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Investigation of Bacterial Biofilm sample taken from Fresh Water and Soil and some Antibiofilm Approaches

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Abstract

Background: Matrix-enclosed populations of bacteria known as Biofilms, stick to one another or to the different surfaces or interface. This definition comprises floccules, adherent populations and microbial clumps within the porous media having pore spaces. This ability of biofilm formation is a special property of bacteria. Multicellular communities existing in the natural environment known as biofilms are having unique architectural features by interstitial voids such as micro and macro colonies. Biofilms are basically an ordered aggregate of microorganisms living within their self-produced extracellular matrix and attach to the surfaces irreversibly but these aggregates are not easy to remove unless rinse quickly. In the attachment stage of biofilm to the surface, formation of (EPS) extracellular polymeric substances occurs. Phylogenetic history of different related biofilms can be find by using different computational techniques like tree viewer and quorum sensing. Biofilm formation is not a good thing in many ways so there must be some ways to stop the formation of biofilm so there are some anti-biofilm approaches and by using them we can stop the growth of biofilms. Some of these techniques include aptamers, enzyme treatment, nanoparticles, photo- dynamic therapy anti adhesion approaches etc. The development of anti-biofilm agents against different microbial targets and their subsequent application as adjuvants with antimicrobial agents seems to be more efficient.

Keywords: Biofilm; Extracellular polymeric substances; Microbial colony; anti-biofilm Approaches; Adhesion; Anti-adhesion; Phylogenetic history; Quorum sensing; Photo dynamic Therapy; Nanoparticles; Aptamers; Adjuvants

Introduction

Biofilms can be defined as aggregates of microbes held together in their self-made extracellular matrix. Biofilms are present in the natural environments cling to each other or to the surfaces. Since the first description about bacterial biofilms, their real importance has gradually emerged and the first recognition of the ubiquitous nature of biofilms [1].

During the fifteen decades,ensuing the discoveries of Louis Pasteur. It has come to be interestingly clear that the biofilms possessed a noticeably different growth phase of bacteria that is extremely diverse from the planktonic growth phase being studied so diligently [2].

Bacterial cells change their phenotypes during the complex process of adherence in response to the proximity of a surface. Sessile bacteria throughout the initial stages in formation of biofilm find themselves in unchanging vicinity with cells of the same species and of other species as single and assorted species micro colonies are then formed.The vivacious extra polysaccharide matrix production in the emerging biofilm and the cellular juxtapositions are the conditions for a micro-environment of every biofilm bacterium. Many biofilm bacteria react to their unique specific micro environmental conditions showing different patterns of growth and a structurally complex mature biofilm gradually develops accordingly. A major factor responsible in shaping the structure of a biofilm and in forming the ultimate associations which will form the mature biofilms well organize enough to attach to the surface is physiological cooperatively [3].

Materials and Methods

Biofilm formation is a characteristic property of bacteria. Biofilms constitute multicellular colonies of microorganisms that are joined together through a matrix. The mechanisms underlying in the formation of biofilms vary for different bacteria that solely depends on different environmental circumstances and strain specificity. In my review, I have emphasized on 4 well-known model organisms so that an overview about how different organisms are involved in biofilm formation can be given: Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Bacillus subtilis. These bacteria are used as an example to argue the salient charactristics of biofilm formation and the process involved in these when extracellular signaling activates them. Formation of biofilm can influence humans in different ways as they form in natural, industrial and medical settings. For example, biofilm development of medical devices such as catheters or implants

