Biofilms Formation and its various effects in the Field of Biotechnology

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***Abstract:-*Biofilms are manifested in nature and the complex surface (bacterial, algal and fungal)require attachment areas which are termed as an Extracellular matrix(ECP) which is made of lipids, proteins, nucleic acids, and polysaccharides etc. The nature of microbes may vary in different habitat and environment. As we know that biofilms are found in planktonic or sessile surfaces, on the basis of that it categories in five phases; Cell attachment, Cell to cell adhesion, Cell proliferation and growth, Cell maturation and Cell detachment and dispersal. The role of Extracellular polymeric substances (EPS) is to immobilize biofilm cells, maintained long-term proximity and allows intense interactions to occur, including; horizontal gene transfer, cell-cell communication and also the formation of synergistic microbial interaction. It was demonstrated that micro-structural and mechanical properties of biofilms can be developed via colloidal self-assembly cells and polymers.**

Keywords-Adhesion, assembly, sessile, dispersal, synergistic

INTRODUCTION

Biofilms

A bacterial cell generally occurs in two different types of growth planktonic cells and Sessile aggregates. The sessile aggregates are termed as Biofilms. Biofilms are ubiquitous in nature. It is attached to aquatic sediments and contaminated soils where it releases some chemicals into the environment. Microorganism living in Biofilm form is more beneficial for cell growth and survival in the protected environment. These adherent cells become attached with extracellular matrix that is made up extracellular polymeric substances (EPS). Overall 90% of all bacteria live in biofilms. The cells within the biofilm produce the EPS components are polysaccharides, lipids, proteins, and DNA. Biofilms can adhere to a surface like a tooth, rocks, and sewage pipelines surface and food products etc. The smallest unit of biofilm is Micro colony. (Toole *et al*., 2000).

Presence of biofilms in different habitats and its application

Biofilms formation is rapidly increased day by day by in medical, food and other industrial systems. It may be caused by both disease-causing and non-disease-causing microbes (Deibel *et al*., 2003) .Some of them is discussed below:-

Biomedical Devices and Clinical settings: Biofilms are commonly found in the biomedical devices and implants within the human body. These organisms typically originate from the skin of a patient or health care worker, or tap water to which the device is exposed Such medical devices include urinary catheters, central venous catheters, prosthetic heart valves and artificial hip prosthesis. Biofilm formation involves in adhesion of fungal cells to retreated substrates and formed growth in the medium (Chandra *et al.*,2008).Microbes in Biofilms gain access to the catheter by migrating externally from the skin along the exterior catheter surface or internally from the catheter port. It has been observed that colonization and biofilm formation can occur within 3 days of inserting the catheter, but biofilm formation on internal surfaces of the catheters is more likely to be present for those that remain in place for longer periods of time. Specific to urinary catheters, biofilms that develop will infect the patient and result in a urinary tract infection. This is more likely to happen in an open system, where the catheter drains into an open collection center than a closed system, where the catheter empties into a plastic bag. Time is also a variable; essentially all patients who have a urinary catheter for more than 30 days get infected with a UTI (Kokare *et al*., 2009). A study demonstrated that bacterial pathogens in biofilms play a role in chronic rhinosinusitis (CRS).Biofilms in mucosal specimens of patients undergoing surgery for CRS. The total number of 30 samples and 4 control samples were studied. By using scanning electron microscopy (SEM) 24 (80%) of the 30 patients were found to have micrographic evidence of Biofilms.The six cryofixation samples showed biofilm structures on SEM micrographs that were correlated with bacterial structures seen at the mucosal surface.Bacterial cultures were positive on all patients was visualized.The biofilm 3-D structure, glycocalyx, and water channels were seen in TEM (transmission electron microscope) cleared that bacteria present in the biofilm. (Sanclement *et al* , 2009).Artificial mature biofilm of *K. Pneumonia* B5055 was made on polycarbonate membranes. The center of biofilm had more inactive cells whereas periphery had more actively dividing cells.

