

Bacterial Biofilm: Beyond Planktonic Life

¹Yostnamayee Panda, ²Alaka Sanghamitra, ³Sonali Sandipta Dash, ⁴Dhananjay Soren

¹Department of Zoology

¹Ravenshaw University, Cuttack-753003, Odisha, India

¹mamapanda94331@gmail.com, ²sanghamitraalka@gmail.com, ³sonalisdash@ravenshawuniversity.ac.in,
⁴dsoren@ravenshawuniversity.ac.in

Abstract—Biofilms represent the predominant mode of microbial life and are formed when microorganisms attach to surfaces and become embedded within a self-produced extracellular polymeric substance (EPS) matrix. This multicellular lifestyle enhances microbial survival, adaptation, and persistence under a wide range of environmental conditions. Biofilm-producing bacteria are ubiquitously distributed across natural ecosystems, host-associated environments, and extreme habitats, where they play crucial ecological, clinical, and biotechnological roles. In natural environments, biofilms contribute to nutrient cycling, biogeochemical processes, environmental remediation, and ecosystem stability through the establishment of complex microbial communities. In contrast, biofilm formation by pathogenic bacteria poses significant challenges to human health by promoting chronic infections, increasing antimicrobial tolerance, and facilitating evasion of host immune defenses. Biofilm-associated infections involving species such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* are particularly difficult to eradicate and contribute substantially to global healthcare burdens. Furthermore, biofilm formation is a key adaptive strategy among extremophilic microorganisms inhabiting environments characterized by extreme temperature, salinity, pH, pressure, radiation, and nutrient limitation. The EPS matrix provides structural integrity and protection against harsh physicochemical stresses, enabling microbial survival in some of the most challenging environments on Earth. This review provides a comprehensive overview of biofilm-producing bacteria across environmental, clinical, and extremophilic settings, highlighting the mechanisms of biofilm development, EPS composition, ecological significance, pathogenicity, and adaptive responses to environmental stress. Comparative analysis of biofilm formation across these diverse habitats reveals both conserved and niche-specific strategies that contribute to microbial resilience. Understanding these mechanisms may facilitate the development of novel antibiofilm therapies, environmental biotechnologies, and industrial applications while advancing our knowledge of microbial adaptation and evolution.

Index Terms—Biofilm, EPS (Extracellular Polymeric Substance), Extremophiles, Antibiotic resistance, Chronic infection

I. Introduction

Microorganisms rarely exist as free-living planktonic cells in nature. Instead, the predominant mode of microbial life is within biofilms, highly organized multicellular communities embedded in a self-produced extracellular polymeric substance (EPS) matrix composed primarily of polysaccharides, proteins, lipids, and extracellular DNA (Flemming and Wingender, 2010). Biofilm formation enables microorganisms to adhere to a wide range of biotic and abiotic surfaces, facilitating survival under adverse environmental conditions while promoting collective metabolic activity, intercellular communication, and genetic exchange (Hall-Stoodley et al., 2004). The transition from planktonic growth to a biofilm lifestyle represents a major adaptive strategy that has contributed significantly to microbial persistence and evolution across diverse habitats.

Biofilms are ubiquitous in natural ecosystems, where they play essential roles in nutrient cycling, organic matter degradation, bioremediation, and maintenance of ecological balance. Environmental biofilms

colonize soil particles, aquatic systems, plant surfaces and industrial infrastructures, forming complex multispecies communities that influence global biogeochemical processes (Flemming et al., 2016). Through the establishment of structured microbial consortia, biofilms provide protection against desiccation, predation, nutrient limitation and other environmental stresses, thereby enhancing microbial fitness and ecosystem resilience.

In contrast to their beneficial ecological functions, biofilms also represent a major challenge in human health. Numerous pathogenic bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, form biofilms that contribute to chronic and recurrent infections. Biofilm-associated infections are frequently observed in wounds, dental plaques, respiratory tracts, urinary tracts, and on implanted medical devices such as catheters, prosthetic joints, and heart valves (Lebeaux et al., 2014). Cells embedded within biofilms exhibit enhanced tolerance to antibiotics, disinfectants, and host immune responses, often resulting in treatment failure and increased healthcare burden worldwide.

