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Enzymes as Anti-biofilm Agents for Efficient Dispersion of Microbial Biofilms

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Abstract: Microbial biofilm is an organized community of bacterial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface. Self-produced extra-polymeric matrix facilitates the survival of microorganisms in an adverse environment. These matrices contain polysaccharides, proteins, and extracellular microbial DNA. The biofilm can consist of one or more microbial (bacterial or fungal) species. Pathogenic bacteria in biofilms are resistant to current therapeutic regimes due to their resistant phenotype. The efficient removal of biofilm is a big challenge in the healthcare sector especially in the living system where harsh chemicals and high temperatures cannot be used. Instead of that milder reagents such as enzymes can be of great importance as enzymes are highly selective and capable of disrupting the structural stability of the biofilm matrix. These enzymes can degrade extra polymeric substance which in turn exposes the pathogenic bacterial cells to antibiotics and subsequently host immune response can also act efficiently to clear the infectious agents. Many enzymes namely DNase I, α-amylase, protease, alginate lyase, and dispersin B have been employed to degrade biofilm. The selection of one enzyme or the combination of enzymes depends on the chemical nature of the biofilm matrix. The present article focuses on the mechanism involved in biofilm formation, types of biofilms and their destruction with the application of various enzymes of microbial origin.

Keywords: Biofilms, extra-polymeric matrix, pathogens, enzymes.

Introduction

A structural community of bacterial cells surrounded in a self-produced polymeric matrix that is attached to an inert or living surface is called microbial biofilm. The self-produced extra-polymeric matrix facilitates the survival of bacterial cells in an adverse environment. The matrices contain polysaccharides, proteins, and extracellular microbial DNA. The biofilm can consist of one or more microbial (bacterial or fungal) species [3]. Biofilms comprise multiple microorganisms that are found to be associated with the biotic and abiotic surfaces. Biofilms can be either single or multilayered and can have either homogenous or heterogeneous populations of bacteria that remain in the matrix made up of extracellular polymeric substances secreted by the constituent population of the biofilm [43]. Biofilms can easily develop on the inert surfaces of medical devices, contact lenses and catheters or living tissues, as on epithelium of the lungs (particularly in cystic fibrosis patients), on the endocardium and wounds 3. Biofilm can also be formed in diseases like endocarditis, periodontitis, rhinosinusitis, and osteomyelitis, but it is mostly seen in medical implants and urinary catheters (Table 1). These infections can often only be treated by removal of the implant, thus increasing the trauma to the patient and the cost of treatment. The formation of microbial biofilms is an important reason for the failure of anti-microbial therapy. The biofilm generally cannot be treated

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Table 1. The major human infections caused due to biofilms generation

Objects	Common biofilm bacterial species	Location
Living objects		
Dentalcaries	Acidogenic Gram-positive cocci (e.g. *Streptococcus*)	Tooth
Periodontitis	Gram-negative anaerobic oral bacteria	Gum
Otitis media	Nontypable strains of *Haemophilus influenza*	Middle ear
Musculoskeletal infections	Gram-positive cocci (e.g. *Staphylococci*)	Soft tissue
Necrotizing fasciitis	Group A *Streptococci*	
Biliary tract infection	Enteric bacteria (eg. *Escherichia coli*)	Biliary tract
Osteomyelitis	Various bacterial and fungal species (mixed)	Bones
Bacterial prostatitis	*E. coli* and other Gram-negative bacteria	Prostate gland
Native valve endocarditis	*Viridans* Group *Streptococci*	Inner surface of heart
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*	Lungs
Meliodiosis	*Pseudomonas pseudomallei*	Lungs, heart
ICU pneumonia	Gram-negative rods	Chest
Exit sites	*S. epidermidis* and *S. aureus*	Anal
Peritoneal dialysis (CAPD) peritonitis	A variety of bacteria and fungi	Site where the catheter is inserted to carry the cleansing fluid
Pentile prostheses	*S. aureus* and *S. epidermidis*	Penis
Non- living objects		
Sutures	*Staphylococcus epidermidis* and *S. aureus*	Surgical site
Arteriovenous shunts	*S. epidermidis* and *S. aureus*	Surface of shunts
Schleral buckles	Gram-positive cocci	Deep behind the eyelids under the muscles
Contact lens	*P. aeruginosa* and Gram-positive cocci	Surface of lens
Urinary catheter cystitis	*S. epidermidis, K. pneumoniae, E. faecalis, Proteus mirabilis*	Surface of catheter
IUDs	*S. epidermidis*, *S. aureus*, *Corynebacterium* sp., *Micrococcus* sp., *Enterococcus* sp., *Candida albicans*, Group B *Streptococci*.	Intra uterine devices
Endotracheal tubes	A variety of bacteria and fungi	Inside the tube
Hickman catheters	*S. epidermidis* and *C. albicans*	Surface of catheter

table 1. (continued).

Objects	Common biofilm bacterial species	Location
Non- living objects		
Central venous catheters	*S. epidermidis*, *S. aureus*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans*	Surface of catheter
Mechanical heart valves	*Viridans streptococci*, *Enterococci*	Surface of valves
Vascular grafts	Gram-positive cocci	Surface of grafted material
Biliary stent blockage	A variety of enteric bacteria and fungi	Inside *biliary stents*
Orthopedic devices	*Hemolytic streptococci*, *Enterococci*, *P. mirabilis*, *Bacteroides* sp., *P. aeruginosa*, *E. coli*	Inside the device

by antibiotic therapy because the microorganisms in it remain unaffected. The biofilm infection indications are recurrent even after several antibiotic therapy cycles and the only successful means of eradicating the cause of the infection is the removal of the implanted device or the surgical removal of the biofilm that has formed on live tissue [3].

Biofilms are ubiquitous in nature therefore, it is difficult to eradicate them. It has been seen that many infectious diseases harbor biofilms of bacterial pathogens as the reservoir of persisting infections which can prove fatal at times [43]. Growing microorganisms cause chronic infections that share clinical characteristics, like persistent inflammation and tissue damage. A large number of chronic bacterial infections include bacterial biofilms, making these infections very hard to be eradicated by conventional antibiotic therapy [3]. Different biofilms differ from their free-living counterparts in their growth rate, constitution, structure, and increased resistance to biocides, antibiotics, and antibodies by upregulation and/or down-regulation of approximately 40 % of their genes. This makes them highly difficult to eradicate with therapeutic doses of antimicrobial agents [99].

The fraction of bacteria evolve as persister cells (metabolically inert, replicate slowly, modulate toxin-antitoxin system, upregulate DNA repair and anti-oxidative machinery, have enhanced phosphate metabolism and exhibit unresponsiveness towards minimal inhibitory concentrations of antibiotics) are genetically similar but are physiologically different compared to parent cells [73]. Majority of biofilm cells and planktonic cells normally killed by drug treatment. However, drug-tolerant persisters repopulate the biofilm, disseminate into single microbial cells and start a new cycle of biofilm development [63,73,137] that increases the duration of treatment of diseases caused by biofilm-forming pathogenic microorganisms. It has been observed that bacteria residing within biofilms is antibiotic tolerant and susceptible to antibiotics or other chemicals upon dispersal from a biofilm which suggests that resilience towards antibiotics is due to phenotypic adaptability and not essentially due to genetic adaptability [6]. Factors such as mechanical stress, enzymatic diges-

tion, pH, oxygen availability, temperature, and limiting nutrition trigger dispersal of cells from the biofilm. Biofilms induced due to low oxygen conditions whereas normoxia decreases biofilm formation [121]. Enhanced bacterial respiration reduces the persisters in the bacterial population [67,126].

The host immune system reacts to various bacterial infections by activating several signaling cascades, complement activation, cytokines, and expressing genes associated with stress management [46,47]. However, host immune responses are not much effective against bacterial biofilms in comparison with their single microbial cell counterpart [104]. Many bacterial pathogens that are initially considered as strictly extracellular can continue to exist inside the host body by the evolution of biofilm through the process of adaptation that results in the evasion of the bacteria from the innate immunity of the host. The evasion of biofilms from host innate response proves harmful to the host, as the inflammatory influx released by the body in response to the bacterial infection may harm the host tissues [7,43]. Sub-population of persister cells is tolerant to high levels of antimicrobial agents. Therefore, antibiotics such as â-lactams which are only active against dividing cells are not very efficient at eradicating biofilm infections [50]. The EPS matrix also acts as a diffusion barrier to delay the infiltration of some antimicrobial agents [135]. The reactive chlorine species in a number of these agents deactivated at the surface layers of the biofilm before they are not able to disseminate into the interior of the biofilm [26]. A study showed that oxacillin, cefotaxime and vancomycin had reduced the penetration throughout *S. aureus* and *S. epidermidis* biofilms [111]. However, with the emergence of multidrug-resistant *S. aureus*, the desire for more effective treatments of biofilm-associated infections becomes imperative [58,98].

