Chapter 18
Potential Use of Biotechnological Tools to Eradicate Microbial Biofilms

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Introduction

Microbial biofilms are aggregates of microorganisms embedded in autogenic extracellular network of proteins and exopolysaccharide materials that adhere to an abiotic or biotic surface. Biofilms are commonly known as the city of microbes and follow the unique pattern of growth to achieve the higher level of organization of free-living microbes. According to Donlan and Costerton (2002) "Biofilm as a structured community of microbial cells enclosed in a self-produced polymeric matrix and adherent to a surface to interface, and to each other" still remains the most appreciated definition of biofilms (Mishra et al., 2020).

Basically, microbial biofilms are complex, dynamic and three-dimensional heterogeneous structures in which cells are interconnected by Extracellular Polymeric Substances (EPS). EPS are a blend of polysaccharides, peptides, nucleic acid and other substances produced by microorganism itself. EPS provides protection to microbial cells under adverse environmental conditions thus encasement acts as a house for cells. Biofilms can withstand metal toxicity, ultraviolet light, lethal effect of antimicrobials and other chemical agents like soaps, detergents, disinfectant and other cleaning agents (Rodríguez-Lázaro et al., 2018).

Nowadays biofilms are a big issue for the food industry, medical field, naval and other industries also. Certain microbes have the capability to aggregate over various surfaces of materials and clinical devices such as medical implants, prosthetic implants, catheters, sutures, intrauterine devices and contact lenses to produce the biofilm.

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F. Ahmad et al. (eds.), *Microbial Biotechnology in the Food Industry*,
https://doi.org/10.1007/978-3-031-51417-3_18

In the food industry, biofilm forming food borne pathogens may contaminate the raw material and food products through secretion and excretion of toxins and enzymes that may create risk for consumer's health. Similarly, medical devices and implantations are also attacked by biofilm forming pathogens that further lead to infections in the human body. Water supply networks are also affected by biofilms that lead to contamination of water, deterioration of water quality and corrosion of water channels by these microbes (Ramirez-Mora et al., 2018). Biofilms are the state in the life cycle of microbes that enhance the attributes of resistance against external attack of antibiotics, chemicals and disinfectants. Future insight the immense need to implicate effective methods for elimination of biofilms from the environment.

Biofilm Formation

Biofilm formation by planktonic cells (free living cells) over a surface is a natural process by which free living microbes attach and aggregate to surfaces and grow into multicellular communities. It is a series of complex process and accomplished mainly in five stages:

- Reversible attachment
- Irreversible attachment or colonization,
- Proliferation
- Maturation
- Dispersion

In this process the life cycle of microbes changed from unicellular to multicellular or planktonic to sessile and this transition between two stages leads to the formation of biofilms. Usually biofilms comprise 10% of dry mass that represent microorganisms while the rest 90% derived from the matrix of biofilm. Indulgent microorganisms in biofilms categorized it as monospecies (formed by a single microorganism) or multispecies (two or more than two types of microorganism) (Satpathy et al., 2016).

The stages of bacterial biofilm formation are given below

Reversible Attachment

The fundamental process in the growth of biofilm begins with reversible adherence of microbial cells to surfaces. It is a complex process and mediated by a series of physical and chemical interactions. Certain surface attributes like surface conditioning, net charge on substrate, hydrophobic surface, surface irregularities, and growth conditions play crucial roles during attachment of bacterial cells. Certain conditions favor the reversible attachment of cells that is attained through delicate interactions

such as Vander Waals and electrostatic forces. The presence of wall and membrane teichoic acid in Gram-positive bacteria and outer membrane phospholipids in Gram-negative bacteria results in a net negative surface charge on the majority of bacterial cells at neutral pH. As a result of charge repulsion, negatively charged substrates inhibit bacterial adhesion, whereas positively charged surfaces promote bacterial attachment and the subsequent formation of biofilm (Verderosa et al., 2019). Finally balancing between attractive forces and repulsive forces determine the attachment of bacterial cell surface over substrate. Secondly, during reversible attachment bacteria usually remain in a random brownian motion leading to detachment of the cell from the surface. The ensuing forces of attraction and repulsion encourage reversible bacterial adhesion to the surface.

Bacteria's ability to sense the abiotic and biotic substratum is facilitated by bacterial appendages that allow them to adhere and form a biofilm. Surface Interaction of flagellar motors triggers a signal cascade that selectively regulates the flagellum biosynthesis pathway while expression of genes that regulate biofilm formation is upregulated. Apart from surface characteristics several other physiochemical factors can influence bacterial biofilm formation such as environmental temperature, osmolarity, pH, nutrient abundance and bacterial cell density. These variables may alter the surface characteristics of both bacteria and the substratum, which would affect bacteria's capacity to adhere to solid surfaces. (Zhang et al., 2015).

Irreversible Attachment or Colonization

In the immediate aftermath of the reversible phase of adhesion, bacteria begin secreting an exopolysaccharide substance, which initiates the irreversible phase of the synthesis of the biofilm matrix. (Abdallah et al., 2014). The EPS matrix's core constituents include a variety of macromolecules such as protein complexes, nucleic acids, lipids, and polysaccharides. During the irreversible attachment forces are stronger to bind bacterial cells through the surface. Bacterial outer membrane proteins, lipopolysaccharides, flagella, and surface adhesions such as fimbraial (including curli and pili) and a fimbrial adhesins mediate irreversible attachment (Srinivasan et al., 2021). The different physical forces and chemical bonding such as hydrogen or covalent bonding as well as electrostatic, ionic, and hydrophobic interactions are also involved in this process. The EPS secretion by bacterial cells is further regulated by quorum sensing mechanism. Bacterial cells aggregate on solid surfaces via intercellular cohesion, whereas their attachment to biotic and abiotic surfaces is mediated by hydrophobic and ionic interactions. (Costa et al., 2018). The bacterial secondary messenger cyclic diguanosine-monophosphate (c-di-GMP) signalling pathway regulates the cellular process responsible for the transition from reversible to irreversible biofilm formation (Toyofuku et al., 2016).