Beyond conventional environments and host-associated niches, biofilm formation is also a critical survival mechanism in extreme habitats. Extremophilic microorganisms inhabiting high-temperature geothermal springs, hypersaline lakes, acidic and alkaline ecosystems, polar ice regions, and deep-sea hydrothermal vents utilize biofilm formation to withstand severe physicochemical stresses. The EPS matrix provides structural stability, retains moisture and nutrients, and protects cells from fluctuations in temperature, salinity, pH, pressure, radiation, and oxidative stress (Parrilli et al., 2022). These adaptations highlight the remarkable versatility of biofilm formation as a universal microbial survival strategy.

Although extensive studies have independently examined environmental, pathogenic, and extremophilic biofilms, a comprehensive comparison of biofilm-forming bacteria across these distinct habitats remains limited. Therefore, this review aims to provide an integrated overview of biofilm-producing bacteria found in natural environments, human-associated pathogenic systems, and extreme ecosystems. Particular emphasis is placed on the mechanisms of biofilm formation, EPS composition, ecological significance, adaptive strategies, and their implications for environmental sustainability, microbial evolution, and human health.

II. BIOFILM PRODUCING BACTERIA FOUND IN NORMAL ENVIRONMENT

Biofilms are ubiquitous in nature. It is a defence mechanism of bacteria / prokaryotes to survive under harsh environment. It provides a sterile environment for interaction among bacteria. Bacteria found in natural environment may be produce biofilm. Three non-pathogenic bacterial strains found in natural environment likes soil, foods were identified as *seudomonas extremorientalis* (strain Lettuce-28), *Paenibacillus peoriae* (strain Lettuce-7), and *Streptomyces cirratus* (strain Geumsan-207). They can grow rapidly and form biofilms within 24hour on stainless steel and ability to show antimicrobial activity against *Salmonella enterica* (Yoonbin Kim et al., 2017). Though biofilm formation is a harmful process but some non- pathogenic *E.Coli* strains (PHL644, probiotic Nissle 1917, PTEE) produce biofilm which may provide field for biocatalysis for the generation of robust biocatalytic platforms (Leech et al., 2020). In a recent investigation of Raipur, Chattisgarh, India was conducted where strains isolated from public water supply samples are *Bacillus tequilensis* (MN889418) and *Pseudomonas beteli* (MN889419) which are non-pathogenic and produce maximum biofilm in public water supply. But the researchers assume that they could be changed into pathogenic strain due to mutation and can cause disease (Rana and Upadhyay 2023). Though *Listeria monocytogenes* is a ubiquitous pathogen which infects human and have the ability to commonly colonize in food processing environments but the research has focused on 6 non-pathogenic *Listeria* strains from food industry and could develop a future perspective to use of potential non-pathogenic *Listeria* strain biofilm as a biocontrol in food industry and used as an inhibitor for biofilm formation by pathogenic *Listeria* bacteria (Hasani et al., 2021). There is a recent study focused on consortium of

