular matrix of biofilm varies in its chemical composition and provides the organic structure of the biofilms as well as facilitates the spatial arrangement of the microbial community within the biofilm. It is composed mainly of proteins, polysaccharides, glycoproteins, extracellular nucleic acids and phospholipids [4,18]. These biomolecules are important in the initial establishment, development, architecture, stability and maintenance of biofilm [17]. The extracellular matrix of microorganisms (fungi and bacteria) differs in terms of composition and components [9]. The composition of the biofilm extracellular matrix is dependent on the microbial strain producing it [17]. Additionally, the nature of the environment where the biofilm was formed determines the type and composition of the extracellular matrix [27]. Availability of nutrients, temperature and pH of the environment affect the secretion of exopolysaccharides and proteins as well as the activity of enzymes during biofilm formation [28].

The extracellular matrix of biofilm produced by *Staphylococcus aureus* and *Staphylococcus epidermidis* composed majorly of extracellular DNA (eDNA), protein and intercellular adhesion polysaccharide [29,30]. Beta-glucan, eDNA, proteins and nucleic acids constitute the biofilm matrix of *Haemophilus influenza* [31]. The biofilm matrix of *Pseudomonas aeruginosa* is made up of alginate exopolysaccharides, protein and eDNA while that of *Propionibacterium acnes* is composed of poly-N-acetyl glucosamine, DNA and proteins [17]. The matrix of *Candida albicans* biofilm consists of protein, polysaccharides (mainly α-1,6 mannan and β-1,6 glucans), lipids and DNA. The extracellular biofilm matrix produced by *Aspergillus fumigatus* is made up of protein, polysaccharides (mainly galactomannan and galactosaminogalactan), lipids, aromatic compounds and DNA [32].

4. Biofilm-producing bacteria

Biofilm-producing bacteria account for approximately 40–80% of whole bacterial species identified on Earth [33]. They can colonize and form biofilms on both natural and synthetic surfaces [21]. Biofilm producers are of concern because they can form biofilms in food processing facilities, water distribution systems, indwelling medical devices (such as urinary catheters and cardiac pacemakers) and tissues of patients leading to food spoilage and persistent infections [11,21,34].

The most frequently detected biofilm-producing bacteria associated with medical devices, wounds and tissues (Fig. 2) include *Lactobacillus* spp., *Prevotella* spp., *Porphyromonas* spp., *Bacteroides* spp., *Fusobacterium* spp., *Streptococcus agalactiae*, *H. influenza* [21], *Streptococcus* spp., *Fusobacterium nucleatum* [3], *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Staphylococcus saprophyticus*, *Klebsiella* species [35], coagulase-negative Staphylococci [36], *P. acnes* [37], *Staphylococcus warneri*, *Staphylococcus lugdunensis* [38], *Enterococcus faecalis*, *Enterococcus faecium* [39], *Staphylococcus haemolyticus*, *Escherichia coli* [40], *Rothia dentocariosa*, *Enterobacter* spp. [41], *Citrobacter* spp., *Proteus* spp. and *Aeromonas* spp. [42].

Bacterial species known to form biofilm in facilities of food industries and associated with food spoilage (Fig. 2) include *Vibrio parahaemolyticus* [21], *L. monocytogenes*, *Clostridium perfringens*, *Campylobacter jejuni*, *Bacillus cereus*, *E. coli*, *Salmonella* spp., *Cronobacter* spp., *Staphylococcus* spp., *Cronobacter* spp. [21,43], *Pseudomonas* spp. [44], and *Geobacillus stearothermophilus* [45]. Biofilm-producing bacteria are also found in potable water distribution systems, where they get dislodged into drinking water. Commonly detected biofilm producers in water (Fig. 2) include *P. aeruginosa*, *Aeromonas hydrophila*, *C. jejuni*, *Klebsiella pneumoniae*, *Legionella*