Mechanism of antibiotic resistance of biofilm-associated bacteria

Three hypotheses have been proposed to explain the possible mechanism of antibiotic resistance of biofilm-associated bacteria:-

1. The first hypothesis suggests that the antibiotic may not be able to penetrate completely into the deep layers of biofilm [116]. Sometimes, if the antibiotic gets degraded while penetrating the biofilm, their action decreases rapidly. Antibiotics may get adsorbed on the extracellular polymeric surfaces of the biofilm which can diminish the penetration of the antibiotic (aminoglycosides) [68,109]. Sometimes, the negatively charged molecules of the biofilm matrix can bind to positively charged antibiotics in nature. This interaction and binding thereby hamper the passage of the antibiotic to the biofilm depth [41,90].

2. The biofilm changes their microenvironment rapidly that resulted in the malfunction of the antibiotics. In deep layers of the biofilm, there is no consumable oxygen left and the niche becomes anaerobic [26]. It has been reported that a class of antibiotics namely aminoglycosides are not effective in anaerobic environmental condition [119]. It has also been found that the increase in the amount of acidic waste accumulation inside a biofilm changes the pH of the environment and subsequently may reduce the action of some antibiotics [116]. The accumulation of toxic waste or limitation of the necessary substrate can lead the bacterial population to remain in a dormant, non-growing form which can protect the bacteria from certain antibiotics like cell wall inhibiting agents and penicillin [123]. The biofilm population decreases the abundance of porins in the bacterial membrane under osmotic stress that consequence in the reduction in the transport of some antibiotics inside the cell [116].

3. It has been proposed that a small population of the bacteria residing in a biofilm may adapt a protective phenotype that results in the development of drug resistance in biofilm population [43]. Antibiotics and chemical treatment my sometimes disturb the gut microflora and cause susceptibility to infection caused by *Clostridium* sp.[18]. The symbiota of gut (probiotics) has an important role in maintaining microbial composition, metabolism, and immunity of gut by immune-modulating systemic immunity and pH [112]. Gut microflora compete with pathogens for binding sites and neutralize toxins released by pathogens. Microbiota as probiotics have potentials for use against biofilms associated with dental plaque, chronic wounds, and urogenital infections [113,127].

The biofilm matrix is composed of DNA, pro-

teins, extracellular polysaccharides and its resistance to antibiotics indicates that the disruption of the biofilm structure could be achieved via the degradation of individual biofilm compounds by various enzymes [3].

The major types of biofilms

Pseudomonas aeruginosa Biofilms

In cystic fibrosis (CF) patients the principal pathogen in the lungs is *P. aeruginosa*. Bacterial chronic colonization leads to progressive lung damage and eventually respiratory failure and death in most CF patients. In *P.aeruginosa*; a complex quorum sensing hierarchy plays a central or very important role in the regulation of virulence and contributes to the late stages of biofilm maturation. Antibiotic therapy in patients colonized with *P. aeruginosa* often gives a measure of relief from symptoms but fails to cure the beset ongoing infection. This is because the antibiotic therapy cannot eliminate the antibiotic-resistant sessile biofilm communities [2].

***Staphylococcal* Biofilms**

Intercellular adhesions of *Staphylococcus epidermidis* within PIA biofilms are a major cause of medical device-related infections [42]. The slime substance PIA is a polysaccharide composed of beta-1, 6-linked N-acetyl glucosamines with partly diacetylated residues, in which the cells are embedded and protected against the host's immune defense and antibiotic treatment. The genetic and molecular basis of biofilm formation in *staphylococci* is multifaceted. Various proteins such as the staphylococcal surface protein (SSP1), the accumulation-associated protein (AAP), the biofilm-associated protein (Bap), and the clumping factor A (Clf A) are involved in biofilm formation of *Staphylococcus epidermidis* [2].

Dental Biofilms

Dental biofilms, commonly called plaque are the most well studied natural biofilm in humans. The development of dental biofilms follows a sequence of events and involves hundreds of species of bacteria. The tooth enamel becomes coated with a variety of proteins and glycoproteins of host origin and this coating is called as acquired pellicle. The primary colonizers, first *streptococci* and later actinomycetes, colonize the surface of the teeth by adhesion molecules and pilli. The bacteria on the pellicle undergo cell to cell interaction via quorum sensing. Several *streptococci*, including *Streptococcus mutans* and related organisms, begin to synthesize insoluble glucan via glucan binding protein. Bridge bacteria (members of the genus *Fusobacterium*) form aggregates with primary colonizers. The late colonizers form aggregate with bridge bacteria. The biofilm primarily consists of nonpathogen at this point. However, in the presence of dietary sucrose and other carbohydrate, acids are produced via fermentation, which leads to demineralization of the tooth enamel, over time, caries. The microbial flora continues to change if the plaque is allowed to remain undisturbed on the teeth for several days. The last colonizers of the biofilm are considered pathogenic because of their role in periodontal disease. The most important pathogens include *Porphyromonas gingivalis, Bacteriodes forsythus, Actinobacillus actinomycetie-mcomitans,* and *Treponema denticola* 102.

***Candida* Biofilms**

Most manifestations of candidiasis are associated with the formation of *Candida* biofilms on surfaces and it is also associated with infections at both mucosal and systemic sites. *Candida* biofilms share several properties with bacterial biofilms. *C. albicans* biofilm formation has 3 distinct developmental phases: early (0-11h), intermediate (12-30h), and mature (38-72h). The detailed structure of mature *C. albicans* biofilms consists of a dense network of yeast, hyphae and pseudohyphae. This mixture of yeast, hyphae, and the matrix material is not seen when the organisms are grown in liquid culture or on an agar surface, which suggests that morphogenesis is triggered when an organism contacts a surface [28,29,100]. The *C. dubliniensis* can adhere to and form biofilms with structural heterogeneity and typical microcolony and water channel architecture similar to bacterial biofilms and *C. albicans* biofilms [93,100]. Indwelling intravascular catheters represent a risk factor that is associated with nosocomial *Candida* infections.

Biofilm formation

Biofilm formation is a dynamic process and different mechanisms are involved in their attachment and growth [103]. The biofilm-forming microorganisms possess specific mechanisms for initial attachment to a surface, the formation of microcolony leading to the development of a three-dimensional structure of mature biofilm. In most biofilms formation, unicellular organisms come together to form a community that is attached to a solid surface and covered in an exopoly-saccharide matrix. The microorganisms account for less than 10 % of the dry mass, whereas the matrix can account for over 90 %. Biofilm growth is guided by a series of physical, chemical, and biological processes [43], and formation can be divided into three main stages: early, intermediate, and mature [3]. Biofilm formation and maturation are sequential, dynamic, and complex processes, which depend on the substratum, the medium, intrinsic properties of the cells, signaling molecules, cellular metabolism, and genetic control. The process of biofilm formation begins with a conditioning layer of organic or inorganic matter on a surface. This conditioning layer alters the surface characteristics of substratum which eventually favors microorganisms to colonize on surface [103].

Process of biofilm formation

The process of formation of biofilms comprises several distinct steps (Fig 1):

Step 1

Initially bacterial cells attach reversibly via weak interactions (such as van der Waal forces) with an abiotic or biotic surface [15,27]. The bacteria cells attach reversibly to a solid living or non-living substratum [92] by van der Waal forces, steric interactions, and electrostatic (double layer) interaction, collectively known as the DLVO (Derjaguin, Verwey, Landau and Overbeek) forces [38]. The surface of the substratum is conditioned by the host matrix proteins (fibrinogen, fibronectin, and collagen), forming a conditioning film that facilitates adhesion by the bacteria [35,98]. In this stage, microbial cells adhere to the surface either by physical forces or by bacterial appendages such as Pilli or flagella. Different factors like surface functionality, temperature, and pressure can modulate the bacterial adhesion greatly. Attachment of a microbial cell to a surface is known as adhesion, whereas the attachment among microbial cells is termed as cohesion.