microbial biofilms which are naturally present in soil, can be used as a biofertilizer and support optimal plant growth. Some species among them which found in soils are *R. Leguminosarum*, *B.subtilis*, *Mesorhizobium ciceri* spp., *Aspergillus* spp., *Azotobacter* etc. The biofilm produce by these strains plays a great role towards increases the plant resistance against biotic and abiotic stress, enhance plant growth and quality of crops (Dewi et al., 2023). Another study which emphasized on both bacteria and fungi collected from environmental samples, hospital ward curtains and medical trays. From that bacterial isolates *Staphylococcus aureus* and *Candida albicans* form highest potential to form biofilm with 30.25 % and 23.25% of total biofilm formation respectively and caused major agent of hospital-based infection (Shaheen and Baqai 2016). *Bacillus* and *Paenibacillus* are main biofilm forming bacteria in the soil whose form soil biofilm and after their formation the microbial respiration rate is increased which responded rapidly to the change of nutrient availability (Wu et al., 2019). Clay minerals and metal oxides as important part of soil matrix perform vital roles towards development of microbial communities and development of biofilm of the representative soil bacteria *Bacillus subtilis* (Ma et al., 2017). Soil and plant growth promoting microbes such as *Bacillus*, *Pseudomonas*, *Rhizobium* form biofilms in soil and on plant surface help in plant growth in multiple ways such as nutrients cycling, mineral solubilization, protecting plants against biotic and abiotic stress (Solanki et al., 2020). Some plant root exudates like citric acid from cucumber root attracted *Bacillus subtilis* N₁₁ and fumaric acid from banana attracted *B. amyloliquefaciens* SQR9 biofilm production (Mhlongo et al., 2018). A local study using water samples from aqua guard and pipe lines to kitchen of S.C.B Medical college hostels found 187 isolates. Out of 187 isolates biofilm was detected in 37 isolates in *Acinetobacter* spp., *Klebsiella* spp., and *Pseudomonas* spp. They show strong biofilm producers and can be causes health risk towards the consumption of pipelines water (Mahapatra et al., 2015). State drinking water pipes lined with biofilms affect the water quality by releasing bacteria from those pipes to the water. Water microbiome contains biofilm forming bacteria are including genera identified as *Sphingomonas*, *Nitrospira*, *Mycobacterium*, and *Hyphomicrobium* (Chan et al., 2019)

III. BIOFILM PRODUCING BACTERIA FOUND IN HUMAN BODY AS PATHOGENS

Biofilms plays a major role in the development and persistence of chronic infections by providing a defensive environment for bacteria and facilitating their ability to evade from immune defences and antimicrobial treatment. Antony van Leeuwenhoek was the first who observed and found biofilm by using his primitive microscope on matter from his own teeth. Niels Hoiby was first who demonstrate the role of biofilms towards causing disease (Niels Høiby. 2014). He observed a connecting link between the aetiology of persistent infection and aggregates of bacteria in cystic fibrosis (Høiby. 2017). *Vibrio cholerae*, who is the main causative agent of diarrheal disease is a biofilm producing human pathogenic bacteria. Through, it's biofilm it can efficiently colonizes to intestinal tract. The bacterial cells reside in biofilm is more infectious than free living planktonic form (Tamayo et al., 2010). Another disease-causing pathogen is *Legionella pneumophila* which causes respiratory tract disease. It's a biofilm producing bacterial pathogens found in drinking water system. Its biofilm thought to cause persistent and chronic infection of respiratory disease (Proctor et al., 2018). Bacteria that causes nosocomial disease involved in health care associated infections are gram positive bacteria e.g. *Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus faecalis*, as well as gram negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* (Donlan. 2001). Rhinosinusitis is chronic infection on the nasal mucosal surface caused by biofilm producing bacteria *Staphylococcus aureus* (Foreman and Wormald 2010). Chronic pharyngitis and laryngitis infections in most cases associated with multiple biofilm forming bacteria like *S. aureus*, *H. influenzae*, *C. albicans*, *Propionibacterium acnes*, *Moraxella nonliquefaciens*, *Neisseria meningitidis*, *S.pneumoniae* (Kinnari et al., 2012). In adult CF patients *P.aeruginosa* biofilm produced alginate and provides high viscosity, lower sheer, and nutrient richness to the patient mucosa (Lyczak et al., 2002). *S. aureus* causes urinary tract infections produces strong biofilm and that's why show high resistance to nitrofurantoin, tetracyclin, erythromycin, ciprofloxacin, compared to non-biofilm producing strain (Yousefi