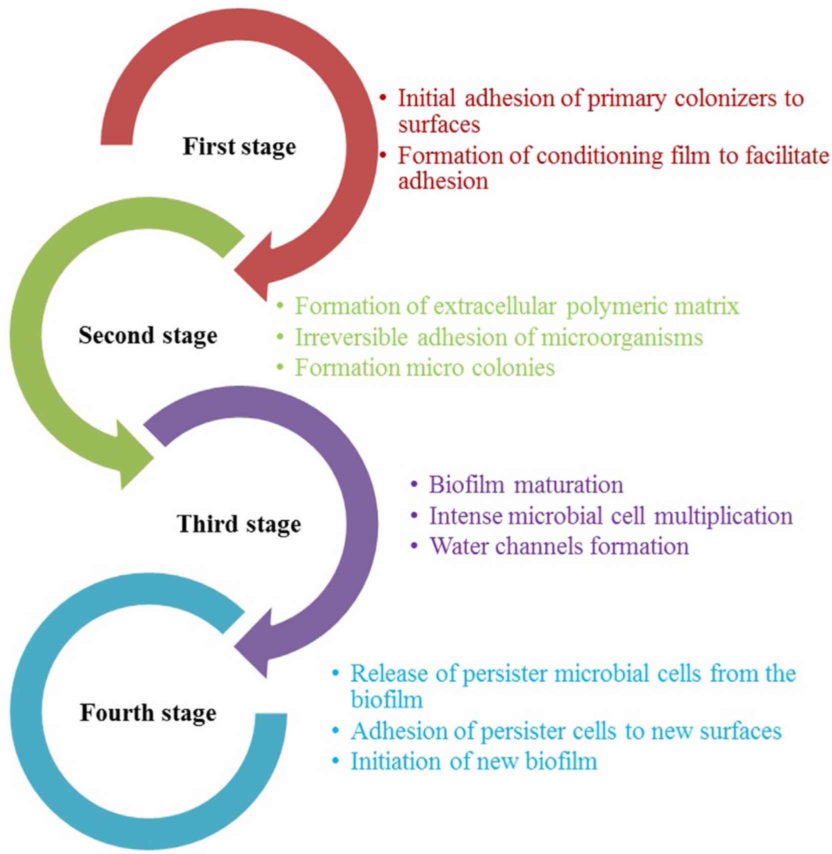

Fig. 1. The four stages of biofilm formation.

pneumophila, Mycobacteria [21,46,47] and *Vibrio cholerae* [48]. Biofilm-producing plant pathogenic bacteria (Fig. 2) include *Pseudomonas syringae pv. syringae*, *P. aeruginosa* [21], *Xanthomonas citri*, *Xylella fastidiosa* [49] and *Ralstonia solanacearum* [50].

5. Biofilm-producing fungi

Biofilm-producing fungal species are also of concern in medical settings, industries and water distribution lines (Fig. 3). Biofilm-producing fungi of clinical importance include *Blastoschizomyces capitatus*, *Malassezia pachydermatis*, *Rhizopus* species, *Candida tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. auris* and *Rhizomucor* species [32], *C. albicans*, *A. fumigatus*, *Cryptococcus neoformans*, *Trichosporon asahii*, *Coccidioides* species, *Pneumocystis* species [32,51], *Fusarium oxysporum* and *F. solani* [52].

Biofilm-producing fungi associated with plants include species of *Botrytis*, *Fusarium*, *Verticillium* and *Didymella* [52]. Species of *Aspergillus*, *Alternaria*, *Botrytis*, *Candida*, *Cladosporium* and *Penicillium* were reported to be associated with biofilms in water systems [53].

6. Types, sources and mechanisms of action of microbial anti-biofilm products

Microorganisms including bacteria, fungi and algae, have been identified as sources of diverse natural products with reported broad-spectrum anti-biofilm activities [19,54]. These microbial products inhibit biofilm formation by acting either individually or synergistically [55] using a diverse mechanism of action such as suppression of cell adhesion and attachment, interruption of extracellular matrix generation biofilm components degradation, inhibition of cell division and survival, prevention of biofilm maturation, deprivation of substrates, down-regulation of quorum sensing molecules leading to blockage of quorum sensing network and development of biofilm [4,26]. These compounds include bioactive compounds, biosurfactants, antimicrobial peptides and enzymes (Table 1).

6.1. Bioactive compounds

Bioactive compounds produced by microorganisms can disrupt biofilm formation [56]. These compounds act by suppressing communication through quorum sensing, interfering with the production of the biosynthetic enzyme [57], interfering and competing with inducers of quorum sensing like acyl homoserine lactone, autoinducers, and autoinducers type 2 to disrupt the quorum sensing mechanism [58]. They also contribute to bacterial adhesion inhibition [59] by interfering with the forces (electrostatic attraction, sedimentation, Brownian movements, and Van der Waals force of attraction) that act by supporting bacterial attachment to different surfaces, which sometimes prevent biofilm growth in its early stages. By altering the biofilm structure, bioactive compounds increase the detachment rate of bacteria and lead to the loss of bacteria biomass from the substratum [9]. These compounds inhibit adherence during biofilm formation by preventing the production of alginate and exopolysaccharide [60]. These compounds can also suppress genes that are involved in biofilm formation [59] through degradation of signaling molecules, blockage of the gene regulation system or competitive inhibition [61]. They also can prevent access to nutrients required for

Fig. 2. Biofilm producing bacterial species associated with medical devices, food industry facilities, water distribution system and plants.

adhesion and bacterial growth, as well as interrupt the extension process [62].