Step 2

The irreversible attachment to the surface via hydrophilic/hydrophobic interactions through several attachment structures (flagella fimbriae, lipopolysaccharides, or adhesive proteins) [15,27]. A number of the reversibly adsorbed cells remain to immobilize and become irreversibly adsorbed as a result of the hydrophobic and hydrophilic interaction between the bacteria and the surface

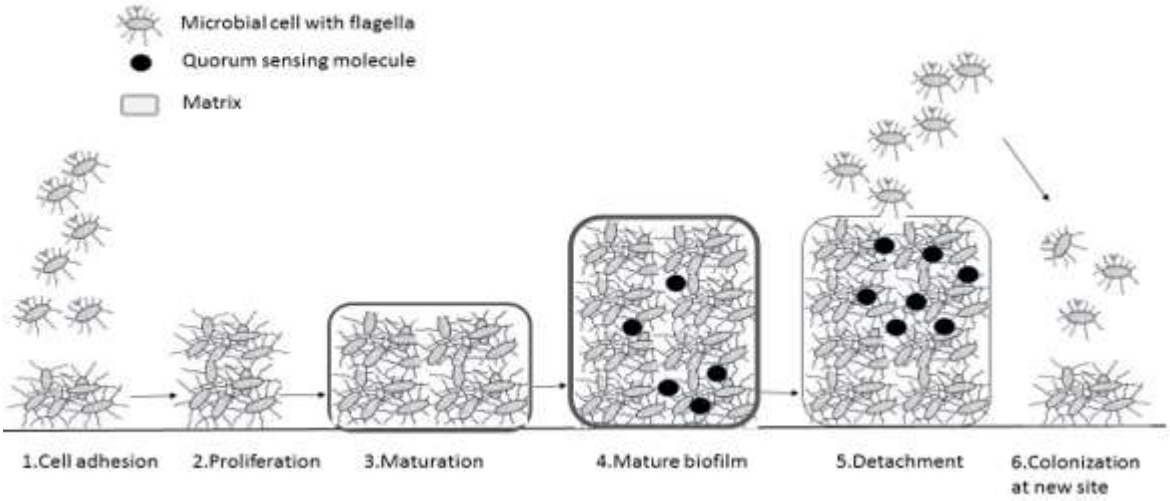

Fig. 1. The process of biofilm formation

[75,98]. The irreversible attachment occurs when the attractive forces are greater than repulsive forces [38]. It has been reported that the physical appendages of bacteria like flagella, fimbriae and pili overcome the physical repulsive forces of the electrical double layer of the cell and the surface and consolidate the interactions between bacteria and the surface [66]. Cell surface hydrophobicity also plays a crucial role in biofilm formation when the bacteria adhere to a hydrophobic nonpolar surface because the hydrophobic interaction between the surface and the bacteria reduces the repulsive force between them [122]. Therefore, in the first and second stages of biofilm development, microbial cells initially loosely associate with the concerned surface, succeeded by specific and strong adhesion [43,45].

Step 3

The proliferation and production of a self-produced extracellular polysaccharide (EPS) matrix mainly composed of polysaccharides, proteins, and extracellular DNA and ultimately the development of the biofilm architecture [17,34]. The microbial cells communicate with each other by the production of autoinducer signals [25,124] that results in the expression of biofilm-specific genes. In this stage, microorganisms secrete a matrix of EPS to stabilize the biofilm network. It was found that *P. aeruginosa* makes and releases three polysaccharides, namely alginate, Pel and Psl which provide stability to the biofilm. Alginate interacts with nutrients and water and supplies nutrients to the biofilm [101]. Pel (glucose-rich polysaccharide) and Psl (pentasaccharide) act as a scaffold for the structure of the biofilm [20,36]. It has been reported that eDNA is also responsible for cellular communication and stabilization of *P. aeruginosa* biofilm [40]. Young *Pseudomonas* biofilms are more susceptible to DNase treatment compared to mature biofilm which suggests the stabilizing role for eDNA during the initial biofilm stages when EPS components are less [132]. The biofilm at this stage becomes multi-layered and their thickness increased up to 10 mm [43].

EPS are responsible for binding of cells and other particulate materials together (cohesion) and to the surface (adhesion) [5,16,118]. The general composition of bacterial EPS comprises polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances [55,118]. According to Tsuneda *et al,* (2003), proteins and polysaccharides account for 75-89 % of the biofilm EPS composition, indicating that they are the major components. Biofilms form a gel phase where microorganisms live inside [118]. The EPS matrices act as a barrier and have a protective effect on biofilm microorganisms against adverse conditions. The EPS matrix delays or prevents antimicrobials from reaching target microorganisms within the biofilm by diffusion limitation and/or chemical interaction with the extracellular proteins and polysaccharides 48,78. Lipids and nucleic acids might significantly influence the rheological properties and thus the stability of biofilms [87]. The extracellular DNA is required for the initial establishment of biofilms by *P. aeruginosa* and possibly for biofilms formed by other bacteria that specifically release DNA [132].

Step 4

The fourth phase in biofilm formation is the maturation phase, bacteria grow, multiply and form microcolonies or mature biofilm [45]. The mature biofilm contains water channels that effectively distribute nutrients and signaling molecules within the biofilm [30,45]. Once microcolonies are formed in optimal growth conditions, the biofilm undergoes the maturation stage where a more complex architecture of biofilm is established with water channels equipped to aid the flow of nutrients into the deep interior of the biofilm. The cells from different regions of a biofilm can show different gene expression patterns due to the different physicochemical conditions in terms of oxygen availability, diffusible substrates and metabolic side products, pH, and cell density [98]. The size of the microcolony at this stage increases and its thickness reaches to about 100 mm. Microcolonies in biofilm quite often consist of diverse microbial communities. Therefore, multispecies micro-consortia function in a relatively complex manner [43]. Their proximity enhances substrate exchange, distribution of metabolic products, and removal of toxic or waste end products [24].

Step 5

The dispersion of microbial cell marks the shedding of the biofilm and return of sessile cells to the motile form [44]. The detachment of biofilm cells takes place individually or in clumps due to intrinsic or extrinsic factors. The biofilm spreads and colonizes to the new surfaces to form biofilm. The microbial community inside the biofilm produces different saccharolytic enzymes that break the biofilm stabilizing polysaccharides and thereby releases surface bacteria residing on the top of the biofilm structure for colonization to a new surface [43]. The *P. fluorescens* and *P. aeruginosa* release various enzymes such as alginate lyase, *E. coli* releases *N*-acetyl-heparosan lyase and *Streptococcus equisimilis* produce hyaluronidase for the breakdown of the biofilm matrix [117]. Moreover, at this stage microorganisms upregulate the expression of the flagella proteins which make the organisms motile, and bacteria can move to a new site. Disruptive forces are also important in biofilm cycle as detachment of cells from the biofilm helps in spreading the infection from the biofilms to other sites [94].

Step 6

Finally the cells get dispersed from biofilms and subsequently colonize at other niches [103,115]. The dispersed bacterial cells from the biofilm, either by physical detachment or signaling events followed by the hydrolysis of EPS, return to the mobile state to enable the occupancy of new niches. The subsequent biofilm formation occurs similarly but at a new site [14,98].

Biofilm degradation by enzymes

Various antibiotics and other chemicals have been involved in the removal of biofilms. In *P. aeruginosa* clarithromycin blocks biofilm matrix formation [136]. The overall thickness of the biofilm reduces by ciprofloxacin and exposes the immature biofilm to phagocytosis by polymorphonuclear neutrophils and the matrix polymer of biofilm in *S. aureus* was dissolved by Streptokinase [85]. The acyl-homoserine lactone interferes with cellular signaling mechanisms that have been used for quorum sensing adversely affects normal biofilm formation [95]. However, due to the antibiotic resistance of biofilm-associated bacteria, alternate and efficient tools are needed to overcome these limitations and the use of different enzyme.

The composition of the EPS matrix has been studied in bacteria such as *P. aeruginosa*, *Bacillus sp, staphylococcus sp, streptococcus spp.* The constituent of the extracellular matrix depends on the environment and the bacteria present within the biofilms. The main component of biofilms is DNA, polysaccharides, proteins, and EPSes. The degradation of matrix components can weaken or disperse biofilms and studies show that the complete and effective disruption of the architecture of the biofilm could be done by various enzymes [32]. The common enzymes used for disruption of the biofilms are deoxyribonucleases, proteases, glycoside hydrolase, lysostaphin, lyase, and lactonase.