Biofilm Proliferation

After the irreversible attachment of bacterial cells over the surface process of proliferation commences. In this phase cells multiply either by binary fission or asymmetric division (Laventie et al., 2019). Proliferation of cells triggers the intercellular communication, activation of secondary messengers and production of EPS. Formation of microcolonies initiated by attachment of bacterial cells to the surface as well one another by secreting microbial EPS that entraps the cells. The enormous productions of EPS lead to formation of multi-layered structure that gradually transformed to 3D structure of bacterial biofilm.

Biofilm Maturation

Process of maturation started after the formation of micro colonies or immature biofilms. Further, cells are aggregating over the micro colonies to form the macro colonies. Intensive cell proliferation and EPS production continues until biofilm acquires an optimal cell density. During the maturation phase, intra-colony channels within the biofilm matrix facilitate the influx of nutrients, oxygen, and various other elements indispensable to bacterial growth, as well as the efflux of waste products and dead cells. Intercellular communication is strong and mainly carried out through quorum sensing. EPS is a multi-layered, three-dimensional bacterial cell structure that accounts for more than 90% of the dry mass in mature biofilms.

Dispersal/Detachment

Detachment or dispersal of biofilms is the end phase of the formation process. After maturation, bacterial cells start to leave the old house and spread over new stratum to form other biofilms. Thus the cycle of biofilm formation is going on in nature to maintain itself. Detachment of microbial cells is a natural and complex process that is influenced by a number of intrinsic and extrinsic factors. It was found that various intrinsic and extrinsic factors like EPS degrading enzymes, nutritional deficiency, mechanical shear forces and environmental factors like temperature, pH, dissolve oxygen can influence biofilm dispersal (Gupta et al., 2016). On the basis of the causal factor of dispersion, it may be of two types either active or passive.

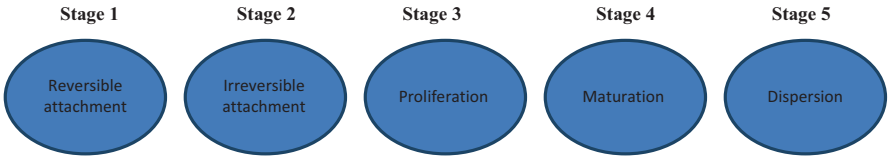
Active Dispersal

In active dispersal immediate dispersal of microbial cells occurs to combat the intrinsic factors like low amount of EPS, nutritional and oxygen deficiency in the internal environment of biofilms. Large number of microbial cells start to slough off

from the center of biofilms to create a hollow cavity inside the three dimensional structure. Active dispersal, a gene regulated mechanism, governed the cell motility by up regulating genes to increase the synthesis of locomotor organs like flagella. The increased movement of bacterial cells inside the biofilm enhances the dispersion. In contrast, genes down regulate the production of EPS and synthesis of attachment appendages like fimbriae to create the instability in the internal environment of biofilm that also favors the active dispersal. The signalling pathway c-di-GMP additionally takes part in the dispersal process; the low concentration of c-di-GMP promotes the detachment of cells. In microbial cells, low levels of oxygen and high levels of glucose diminishes the intracellular level of c-di-GMP and in turn enhances the dispersal process (Kostakioti et al., 2013).

Passive Dispersal

In passive dispersal, release of small portions of bacterial cells under the influence of mechanical shearing force from the biofilm take place.



Stages of biofilm formation

Components of Biofilms

The main constituents of microbial biofilms are microbes themselves, extracellular polymeric substances secreted by microbes, water containing structures inside the matrix pores and channels and extracellular DNA.

Microbial Cells

Microbial cells are the main players that form the biofilms. Among the microbes, bacterial cells have the special capability to adhere on the surface and produce the biofilm. Different bacterial genus likes*Pseudomonas, S. aureus, Listeria* and *E. coli* varied in potential to adhere on the surface and to produce biofilms. External appendages over the surface of bacteria like pilli, fimbriae and flagella are important organelles that facilitate biofilm formation.

Extra Cellular Polymeric Substances (EPS)

Extracellular polymeric substances (EPS) also known as extracellular matrix (ECM) produced by microbial cells and also embedded themselves to acquire the protection from adverse conditions. Equity of EPS in biofilm varies and ranges from 90–99% of dry mass of biofilms. EPS production capability also varies from microbe to microbe. EPS differs in its composition, formation and structure and variations are usually due to type of bacterial species and its surrounding environment. Biomolecules like polysaccharides, proteins, lipids and extracellular DNAs (eDNA) are the main constituents of EPS and among these major ones are polysaccharides. Protein part of EPS comprises enzymes and external appendages like fimbriae and pili. In Gram-positive bacteria polysaccharides are mainly cationic while neutral or polyanionic in Gram-negative bacteria (Flemming et al., 2016).

The main function of EPS is to ensure protection to microbial cells and alongside rigidity to 3D biofilm structure. Thus, physical functions are adhesion, cohesion, stability and scaffolding. EPS act as defensive layer for microbial cells against natural and synthetic antibiofilm agents. Important one listed as frequently used disinfectants, sanitizers and antimicrobials in food processing plants. Inspite of protection other requirements like availability of nutrients, quorum sensing and conducive environment facilitated for microbial cells (Costa et al., 2018).

Water Filled Structures

Channels and pores are water-filled structures in a matrix of biofilm. Channels are long and relatively narrow structures connecting two places to facilitate transport and in line also known as "rudimentary circulation systems in biofilms" while pores can serve as storage and buffering pools and are distinguished from channels (Quan et al., 2022). Channel and pore development and function are governed by fundamentally separate systems, and both may be differentiated according to their formation process, functionality, and dimensions. Main function of channels is to allow transport of nutrients, signalling molecules, biomolecules, antimicrobials and waste products.

Extracellular DNA (e DNA)

The eDNA in biofilms is an important component and it provides the structural stability to 3D structure of biofilms. Simultaneously, it promotes the EPS production and gene transfer through transformation. Number of bacteria like *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*,*Staphylococcus* species (*S. aureus, S. epidermis*), *Enterococcus faecalis*, *Helicobacter pylori, and Campylobacter jejuni* release eDNA in their biofilm (Yin et al., 2019).