that often results in difficulty in order to treat chronic infections. Besides this, infections have been related with biofilm formation on human surfaces such as skin, urinary track and teeth. In spite of that biofilms on human surfaces are not always harmful.For instance, [4] dental plaque biofilms consists of dozens of species and their composition immediately inform either the disease is present or absent. There is a progression of colonization in dental plaque but the presence of valuable species opposes settlement by harmful organisms.But biofilms are ubiquitously found universally. Like, biofilms are formed on the hull of ships and inside pipes also where they cause severe damage. Biofilms are also formed in many natural settings but there they allow mutualistic symbioses. For example in order to allow ants to maintain pathogen free fungal gardens, the Actinobacteria often grow on ants. There a large number of welfares and damages that a biofilm can converse, so it is important for us to know that how bacteria develop in these communities.[5]From biofilm formation, a bacterial community mayget a number of benefits. Biofilms provide confrontation to many anti-microbials and protection against host defenses. Within the biofilm arise in the percentage of persisted cells appears to be one of the possible reasons behind the increased resistance against environmental stresses. Persisted cells are non-dividing and are resistant to many antibiotics even though of the fact that being hereditarilyalike to the rest of the population. Persisted cells are supposed to be protected from the antibiotic actions because they express such toxin antitoxin systems which through their toxin modules block the target of antibiotics. In addition to persisters, presences of extracellular matrix provide protection to constituent cells from external harms. These extracellular matrices also provide diffusion barrier to small molecules. In relation to this, some of the cells in bacterial community are metabolically inactive due to slower diffusion of vitamins, nutrients and co factors in biofilms [6].

Processing

By using different techniques of molecular biology and bioinformatics now we are able to know that how different biological species are working in special conditions and by using most advanced class of biology which is bioinformatics we can find Insilco determination. Having knowledge about bioinformatics means we can have knowledge about their ancestors as well that how specific biological specie is related to any other species. In order to study bacterial biofilms it is important to about their evolutionary history and to know about what is the association between different organisms we can use a technique of bioinformatics known as tree viewer in which we can draw phylogenetic tree which will enable us to find out the relationship between different biofilms which have common ancestor [7].

Quorum sensing is also an important technique to find the evolutionary history of biofilms. Once supposed as organisms which are infrequently interrelate, bacteria known as to lead extremely common lives. Essential to this sociality is a capability to detect local cell density and thus coordinate group activities. This capability is termed as quorum sensing, function through the secretion as well as recognition of auto-inducer molecules, which gather in a cell density-dependent way. As biofilms as well work as an auto-inducer so these can also be determined by using this technique. The effects of quorum sensing though, are highly flexible and depend upon both the species under surveillance and the experimental circumstances.

Biofilm constitutes a complex assembly of DNA, protein and polysaccharide in their self-produced EPM (extracellular polysaccharide matrix) and was naturally found on various surfaces including living tissues, potable water system or natural aquatic, medical devices etc. Bacterial biofilms are well studied in avoiding phagocytosis, antibiotics and other disinfectant components. The interstitial voids such as micro and macro colonies found in biofilms allowed the diffusion of gases, antimicrobial agents and nutrients through the biofilms; however, biofilm changes their architecture in response to these changes occurring in the internal and external process. As the cells are in proximity, they exchange their extra chromosomal plasmid, their quorum sensing molecules and show distinct character in each biofilm community [8].

Despite of all the detailed studies on architectural features of biofilms, its composition, mechanisms, benefits and detriments, our review specifically focuses on the steps that are involved in Biofilm formation and Antibiofilm approaches in detail [9-11].

Formation of Biofilm:

The fact that in a biofilm if cells are confined to a limited space that will influence the bacterial growth rate. This condition is very much similar to the stationary phase that is produced in laboratory conditions. Hence, biofilm formation basically represents a natural stationary stage of bacterial growth. With the increase making of secondary metabolites such as pigments, antibiotics and other small molecules, bacteria greatly change their physiology during stationary phase. Secondary metabolites function as signaling molecules either to start the biofilm formation procedure or to inhibit it by other organisms that live in the same habitat [12].

Preparation

Formation of biofilm occurs in many steps as per genetic studies. Quorum sensing between the cells of microorganisms is a special type of signaling and it is a must requirement for the biofilm formation. In comparison with planktonic forms of the same microbes, transcription of different set of genes are required here. The viscous and elastic features of extracellular matrix attribute mechanical stability to a biofilm [13].

Formation of Biofilms is a difficult procedure but it happens in few common steps according to different researcher's i.e.

- Attachment to the Surface or Initial Contact
- Formation of Micro-colony
- Architecture and Maturation of Biofilms
- Dispersion or Detachment of the biofilm.