Deoxyribonucleases

Deoxyribonuclease was found to be effective against the biofilms formed by both Gram +ve (*S. aureus* and *S. pyogenes*) and Gram –ve (*Acinetobacter baumanii, H. influenza, K. pneumonia, E. coli* and *P. aeruginosa*) bacteria (Table 2). Researchers showed that the DNase is highly effective at the concentration of 5 mg/ml and able to significantly degrade 24h active biofilms biomass by approximately 40 % [120]. They also notice the synergistic effects of DNase1with antibiotics (azithromycin, rifamycin, levofloxacin, ampicillin). Table 2 summarizes many of the DNase that has been shown to have biofilm-disrupting activity.

Proteases

Proteinase cleaves the matrix or surface proteins and inhibits dispersal of established biofilms or biofilm formation [98]. Extracellular proteins are a major EPS component that can represent a substantial portion of the biofilm's dry mass [56,70,83,114]. The *S. aureus* alone, secrete ten proteases, four of those [V8 serine protease (SspA), two staphopains (SspB and ScpA), and aureolysin (Aur)] be involved in biofilm disruption [1,77,79,82,107]. Exoproteins are essential for the ability of microbes to sustain and modify the EPS [61,138] and certain proteins, such as DNA-binding proteins (DNABPs), functional amyloids/amyloid-like

Table 2. DNase that disperse biofilms

No.	Enzyme	Source	Effective against	References
1	DNase I	Pancreatic DNase	*P. aeruginosa*, *V. cholerae*, *E. coli*, *S. pyogenes*, *S. aureus*, *S. heamolyticus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Aggregatibacter actinomycetemcomitans*, *Shewanella oneidensis*, *Bordetella pertussis*, *Bordetella bronchiseptica*, *Campylobacter jejuni*, *H. influenza*, *B. bacteriovorus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Candida albicans* and *Aspergillus fumigatus*	37,81,105, 129,132
2	DNase 1L2	Keratinocytes	*P. aeruginosa* and *S. aureus*.	31
3	Dornase alpha	Recombinant human DNase I	*S. aureus* and *S. pneumonia*	44,60
4	λ Exonuclease	viral DNase	*V. cholera*	105
5	NucB	*B. licheniformis*	*B. licheniformis*, *S. aureus*, *S. epidermidis*, *S. salivarius*, *S. constellatus*, *S. lugdunesis*, *S. anginosus*, *S. intermedius*, *E. coli*, *Micrococcus luteus* and *B. subtilis*	91,106,108
6	Streptodornase	Streptococcal DNase	*P. aeruginosa*	86

Table 3. Proteases that disperse biofilms

No.	Enzyme	Source	Effective against	References
1	Aureolysin (Aur)	Staphylococcus	*S. aureus*	77,79
2	LapG Protease	*Pseudomonas putida*		39
3	Proteinase K	*Engyodontium album*	*S. aureus*, *Listeria monocytogenes*, *S. lugdunensis*, *S. heamolyticus*, *Gardnerella vaginalis*, *E. coli*, *Heamophilus influenza* and *Bdellovibrio bacteriovorus*	19,23,37,54, 81,88,96,110
4	Spl Proteases	Staphylococcus serine proteases	*S. aureus*	13,71
5	Staphopain A (ScpA), Staphopain B (SspB)	Staphylococcal cysteine proteases	*S. aureus*	77,82
6	Streptococcal Cysteine Protease (SpeB)	*Streptococcus pyogenes* cysteine protease	*S. aureus*	21,84
7	Surface-protein-releasing enzyme (SPRE)	Streptococcal protease	*S. mutans*	72
8	Trypsin	Pancreatic serine protease	*P. aeruginosa*, *S. epidermidis, S. mitis*, *Actinomyces radicidentis* and *Gardnerella vaginalis*	10,19,89,96
9	V8 Serine Protease (SspA)	Staphylococcal serine protease	*S. aureus*	79,80

proteins (FA/ALPs) and other biofilm-associated proteins (Baps), are vital contributors to surface and EPS adhesion and the overall physical stability of the biofilm matrix [70]. Thus, enzymatic degradation of EPS exoproteins has the potential to cause a massive dispersal event. Table 3 summarizes many of the proteases that have been shown to have anti-biofilm activity.

Glycoside hydrolase

Most biofilms are highly dependent on the presence of secreted extracellular polysaccharides, or exopolysaccharides, as major EPS constituents [9,33,133]. They perform many important functions for the establishment and persistence of biofilms including, structural stability, physical and chemical defense against antimicrobials and the host immune system, adhesion and aggregation of microbial cells, desiccation tolerance, sorption of organic and inorganic compounds and can provide a carbon source in times of nutrient starvation [33,74,130]. A significant amount of research into targeting exopolysaccharides with glycoside hydrolases as a means for dispersing biofilms has been performed due to their importance for the establishment and maintenance of biofilm architecture. The α-amylase is one of the examples of glycoside hydrolases and its biological function was investigated for inhibition and removal of *S. aureus* biofilms [22]. The results indicate that amylase could be used shortly to control of *S. aureus* biofilm infection [103]. Cellulase from *Penicillium funiculusum* was effective in degrading mature biofilms of *P. aeruginosa*; and it was also found to be useful in degrading the exopolysaccharides of *P. fluorescens* [76,125]. Dispersin B, a biofilm-releasing enzyme produced by the Gram-negative periodontal pathogen *Actinobacillus actinomycetecomitans* could eliminate the biofilm in half of the catheter tested in a sheep model for port-related bloodstream infection [62]. The lists many of the glycoside hydrolases that have exhibited biofilm-disrupting ability has been summarized in Table 4.

Lysostaphin

Lysostaphin is a naturally occurring enzyme that can effectively invade into biofilms [12]. The activity of lysostaphin toward biofilms was investigated [128] on clinical and reference strains of *S. aureus* and *S. epidermidis*. Their findings suggest that lysostaphin is capable of eradicating biofilms of all *S. aureus* and *S. epidermidis* strains effectively [103]. Lysostaphin is a natural staphylococcal endopeptidase that can penetrate bacterial biofilms [12]. Lysostaphin is a glycyl-glycine endopeptidase which specifically cleaves the pentaglycine cross-bridge in the staphylococcal peptidoglycan and disrupts the extracellular matrix of *S. aureus* biofilms. The lysostaphin markedly reduced biomass thickness when applied to biofilms of *S. aureus* clinical isolates grown *in vitro* [65,134.] It has been demonstrated that lysostaphin is effective in the treatment of established biofilm infections on implanted jugular veins catheters in mice, particularly in combination with nafcillin [98]. The antimicrobial properties of lysostaphin were analyzed by Walencka *et al,*[128,] and biofilm inhibitory concentration (BIC) of the enzyme for 13 *S. aureus* and 12 *S. epidermidis* clinical strains were also determined 3.

Lyase

The co-administration of a lyase with an antibiotic was tested to inhibit and eradicate biofilms [4]. The researchers assessed a combined effect of alginate lyase (20 μg/mL) and gentamycin (64 μg/mL) on a biofilm of 2 mucoid *P. aeruginosa* strains. Their results revealed that the combined treatment caused liquefaction of the biofilm matrix and complete eradication of the biofilm structure and living bacteria within 96h [103].

Lactonase

Lactonase as a potential antibiofilm enzyme was also examined and it was found that treatment with 1 unit of lactonase reduced biofilm formation by 4 *P. aeruginosa* strains [64]. Also, lactonase treatment disrupted biofilm structure and increased sensitivity to antibiotics ciprofloxacin and genta-mycin [64,103]. Aleksandra *et al*,[3] also established the role of lactonase as a potential antibiofilm agent.