Factors Affecting Biofilm Formation

Various intrinsic and extrinsic factors affect the biofilm formation on abiotic and biotic surfaces. Factors like temperature, nutrient availability, oxygen tension, alkalinity and the physicochemical properties of the substratum of surface, especially texture and hydrophobicity influenced the process of aggregation of cells to form biofilms.

Sectors Affected by Microbial Biofilms

Microbial Biofilms in Food Industry

Foodborne pathogen forms the biofilm on surfaces contacting with foods. Data revealed that more than 60% foodborne outbreaks are related to biofilm forming microbes. Biofilms seems to be a great challenge in food industry especially dairy sector. Environmental contaminants, food handlers and food processing plants are the main source of food borne pathogens over the contact surfaces. Remnants of food attract the microbes and provide the nutrients for multiplication and promote the biofilm formation. Matured biofilms act as continuous source of pathogen that may lead to food spoilage and risk to consumers health. In food industry list of common biofilm-forming food borne pathogen and spoiling organism include *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* O157:H7, *Pseudomonas* spp., *Vibrio parahaemolyticus, Clostridium perfringens*, *Campylobacter jejuni*, *S. aureus*, *Shewanella putrefaciens, Cronobacter* spp., *Geobacillus stearothermophilus*. These microbes either produced monospecies or multispecies biofilms, however multispecies are more common and more difficult to eradicate (Berlanga & Guerrero, 2016). Inspite of food spoilage and food poisoning to consumers biofilms cause damage to equipment surfaces of food processing plants by corrosion. it also reduces the production efficiency by increasing the fluid frictional resistance to surfaces may decrease heat transfer across the equipment. Thus, biofilms in food industry are a big challenge to consumers, food products and processing plants also.

Microbial Biofilms in Medical Field

In the medical field biofilms observed inside the living tissue of human body (teeth, ear and lungs etc), dead tissues and on medical devices (catheter, transplantation devices, contact lenses, prosthetic heart valves, stents, pacemakers, shunts and artificial joints or limbs). The commonly isolated bacteria from medical devices are *Staphylococcus aureus, Staphylococcus epidermidis,Pseudomonas aeruginosa, Haemophilus influenza, Pseudomonas aerobicus* and *Fusobacterium nucleatumand* among these *Staphylococcus spp* is more common.The Biofilm loaded devices can

affect virtually any organ or system of the human body and may cause infective endocarditis, cystic fibrosis, urinary tract infections, periodontitis, osteomyelitis and chronicwounds (Karygianni et al., 2020). Reports revealed approximately 65% of microbial infections of above clinical condition are related to biofilms. Resistance to antimicrobial agents and host defense systems is also enhanced by the attribute of biofilms. Thus biofilms create a considerable impact on human health and health care facilities.

Microbial Biofilms in Other Environment

Apart from food industry and medical field many other industries like paper manufacturing units, water treatment plants, drinking water channels, petroleum, nuclear power plant and marine industries are also affected by biofilms (Carniello et al., 2018). In fact, these industries are influenced directly and indirectly by biofilms that cause deterioration in machinery, equipment and quality of materials. Like presence of biofilms in water distributing pipes and channels lead to the contamination of water.

Tools to Combat Microbial Biofilm

Only because modern science has learned so much about the physiology of biofilms, it has now conceivable to develop efficient bacterial inhibition/dispersal strategies. Possible control strategies for bacterial biofilm may include preventing planktonic cell adhesion to surface and producing biofilm at first place or elimination of already formed biofilms (Van Holm et al., 2023). To limit microbial colonization on surfaces, the early attempt by bacterial planktonic cells to cling to surfaces must be inhibited before they organise into full fledge biofilm structure. This can be achieved either through surface treatment or by killing bacterial planktonic cells. Further, biofilm maturation can be avoided by controlling transcription of gene associated with the development of biofilm. Modern methods for removing biofilms often include antagonising QS signals, biofilm lattice inhibition, or killing the biofilm associated bacteria. Biofilms that have already developed can be eliminated by unsettling them and triggering their detachments.

Mechanical Disruption

Water-based sprays have been employed to mechanically disrupt biofilms, resulting in shear stresses. Ultrasound-induced biofilm dispersion is useful in the destruction of the bacterial biofilm when applied to solid metal surfaces like stainless steel.

Treatment with ultrasound changes the biofilm's shape and makes it more susceptible to antibiotics. Another method for reducing biofilm biomass by creating liquid shear pressures is laser-induced shockwaves (Burzell, 2022). Biofilms that have developed on biomedical apparatus can be disturbed by these shockwaves. Antibiotics are more likely to kill biofilms that remain following shockwave exposure. Another approach for passively disrupting biofilms is to apply a modest electrical current to the biofilm, which causes it to detach from the surface. The application of an electric current to electrolyze water molecules into hydrogen and oxygen gas bubbles at the corresponding electrode, enabling the biofilm to be disrupted.

Photodynamic Therapy (PDT)

The effectiveness of Photodynamic Therapy (PDT) against biofilms of Gram-negative and Gram-positive bacteria and fungi has been demonstrated in numerous investigations. Photosensitizing substances are used in PDT to activate singlet oxygen when exposed to light of a specified wavelength that the compound can absorb. A toxic-free dye and low-intensity visible light are used to create photosensitizing agents, which when combined with oxygen, form cytotoxic free oxygen radicals that induce photooxidation of many biological components (Hamblin & Hasan, 2004). There are many photosensitive agents, however only few of them are selected based on stringent criterion viz. should be non-poisonous, photostable and offer large quantum yield. Photosensitizers can be porphyrin derivatives (benzoporphyrins, trihydroxyanthraquinone, texaphyrin, phthalocyanines, naphthalocyanines, and protoporphyrin IX), tetrapyrroles derivatives (chlorins, bacteriochlorins and phthalocyanines, phthalocyanine) and phenothizine derivatives (Thioproperazine, Trifluoperazine Hydrochloride, Alimezine, Thioridazine Hydrochloride, Levomepromazine Hydrochloride, Promethazine Hydrochloride, Periciazine, Chlorpromazine Hydrochloride) (Oleinick et al., 2002).