et al., 2016). Several study correlates with biofilm forming uropathogenic *E.Coli* (UPEC) and *S.aureus* with genitourinary tract infections (Karigoudar et al., 2019). Colonization and formation of biofilm of *H.pylori* within gastric mucosa can be linked with variety of digestive tract disease like peptic ulcer disease, corpus predominant gastric and possibly also oesophageal adenocarcinoma (Brown. 2000). Our skin also composed of diverse biofilm producing bacteria. Number of biofilms producing bacteria causes variety of skin wounds are staphylococcal scatted skin syndrome caused by *S. aureus*, chronic ulcers caused by Bacterioides, Clostridium streptococcus and chronic wounds caused by *Pseudomonas aruginosa*, acne vulgaris caused by *P.acne* (Brandwein et al., 2016). Otitis Media is an infection occurs in middle cavity of ear. It may chronic or acute. The common Acute otitis media including non-typeable *H.influenzae*, *S.pneumoniae*, *M. catterhalis* were identified in the middle year of otitis prone children. Mixed bacterial infections allergic rhinitis and biofilm seems to be associated with otitis prone children (Ioannidis et al., 2023). The majority of bacteria species in both COME (chronic media with effusion) and RAOM (recurrent acute otitis media) cases seemed to produce biofilms. Total isolated strains 83% positive for biofilm production on silicon discs. Chronic rhinosinestis (CRS) is a debilitating condition characterized by long lasting inflammation of the paranasal sinuses. Bacterial infections and biofilms have emerged as a key contributor to the chronicity of sinusitis. Microbial interplay between *S.aureus* and *P.aeruginosa* and their interactions interaction in chronic infections influenced disease progression (Vanderpool and Rumbaugh 2023). Micrococcus species which usually contaminates from skin and mucous membrane and rarely cause infectious disease have capacity to form biofilm in prosthetic material and hence cause disease like prosthetic valve endocarditis (Rodriguez-Nava et al., 2020). Treatment of chronic bacterial prostatitis is complicated due to presence of multi drug resistance bacteria ESBL (Extended – spectrum beta lactamase) producing *E. coli* biofilms and shifts in bacterial etiology (Xiong et al., 2020). In case of vaginal biofilm producing bacteria *G. vaginalis* as the initial colonizer that provides scaffold to other bacteria as secondary colonizers attach in order to established mature biofilm. *A.vaginae* acts as secondary colonizers and also linked to bacterial vaginosis (Hardy et al., 2016).

IV. BIOFILM PRODUCING EXTREMOPHILES

Extremophiles is an organism that is able to live in extreme environment. Bacteria and archaea can inhabit a variety of extreme environment such as radiation, oxidation, desiccation, alkalinity, acidity, salinity, high pressure etc. Depending on their extreme condition tolerance, such extremophile bacteria are called thermophiles, hyperthermophiles, psychrophiles, halophiles, alkaliphiles, acidophiles, piezophiles, or radiophiles. They have developed compatible genetic and defence mechanisms through evolution, which allow them to adapt to such extreme conditions (Mehta and Satyanarayana. 2017).

Biofilm producing thermophilic bacteria:

Microorganism who found in higher temperature are known as thermophiles (growth temperature 45–75 °C) (Luke et al., 2007). Thermophiles that producing biofilms are found mainly in marine and terrestrial hot spring environment. In this environment bacteria experience high pressure, high temperature and high concentrations of toxic elements. That's why they produce more and more EPS (Chaudhari et al., 2014). A highly thermostable EPS, which can hydrolyze only at 280 °C, is produced by a bacterium *G. tepidamans* residing in a terrestrial hot spring. EPS producer thermophilic bacteria mainly found in hot marine shallow vents are *Geobacillus thermodenitrificans*, *Geobacillus tepidamans*, *Bacillus thermoantarcticus*, and *Bacillus licheniformis* (Kambourova et al., 2009). A study of 692 strains collected from deep sea hydrothermal vents of IFREMER (French Research Institute for Exploitation of the Sea) 38 strains were selected for EPS production at laboratory scale Among 38 strains selected for EPS production seven strains secreted only limited amounts of EPS. Therefore, further chemical and physico-chemical analyses were carried out on 31 EPS recovered with higher yields. In parallel, identification of the EPS-producing strains was performed based on 16S rRNA gene analyses (Zykwinska et al., 2019). Glc,