Formation

As this is the initial step in formation of biofilm it requires attachment with a surface, microbial cells get attached to the surface with the help of their adjuncts like Pilli and flagella and they may as well get attached to the other physical forces like electrostatic interactions or Vander Waal's forces etc. One cause for growth and attachment of microbes in a biofilm is solid-liquid interface. The strength needed for the contact between the surface of attachment and bacteria is given by flagella, fimbriae and Pilli. [14] Other reason behind the attachment of microbes is hydrophobicity of the surface as it greatly decreases the repulsion force between surface and bacteria. Microbes more likely to attach to Teflon or plastics as they are hydrophobic and non-polar surfaces rather than on metals or glass because they are polar and hydrophilic surfaces.

Micro-colony

Afterward the stable attachmentphase of microbes to a living or a nonliving surface, microbial cells start the procedure of division and multiplication which is introduced through specific chemical signaling occurring within the Extracellular polysaccharide matrix. This will ultimately lead to the micro colonies formation. In a biofilm, bacterial colonies are of many micro communities and these communities interact with each other in multiple ways .In distribution of important metabolic products, exchange of substrate andelimination [14] of the metabolic end-products, this microbial coordination plays a vital role. For example throughoutthe anaerobic digestion, the complex organic matter is converted to methane and carbon dioxide, 3kinds of bacterial association is required i.e.

- From organic compounds production of acid and alcohol is start by the fermentative bacteria while depending upon the dissimilation of complex organic compounds.
- Acetogenic bacteria then consumed these as their substrates.
- By converting the acetate, hydrogen and carbon dioxide into methane, methanogens get energy. For the development of a syntrophic association, the need of a complete environment is being fulfilled by biofilm [15].

In this phase, with the help of auto-inducer signals microbial cells coordinate with each other. To achieve required microbial density cell-cell coordination is an essential procedure. This coordination ultimately leads toexcretion of auto inducers, the signaling molecules. Quorum sensing is facilitated by these signaling molecules. At this stage of biofilm maturation, in order to form EPS expressions of certain gene products arerequired. Since EPS maintains the 3D structure of a biofilm, then interstitial spaces are beingformedwithin the matrix. To eradicate theleftover from the populations of micro colonies and to distribute essential nutrients among the communities of a biofilm, a circulatory system is required and to accomplish that purpose these channels are occupied with water [16].

Dispersion or Depression Stage of a Biofilm

In the detachment stage, in the biofilm the microbial cells quickly multiply and disperse in order to change from sessile to motile form. Then dissociation happens in a usual phenomenon. Bacterial cells of some bacteria directly disperse into the environment because these kinds of the bacteria do not form EPS but motorized pressure might too get involved in this procedure sometimes. Within the biofilm throughout the dispersion stage the microbial colonies release diverse sacchrolytic enzymes in order to discharge the superficial of microorganisms to a novel extent for the settlement purpose. For instance P flouresence and Pseudomonas aeruginosayield alginate lyase, Escherichia coli produce N-acetyl-heparosanlyase and S. [17] equiyieldshyaluronidase for the breakdown of Extracellular polysaccharide medium and later dispersion. In thisstageupregulation of the manifestation of some proteins is carried out by microbial cells because these proteins are essential to flagella formation which will ultimately allow the bacteria to transfer into a novel place. Detachment of the microbial cells and then move to a new place will be helpful in scattering of the infections.

Biofilm

Antibiofilm approaches include the natural and induced process that leads to lessen the bacterial biomass through modifications done in biofilm formation durability and orquality". Antibiofilm approaches can target either theadhesion stage of biofilms or mature biofilms. A biological response to a biomedical device solely depends on the structure and the surface capabilities of the material used in it and most likely device-associated [18] infections are originate from surface material contamination at time of implantation. Thus, the surface capabilities or compositions of biomaterials are modified in order to achieve appropriate results. To magnify functionality and biocompatibility, surface engineering of materials can be done which ultimately reduce the microbial contamination and put a stop to biofilm infections.Besides this, different approaches that target microbial biofilms' adhesion or maturation are being developed. Adjuvants in combination with antimicrobial agents can be used as antimicrobial agents.