Amikacin antibiotic ($40\ \mu g\ ml^{-1}$) susceptibility was determined and it was found that cells in younger biofilm were more susceptible as compared to cells in the older biofilm. The thickness and heterogeneity of biofilm increased from 0.093 to 0.231mm with time and the effectiveness of antibiotic decreases (Singhla *et al*, 2014).In another study biofilm formation by 115 clinical uropathogenic *E.coli* strains under different growth, conditions were studied using spectrophotometer (A_{531}) after (crystal violet) staining and correlated with bacterial growth (A_{600}). The live and dead cells in biofilm formation were observed on the glass surface by an epifluorescence microscope. It was concluded that biofilm was maximum in the rich medium after 24 h and its level has not changed in time. When biofilm level was compared to bacterial growth it shows that in the minimal medium growth was higher. The results suggested that bacteria prefer to grow in the biofilm community (Bialek *et al.*, 2015). It is a known fact that biofilms that occur on dry surfaces have increased tolerance to disinfectants. A study was conducted in which formulated and non formulated disinfectants were tested against *Staphylococcus aureus* species grown in the form of dry surface biofilms (DSB) in the bioreactor with alternate hydration and dehydration cycles. The efficacy of treatment was detected both in the presence or absence of organic soil. Biofilms were treated with disinfectants like peracetic acid, hydrogen peroxide, and chlorine and the residual biofilm viability and mass were calculated by plate culture and protein assay respectively. The results were obtained showed that the chlorine-based products reduced the viability of biofilms by $2.8\log_{10,}$ and $2\log_{10}$ for proxitane but products failed to reduced biofilms in the soil. Surfex disinfectant completely inactivated biofilm ($6.3\log_{10}$). Hydrogen peroxide products showed minimal efficacy against dry surface biofilm. So it may be concluded that formulated disinfectants with active ingredients increase biofilms degradation (Chowdhury *et al.*, 2018).

Biofilms in the Oral Cavity: In saliva, Salivary micelle-like globules (SMGs) present in enamel determines the adhesive interactions that cause a specific organism to adhere to the pellicle. Dental biofilm occurs primarily as microcolonies. The acquired pellicle attracts gram-positive cocci such as *Str. mutans* and *Streptococcus sanguis,* organisms in plaque formation. Subsequently, a filamentous bacterium such as *Fusobacterium*

nucleatum and slender rods adhere to primary colonizers. *Vibrios* and spirochetes appear as the biofilm thickens. Calcified dental biofilm is termed as calculus. The precipitation of calcium phosphates within the organic plaque matrix, which depends on plaque, pH, and phosphate, local saturation of calcium and availability of fluoride ions and biological factors such as crystallization nucleators/inhibitors from either bacteria or oral fluids. (Listgarten,1999).A study was conducted on oral pathogens like Streptococcus mutans which are retained on toothbrushes and form biofilms which may infect users. The professional dentist rejects the use of toothbrushes covers from e external contaminant from minimizing exposure to air. This study suggested that increasing the ventilation of tooth brushes covers will reduce the retention of *S.mutans.* There were 12 samples of brushes there out of which 4 modified toothbrushes covered, 4 unmodified toothbrushes and 4 uncoverd.Toothbrushes were incubated in Brain heart infusion (BHI) broth at 37degree Celsius for 48 hours stained with crystal violet and colonies counted under a stereoscopic microscope. The study concluded that as ventilation is increased .There is retention of biofilms which would result in a safe dental product. (King, 2004).The antibiotic resistance and biofilm formation interested among a collection of 51 clinical isolates of *Staphylococcus pseudointermedius* collected from canine pyoderma. All isolates were tested for the susceptibility of 14 antimicrobial agents by the disk diffusion method in Mueller-Hinton agar. Oxacillin resistance was detected by subculture on oxacillin screening agar base. Biofilm formation was investigated by the Microtitre Plate test (MtP) and for some strains by transmission electron microscopy (TEM). Antibiotic resistance profiling demonstrated that 45/51 *Staphylococcus pseudointermedius* isolates had a multi drug resistant (MDR) phenotype, exhibiting simultaneous resistance to at least 3 antibiotics categories; whereas 6 isolates showed a non-MDR phenotype. Thirty strains (59%) were resistant in oxacillin resistant screening agar, the same strains were also positive for mecA by PCR assay. All *Staphylococcus pseudointermedius* isolates showed biofilm production by MtP method. Seventeen out of 51 isolates were classified as weakly adherent, 26 as moderately adherent, and 8 as strongly adherent. Moreover, no difference in biofilm formation between methicillin-resistant *Staphylococcus pseudointermedius* (MRSP) and methicillin-susceptible *Staphylococcus*

pseudointermedius (MSSP) (P value > 0.05) was noted. The antimicrobial resistance mechanisms and biofilm formation could explain the difficulty in treating *Staphylococcus pseudointermedius* canine infections, chemotherapeutic failure, and consequently persistent infections. (Stefanetti *et al*., 2017).