Conclusion

Biofilm formation enables microorganisms to

Table 4. Glycoside hydrolases that disperse biofilms

No.	Enzyme	Source	Effective against	References
1	Alginate lyase	*Laminaria hyperborean*	*P. aeruginosa*	4,11,49,69
2	α-Amylase	Mammalian pancreas	*V. cholerae*, *S. aureus* and *P. aeruginosa*	22,32,59,130
3	α-Mannosidase	*Arthrobacter* sp.	*P. aeruginosa*	10
4	β-Mannosidase	*Bacillus* sp.	*P. aeruginosa*	10
5	Cellulase	*A. niger* and *Trichoderma* sp.	*S. aureus* and *P. aeruginosa*	32
6	Dispersin B	*A. actinomycetemcomitans*	*S. aureus*, *A. actinomycetemcomitans*, *S. epidermidis*, *A. baumannii*, *K. pneumoniae*, *E. coli*, *Burkholderia*s pp., *A. pleuropneumoniae*, *Yersinia pestis* and *Pseudomonas fluorescens*.	52,53,62,129
7	Hyaluronidase	Testis of mature bulls	*S. aureus* and *S. intermedius*	51,97
8	PelAh	Glycoside hydrolase	*P. aeruginosa*	8
9	PslGH	Glycoside hydrolase	*P. aeruginosa*	8

endure situations such as immune defenses and conventional antimicrobial therapies. The biofilms are the dominant lifestyle of microorganisms in all environments, either natural or manmade, and remain a serious concern in the healthcare, food, and marine industries. This ability has challenged the treatment of infections caused by such microorganisms. The development of effective strategies to combat biofilms is a challenging task. The rise of antibiotic resistance has led to a decrease in the efficacy of treatments for the elimination of biofilm infections. The researchers and clinicians have begun concentrating their efforts on coupling biofilm destruction with antimicrobial therapy as the increased tolerance of biofilm-embedded pathogens to antibiotics and the fact that as many as 80 % of all human bacterial infections are biofilm-associated.

The new approaches such as enzyme treatments gaining more attention that weaken the structure of the biofilm and target each important component of biofilm. These seem to be better strategies for biofilm dispersal as it can more effectively release biofilm-associated microbes from the protection of the EPS. Enzyme that can target the EPS on a molecular scale, or cause the microbes themselves to actively degrade their biofilms, may represent the next logical step towards total eradication of biofilm-afforded protection of infectious microorganisms.

Future prospective

The major role of biofilm is in providing antimicrobial resistance, in chronic diseases and biofilm itself as a reservoir for pathogenic organisms. Microbial biofilm research is proceeding on many fronts with particular emphasis on elucidation of the genes specifically expressed by biofilm-associated organisms. More research is needed that should focus on the development of new methods of degradation of biofilms. Researches in the fields of food and water, clinical, environmental, and industrial microbiology have begun to investigate microbiological processes from a biofilm perspective.

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References

1. **Abraham, N.M., Jefferson, K.K. (2012).** *Staphylococcus aureus* clumping factor B mediates biofilm formation in theabsence of calcium. Microbiology. 158: 1504-1512.
2. **Aprana, M.S., Yadav, S. (2008).** Biofilms: Microbes and Disease. Brazilian Journal of Infectious Disease. 12: 526-530.
3. **Aleksandra, T., Grzegorz, F., Mariusz, G., Joanna, N. (2012).** Innovative strategies to overcome biofilm resistance. BioMed Research International. 2013: 1-13.
4. **Alkawash, M.A., Soothill, J. S., Schiller, N.L. (2006).** Alginate lyase enhances antibiotic killing of mucoid *Pseudomonas aeruginosa* in biofilms. Acta Pathologica, Microbiologica et Immunologica Scandinavica. 114: 131-138.
5. **Allison, D.G. (2003).** The biofilm matrix. Biofouling. 19: 139-150.
6. **Anwar, H., Biesen, T., Dasgupta M., Lam K., Costerton, J.W. (1989).** Interaction of biofilm bacteria with antibiotics in a novel *in vitro* chemostat system. Antimicrobial Agents and Chemotherapy. 33: 1824-1826.
7. **Archer, N.K., Mazaitis, M.J., Costerton, J.W., Leid, J.G., Powers, M.E., Shirtliff, M.E. (2011).** *Staphylococcus aureus* biofilms properties, regulation and roles in human disease. Virulence. 2: 445-459.
8. **Baker P., Hill, P.J., Snarr, B.D., Alnabelseya N., Pestrak, M.J., Lee, M.J., Jenning, L. K., Tam, J., Melnyk, R.A., Parsek, M.R. (2016).** Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms. Science Advances. 2: e1501632.

9. **Bales, P.M., Renke, E.M., May, S.L., Shen Y., Nelson, D.C. (2013).** Purification and characterization of biofilm-associated eps exopolysaccharides from escape organisms and other pathogens. PLoS ONE 8: doi: 10.1371/journal.pone.0067950.
10. **Banar, M., Emaneini, M., Satarzadeh, M., Abdellahi, N., Beigverdi, R., Leeuwen, W.B., Jabalameli, F. (2016).** Evaluation of mannosidase and trypsin enzymes effects on biofilm production of *Pseudomonas aeruginosa* isolated from burn wound infections. PLoS ONE 11: e0164622, doi: 10.1371/journal.pone.0164622.
11. **Bayer, A.S., Speert, D.P., Park, S., Tu, J., Witt, M., Nast, C.C., Norman, D.C. (1991).** Functional role of mucoid exopolysaccharide (alginate) in antibiotic-induced and polymorphonuclear leukocyte-mediated killing of *Pseudomonas aeruginosa*. Infection and Immunology. 59: 302-308.
12. **Belyansky, I., Tsirline, V.B., Montero, P.N., Satishkumar, R., Martin, T.R., Lincourt, A. E., Shipp, J.I., Vertegel, A., Heniford, B.T. (2011).** Lysostaphin coated mesh prevents *Staphylococcal* infection and significantly improves survival in a contaminated surgical field. American Surgeron. 77: 1025-1031.
13. **Boles, B.R., Horswill, A.R. (2008).** Agr-mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathogen. 4: e1000052.
14. **Boles, B. R., Horswill, A.R. (2011).** *Staphylococcal* biofilms disassembly. Trends in Microbiology. 19: 449-455.
15. **Bos, R., Vander, M. H.C., Busscher, H.J. (1999).** Physico-chemistry of initial microbial adhesive interactions-its mechanisms and methods for study. FEMS Microbiology Reviews. 23: 179-230.
16. **Boyle, J. (1989).** Structure and function of biofilms. Characklis, W. G., Wilderer, P. A. Editors John Wiley and Sons, Chichester. pp. 369-371
17. **Branda, S.S., Vik, S., Friedman, L., Kolter, R. (2005).** Biofilms: the matrix revisited. Trends in Microbiology. 13: 20-26.
18. **Buffie, C.G., Jarchum, I., Equinda M., Lipuma L., Gobourne A., Viale A., Ubeda C., Xavier J., Pamer, E.G. (2012).** Profound alterations of intestinal microbiota following a single dose of *clindamycin* results in sustained susceptibility to *Clostridium difficile* induced colitis. Infection and Immunology. 80: 62-73.
19. **Chaignon, P., Sadovskaya, I., Ragunah, C., Ramasubbu, N., Kaplan, J. B., Jabbouri, S. (2007).** Susceptibility of *staphylococcal* biofilms to enzymatic treatments depends on their chemical composition. Applied Microbiology and Biotechnology. 75: 125-132.
20. **Colvin, K. M., Gordon, V. D., Murakami, K., Borlee, B.R., Wozniak, D.J., Wong, G.C., Parsek, .R. (2011).** The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. PLoS Pathogen. 7: e1001264
21. **Connolly, K.L., Roberts, A.L., Holder, R.C., Reid, S.D. (2011).** Dispersal of group a *streptococcal* biofilm by the cysteine protease SpeB leads to increased disease severity in a murine model. PLoS ONE 6: e18984.
22. **Craigen, B., Dashiff, A., Kadouri, D.E. (2011).** The use of commercially available alpha-amylase compounds to inhibit and remove *Staphylococcus aureus* biofilms. Open Microbiology Journal. 5: 21-31.
23. **Cui, H., Ma, C., Lin, L. (2016).** Co-loaded proteinase K/thyme oil liposomes for inactivation of *Escherichia coli* O157:H7 biofilms on cucumber. Food and Function. 7: 4030-4040.
24. **Davey, M.E., O'toole, G.A. (2000).** Microbial biofilms: from ecology to molecular genetics. Microbiology and Molecular Biology Review. 64: 847-867.
25. **Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., Greenberg, E.P. (1998).** The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. 280: 295-298.
26. **de Beer, D., Stoodley, P., Roe, F., Lewandowski, Z. (1994).** Effects of biofilm structure on