Carolacton produced by a myxobacterium called *Sorangium cellulosum* exhibited anti-biofilm activity against *Streptomyces mutans* by affecting several regulatory systems of the organism [63]. Anti-biofilm activity of macrotetrolides (monactin, dinactin and trinactin) isolated from *Saccharomyces cerevisiae* against *C. albicans* strains was reported by Tebbets et al. [64]. In addition, the polymer produced by *Trichosporon montevideense* inhibited the formation of biofilm by *C. albicans* and *C. krusei* [65]. Formation of biofilms by bacterial isolates namely *S. aureus*, *Bacillus subtilis* and *E. faecalis* was inhibited by sartorypyrone A and aszonapyrone A produced by *Neosatorya fischeri* KUTC 6344 [66]. Biofilm formation by Methicillin-Resistant *S. aureus* (MRSA) was inhibited by cytosporone E isolated from *Leucostoma persoonii* as observed in a study conducted by Beau et al. [67]. Patulin and penicillanic acid isolated from *Penicillium coprobium* and *P. radicicola* respectively exhibited quorum-sensing inhibitory activity. These compounds act by interfering with RhlR and LasR proteins, which regulate the expression of virulence genes in *P. aeruginosa* [68].

Biofilm formation by *S. aureus* and *S. epidermidis* was prevented by gallidermin, a lantibiotic produced by *Staphylococcus gallinarum* [69]. Transcriptional analysis conducted by Saising et al. [69] revealed that gallidermin appreciably reduces the transcription of *ica* and *atl* genes in both *S. aureus* and *S. epidermidis*. The *atl* gene codes for the major autolysin involved in surface attachment during biofilm formation while the *ica* gene codes for an exopolysaccharide called polysaccharide intercellular adhesin (PIA) [69]. Amino acids and their derivatives isolated from microorganisms have been reported to possess anti-biofilm activities. Cyclo(L-Tyr-L-Leu) isolated from *Penicillium* sp. was reported to inhibit biofilm production by *S. epidermidis* [70]. Furthermore, cyclo(L-Leu-L-Pro) isolated from *Penicillium commune* exhibited activity against the biofilm of *S. aureus* [71].

Another group of bioactive anti-biofilm compounds are the extracellular polysaccharides. Aside from being an essential compound of biofilms, they have been recognized to inhibit the biofilm formation of other microorganisms [72]. EPS-273, an extracellular polysaccharide sourced from the marine bacterium *Pseudomonas stutzeri* 273, prevents biofilm formation by *P. aeruginosa*. It inhibits biofilm production in *P. aeruginosa* by decreasing the production of