PDT has become a popular alternative strategy for eliminating biofilms and offers a number of benefits over other methods. The actions of PDT include the rapid destruction of bacterial cells, reduction in biofilm thickness, and disintegration of the EPS structure (Dogsa et al., 2005). They work across a wide spectrum and are equally effective against drug resistant bacteria. PS-generated ROS have a short lifetime, and their efficiency decreases dramatically if the target is located far from the site of ROS formation due to diffusion hindrance. The yield of ROS is greatly influenced by the type of PS and hence creating great hindrance in achieving homogeneity affect. The efficiency of PDT in biofilms is also diminished by PS's inability to accumulate in biofilms or to penetrate to the bottom of EPS layers. The cationic PS display tenfold better effectiveness as compared to the anionic PS because they are trapped in the EPS matrix as a result of ionic or hydrophobic interaction (Ghorbani et al., 2018).

Photothermal Therapy (PTT)

A type of treatment known as photothermal therapy (PTT) employs the strong absorption of particular metallic nanoparticles and nanomaterials to locally heat a region. The hyperthermia generated by PTT compounds is largely employed to damage bacterial integrity or biofilm structure. Near infrared (NIR) wavelengths between 650 and 900 nm are the most effective for PTT, where it may penetrate the biofilm profoundly with little harm to the surrounding areas. PTT breaks down metabolic signals, denatures proteins and enzymes, and impairs membrane permeability to kill infections. PTT provides a number of benefits, including being effective, barely intrusive, and remotely controllable. In addition to having a broad antibacterial range, PTT does not result in bacterial mutations.

Photothermal agents are categorized as metal nanoparticles, carbon-based nanocomposites, and polymers. Metal nanostructures of various types, such as nanorods, nanostars, nanobipyramids, nanowires, and nanoworms (NWs), have been used as PTT agents. Among carbon-based nanocomposites, carbon nanotubes (CNs), and carbon quantum dots (CQDs) has been extensively studies. When combined with other treatments, such as photodynamic, PTT improves efficacy by providing synergistic antibacterial effects.

Microbial Enzymes

Anti-biofilm enzymes are regarded as novel and environmentally safe biofilm management agents, due to their ability to degrade extracellular matrix and promote biofilm dissociation. Bacterial biofilm extracellular matrix is consisting of nucleic acids, proteins, and polysaccharides hence, destruction of lattice of biofilm is possible by employing enzymatic lysis. A wide range of bacterial enzymes, for example proteases, glycosidases, and DNases, aid in the dispersal of active biofilms and increase cellular susceptibility to antimicrobials (Chew et al., 2019.). Formulations that contain enzymes capable of degrading microbial DNA, extracellular polysaccharides, proteinaceous components, and quorum-sensing molecules can more efficiently eliminate complex biofilms.

Exopolysaccharides are an essential element of bacterial biofilms and have a significant impact in growth and maintenance of the biofilm's integrity. Apart from being a nutrition binding matrix, it also helping with initial surface adhesion of bacterial cell, bacterial cell aggregation, water retention, mechanical stability, nutrient absorption, nutrient storage, enzyme binding, and functioning as a barrier against environmental stressors and antimicrobial chemicals derived from microorganisms. Therefore, glucosidase has broad applicability in managing biofilm infections by active polysaccharide breakdown. Glycosylated linkages between two or more carbohydrates are hydrolyzed by Dispersin B and other glycoside hydrolases (GHs).

Proteases produced by microbes control biofilms' dynamic architecture. This dynamic structure is essential for controlling biophysical processes associated with biofilm synthesis and maturation such as matrix remodelling and biofilm dispersal. One of the most efficient ways to disperse biofilms is by hydrolyzing the proteolytic adhesion of bacterial cells to solid surfaces, which also interferes with bacterial quorum sensing by disrupting signalling peptides. These proteases have a substantial effect on how biofilms are regulated in the organisms in which they are expressed and may also have an impact on biofilms from other species. Dispersal of bacterial biofilms is aided by metalloproteases and serine proteases produced by several bacterial species.

The enzyme oxidoreductase is produced by a number of wood-degrading fungal species, and it causes the oxidative degradation of glycans and oligosaccharides to produce the necessary lactones. These lactones hydrolyze on their own to form an unstable, ring-opened carboxylic acid. Reactive oxygen species (hydrogen peroxide) are formed as a result of this oligosaccharide oxidation process, and their accumulation has an antimicrobial effect. Extracellular DNA (eDNA), a vital and frequently sticky component, is also present in bacterial and fungal biofilms. EDNA-binding proteins, encourage the production of biofilms and crosslink the biofilm matrix to increase its stability by holding the bacterial extracellular DNA (Devaraj et al., 2019). Extracellular DNA's phosphodiester backbone is broken up into shorter sequences by the micrococcal nuclease enzymes, which reduces the DNA's sticky properties. Microbial lipase has a broad pH and temperature operating range, as well as high stability and activity. It has the ability to gradually hydrolyze triglycerides into glycerol and fatty acids and is recognised to be essential in the clearance of biofilm. Knowing that proteins, DNA, and polysaccharides are chief constituent of extracellular polymeric biofilm components, anti-biofilm enzyme mixture or anti-biofilm enzyme in combination with other substances may be a superior method of managing and eliminating biofilms (Bi et al., 2021). Used extensively in the biomedical, food, and healthcare industries, these cocktail enzyme formulations have already proven to be commercially viable (Table 18.1).