oxygen distribution and mass transport. Biotechnology and Bioengineering. 43: 1131-1138.
27. **Donlan, R.M. (2002).** Biofilms: microbial life on surfaces. Emerging Infectious Diseases. 8: 881-890.
28. **Douglas, L.J. (2002).** Medical importance of biofilms in *Candia* infection. Revista Iberamericana de Micologia. 19: 139-43.
29. **Douglas, L.J. (2003).** Candida biofilms and their role in infection. Trends in Microbiology. 11: 30-36.
30. **Dufour, D., Leung, V., L´evesque, C.M. (2012).** Bacterial biofilm: structure, function, and antimicrobial resistance. Endodontics Topics Banner. 22: 2-16.
31. **Eckhart, L., Fischer, H., Barken, K.B., Tolker-Nielsen, T., Tschachler, E. (2007).** DNase1L2 suppresses biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The British Journal of Dermatology. 156: 1342-1345.
32. **Fleming, D., Chahin, L., Rumbaugh, K. (2017).** Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. Antimicrobial Agents and Chemotherapy. 61: e01998-16.
33. **Flemming, H.C., Wingender, J. (2010).** The biofilm matrix. Nature Reviews Microbiology. 8: 623-633.
34. **Flemming, H.C., Neu, T.R., Wozniak, D.J. (2007).** The EPS matrix: the house of biofilm cells. Journal of Bacteriology. 189: 7945-7947.
35. **Francois, P., Schrenzel, J., Stoerman-Chopard, C., Favre, H., Hermann, M., Foster, T. J., Lew, D.P., Vaudaux, P. (2000).** Identification of plasma proteins adsorbed on hemodialysis tubing that promote *Staphylococcus aureus* adhesion. Journal Laboratory and Clinical Medicine. 135: 32-42.
36. **Franklin, M.J., Nivens, D.E., Weadge, J.T., Howell, P.L. (2011).** Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. Frontier in Microbiology. 22: 167.
37. **Fredheim, E.G., Klingenberg, C., Rohde, H., Frankenberger, S., Gaustad, P., Flaegstad, T., Sollid, J.E. (2009).** Biofilm formation by *Staphylococcus haemolyticus*. Journal of Clinical Microbiology. 47: 1172-1180.
38. **Garrett, T.G., Bhakoo, M., Zhang, Z. (2008).** Bacterial adhesion and biofilms on surfaces. Progress in Natural Science. 18: 1049-1056.
39. **Gjermansen, M., Nilsson, M., Yang, L., Tolker-Nielsen, T. (2010).** Characterization of starvation-induced dispersion in *Pseudomonas putida* biofilms: genetic elements and molecular mechanisms. Molecular Microbiology. 75: 815-826.
40. **Gloag, E.S., Turnbull, L., Huang, A., Vallotton, P., Wang, H., Nolan, L.M., Mililli, L., Hunt, C., Lu, J., Osvath, S.R., Monahan, L.G., Cavaliere, R., Charles, I.G., Wand, M. P., Gee, M.L., Prabhakar, R., Whitchurch, C.B. (2013).** Self-organization of bacterial biofilms is facilitated by extracellular DNA. Proceedings of National Academy of Science. 110: 11541-11546
41. **Gordon, C.A., Hodges, N.A., Marriott, C. (1988).** Antibiotic interaction and diffusion through alginate and exopolysaccharide of cystic fibrosis-derived *Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy. 22: 667-674.
42. **Gotz F. (2002).** *Staphylococcus* and biofilms. Molecular Microbiology. 43: 1367-78.
43. **Gupta, P., Sarkar, S., Das, B., Bhattacharjee, S., Tribedi, P. (2016).** Biofilm, pathogenesis and prevention-a journey to break the wall: a review. Archives of Microbiology. 198: 1-15.
44. **Hall-Stoodley, L., Costerton, J.W., Stoodley, P. (2004).** Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology. 2: 95-108.
45. **Hall-Stoodley, L., Nistico, L., Sambanthamoorthy, K., Dice, B., Nguyen, D., Mershon W.J., Johnson, C., Hu, F.Z., Stoodley, P., Ehrlich, G.D. (2008).** Characterization of biofilm matrix, degradation by DNase treatmentand evidence of capsule downregulation in *Streptococcus pneumo-*

niae clinical isolates. BMC Microbiology. 8: 173.
46. **Hartmann, A., Rothballer, M., Hense, B.A., Schroder, P. (2014).** Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. Frontiers in Plant Science. 5: 131.
47. **Hartmann, A., Schikora, A. (2012).** Quorum sensing of bacteria and trans-kingdom interactions of *N*-acyl homoserine lactones with eukaryotes. Journal of Chemical Ecology. 38: 704-713.
48. **Heinzel M. (1998).** Phenomena of biocide resistance in microorganisms. International Biodeterioration and Biodegradation. 41: 225-234.
49. **Hisano, T., Nishimura, M., Yonemoto, Y., Abe, S., Yamashita, T., Sakaguchi, K., Kimura, A., Murata, K. (1993).** Bacterial alginate lyase highly active on acetylated alginates. Journal Fermenttation and Bioengineering. 75: 332-335.
50. **Hoiby, N., Bjarnsholt T., Givskov, M., Molin, S., Ciofu, O. (2010).** Antibiotic resistance of bacterial biofilms. International Journal of Antimicrobiology Agents. 35: 322-332.
51. **Ibberson, C.B., Parlet, C.P., Kwiecinski, J., Crosby, H.A., Meyerholz, D.K., Horswill, A.R. (2016).** Hyaluronan modulation impacts *Staphylococcus aureus* biofilm infection. Infection and Immunology. 84: 1917-1929.
52. **Itoh, Y., Wang, X., Hinnebusch, B.J., Preston, J.F., Romeo, T. (2005).** Depolymerization of beta-1, 6-N-acetyl- D-glucosamine disrupts the integrity of diverse bacterial biofilms. Journal of Bacteriology. 187: 382-387.
53. **Izano, E.A., Wang, H., Ragunath, C., Ramasubbu, N., Kaplan, J.B. (2007).** Detachment and killing of *Aggregatibacter actinomycetemcomitans* biofilms by dispersin B and SDS. Journal of Dental Research. 86: 618-622.
54. **Izano, E.A., Shah, S.M., Kaplan, J.B. (2009).** Intercellular adhesion and biocide resistance in non-type able *Haemophilus influenzae* biofilms Microbial Pathogensis. 46: 207-213.
55. **Jahn, A., Nielsen, P.H. (1998).** Cell biomass and exopolymer composition in sewer biofilms. Water Science and Technology. 37: 17-24.
56. **Jiao, Y., Cody, G.D., Harding, A.K., Wilmes, P., Schrenk, M., Wheeler, K.E., Banfield, J.F., Thelen, M.P. (2010).** Characterization of extracellular polymeric substances from acidophilic microbial biofilms. Applied and Environmental Microbiology. 76: 2916-2922.
57. **John, G.T., Donale, C.L. (2007).** Biofilms: architects of disease. In: Connie R.M., Donald C.L., George M., editors. Textbook of diagnostic microbiology. 3rd ed. Saunders. p. 884-95.
58. **Kalia, V.C., Purohit, H.J. (2011).** Quenching the quorum sensing system: potential antibacterial drug targets. Critical Reviews in Microbiology. 37: 121-140.
59. **Kalpana, B.J., Aarthy, S., Pandian, S.K. (2012).** Antibiofilm activity of α-amylase from *Bacillus subtilis* S8-18 against biofilm forming human bacterial pathogens. Applied Biochemistry and Biotechnology. 167: 1778-1794.
60. **Kaplan, J.B., LoVetri, K., Cardona, S.T., Madhyastha, S., Sadovskaya, I., Jabbouri, S., Izano, E.A. (2012).** Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in *Staphylococci*. Journal of Antibiotics. 65: 73-77.
61. **Kaplan, J.B. (2010).** Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. Journal of Dental Research. 89: 205-218.
62. **Kaplan, J.B., Ragunath, C., Velliyagounder, K., Fine, D.H., Ramasubbu, N. (2004).** Enzymatic detachment of *staphylococcus epidermidis* biofilms. Antimicrobial Agents and Chemotherapy. 48: 2633-2636.
63. **Keren, I., Minami, S., Rubin, E., Lewis, K. (2011).** Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters. American society for Microbiology. 2: e00100-e00111.
64. **Kiran, S., Sharma, P., Harjai, K., Capalash, N. (2011).** Enzymatic quorum quenching increases antibiotic susceptibility of multidrug resistant *Pseudomonas aeruginosa*. Iraian Journal of