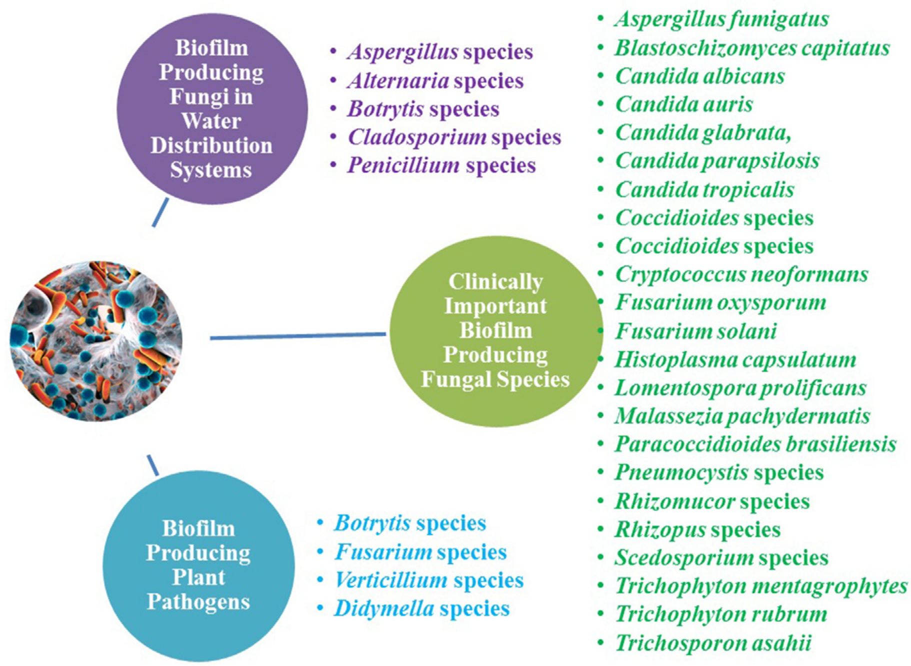

Fig. 3. Biofilm producing fungal species.

Table 1
Types and examples of anti-biofilms derived from microorganisms.

Types of microbial anti-biofilm	Examples	Microbial sources	Reported activities	References
Bioactive compounds	Carolacton	*Sorangium cellulosum*	Exhibits anti-biofilm activity against *Streptomyces mutans*	Kunze et al. [63]
	Macrotetrolides	*S. cerevisiae*	Exhibits anti-biofilm activity against *C. albicans* strains	Tebbets et al. [64]
	Cyclo(L-Tyr-L-Leu)	*Penicillium* sp.	Inhibits biofilm production by *S. epidermidis*	Scopel et al. [70]
Biosurfactants	*Lactobacillus* derived biosurfactant	*Lactobacillus gasseri*	Inhibits biofilm formation in some strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA)	Giordani et al. [86]
	Lipopeptide biosurfactant	*Acinetobacter junii* B6	Disrupts biofilm of *Proteus mirabilis, S. aureus* and *P. aeruginosa*	Ohadi et al. [88]
	Biosurfactant	*Cobetia* sp.	Interferes with the lipophilic signals involved in intercellular communication.	Ibacache-Quiroga et al. [90]
Antimicrobial peptides	pepR	Dengue virus capsid protein	Induces membrane permeabilization in *S. aureus*.	Pinto et al. [98]
	Bacteriocin-Like Substance (BLS)	*Bacillus* species P34	Inhibits biofilm formation by *S. aureus* and *E. faecalis*.	Costa et al. [99]
Microbial enzymes	Amylase	*Bacillus cereus*	Exhibits inhibitory activity against biofilms produced by *P. aeruginosa* and *S. aureus*	Vaikundamoorthy et al. [103]
	Pectinase	*Pseudomonas stutzeri*	Exhibits inhibitory activity against biofilms of *E. faecalis, P. aeruginosa* and *S. aureus*	Nasayif et al. [104]

pyocyanin (a virulence factor) and inhibits the release of eDNA (required for stable biofilm production) [73]. Two polysaccharides produced by *P. aeruginosa* PAO1 namely Pel and Psl, were reported to reduce biofilm formation by *S. epidermidis* [74]. Inhibition of biofilm formation and disruption of biofilms formed by *Salmonella enterica* and *Vibrio harveyi* were observed using sulfated polysaccharides produced by *Chlamydomonas reinhardtii*. These polysaccharides disrupt biofilms by degrading the eDNA component of the EPS matrix [75].

Bioactive compounds isolated from *Paenibacillus* species 139SI exhibited anti-biofilm activities against Gram-positive and Gram-negative bacterial isolates. These compounds were amino acid antibiotic (Leucine 2-(hydroxymethoxyphosphinyl)-2-methylhydrazide), phospholipase A2 inhibitor (4-hydroxy-5-(hydroxymethyl)-3-(14-methylpentadecanoyl) tetronicacid-2(5H)-furanone) and antibacterial agent (6-(hydroxymethyl)-1-phenazinecarboxyamide). The anti-biofilm activity of the amino acid antibiotic was proposed to be through modulation of microbial surfaces thereby inhibiting cell–cell interaction and cell-surface attachment [76]. The *Paenibacillus* species 139SI produced by phospholipase A2 inhibitor is structurally similar to (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone isolated from *Delisea pulchra*, which was reported to inhibit *E. coli* biofilm formation by acting as quorum sensing antagonist [76,77].