GalA, GlcA, GalNAc and GlcNAc were also the main constituents of the EPS secreted by a marine bacterium, *Vibrio neocaledonicus*. (Chalkiadakis et al., 2013). Another study was performed where thermophilic *Bacillus haynesii* CamB6, Species isolated from Chilean Hot Spring was studied for the formation of different stress response molecules on exposure to temperatures ranges from (50-60 °C), PH (5-7) and sources of nitrogen and carbon (1-5gL⁻¹) the bacteria responded with a biofilm network formation in hydrophobic polystyrene surface and also fed-batch growth condition. Under stress condition the cellular morphology significantly changed being smaller in size and in network of eps matrix formation (Marin-Sanhueza et al., 2022). *Geobacillus* sp. WSUCF1 is a Gram +ve spore forming thermophilic bacterium isolated from soil sample. This study indicates the strain WSUCF has a thermophilic EPS producer for a broad range of industrial application. Also, this study focuses on EPS biosynthesis and transportation (Wang et al., 2019). Another study depicts production and characterization of EPS produced by *Staphylococcus* sp BSP3, isolated from a Chilean hot spring san pedro as a thermotolerant, antioxidant which can provide a large set of applications as a potential biotechnological additive (Banerjee et al., 2024). An old research based on thermophilic strain isolated sea sand at Maronti, near Sant' Angelo (Ischia) grows at optimal temperature 60°C at pH 7 thermophilic bacterium named strain 4004 produces exocellular polysaccharides (EPS) in yields. Higher EPS yield was obtained from sole carbon source with sucrose or tetrahalose. They found 3 EPS fractions EPS1, EPS2, EPS3 after purifications. Quantitative monosaccharides analysis revealed the presence of mannose:glucose:galactose in relative ratio of 0.5:1.0:0.3 in EPS1. mannose:glucose:galactose in a relative ratio of 1.0:0.3:trace in EPS2, and galactose:mannose:glucosamine:arabinose in a relative ratio of 1.0:0.8:0.4:0.2 in EPS3. It has been hypothesized that the production of EPS by thermophilic marine bacterium could serve to form a boundary between the bacterial cell and its immediate environment (Schiano et al., 2003).

Biofilm producing halophilic bacteria:

Halophile is an extremophilic bacteria that lives in high salt concentrations. For the protection of cell membrane integrity from the saline environment and tonicity breakdown from osmotic pressure, halophilic bacteria have EPS capsules encasing them (Kunte. 2006). *Halomonas eurihalina*, a moderately halophilic bacterium, produces an anionic EPS with industrial applications (Llamas. 2002). *H. eurihalina* produced an exopolysaccharide which Chemical analysis suggested as major components rhamnose, glucose and mannose, with a higher content of uronic acids, acetyl and sulphate groups (Bejar et al., 1996). *Halomonas* sp. OKOH was isolated from the bottom sediment of Algoa Bay of South Africa. The bacterium was reported to produce an exopolysaccharide. The phenol-sulphuric acid method revealed a high carbohydrate content, suggesting the polysaccharidic nature of EPS (Mabinya et al, 2011). Another halophilic bacterium is *Halomonas* spp. AAD6 (JCM15723) which produces EPS in its growth medium. Levans are polymers constituted by β (2->6) fructo-furanosyl residue (Poli et al., 2009). This Levan has antibacterial, anti-cancer and antioxidant property with probiotic potential (Byun et al., 2014). *Halomonas alkaliantarctica* strain CRSS, isolated from salt sediments near the Salt Lake in Cape Russell in Antarctica, has been shown to produce different EPSs when grown in different condition. the glycosyl composition of EPS indicated the presence of neutral polysaccharide (Poli et al., 2004).