Phages

Phages are well adapted to break up biofilms since they are bacteria's natural enemies and can do so by entering the biofilm, disrupting the extracellular matrix, and infecting the bacteria. Phage therapy may work better and kill more biofilm bacteria if enzymes are used beforehand to dissolve the biofilm matrix. Additionally, the extracellular matrix is broken down by EPS-degrading enzymes produced by the host bacteria. The EPS breakdown aids phage penetration, growth, and pahge-mediated lysis of the bacterium. Lytic phages express the enzyme polysaccharide depolymerases. Polysaccharide depolymerases breakdown the polysaccharide framework and proteins in the biofilm (Srinivasan et al., 2021). Bacterial dispersion

Table 18.1 List of Microbial enzyme having antibiofilm activity

Class	Enzyme	Source	Target
Glycosidases hydrolases Hexosaminidases	Dispersin B	*Aggregatibacter actinomycetemcomitans*	Gram-positive & Gram-Negative bacteria biofilm
	Glycosidase pectinase	*K. oxytoca* af-G4	*Pseudomonas aeruginosa*
	Glycoside hydrolase Sph3h	Fungal origin	Activity against Pel and Pel-mediated biofilms
	Alginate lyase	Various algae	*Escherichia coli, Enterobacter aerogenes, Vibrio sp., Shigella flexneri*
	Pectinase	*Rhizopus sp., Siphoneugena densiflora (Myrtaceae)*	*S. aureus*
	Amyloglucosidase	*A. niger,*	*S. aureus* from polymicrobial biofilms
	Inulinase	*A. niger, Acinetobacter baumannii*	*Pseudomonas aeruginosa*
	Xylanase	*A. oryzae*	*P. aeruginosa* strains, PAO1
	A-amylase	*Bacillus strains*	*V. cholerae* and MRSA strains
	Cellulase	*A. niger*	*Burkholderia cepacia*Biofilms
Nucleases DNases	Dnase (nucb)	*Bacillus licheniformis*	Gram-negative and Gram-positive bacteria
Bacterial proteases proteases	Serine protease Esp	*Staphylococcus epidermidis*	*S. aureus* biofilm
	Neutrase	*B. amyloliquefaciens*	*S. aureus* and *S. epidermidis*
	Protease B	*B. licheniformis*	*Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae*
	Subtilisin A(alkalase)	*B. licheniformis*	*S. marcescens* biofilms
	Metalloprotease serratopeptidase (SPEP)	*Serratia marcescens*	*P. aeruginosa* and *S. epidermidis*
	Subtilisin A	Bacillus genus	*Escherichia coli* staphylococcal biofilms
	Lasb elastase	*P. aeruginosa*	*Escherichia coli* staphylococcal biofilms
	Proteinase K	Engyodontium album	*Escherichia coli* staphylococcal biofilms

(continued)

Table 18.1 (continued)

Class	Enzyme	Source	Target
Endopeptidase	Bacteriolysin, lysostaphin	Staphylococci	Antibiotic-resistant *S. aureus strains*
Oxidoreductases	Cellobiose dehydrogenase	***Lignocellulolytic fungi***	Clinical S. epidermidis and Pseudomonas strains
	Hexose oxidase	Yeast Hansenula polymorpha	Staphylococcus aureus, methicillin-resistant S. aureus and Pseudomonas strains
	Glucose oxidase	Aspergillus species	Staphylococcus aureus, methicillin-resistant S. aureus and Pseudomonas strains

from biofilm is initiated as a result of localised bacterial lysis caused by a phage, as well as the accompanying enzymes degrading the bacterial cell wall and EPS.

The defence mechanisms of biofilms can prevent phage infection by modulating phage adsorption, entry, dispersion, and multiplication within biofilms. Factors such as biofilm framework organization, thickness of biofilm matrix, biofilm maturation stage, and the type of the constituent bacterial strains may limit phage infection and biofilm activity. In order to prevent phage infection, bacteria use the restriction-modification (R-M) mechanism, which involves specifically identifying and destroying phage nucleic acids Phages can penetrate the inner layers of a biofilm and can also reversibly bind to the bacterial adhesins in order to gain entry.

Monophages often have a limited host range since they are usually specific for a few strains of a bacterial species. Therefore, phage combinations, bioengineered phages, and phage-derived enzymes have all been employed to increase effectiveness and widen the spectrum (Maciejewska et al., 2018). The prerequisite for removing bacterial biofilms is the use of lytc bacteriophages that are incapable of lateral gene transfer of any virulence, toxin, or antibiotic resistance genes, and they should not be able to transduce infected bacterial cells.

By selecting phages with increased specificity, lysis capacity, reduced resistance, or avoiding lysogenic strains, the overall efficacy of phage therapy can be increased significantly. Phage application rate is critical since greater phage dosages result in a considerable decrease in phage output. Similarly, limited phage application may result in inadequate phage progress into biofilms.Genetic engineering has permitted the development of phages that encode peptidoglycan hydrolases that facilitate phage adsorption by unmasking receptors, penetration, and diffusion through the EPS-matrix for biofilm eradication (Clokie et al., 2009). Examples of phage-derived products that are easier to use than phages themselves are lysins and depolymerases. Phage cargoes can also be tailored to include nucleic acids, nanomaterials, pharmaceuticals, and diagnostic probes. The temperate phages might be employed as carriers for CRISPR-associated nuclease to reverse plasmid-mediated antibiotic resistance. Lytic phages along with their derivatives are typically used in tandem with antibiotics in combination treatment (Table 18.2).

Table 18.2 Advantage and Disadvantages of phage therapy

Advantages	Disadvantages
No toxicities or side effects	Narrow host ranges
Bactericidal rather than bacteriostatic in action	Anti-phage adaptive immune responses
Minimal impact on normal flora bacteria	Horizontal transmission of potential virulence factor
Effective against antibiotic resistant bacteria	Differences in pharmacokinetic behavior
Genetic modification is possible	Unknown safety or therapeutic efficacy
Less impact of phages on environments	Absence of regulatory framework for phage therapy
Relatively low concentration dosing	Challenging to identifying suitable phages

Antibiofilm agents (ABA)

Antibiofilm agents (ABA) are inorganic or organic chemicals that can inhibit or check the growth of microbial biofilms and broadly classified in two categories natural and synthetic.

Natural Antibiofilm Agents

Plant-Based Antibiofilm Agents

Antibiofilm agents derived from terrestrial and aquatic plants as well as microorganisms have been identified. Among the compounds on the list are phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes (Bashir & Kumar, 2021). Numerous bioactive compounds with anti-biofilm activity have been identified from Indian medicinal plants, active against a variety of Gram positive and Gram negative organisms. These phytochemicals primarily disrupt the quorum sensing network by blocking quorum sensing inducers (Table 18.3).