Anti-adhesion strategies can cause either general or specific inhibition of adhesion depending on its target. Nonspecific inhibition of adhesion is conferred throughthe modification of surfaces chemistry or topography. Engineering surface topography or its manipulation at micro and nanoscale seems to be an advantageous approach. It is non-toxic and independent of material type. Moreover, it can also be chemically modified. [19] Yet, this approach has not been fully explored. Existing studies infer that there is no rule concerning the effect of nanoscale topographical modifications on the bacterial supplements studied the properties of superficialstructures on the Candida albicans. Biofilm formation outsides were coated

with particles of different sizes of polydimethylsiloxane (PDMS) solids. The study reported that higher biofilm formation was observed on those surfaces which were coated with the particles whichhave size rangeof 4 to 8µm when compared to the surfaces that were coated with particles which have a size range of 0.5 to 5 micrometer reported that biofilms grown on spatiallypreparedmicro topographicexternalshapesproduced on polydimethylsiloxane reduced the adhesion of 3 strains of bacteria (S. epidermidis, E. coli and Bacillus subtilis) by 30 to 45% more when compared to smooth control surfaces.The inhibitory effect of surface topography has been attributed to the presence of fewer binding sites when compared to flat surfaces. The presence of similar curvature between the solid surface and the microorganism can also make the adhesion process more challenging. Additionally, the topography of a solid surface can trap air which in return reduces the access of microorganisms to the solid.

Photo-Dynamic Therapy

PDT (Photodynamic therapy) is based on application of a nontoxic PS (photosensitizer)that can be activated upon exposure to a specific wavelength. Such activationresults in directly damaging the sub cellular components by producing cytotoxic reactive oxygen species. PDT has a widerangeaction against biofilm microorganismswhich includesresilient pathogens. Photosensitizers have beensuggested to exert their effect by destroying either thecomponents of the biofilm matrix, cell surface or intracellular damage after penetrating the cytoplasmic membranes. Conjugated a photosensitizer toluidine blue O (TBOToluidine blue O)with AgNP (silver nanoparticles). The conjugate inhibited S. mutans biofilm upon exposure to laser light (630 nm). Upon comparison to TBO when applied alone, the conjugate increased the leakage ofcellular constituents and resulted in more evident down regulation of biofilm related genes characterized the effect of sublethal doses of PDT using MB (methylene blue), ICG (indocyanine green) and TBO (toluidine blue O) on E. faecalis biofilms. The sub pernicious doses lessen formation of biofilm up to 22.6%, 19.5% and 42.8% respectively. The obtained results indicate that ICG-PDT demonstrated higher antibiofilm activity when compared to other photosensitizer tested the effects of ICG (Indocyanin Green)on biofilms formed by E. faecalis. Photodynamic therapy mediated through ICG significantly reduced bacterial counts and inhibited biofilm formation [20].

Nanoparticles

Nanoparticles are defined as materials whose basic unit in the three dimensional space is in this range (1-100 nm) or having one measurement on nanometer scale range. Nano particles are having anti-bacterial activity against the gram negative and gram positive bacteria above a wide spectrum range which is because of their huge surface area to volume proportion as well as to their distinctive advance chemical and physical qualities. In addition to their antibacterial activity, nanoparticles have recently become a promising approach to control or prevent biofilms .

Silver nanoparticles developed through reduced the protein and carbohydrate content of biofilm matrix which weakened the biofilm and allowed the penetration of drugs. The Au nanoparticles loaded with gentamicin (GPA NPs) produced by effectively damaged the established biofilms of gram positive bacteria i.e. S. aureus and L. monocytogenes and gram negative bacteriai.eP.aeruginosa , S. Typhimurium and Escherichia coli. Moreover, nanoparticles didn't demonstrate toxicity to RAW 264.7 cell line. produced nanoparticles from the non-toxic poly chitosan. The particles demonstrated bactericidal activity and anti-adhesive activity. Moreover, they lessen the biofilm developmentthrough Methicillin resistant and Methicillin susceptible Staphylococcus aureus strains. The silver nanoparticles produced by Kyaw et al. 2017 were capable to hinder the production ofbiofilm byEscherichia coli ,P. aeruginosa , B. subtilis and S. Typhimurium .DH-5α at concentration equivalent to 6.25 ppm. Moreover, they destroyed Salmonella, Pseudomonas, and B. subtilis biofilms at concentrations ranging from 25-50 ppm. assessed the antibiofilm activity of silver nanoparticles in contradiction ofEscherechia coli,A. baumanni, K. pneumonia,P. mirabilism and P. auruginosa. The AgNPs successfully confined biofilm formation of the tested bacteria within range of about 12.5-100 µg/ml. also tested AgNPscreatedby using V. zizanioides aqueous root extract which turned out to be aperfect anti-QS and anti-biofilm agent against S. marcenscens. prepared AgNPs using catechin, cat-borax or polycat. Silver nanoparticles prepared using polycat demonstrated superior antibacterial and intensify the anti-biofilm action against Pseudomonasaeruginosa biofilms reported a novel polymeric NPs (block copolymer Nanoparticles) that can diffuse into the biofilms, and cause dispersal of preformed biofilms upon binding to the bacterial cells of various clinically gram positive bacteria that are resistant to many drugs including E. faecalis ,S. aureusand Enterococci tested the effects of NO (nitric oxide) releasing silica nanoparticles shape onPseudomonas aeruginosaandStaphylococcus aureus biofilms. The study reported that the rod shaped nanoparticles were more effective in delivering nitric oxide to the biofilms and induced greater antibacterial action when compared to spherical shaped ones. The antibiofilm [15] effect of nanoparticles is attributed to their antibacterial characteristics as well as to other properties (extra small sizes, shapes and surface charges) which result in increased penetration ability and makes them potent drug delivery agents.