Biofilm producing psychrophilic bacteria:

The bacteria which can grow and multiply in cold environment within temperatures ranges from 15°C or lower than that are known as Psychrophiles. In order to face extreme cold temperature. Dairy origin psychrophilic bacteria strain (previously identified from Chinese raw milk). They are *Pseudomonas azotoformans*, *Serratia quinivorans* and *Pseudomonas libanensis*. Biofilm formation by psychrotrophic bacteria is of particular concern because of their ability to form biofilms on the surface of milk storage and transportation tankers with subsequent production of spoilage enzymes within biofilms leading to raw

milk contamination (Yuan et al., 2018). *Pseudomonas* sp. BGI-2 is a psychrotrophic bacterium isolated from the ice sample collected from Batura glacier, Pakistan. In this study the researchers have optimized the growth and production of exopolysaccharides by cold adapted *Pseudomonas* spp BGI-2 using different nutritional and environmental condition. The strain able to grow in 4-35°C temperatures, pH 5-11 and salt concentration 1-5%. The preferable carbon sources for growth and EPS production are glucose, galactose, mannose, mannitol, glycerol. And this EPS prove to major cryo-protection for a mesophilic bacteria *E. coli* K12 comparable to glycerol. Increased EPS production at low temperatures, freeze thaw tolerance of the EPS producing strain, and increased survivability of *E. coli* in the presence of EPS as cryoprotective agent supports the hypothesis that EPS production is a strategy for survival in extremely cold environments such as the glacier ice (Ali et al., 2020). *Pseudomonas* sp. ID1 is a cold-adapted bacterium isolated from a marine sediment sample collected from South Shetland Islands (Antarctica) that is noted for the highly mucous appearance of its colonies. The researchers characterized an exopolysaccharide (EPS) produced by this strain, which is mainly composed of glucose, galactose and fucose, and has a molecular mass higher than 2×10^6 Da. And also studied its potential biotechnological applications as an emulsifier and cryoprotectant agent (Carrión et al., 2015). Old research conducted with collecting the strain from sea water and sea ice in the Southern Ocean. The EPS producing bacterial strain which belongs to genus *Pseudoaltermonas*. This strain produces EPS 30 folds higher at -2 to 10°C than at 20°C. This EPS composed of primarily neutral sugar and uronic acid with sulfate (Mancuso Nichols et al., 2004). Another Psychrotolerant bacterium isolated from deep sea sediments is *Pseudoalteromonas* sp. SM9913. This work studied the structural characterization and ecological roles of the exopolysaccharide (EPS) secreted by this strain. The EPS production by this strain increased when the temperature decrease. This EPS could enhance the stability of the cold-adapted protease MCP-01 secreted by the same strain through preventing its autolysis. It could bind many metal ions, including Fe²⁺, Zn²⁺, Cu²⁺, Co²⁺ (Qin et al., 2007). EPS produced by psychrophile *Pseudoalteromonas* sp. has been reported to be highly relevant for metals and radionuclide biosorption (Caruso et al., 2018). A recent study focused on the exploration of microbial communities found in extreme cold environment of passu and pisan glaciers of Pakistan. From 25 initially screened strains five were found to be produce EPS. Out of five one strain CUI-P1 displaying highest yield of EPS production which has both cryoprotectant and emulsifying activity and can be used in biotechnological industry (Ferheen et al., 2023).

V. CONCLUSION

Biofilm formation represents one of the most successful and evolutionarily conserved microbial survival strategies, enabling bacteria to colonize and persist in a remarkable diversity of habitats ranging from natural ecosystems and host-associated environments to some of the most extreme conditions on Earth. Despite the vast differences in environmental pressures encountered by environmental, pathogenic, and extremophilic microorganisms, biofilm formation remains a common adaptive mechanism that enhances cellular protection, nutrient acquisition, intercellular communication, and long-term survival. The extracellular polymeric substance (EPS) matrix serves as the structural and functional foundation of biofilms, providing resistance against physical, chemical, and biological stresses while facilitating community-level interactions.