Other compounds produced by microorganisms with reported anti-biofilm activities include α-D-galactopyranosyl-(1→2)-

glycerol-phosphate produced by *Bacillus licheniformis*, Ophiobolin K isolated from *Emericella variecolor*, Flavipesin A produced by *Aspergillus flavipes*, Usnic acid produced by *Chondrus crispus* and Juglone produced by *C. crispus* [56]. Inhibitory activities of 1-hydroxy-1-norresistomycin isolated from *Streptomyces variabilis* against biofilm formation in clinical isolates of *V. cholerae*, *E. coli*, and *S. aureus* were also reported by Ramalingam et al. [78].

6.2. Biosurfactants

Biosurfactants are a heterogeneous group of surface-active amphiphilic compounds that are produced mainly by microorganisms that accumulate in between liquid phases and therefore reduce the surface and interfacial tensions [79,80]. Microorganisms produce diverse groups of biosurfactants possessing antifungal, antibacterial, and anti-biofilm properties [81]. The mode of action of biosurfactants is by reduction of adherence ability of the biofilm to surfaces. Thus, biosurfactants alter the ability of the cell to adhere to surfaces by decreasing the hydrophobicity of the cell surface, rupturing the membrane, blocking the electron transport chain, and reducing the energy requirements of the cell [82]. Disruption of the membrane results from the insertion of biosurfactant molecules into the cell membrane of microorganisms, leading to altered membrane permeability. This eventually results in disruption of the cell membrane and death [83,84]. Biosurfactants were also reported to inhibit autoinducer-2 (AI-2) signaling molecules, accessory gene regulator (agr A), and staphylococcal accessory regulatory (sar A) gene expression [85]. They are good coating agents for medical implants such as bone implants and urinal catheters by preventing pathogenic organisms from forming biofilms without using synthetic drugs [55].

Biosurfactants from *Lactobacillus gasseri* were found to inhibit biofilm formation in some strains of Methicillin-Resistant *S. aureus* (MRSA) [86]. Dispersion of the preformed biofilm by biosurfactant at the concentration of 100 mg/ml was reported by Sambanthamoorthy and co-workers [87]. Lipopeptide biosurfactant produced by *Acinetobacter junii* B6 was reported to disrupt biofilm produced by *Proteus mirabilis, S. aureus* and *P. aeruginosa* [88]. They were indicated to be involved in regulating quorum sensing and the motility of bacteria [89]. The ability of biosurfactants to block quorum sensing was observed in the biosurfactants produced by *Cobetia* sp. that interfere with the lipophilic signals involved in intercellular communication. This leads to the repression of some genes involved in biofilm formation [90].

A study conducted by Singh and Sharma [91] revealed the anti-biofilm property of lipopeptide biosurfactant produced by *Bacillus tequilensis* strain SDS21. The biosurfactant was able to eradicate >99% of the biofilms formed by *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *Salmonella* Typhi, and *Salmonella* Typhimurium biofilm on different types of surfaces. Inhibition of *S. aureus* biofilm formation by mannosyl erythritol lipids isolated from *Pseudozyma aphidis* DSM 70725 was reported by Shu et al. [92]. Inhibition of *S. aureus* biofilm formation by this biosurfactant is through disruption of bacterial adhesion to surfaces.

Biofilm formation by *S. aureus* and *B. subtilis* was disrupted by sophorolipid produced by *Candida bombicola*. This biosurfactant exhibited anti-biofilm activity against these bacteria both singly and when cultured together [93]. Biosurfactant produced by *Lactobacillus casei* was reported to inhibit biofilm formation by the oral strain of *S. aureus* [94]. Pontifactin, a biosurfactant produced by a marine bacterium *Pontibacter korlensis* exhibited anti-biofilm activity against isolates of *S. aureus*, *Salmonella* Typhi, *V. cholerae*, and *B. subtilis* [95]. These biosurfactants disrupt biofilm production by increasing or altering the permeability of bacterial membranes [72].

6.3. Antimicrobial peptides (AMP)

Antimicrobial peptides (AMP) are compounds that act on bacterial cell membranes by electrostatic interaction with membrane phospholipids when inserted into the membrane thereby killing the bacteria. They are found in animals, humans, microbes and plants [55]. Antimicrobial peptides and antimicrobial compounds have been demonstrated to block some molecular processes involved in the production of biofilms when used in combination [96].

Antimicrobial peptides are being employed to prevent the formation of biofilm or to eradicate matured ones. The four major processes by which antimicrobial peptides destroy biofilms are (i) degradation of the potential membrane of biofilm-embedded cells; (ii) degradation of the biofilm-associated polysaccharide and the matrix; (iii) down-regulation of genes that code for biofilm formation and (iv) interrupting the bacterial quorum sensing [97].