Microbiology. 3: 1-12.
65. **Kokai-Kun, J.F., Chanturiya, T., Mond, J.J. (2009).** Lysostaphin established *Staphylococcus aureus* biofilms in jugular vein catheterized mice. Journal of Antimicrobiology Chemotherapy. 64: 94-100.
66. **Kumar, C.G., Anand, S.K. (1998).** Significance of microbial biofilms in food industry: a review. International Journal of Food Microbiology. 42: 9-27.
67. **Kumar, A., Alam, A., Rani, M., Ehtesham, N.Z., Hasnain, S.E. (2017).** Biofilms: Survival and defense strategy for pathogens. International Journal of Medical Microbiology. 307: 481-489.
68. **Kumon, H., Tomochika K.I., Matunaga, T., Ogawa, M., Ohmori H. (1994).** A sandwich cup method for the penetration assay of antimicrobial agents through *Pseudomonas* exopolysaccharides. Microbiology and Immunology. 38: 615-619.
69. **Lamppa, J.W., Griswold, K.E. (2013).** Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy. Antimicrobiology Agents and Chemotherapy. 57: 137-145.
70. **Lasa, I., Penades, J.R. (2006).** Bap**:** A family of surface proteins involved in biofilm formation. Resereach in Microbiology. 157: 99-107.
71. **Lauderdale, K.J., Boles, B.R., Cheung, A.L., Horswill, A.R. (2009).** Interconnections between sigma B, agr, and proteolytic activity in *Staphylococcus aureus* biofilm maturation. Infection and Immunity. 77: 1623-1635.
72. **Lee, S.F., Li, Y. H., Bowden, G.H. (1996).** Detachment of *Streptococcus mutans* biofilm cells by an endogenous enzymatic activity. Infection and Immunology. 64: 1035-1038.
73. **Lewis, K. (2010).** Persister cells. Annual Review of Microbiology. 64: 357-372.
74. **Limoli, D.H., Jones, C.J., Wozniak, D.J. (2015).** Bacterial extracellular polysaccharides in biofilm formation and function. Microbiology Spectrum 3(3) doi:10.1128/microbiolspec. MB-0011-2014.
75. **Liu, Y., Yang, S.F., Li, Y., Xu, H., Qin, L., Tay, J. (2004).** The influence of cell and substratum surface on hydrophobicities on microbial attachment. Journal Biotechnology. 110: 251-256.
76. **Loiselle, M., Anderson, K.W. (2003).** The use of cellulose ininhibiting biofilms formation from organisms commonly found on medical implants. Biofouling. 19: 77-85.
77. **Loughran, A.J., Atwood, D.N., Anthony, A.C., Harik, N.S., Spencer, H.J., Beenken, K.E., Smeltzer, M.S. (2014).** Impact of individual extracellular proteases on *Staphylococcus aureus* biofilm formation in diverse clinical isolates and their isogenic sarA mutants. Microbiology Open. 3: 897-909.
78. **Mah, T.F., O'Toole, G.A. (2001).** Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology. 9: 34-39.
79. **Marti, M., Trotonda, M.P., Tormo-Mas, M.A., Vergara-Irigaray, M., Cheung, A.L., Lasa, I., Penades, J.R. (2010).** Extracellular proteases inhibit protein-dependent biofilm formation in *Staphylococcus aureus*. Microbes Infection. 12: 55-64.
80. **McGavin, M.J., Zahradka, C., Rice, K., Scott, J.E. (1997).** Modification of the *Staphylococcus aureus* fibronectin binding phenotype by v8 protease. Infection and Immunity. 65: 2621-2628.
81. **Medina, A.A., Kadouri, D.E. (2009).** Biofilm formation of *Bdellovibrio bacteriovorus* host-independent derivatives. Resereach in Microbiology. 160: 224-231.
82. **Mootz J.M., Malone C.L., Shaw L.N., Horswill A.R. (2013).** Staphopains modulate *Staphylococcus aureus* biofilm integrity. Infection and Immunology. 81: 3227-3238.
83. **Muthukrishnan, G., Quinn, G.A., Lamers, R.P., Diaz, C., Cole, A.L., Chen, S., Cole, A.M. (2011).** Exoproteome of *Staphylococcus aureus* reveals putative determinants of nasal carriage. Journal of Proteome Research. 10: 2064-2078.
84. **Nelson, D.C., Garbe, J., Collin, M. (2011)**. Cysteine proteinase SpeB from *Streptococcus pyogenes*-A potent modifier of immunologically important host and bacterial proteins. Bilogical

Chemistry. 392: 1077-1088.
85. **Nemoto K., Hirota K., Ono, T., Murakami K., Nagao, D., Miyake, Y. (2000).** Effect of varidase (streptokinase) on biofilm formed by *Staphylococcus aureus* Chemotherapy. 46: 111-115.
86. **Nemoto, K., Hirota, K., Murakami, K., Taniguti, K., Murata, H., Viducic, D., Miyake, Y. (2003).** Effect of varidase (streptodornase) on biofilm formed by *Pseudomonas aeruginosa*. Chemotherapy. 49: 121-125.
87. **Neu, T.R. (1996).** Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiological Reviews. 60: 151-166.
88. **Nguyen, U.T., Burrows, L.L. (2014).** DNase I and proteinase K impair *Listeria monocytogenes* biofilm formation and induce dispersal of pre-existing biofilms. International Journal of Food Microbiology. 187: 26-32.
89. **Niazi, S.A., Clark, D., Do, T., Gilbert, S.C., Foschi, F., Mannocci, F., Beighton, D. (2014).** The effectiveness of enzymic irrigation in removing a nutrient-stressed endodontic multispecies biofilm. International Endodontic Journal. 47: 756-768.
90. **Nichols, W.W., Dorrington, S.M., Slack, M.P., Walmsley, H.L. (1988).** Inhibition of tobramycin diffusion by binding to alginate. Antimicrobial Agents and Chemotherapy. 32: 518-523
91. **Nijland, R., Hall, M.J., Burgess, J.G. (2010).** Dispersal of biofilms by secreted, matrix degrading, bacterial DNase. PLoS ONE. 5: e15668.
92. **O'Neill, E., Pozzi, C., Houston, P., Humphreys, H., Robinson, D.A., Loughman, D.A., Foster, T.J., O'Gara, J.P. (2008).** A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. Journal of Bacteriology. 190: 3835-3850.
93. **O'Toole, G., Kaplan, H.B., Kolter, R. (2000).** Biofilm formation as microbial development. Annual Review of Microbiology. 54: 49-79.
94. **Otto M. (2013).** *Staphylococcal* infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Annual Review of Medicine. 64: 175-188.
95. **Parsek, M.R., Greenberg, E.P. (2000).** Acyl-homoserine lactone quorum sensing in gram negative bacteria: a signaling mechanism involved in associations with higher organisms. Proceedings of National Academy of Science. 97: 8789-8793.
96. **Patterson, J.L., Girerd, P.H., Karjane, N.W., Jefferson, K.K. (2007).** Effect of biofilm phenotype on resistance of *Gardnerella vaginalis* to hydrogen peroxide and lactic acid. American Journal Obstetric Gynecology. 197: 170.e1-170.e7.
97. **Pecharki, D., Petersen, F.C., Scheie A.A. (2008).** Role of hyaluronidase in *Streptococcus intermedius* biofilm. Microbiology. 154: 932-938.
98. **Pooi, Y.C., Yien, S.T. (2014).** Anti-biofilm agents: recent breakthrough against multi-drug resistant *Staphylococcus aureus*. Pathogens and Disease. 70: 231-239.
99. **Prakash, B., Veeregowda, B.M., Krishnappa, G. (2003).** Biofilms: A survival strategy of bacteria. Current Science. 85: 1299-1307.
100. **Ramage, G., VandeWalle, K., Wickes, B.L., Lopez Ribod, J.L. (2001).** Biofilm formation by *Candida dubliniensis*. Journal Clinical Microbiology. 39: 3234-40.
101. **Rasamiravaka, T., Labtani, Q., Duez, P., ElJaziri, M. (2015).** The formation of biofilms by *Pseudomonas aeruginosa*: a review of the natural and synthetic compounds interfering with control mechanisms. BioMed Resereach International. 759348.
102. **Rosan, B., Lamont, R.J. (2000).** Dental plaque formation. Microbes and Infection. 2: 1599-1607.
103. **Sadekuzzaman, M., Yang, S., Mizan, M.F. R., Ha, S.D. (2015).** Current and Recent Advanced Strategies for Combating Biofilms. Comprehensive Reviews Food Science and Food Safety. 14: 491-505
104. **Schultz, G., Phillips, P., Yang, Q., Stewart, P.S. (2010).** Biofilm maturity studies indicate

sharp debridement opens a time-dependent therapeutic window. Journal of Wound Care. 19: 320-328