In natural environments, biofilm-forming bacteria contribute significantly to nutrient cycling, ecosystem stability, and biogeochemical processes. Conversely, in human-associated systems, biofilms are major contributors to chronic infections, antimicrobial resistance, and treatment failure, posing substantial clinical and economic challenges. Extremophilic biofilms further demonstrate the extraordinary adaptability of microbial life, revealing unique mechanisms that permit survival under extreme temperature, salinity, pH, pressure, radiation, and nutrient-limited conditions. Comparative analysis across these diverse habitats highlights both conserved and habitat-specific biofilm formation strategies, emphasizing the dynamic relationship between microbial communities and their surrounding environments.

Recent advances in genomics, transcriptomics, proteomics, metabolomics, and imaging technologies have greatly expanded our understanding of biofilm architecture, regulation, and function. However, significant knowledge gaps remain regarding the molecular mechanisms governing biofilm adaptation, interspecies interactions, EPS heterogeneity, and stress-response pathways across diverse ecological niches. Future studies integrating multi-omics approaches, systems biology, and artificial intelligence-driven analyses will be essential for unraveling the complexity of biofilm ecosystems. Such insights may facilitate the development of innovative antibiofilm therapies, improved environmental biotechnologies, and novel bio-inspired materials derived from extremophilic microorganisms.

Overall, understanding biofilm-producing bacteria across environmental, clinical, and extreme habitats provides a holistic perspective on microbial adaptation and resilience. Continued exploration of these microbial communities will not only advance fundamental microbiology but also contribute to addressing critical challenges in medicine, industry, environmental sustainability, and biotechnology.

References

- [1] Flemming HC, Wingender J. 2010. The biofilm matrix. *Nature Reviews Microbiology* 8:623–633.
- [2] Hall-Stoodley L, Costerton JW, Stoodley P. 2004. Bacterial biofilms: From the natural environment to infectious diseases. *Nature Reviews Microbiology* 2:95–108.
- [3] Flemming HC, Wingender J, Szewzyk U, et al. 2016. Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology* 14:563–575.
- [4] Lebeaux D, Ghigo JM, Beloin C. 2014. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiology and Molecular Biology Reviews* 78:510–543.
- [5] Parrilli E, Tutino ML, Marino G. 2022. Biofilm as an adaptation strategy to extreme conditions. *Rendiconti Lincei. Scienze Fisiche e Naturali* 33:527–536.
- [6] Kim Y, Kim H, Beuchat LR, Ryu JH. Development of non-pathogenic bacterial biofilms on the surface of stainless steel which are inhibitory to *Salmonella enterica*. *Food Microbiol.* 2018 Feb;69:136-142. doi: 10.1016/j.fm.2017.08.003. Epub 2017 Aug 13. PMID: 28941894.
- [7] Leech, J., Golub, S., Allan, W. et al. Non-pathogenic *Escherichia coli* biofilms: effects of growth conditions and surface properties on structure and curli gene expression. *Arch Microbiol* 202, 1517–1527 (2020). <https://doi.org/10.1007/s00203-020-01864-5>
- [8] Rana S, Upadhyay LSB. Utilization of non-pathogenic bacteria to obtain optimum biofilm production for beneficial applications. *Braz J Microbiol.* 2023 Sep;54(3):1875-1883. doi: 10.1007/s42770-023-01044-9. Epub 2023 Jun 30. PMID: 37389797; PMCID: PMC10484866.
- [9] Hasani, E., Labidi, S., Mohácsi-Farkas, C. and Kiskó, G., 2021. Comparison of biofilm formation between non-pathogenic *Listeria* strains under different stress conditions. *Progress in Agricultural Engineering Sciences, 16(S2)*, pp.73-80.
- [10] Dewi, W.S., Amalina, D.D. and Romadhon, M.R., 2023, April. Microbial Biofilm for Soil Health, Plant Growth, and Productivity under Multi Stress. A Review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1162, No. 1, p. 012008). IOP Publishing.
- [11] Shaheen, A. and Baqai, R., 2016. Biofilm formation by environmental microbes isolated from hospitals in Karachi, Pakistan. *American scientific research journal for engineering, technology, and sciences, 15(1)*, pp.240-251.