Marine Natural Products

Marine flora and fauna are source of several natural materials, which have been tested for antibiofilm activity. Flustramine C analogues derived from the bryozoan Flustra foliacea inhibited Methicillin Resistant Staphylococcus aureus (MRSA), *A. baumannii*, and *E. coli* biofilm synthesis. Brominated guanidinium oxazolidinones, known as synoxazolidinones, were identified in arctic permafrost from *Synoicum pulmonaria* with strong action against Gram-negative acterial biofilm (Tadesse et al., 2010). Bufotenine, discovered in the Mediterranean coralline algae *Paramuricea clavate*, has been demonstrated to inhibit the adherence of the marine

Table 18.3 Natural Antibiofilm agents

Compound	Active ingredient	Source	Antibiofilm activity
Anthraquinone	Emodin	Roots and barks of numerous plants, molds and lichens	*P. aeruginosa and Stenotrophomonas maltophilia*
Flavonoids	Phloretin	Apples	*E. coli O157:H7 biofilm*
	Baicalin	Roots of Scutellaria baicalensis	*Burkholderia cenocepacia*
	Naringenin 2,	Citrus fruits	*V. harveyi and E. coli*
	Proanthocyanidins,	Cranberry plants	*P. aeruginosa*
	Stilbenoid resveratrol	Skin of grapes and berries	*Vibrio cholerae*
	Ajoenes	Extracts of garlic	*P. aeruginosa*
	Gingerols	Extracts of ginger	*P. aeruginosa strain* PA14
	Hyperforin	Hypericum perforatum St. John's Wort	*S. aureus* ATCC 29213,MRSA, *Enterococcus faecalis* ATCC 29212
	7-Epiclusianone	Rheedia brasiliensis	*Streptococcus mutans*
Triterpenoid	Isolimonic acid	Citrus plants	*Vibrio harveyi, E. coli* O157:H7
	Chelerythrine	Chelidonium majus	*S. aureus ATCC 6538P and S. epidermidis ATCC 35984*
	Casbane diterpene	Croton nepetaefolius	*Gram-positive* and *Gram-negative bacteria*
	Proanthocyanidin A2-phosphatidylcholine	Krameria lappacea	*Staphylococcus*
Polyphenolic compound	Tannic acid	Teas and other plant-derived foods	*S. aureus biofilm*
	Ginkgolic acid	Ginkgo biloba	*E. coli O157:H7*
	Quercetin	Fruits, vegetables and grains	*Streptococcus pneumoniae*
Essential oils	Carvacrol	Oregano	*S. aureus and S. epidermidis, S. typhimurium*
	Thymol	Oregano	*S. aureus, E. coli*
Plant alkaloid	Bgugaine	Arisarum vulgare	*P. aeruginosa*

bacterium *Pseudoalteromonas* spp. (Ponti et al., 2014). Ageloxime D, a diterpene alkaloid derived from the marine sponge *Agelas nakamurai*, blocks *S. epidermidis* from forming biolms (Choi et al., 2020). Darwinolide, a derivative of the Antarctic coral *Dendrilla membranosa*, inhibits MRSA biofilm formation. Bromoageliferin, derived from sea sponges, inhibited the production of biofilm by *P. aeruginosa,A. baumannii,S. aureus*, and *Bordetella bronchiseptica*. Meridianins are secondary chemicals produced from the sea mollusk *Aplidium meridianum* that

inhibit the growth of MRSA biofilms. *Delisea pulchra*, a marine macroalga rich in halogenated furanones, has been shown to interfere with quorum sensing by competing with LuxR-type receptors known to inhibit the development of *S. enterica* and *P. aeruginosa*.

Biosurfactants

Biosurfactants are a diverse category of amphiphilic chemicals generated mostly by microorganisms that aggregate at the interface between liquid phases, reducing surface and interfacial tension. They have acclaimed anti-adhesive, antibacterial, and biofilm disrupting abilities. Biosurfactants are preferred choice owing to highly selective action, selectivity, low cytotoxicity, great biocompatibility, high biodegradibility, and effectiveness at extreme pH and temperature (da Silva et al., 2021). Biosurfactants are frequently found in mixtures with isomers hence their purification labor-intensive or expensive. Tetrasodium EDTA and thiazolidione derivatives are the two most often used biosurfactant antibiofilm agents (tEDTA). Glycolipids are among the most researched categories of biosurfactants in other domains, despite the fact that they are underused as biofilm dispersion agents. N-acetylcysteine disrupts existing biofilms in order to exert their effects (Maier, 2003) (Table 18.4).

Antimicrobial Peptides

The natural antimicrobial/host defence peptides or small synthetic peptides are a separate class from antimicrobial peptides. Several new antibiofilm peptides have been identified that target numerous types of bacteria in biofilms, including significant clinically important antibiotic-resistant Gram-negative and Gram-positive bacteria. Many antimicrobial peptides have antibiofilm action in addition to their

Table 18.4 Biosurfactants used for antibiofilm activity

Compound	Active ingredient	Source	Antibiofilm activity
Lipopeptides	Fengycin-like lipopeptides	*B. subtilis and B. licheniformis*	*S. aureus and Escherichia coli*
	Putisolvin	*P. putida*	Pathogenic Pseudomonas sp. strains
	Pseudofactin	*P. fluorescens*	*Enterococcus faecalis*, *E. coli*, *Staphylococcus epidermidis*, *Enterococcus hirae* and *Proteus mirabilis*.
Cyclic peptide heptamer	Surfactin	*B. subtilis*	Salmonella sp.
Glycolipids	Rhamnolipids	*P. aeruginosa*	*Bordetella bronchiseptica*, *Bacillus pumilus*, *Candida tropicalis*
	Sophorolipids	*Candida sp.*	*Bacillus subtitlis*