The AMPs are mostly cationic amphiphilic compounds; however, anionic and neutral peptides are in existence as well [18]. The cationic site of the peptide interacts with anionic bacterial moiety, which may be lipoteichoic acid in Gram-positive bacteria or lipopolysaccharides in Gram-negative bacteria. This interaction leads to the disruption of the cytoplasmic membranes, resulting in cell lysis and leakage of ATPs [97].

The AMPs could inhibit the creation of biofilms by increasing twitching motility in *P. aeruginosa* through the promotion of gene expression needed for type IV pili biosynthesis and function [97]. A viral-derived peptide (pepR) was reported to induce membrane permeabilization of *S. aureus*, which is a significant pathogen in biofilm formation. In addition, pepR was observed to establish an ionic interaction with the biofilm matrix resulting in its dispersion [98]. Bacteriocin Like Substance (BLS P34), an antimicrobial peptide isolated from *Bacillus* species P34 was reported to inhibit biofilm formation by *S. aureus* and *E. faecalis* as well as eradicate the biofilms formed [99]. Microcin-B17, a post-translationally modified peptide sourced from *E. coli* exhibited anti-biofilm activity by inhibiting the division and survival of bacterial cells [72].

Three models have been used to explain this mode of interaction that leads to lysis: (i) barrel-stave model in which, the AMP amphipathic moiety interacts with the membrane lipids, while hydrophilic residues generate a cavity in the membrane; (ii) toroidal model; where the hydrophobic-hydrophilic bilayer arrangement is disrupted when the peptide induces curvature in the bilayer, thereby generating a ring-shape pore; (iii) carpet model; here the AMPs are adsorbed to adhere and cover the entire surface of the cell until a certain concentration is reached and that leads to the loss of integrity of the membrane, resulting to its disruption [100]. Other mechanisms of action include inhibiting the synthesis of DNA, RNA, and cell wall. These modes of action result from the translocation of small AMPs across the cell to interfere with transcriptions and or translations of particular genes [16] as well as inhibition of enzymes required for nucleic acid replication [9].

The AMP has also been reported to be effective in breaking the extracellular matrix by increasing its stiffness and roughness. Also, AMP interferes with the cell cycle of fungal cells, leading to apoptosis [101]. Certain AMPs inhibit biofilm formation by preventing the accumulation of alarmones (namely: guanosine tetraphosphate and guanosine pentaphosphate), which are an important part of the bacterial stringent response to nutritional stress [9,102]. AMPs can penetrate deep into the biofilm and interfere with the integrity of lipopolysaccharides of the bacterial cell thereby disrupting and killing the bacteria [9].

6.4. Microbial enzymes

Enzymes produced by microorganisms have been reported to exhibit anti-biofilm activities through the degradation of EPS components namely proteins, carbohydrates and lipids, this degradation results in a compromise of the EPS integrity [105]. The microbial enzymes degrade and solubilize the components of the biofilm matrix, thereby disassembling the biofilm and exposing the microorganisms within it. Disruption of this protective matrix disperses the microbial cells into the environment [9]. The use of these enzymes, therefore, increases the permeability and effectiveness of antimicrobial agents [1].

Arthrobacter sp. CW01 produced anti-biofilm enzymes known as amylase and protease. These enzymes were reported to have anti-biofilm activity due to the presence of the hydrolase group responsible for degrading the physical integrity of EPS and digesting slime layers of the biofilm, resulting in the breaking of mature biofilm structure [106]. Another research finding by Li and co-workers [107] reported the ability of microbial enzymes such as proteinase K, lipase, and cellulase to eradicate biofilm. In the findings, it was reported that the enzymes worked in synergy to effectively inhibit the stages of formation of biofilm and to remove the mature one. The mechanism of action of the combined enzymes reported in the study was their ability to regulate the exopolysaccharide matrix production and synthesis of biomolecules.

The inhibitory activity of amylase produced by *Bacillus cereus* against biofilms produced by *P. aeruginosa* and *S. aureus* was reported by Vaikundamoorthy et al. [103]. *Pantoea agglomerans* produced α-amylase that disrupted biofilms formed by food-borne pathogenic bacteria namely *Bacillus cereus*, *L. monocytogenes*, *Serratia marcescens*, *Salmonella* Typhi and *V. parahaemolyticus*. Amylases disrupt biofilms by hydrolyzing the EPS components of the biofilm [108]. *Bacillus subtilis* S8-18 produced α-amylase, which degrades mature biofilm formed by methicillin-resistant *S. aureus* thereby disrupting the EPS matrix [109].