105. **Seper, A., Fengler, V.H., Roier, S., Wolinski, H., Kohlwein, S.D., Bishop, A.L., Camilli, A., Reid, l.J., Schild, S. (2011).** Extracellular nucleases and extracellular DNA play important roles in *Vibrio cholerae* biofilm formation. Molecular Microbiology. 82: 1015-1037.
106. **Shakir, A., Elbadawey, M.R., Shields, R.C., Jakubovics, N.S., Burgess, J.G. (2012).** Removal of biofilms from tracheoesophageal speech valves using a novel marine microbial deoxyribonuclease. Otolaryngology Head and Neck Surgery:Offical Journal of American Academy of otolaryngology- Head and Neck Surgery. 147: 509-514.
107. **Shaw, L., Golonka, E., Potempa, J., Foster, S.J. (2004).** The role and regulation of the extracellular proteases of *Staphylococcus aureus*. Microbiology. 150: 217-228.
108. **Shields, R.C., Mokhtar, N., Ford, M., Hall, M.J., Burgess, J.G., El-Badawey, M.R. Jakubovics, N.S. (2013).** Efficacy of a marine bacterial nuclease against biofilm forming microorganisms isolated from chronic rhinosinusitis. PLoS ONE 8: e55339.
109. **Shigeta, M., Tanaka, G., Komatsuzawa, H., Sugai, M., Suginaka, H., Usui, T. (1997).** Permeation of antimicrobial agents through *Pseudomonas aeruginosa* biofilms: a simple method. Chemotherapy. 43: 340-345.
110. **Shukla, S.K., Rao, T.S. (2013).** Dispersal of Bap-mediated Staphylococcus aureus biofilm by proteinase K. Journal of Antibiotic. 66: 55-60.
111. **Singh, R., Ray, P., Das, A., Sharma, M. (2010).** Penetration of antibiotics through *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. Journal of Antimicrobiology Chemotherapy. 65: 1955-1958.
112. **Singh, Y., Ahmad, J., Musarrat, J., Ehtesham, N.Z., Hasnain, S.E. (2013).** Emerging importance of holobionts in evolution and in probiotics. Gut Pathogens. 5: 12.
113. **Singh, Y., Hasnain, S.E. (2014).** Holobionts: emerging strategy for interventions against infectious diseases, metabolic disorders and cancers. Indian Journal of Medical Resereach. 140: 11-14.
114. **Speziale, P., Pietrocola, G., Foster, T.J., Geoghegan, J.A. (2014).** Protein-based biofilm matrices in *Staphylococci*. Frontier in Cellular and Infection Microbiology. 4: 171.
115. **Srey, S., Jahid, I.K., Ha, S. (2012).** Biofilm formation in food industries: a food safety concern. Food Control. 31: 572-85.
116. **Stewart, P.S., Costerton, J. W. (2001).** Antibiotic resistance of bacteria in biofilms. Lancet. 358: 135-138.
117. **Sutherland I.W. (1999).** Polysaccharases for microbial exopolysaccharides. Carbohydrate Polymer. 38: 319-328.
118. **Sutherland, I.W. (2001).** The biofilm matrix-an immobilized but dynamic microbial environment. Trends in Microbiology. 9: 222-227.
119. **Tack, K.J., Sabath, L.D. (1985).** Increased minimum inhibitory concentrations with anaerobiasis for tobramycin, gentamicin, and amikacin, compared to latamoxef, piperacillin, chloramphenicol, and clindamycin. Chemotherapy. 31: 204-210.
120. **Tetz, G.V., Artemenko, N.K., Tetz, V.V. (2009).** Effect of DNase and antibiotics on biofilm characteristics. Antimicrobial Agents and Chemotherapy. 53: 1204-1209.
121. **Totani, T., Nishiuchi, Y., Tateishi, Y., Yoshida, Y., Kitanaka, H., Niki, M., Kaneko, Y., Matsumoto, S. (2017).** Effects of nutritional and ambient oxygen condition on biofilm formation in *Mycobacterium avium* subsp. *hominissuis* via altered glycolipid expression. Science Report. 7: 41775.
122. **Tribedi, P., Sil, A.K. (2014).** Cell surface hydrophobicity: a key component in the degradation of polyethylene succinate by *Pseudomonas* sp. AKS2. Journal of Applied Microbiology. 116:

295-303.

123. **Tuomanen, E., Cozens, R., Tosch, W., Zak, O., Tomasz, A. (1986).** The rate of killing of *Escherichia coli* by beta-lactam antibiotics is strictly proportional to the rate of bacterial growth. Journal General Microbiology. 132: 1297-1304.
124. **Vasudevan, R. (2014).** Biofilms: microbial cities of scientific significance. Journal of Microbiology Expperimentation. 1, doi: 10.15406/jimen.2014.01.00014.
125. **Vickery K., Pajkos, A., Cossart, Y. (2004)**. Removal of biofilms from endoscope: evaluation of detergent efficacy. American Journal of Infection Control. 32(3): 170-176.
126. **Vilcheze, C., Hartman, T., Weinrick, B., Jain, P., Weisbrod, T.R., Leung L.W., Freundlich, J.S., Jacobs, W.R. (2017).** Enhanced respiration prevents drug tolerance and drug resistance in *Mycobacterium tuberculosis*. Proceding of National Academy of Science of the United State of America. 114: 4495-4500
127. **Vuotto, C., Longo, F., Donelli, G. (2014).** Probiotics to counteract biofilm-associated infections:promising and conflicting data. International Journal of Oral Science. 6: 189-194.
128. **Walencka, E., Sadowska, B., Rozalska, S., Hryniewicz, W., Rozalska, B. (2005).** Lysostaphin as a potential therapeutic agent for *staphylococcal* biofilm eradication. Polish Journal of Microbiology. 54: 191-200.
129. **Waryah, C.B., Wells, K., Ulluwishewa, D., Chen-Tan, N., Gogoi-Tiwari, J., Ravensdale, J., Costantino, P., Gokcen, A., Vilcinskas, A., Wiesner, J. Mukkar, T. (2017).** *In vitro* antimicrobial efficacy of tobramycin against *Staphylococcus aureus* biofilms in combination with or without DNase I and/or Dispersin B: A preliminary investigation. Microbial Drug Resistance. 23: 384-390.
130. **Watters, C., Fleming, D., Bishop, D., Rumbaugh, K.P. (2016).** Host responses to biofilm. Progress in Molecular Biology and Translational Science. 142: 193-239.
131. **Watters, C.M., Burton, T., Kirui, D.K., Millenbaugh, N.J.** (**2016).** Enzymatic degradation of in vitro *Staphylococcus aureus* biofilms supplemented with human plasma. Infection and Drug Resistance. 9: 71-78.
132. **Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C., Mattick, J.S. (2002).** Extracellular DNA required for bacterial biofilm formation. Science. 295: 1487.
133. **Wingender, J., Strathmann, M., Rode, A., Leis, A. (2001).** Isolation and biochemical characterization of extracellular polymeric substances from *Pseudomonas aeruginosa*. Methods in Enzymology. 336: 302-314.
134. **Wu, J.A., Kusuma, C., Mond, J.J., Kokai-Kun, J.F. (2003).** Lysostaphin disrupts *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms on artificial surfaces. Antimicrobial Agents and Chemotherapy. 47: 3407-3414.
135. **Xu, K.D., McFeters, G.A., Stewart, P.S. (2000).** Biofilm resistance to antimicrobial agents. Microbiology 146: 547-549.
136. **Yasuda, H., Ajiki, Y., Koga, T., Kawada, H., Yokota, T. (1993).** Interaction between biofilms formed by *Pseudomonas aeruginosa* and clarithromycin. Antimicrobiology Agents and Chemotherapy. 37: 1749-1755.
137. **Zhang, Y. (2014).** Persisters, persistent infections and the Yin-Yang model. Emerging Microbes and Infection 3, doi:10.1038/emi.2014.3.
138. **Zhang, X., Bishop P.L.** (**2003).** Biodegradability of biofilm extracellular polymeric substances. Chemosphere. 50: 63-69.