effectiveness against planktonic bacteria. Antimicrobial peptides' antibiofilm actions include blocking bacterial cell adhesion at the start of the biofilm, decreasing biofilm maturation, removing already-formed biofilms, and/or dispersing the cells inside the biofilm. Additionally, antimicrobial peptides have the ability to disrupt the bacterial cell signalling system and degrade the extracellular polymeric matrix of bacterial biofilms(Huan et al., 2020). In addition to targeting a severe stress response in both Gram-negative and Gram-positive bacteria, antibiofilm peptides can also downregulate genes essential for biofilm formation and the movement of binding proteins(Fong & Yildiz, 2015). Based on the net charge they carry, AMPs can be categorised as either anionic or cationic AMPs. Interestingly, the great majority of bactericidal AMPs are cationic. These cationic AMPs attach to the anionic bacterial cell surface, causing bacterial cell lysis via membrane breakdown, impairment of cell wall synthesis, cell division, and suppression of LPS transport. The development of biofilms in bacteria is regulated by guanosine tetraphosphate (p) ppGpp, which is also involved in controlling growth and a number of other stress responses. When the AMPs reach the bacterial cell, they attach to the (p)ppGpp and cause it to degrade. Numerous peptides have been identified which disrupt the framework of biofilms by inhibiting matrix formation or promoting matrix breakdown. Most clinical strains of bacteria are typically sensitive to one class of AMPs or another, and resistance crossover to AMPs appears to be rare.

To avoid being destroyed by antimicrobial peptides, bacterial species have developed a variety of coping mechanisms. Gram-negative bacteria can release a number of compounds that can serve as a trap for antimicrobial peptides, such as alginate. The majority of the molecules that make up EPS have a negative charge, which may keep AMPs away from the biofilm by repelling them electrostatically through the positively charged peptides. The alternation of net-negative charge on the bacterial cell surface may interfere with electrostatic attraction to cationic AMPs. This can either be done by suppressing and/or changing the production of LPS or by functionalizing the part of lipid A by adding a phosphoethanolamine moiety. Esterification with a lysine residue and the addition of phosphatidylglycerol to teichoic acids can have a similar impact on Gram-positive bacteria (Brown et al., 2013). Increased resistance to antimicrobial peptides may result from modification of the phosphatidylglycerol (PG) group linked with the peptidoglycan sacculus in Gram-positive bacteria, which is mediated by bacterial membrane protein. AMP-EPS interaction may alter their antimicrobial effectiveness, posing a barrier to their development as antibiofilm medicines. A number of bacterial proteases have been discovered that can degrade AMPs. Several extracellular proteins of bacterial origin that can inactivate AMPs by binding to key metabolic enzymes have been identified (Bahar & Ren, 2013). Gram negative bacterial Outer membrane vesicles (OMV) are spherical bilayer structure produced in response to stress. These OMP can sequester free AMPs, before they can interact with bacterial cell. Also in many bacteria functional bacterial efflux mechanisms will efficiently flush out AMPs out of bacterial cells. By inhibiting their synthesis or increasing the production of host proteases that break down HDPs, certain bacteria can alter how HDPs are expressed in host cells.

Despite its numerous benefits, therapeutic use of antimicrobial peptides is fraught with complications. The protocol for synthesis and usage of AMP is still in its infancy, and optimization is required to realise its full potential. Host proteases' ability to break down AMP might prevent it from working properly. The AMP molecules have an innate tendency to form molecular aggregates, rendering them useless. The concentration of AMPs at the site of action is decreased by the spontaneous production of binding proteins by certain bacterial species. Antibiofilm peptides are presently only used to treat skin and soft tissue infections due to the fact that the safety profile of AMP therapy is still being studied (Table 18.5).

Metabolite Molecule

Marine species, particularly Alcyonacea and ahermatypic coral, sessile marine sponges, marine plants and macroalgae, produce secondary metabolite that has inhibitory effects on biofilm. Numerous metabolites from various marine species have been isolated, described, and shown to be excellent candidates for use as antibiofilm agents. In addition, the marine symbiotic bacteria are also known to produce some of the inhibitory compounds. These secondary metabolites have several important functions, one of which is to prevent the growth of biofilms by deactivating quorum sensing signals. Some of these metabolites have enzymatic activity, which aids in the degradation of biofilm-polymer by disrupting the signals. They interfere with the formation and integrity of biofilm and reducing the bacterial growth density.

Synthetic Antibiofilm Agent

Nanoparticles

Most antibacterial medications are rendered completely ineffective by the biofilm EPS matrix. The application of nanoparticles is one strategy to overcome this disadvantage by helping to penetrate biofilm armour. The majority of these nanoparticles are formed of inorganic materials, such as metal oxide nanoparticles; however, due to the flexibility of their design, organic nanoparticles are popular choice as delivery systems for antibiotics with sustained drug release. These nanocarriers successfully capture medicinal molecules, preserving them from biodegradation and increasing their efficiency. As biofilm-targeting agents, nanosystems with intrinsic antibacterial activity can be utilised. Due to their substantial total surface areas and direct interaction with microorganisms, nanoparticles exhibit effective antibacterial activity. Following nanoparticle attachment, the bacterial cytoplasmic membrane is pierced and the nanoparticles kills bacteria by interfering with protein synthesis mechanism either by DNA damage, disrupting process of translation, and/or upsetting transcription after penetrating. Utilizing nanoparticle therapy in conjunction