Alkaline phosphatase produced by *Cobetia amphilecti* KMM296 was reported to exhibit anti-biofilm potential against the biofilms of *P. aeruginosa* and *S. aureus* [110]. Purified pectinase produced by *P. stutzeri* showed promising activity against biofilms of *E. faecalis*, *P. aeruginosa* and *S. aureus* [104]. Extracellular serine protease (Esp) produced by the commensal bacterium *S. epidermidis* was reported to inhibit and destroy the biofilm formation of *S. aureus* [111]. Another example of a matrix-degrading microbial enzyme is dispersin B, a β-*N*-acetylglucosaminidase produced by *Actinobacillus actinomycetemcomitans*. This enzyme was able to disintegrate matured *S. epidermidis* biofilms [112]. Proteolytic and carbohydrate degrading enzymes produced by fungal isolates namely *Aspergillus niger*, *Trichoderma viride*, and *Penicillium* spp. exhibited biofilm removal activity against *Pseudomonas fluorescens* biofilm [113].

7. Advantages of microbial anti-biofilm

Microbial anti-biofilm prevents colonization and successive formation of biofilm. Protein-carbohydrate-mediated interaction is an important factor for microbial biofilm formation and subsequent infection [114]. Therefore, among the objectives of microbial anti-biofilm is to impede such interaction for the prevention of numerous infectious diseases [115]. The use of microbial anti-biofilms creates less selective pressure and hence there is less possibility of resistance development [114]. Other merits of microbial anti-biofilm are that they can resist harsh environmental conditions while retaining their efficacy, and they maintain their activities without being toxic to the host [116].

8. Challenges and limitations of anti-biofilm strategies

Most chronic and biofilm-related infections are caused by biofilm from polymicrobial sources. Meanwhile, this is not taken into account as current and conventional anti-biofilm strategies are usually aimed at a single species of bacteria [117,118]. Also, the lack of adequate techniques to deliver the microbial anti-biofilm compounds to affected sites coupled with the possible presence of several bacterial biofilms in the sites pose a serious challenge to the use of anti-biofilm [118]. The large size of some antimicrobial peptides and polysaccharides makes them inappropriate for therapeutic use [9].

Existing *in vivo* and *in vitro*, anti-biofilm assays have been unable to efficiently predict the outcome of anti-biofilm action in humans, although several medical devices with anti-biofilm claims have been presented to relevant institutions such as the Food and Drug Administration (FDA) [119]. There is the possibility of anti-biofilm degradation, inactivation or inhibition by the host. Cytotoxic effects might also occur following treatment of the host with microbial anti-biofilms, especially when they are administered at high doses and for prolonged periods [9,72]. The development of consistent and reliable alternatives to clinical investigations is therefore needed, which uses standardized procedures to evaluate and validate techniques that correlate with results from clinical investigations. The mechanisms of action for various anti-biofilm and other known antibacterial agents are not properly understood. Due to the continuous need to develop delivery applications of certain drugs, the transformation of microbial anti-biofilm compounds into a commercial commodity remains another existing challenge for researchers in the field [118]. Treatment of biofilms with anti-biofilms does not always result in the complete elimination of microbial cells. The residual microorganisms left might facilitate re-colonization and biofilm re-installation after treatment withdrawal [72]. Another concern is the possible adhesion of epitomes of anti-biofilm with human cells as well as the low affinity of microbial anti-biofilm receptors to ligands of bacteria. There is also the possibility of the occurrence of bacterial mutations that can affect the effectiveness of the microbial anti-biofilm and the ability of the pathogen to adhere to the host receptor [116].

Peptides from microbial sources are known to have potential anti-biofilm properties. However, the high production cost, and poor stability *in vivo* coupled with the long time it takes for synthesis of the peptides pose limitations for various applications in industries, medicine as well as in the environment [1]. Another disadvantage of using AMPs is that some peptides are highly susceptible to degradation by bacterial protease [55] and they often have proinflammatory effects [7].