Water Systems: -Biofilms occurs in water distribution systems can protect pathogenic (disease-causing) microbes from disinfection and they can also a threat to public health. When microorganisms enter the inner surfaces of drinking water treatment systems, storage containers, and downstream distribution plumbing, the biofilms become a potential source of microbial (i.e. **regrowth**) contamination of water. Public health problems associated with biofilms included a microorganism in Flint's drinking i.e. *Legionella*.A study was determined that the kinetic ability of two strains serotypes *L.pneumophila serotype1* and serotype 2-15 to adhere and form biofilm on three different surfaces like stainless galvanised, copper and polyethylene commonly distributed in hot water system at Morocco at three different temperatures ie.20°,37°,44°C *L.pneumophila* serogroup 2-15 revealed high capability to adhere and form biofilm on stainless galvanised surface and polyethylene serotype 1rather than copper at 37° then 20° then 44° C.(Tai *et al* al,2012).Filamentous fungi have been constantly recovered from diverse aquatic environments including drinking water distribution systems. Although most of the works are focused on the study of planktonic form, recent researches have shown that fungi develop biofilm within these systems. In a study, *Aspergillus sp. (section Nigri), Aspergillus sp. (section Flavi), Alternaria sp., Botrytis sp., Cladosporium sp., and Penicillium sp*. recovered from water biofilms and capability to grow as biofilms under laboratory conditions was evaluated. All six isolates were able to form a biofilm, though different patterns of development were reported. Only Alternaria sp. formed biofilm in water over 24 h of analysis. Malt extract broth (MEB) was shown to be the best culture media for biofilm formation. A direct correlation between biomass and cell activity was not observed, but biomass values and EPS production were directly correlated. (Virgina and Lima, 2013).

Biofilms and food industry:-Biofilms in the food industry are of importance because they have the potential to persist in food sources or from transmission

of diseases. Poor sanitation conditions, food contact part, processing environment, and equipment etc have an essential factor in foodborne diseases and microorganisms involving are *Salmonella* and *Listeria monocytogens*. Improperly unhygienic surfaces enhance and the presence of moist content will contribute to producing the pathogenic microorganisms (Peterman *et al*., 1997). The formation of biofilms in food substances is very complex processes. Biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* were observed to be in the pathophysiology of chronic rhinosinusitis (CRS) .*In vitro* effect of honey against biofilms produced by *Pseudomonas aeruginosa* and *Staphylococcus aureus* .To assess antibacterial activity of honey against 11 methicillin-susceptible and resistant *Staphylococcus aureus* and 11 *Pseudomonas aeruginosa* isolates. Honey was tested against both planktonic and biofilm-grown bacteria. It was found to be effective in killing 100 percent of the isolates in the planktonic form. The bactericidal rates for the Sidr and Manuka honeys against MSSA, MRSA, and *Pseudomonas aeruginosa* biofilms were 63-82 percent, 73-63 percent, and 91-91 percent, respectively. These rates were significantly higher ($P < 0.001$) than those seen with single antibiotics commonly used against *Staphylococcus aureus* .Honey, which is a natural, nontoxic and inexpensive product, is effective in killing *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial biofilms. This observation may have important clinical implications and could lead to a new approach for treating refractory chronic rhinosinusitis. (Alandejani *et al.*, 2009). In this study was to assesses through a fractional experimental design and environmental factors that could affect the survival of *L.monocytogenes* cells on the surface prevent the persistence of this pathogen while on culture with salmon juice or meat exudate medium used with different hygiene status .Biofilm of *L.monocytogenes* pure culture or dual culture with a *Pseudomonas fluoroscens* strain application to drying cleaning and disinfection and comparison of *L.monocytogenes*.Bacterial survival was assessed by culture, qPCR to quantify total cells, and propidium monoazide coupled with qPCR to quantify viable cells and highlight viable but non-culturable (VBNC) cells. Our results showed that faliure to apply cleaning and disinfection cause cell persistence on surfaces. Moreover, the sanitation procedure leads only to a loss of culturability and appearence of VBNC populations. However, an additional daily drying step

after cleaning and disinfection optimises the effectiveness of these procedures to reduce culturable population. (Overney, *et al*., 2017).

Conclusion

Biofilms are manifested in nature and the complex surface (bacterial, algal and fungal)require attachment areas which are termed as an Extracellular matrix(ECP) which is made of lipids, proteins, nucleic acids, and polysaccharides etc. The nature of microbes may vary in different habitat and environment.The biofilms play a crucial role in medical implantation, industrial wastes, dental caries, and food industries etc. The various novel research strategies are being explored further day by day of formation or eradication of biofilms.

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