Table 18.5 List of bioactive antimicrobial peptides

Peptide	Source	Target	Action
Protegrin 1	Leukocytes	Board spectrum of pathogens including multi-drug resistance bacteria	Membrane disruption by forming a pore/channel
Pleurocidin	Skin mucous secretions	Gram-negative bacteria	Bacterial cell membrane damage
Piscidin 3	Fish peptide	Gram-positive and Gram-negative bacterial pathogens	Degradation of extracellular DNA
Indolicidin	Bovine neutrophils	Gram-positive and Gram-negative bacteria as well as fungi	Inhibits DNA synthesis
SMAP-29	Sheep leukocytes	Burkholderia thailandensis	Pore formation on bacterial cell membranes
β defensin 3	Skin, tonsils, oral/saliva,	Gram-positive and Gram-negative bacteria as well as fungi	Reduce the expression of polysaccharide intracellular adhesin (PIA)
Nisin A	Lactococcus and Streptococcus species	Gram-positive bacteria and is particularly effective against bacterial spores	Degrade the membrane of biofilm-embedded cells
Cathelicidin LL-37	Secondary granules of neutrophils	P. aeruginosa biofilm	Affect the bacterial cell signaling system
Hepcidin 20	Liver	Wide range of fungi, bacteria and viruses	Inhibits iron transport by binding to the iron export channel ferroportin
Cecropin A	Haemolymph of giant silkworms, *M. domestica*, arge roundworm *Ascaris suum*	Gram-positive and Gram-negative bacteria as well as fungi	Pore-formation
Melittin A	Honeybee *Apis mellifera*	Methicillin-resistant Staphylococcus aureus (MRSA) biofilm	Pore-formation
Pyrrhocoricin	*Pyrrhocoris apterus* (Sap sucking bug)	Gram-negative bacteria	Prevention of protein folding aided by chaperones
Temporin L	Skin secretions of the European red frog *Rana Temporaria*	Gram-positive and Gram-negative bacteria	Destabilize microbial cytoplasmic membrane
Buforin II	Stomach tissue of the Asiatic toad *Bufo bufo gargarizans*	Gram negative bacteria	Inhibits the cellular functions by binding to DNA and RNA of cells
Protegrin-1	Porcine leukocytes	Gram-positive and gram-negative bacteria	Induces membrane disruption by forming a pore/channel

with external stimuli including pH, light, and magnetic fields can enhance the antibiofilm action.

Following nanoparticle buildup in the biofilm area, it adheres to the external surface of biofilm matrix and migrates milieu interieur. The physicochemical features of the EPS, the milieu surrounding the biofilm, and the zeta potential of the nanoparticles all have a significant impact on the EPS-nanoparticle interaction. Electrostatic attraction allows a negatively charged matrix to easily interact with cationic nanoparticles. Nanoparticles are distributed and diffuse into the biofilm after entering the matrix of extracellular polymeric substances (EPSs). The dispersal of nanoparticles inside the biofilm is influenced by matrix pore size, the existence of aqueous pores, ambient lipophilicity, and the polarity of the EPS and nanoparticles. Ion concentrations differ in the aqueous channels of biofilms. The ion composition and concentration determine nanoparticle penetration into the biofilm.

The specific advantages of nanosystems includes have high drug encapsulation efficiency, release of drug over extended period of time, improved stability, better drug bioavailability, and greater accumulation at biofilm scaffold. Bioengineered nanoparticles can be designed in such a manner that activation by different stimuli causes the photothermal or photodynamic erosion of biofilm matrix. Bacterial colonisation in biofilms has been effectively prevented or treated using nanoparticles as nanocarriers for antibiofilm agents. Due to the presence of antibacterial components such as molecular oxides and macrocyclic surfactants, certain nanoparticles can also have antibiofilm properties on their own. There are several types of nanoparticles used with the intention of biofilms destruction.

Inorganic Metal-Based Nanoparticles

Metals and metal oxides are examples of rigid particles comprised of diverse materials. Some inorganic nanoparticles can interact with EPS due to charged functional groups present on surface or electrostatic interactions, while others can significantly alter the microbial EPS through targeted release of ions. Inorganic nanoparticles exerts its antibacterial mechanisms through mechanical membrane damage caused by electrostatic contact, oxidative stress caused by ROS formation, lipid peroxidation, mechanical destruction of the EPS architecture, and interference with protein function caused by metal ion release.

Organic Polymer Nanoparticles

Several organic compounds like lactic acid, glycolic acid, caprolactone, ethyleneimine, acrylic acid, glutamic acid, and cellulose have been used to make polymeric nanoparticles scaffolds for therapeutic or drug delivery purposes. Multiple medications may be contained by polymeric nanocarriers, which makes synergic treatment possible. The nanostructure should either be physically loaded with these

medications or covalently coupled to them. Polymeric nanocarriers containing antibiotics are widely used to treat biofilm infections. It has been shown that polymeric nanosystems naturally inhibit biofilm growth. Because the outer layers of EPS have negative charges, these nanoparticles function through electrostatic interactions. Polymeric nanoparticles are distinguished by their controlled qualities matched to a specific payload and to the suitable size, as well as their ease of functionalization. Polymers have minimal toxicity and good biocompatibility when it comes to medication delivery.

Lipid-Based Nanoparticles

Lipid-based nanoparticles contain lipid-rich nanosystems either on the surface or in the core matrix of the particle. Based on the nature of the internal matrix, they are classed as solid lipid nanoparticles (SLNs) or nanostructured lipid carriers (NLCs). Solid lipid nanoparticles (SLNs) are nanocarriers with solid lipid cores in the 10–1000 nm range that can hold medicinal active substances that are both hydrophobic and hydrophobic at room and body temperatures. Solid and liquid lipids that are compatible and biodegradable, as well as hydrophilic emulsifiers, make up nanostructured lipid carriers, or NLCs.

Conclusion

The biofilm-forming ability of bacteria is viewed as a serious concern in several domains related to the food and healthcare industries. Bacterial biofilm has the ability to contribute to disease pathogenesis in numerous possible ways, either by increasing bacterial resistance to the body's defensive mechanisms or by making them resistant to antibiotic therapy. Improved understanding of the molecular process of biofilm formation has resulted in the development of novel biofilm remediation technologies and the identification of several potential biofilm removal agents, which have significantly aided in the medical management of biofilm-related complications. The comprehensive characterization of extracellular polymeric compounds will be more precisely conducted by employing optical imaging techniques like confocal microscopy. Nanobots and nanorobotics in conjunction with MALDI-MS are promising future areas to unearth the mysteries of complex biofilm microenvironments. In order to monitor variability in the biofilm milieu, the majority of studies investigating biofilm mechanisms use transcriptomics and proteomics approaches, which involve the use of enormous and complex data sets. Machine learning and artificial intelligence will be more widely used to scale up data analysis with much higher accuracy and speed.

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