Similarly, the use of anti-biofilm enzymes from natural sources especially microorganisms has been limited by their ease of inactivation, unstable nature, high cost of purifying the enzymes and poor permeability. Although in recent years, simulated enzymes with characteristics such as ease of production on a large scale, good stability, easily amenable catalytic activity and high permeability have been studied extensively to solve the problems associated with natural enzymes [1,120]. The simulated biocatalysts mainly include artificial enzymes with DNase-mimicking [121] and peroxidase-mimicking activities [122]. Enzymes are known to efficiently destroy specific major EPS constituents (Fig. 4). Hence, owing to the specificity of enzyme activity, the elimination of biofilm using enzymes cannot be applied to a wide range of microorganisms [1]. Thus, the use of microbial anti-biofilms in human hosts could elicit inflammatory and allergic reactions, hence limiting their use for therapeutic purposes [123].

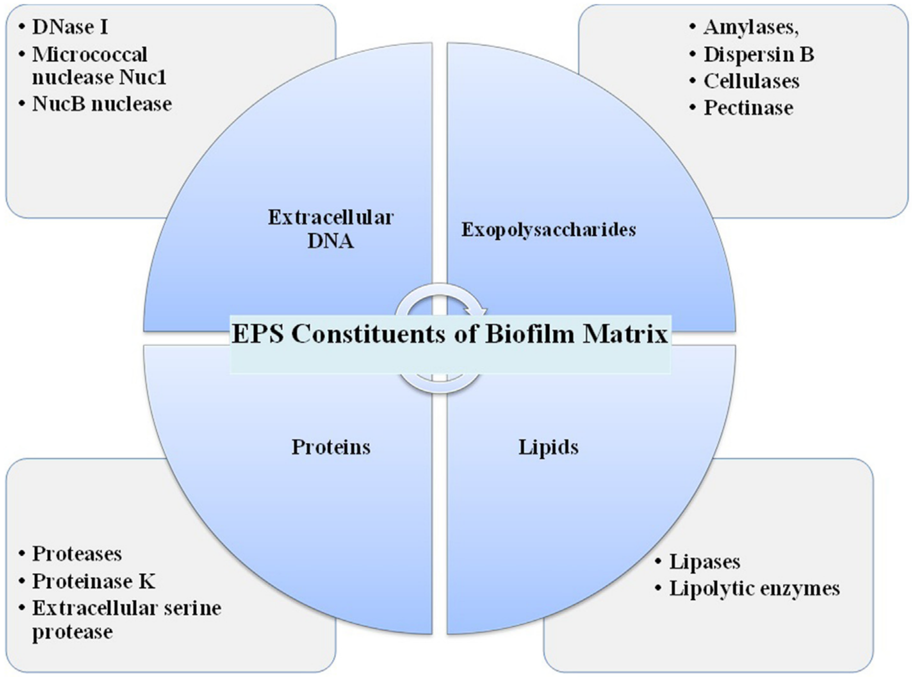

Fig. 4. Examples of EPS constituents degrading microbial enzymes.

9. Conclusion

The incidence of biofilm is an important dilemma for human, animal and environmental health as well as in water treatment, sewage management, food and other related industries and their prevention is inevitable. The formation of microbial biofilm is an intricate multifaceted process that is not well understood and conventional techniques have mostly lost their efficacy. Consequently, this calls for the development of novel, effective and efficient anti-biofilm strategies. There is no current ideal resolution for the total elimination of biofilm, but a potential solution is the concurrent deployments of various microbial anti-biofilm types of machinery with prospective synergistic mechanisms that will upset the constitution of the biofilm and at the same time kill the microorganisms. Hence, there is a need for multidisciplinary research to decipher and understand the essential fundamental networks of the intricate communities involved in microbial biofilm and to assign them to their evolutionary and ecological positions. It is therefore essential to employ bioinformatics tools such as proteomics, metagenomics, and metabolomics, to achieve this task. Exploration of the potentials of microbial anti-biofilm will go a long way to aid both academic and industrial communities to establish a framework for solving problems associated with health, nutrition, environment and industry.

The gene expression of pathogens within a biofilm can be altered using their probiotic complement organisms, which may present a potential innovative anti-biofilm development. This will decrease microbial colonization and prevent the ensuing development of a biofilm. Further investigations are needed to evaluate the benefits of various microbial anti-biofilm compounds to ascertain their effective, efficient, specific and eco-friendly and safe therapeutic and prophylactic applications. Meanwhile, their use, benefits and possible risk in the health sector should be vigorously considered especially their potential consequences on the useful microbiota of the host.

Declaration of competing interest

The authors have no conflict of interest to declare.

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