Aptamers

Aptamers consists of single stranded RNA or DNA sequences that can specifically bind to their targets and often inhibit them .Very rare studies have examined the aptamers as anti-biofilm agents. In an approach to block the flagella motility as a promising strategy to hinder biofilm formation, developed a single stranded DNA aptamer that specifically targeted S. Choleraesuisflagellin protein. The characterized aptamer inhibited the early attachment by restricting cellular aggregation and production of mature biofilms. Moreover,[13] flagellinaptamer demonstrated synergistic effect with ampicillin antibiotic further upgraded the flagella targeting aptamer by linking it with ampicillin. The conjugate had a distinctive

antibacterial activity and higher anti-biofilm activity when compared to those when either component were applied separately. The aptamer is thought to ensure facilitated entry of ampicillin into the biofilm which decreased its tolerance to the antibiotic. Moreover, loss of bacterial motility due to fliaptamer can also result in decreased adherence to the matrix surface. Moreover, the developed aptamer might have also served as an antibiotic carrier that can help ampicillin to penetrate the biofilm, eradicate its cells and overcome biofilm tolerance to drugs. developed an aptamer that targeted P. aeruginosa biofilms. The aptamer which acted as a targeted delivery agent was used to develop two complexes, aptamer SWNT (Single-walled carbon nanotubes) and aptamerciprofloxacin-SWNT. The former complex caused a higher biofilm inhibition by 36% when compared to SWNT alone. [21] The three-component complex demonstrated higher anti-biofilm activity than that when the complex components applied separately or as a two component complex targeted S. Typhimurium biofilms with Graphene oxide and Graphene oxide aptamer conjugates. The ST-3-GO conjugate inhibited and dispersed biofilms within 93.5% and 84.6% respectively. ST-3 aptamer might have facilitated the entry of GO and caused a decrease the cellular membrane potential. Hence, by this way biofilms can be removed.

Conclusion

Biofilms are defined as microbial communities stick with one another or to various surfaces also entrenched within a self-produced extracellular matrix. This also includes floccules, adherent populations and microbial aggregates within the porous media. Bacterial sample can be taken from different sources like soil sewage water etc. and can check the aggregation of different bacterial colonies and can check the formation of biofilm. We can check the formation of biofilms through different sources; we can perform different physical and chemical tests to check the formation of different biofilms. Relation of different biofilms can be finding out by using different computational techniques like tree viewer in which we can draw a phylogenetic tree which will assist to find out the relation between different bacterial biofilms. Quorum sensing is a novel technique which can also be used to find the evolutionary history. Biofilm formation is not a good thing in many ways so there must be some ways to stop the formation of biofilm so there are some anti-biofilm approaches and by using them we can stop the growth of biofilms. Some of these techniques include aptamers, enzyme treatment, nanoparticles, photo- dynamic therapy anti-adhesion approaches etc. The development of anti-biofilm agents against different microbial targets and their subsequent application as adjuvants with antimicrobial agents seems to be more efficient.

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