- [12] Wu Y, Cai P, Jing X, Niu X, Ji D, Ashry NM, Gao C, Huang Q. Soil biofilm formation enhances microbial community diversity and metabolic activity. *Environ Int.* 2019 Nov;132:105116. doi: 10.1016/j.envint.2019.105116. Epub 2019 Sep 1. PMID: 31479959.
- [13] Ma, W., Peng, D., Walker, S.L., Cao, B., Gao, C.H., Huang, Q. and Cai, P., 2017. *Bacillus subtilis* biofilm development in the presence of soil clay minerals and iron oxides. *npj Biofilms and Microbiomes*, 3(1), p.4.
- [14] Solanki, M.K., Solanki, A.C., Kumari, B., Kashyap, B.K. and Singh, R.K., 2020. Plant and soil-associated biofilm-forming bacteria: Their role in green agriculture. In *New and future developments in microbial biotechnology and bioengineering: Microbial biofilms* (pp. 151-164). Elsevier.
- [15] Mhlongo, M.I., Piater, L.A., Madala, N.E., Labuschagne, N. and Dubery, I.A., 2018. The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Frontiers in plant science*, 9, p.314703.
- [16] Mahapatra A, Padhi N, Mahapatra D, Bhatt M, Sahoo D, Jena S, Dash D, Chayani N. Study of biofilm in bacteria from water pipelines. *J Clin Diagn Res.* 2015 Mar;9(3):DC09-11. doi: 10.7860/JCDR/2015/12415.5715. Epub 2015 Mar 1. PMID: 25954617; PMCID: PMC4413065.
- [17] Chan, S., Pullerits, K., Keucken, A. *et al.* Bacterial release from pipe biofilm in a full-scale drinking water distribution system. *npj Biofilms Microbiomes* 5, 9 (2019). <https://doi.org/10.1038/s41522-019-0082-9>
- [18] Niels Høiby, A personal history of research on microbial biofilms and biofilm infections, *Pathogens and Disease*, Volume 70, Issue 3, April 2014, Pages 205–211, <https://doi.org/10.1111/2049-632X.12165>
- [19] Høiby, N., 2017. A short history of microbial biofilms and biofilm infections. *Apmis*, 125(4), pp.272-275.
- [20] Tamayo R, Patimalla B, Camilli A. Growth in a biofilm induces a hyperinfectious phenotype in *Vibrio cholerae*. *Infect Immun.* 2010 Aug;78(8):3560-9. doi: 10.1128/IAI.00048-10. Epub 2010 Jun 1. PMID: 20515927; PMCID: PMC2916270.
- [21] Proctor, C.R., Reimann, M., Vriens, B. and Hammes, F., 2018. Biofilms in shower hoses. *Water research*, 131, pp.274-286.
- [22] Donlan, R.M., 2001. Biofilms and device-associated infections. *Emerging infectious diseases*, 7(2), p.277.
- [23] Foreman, A. and Wormald, P.J., 2010. Different biofilms, different disease? A clinical outcomes study. *The Laryngoscope*, 120(8), pp.1701-1706.
- [24] Kinnari TJ, Lampikoski H, Hyyrynen T, Aarnisalo AA. Bacterial Biofilm Associated With Chronic Laryngitis. *Arch Otolaryngol Head Neck Surg.* 2012;138(5):467–470. doi:10.1001/archoto.2012.637
- [25] Lyczak, J.B., Cannon, C.L. and Pier, G.B., 2002. Lung infections associated with cystic fibrosis. *Clinical microbiology reviews*, 15(2), pp.194-222.
- [26] Yousefi, M., Pourmand, M.R., Fallah, F., Hashemi, A., Mashhadi, R. and Nazari-Alam, A., 2016. Characterization of *Staphylococcus aureus* biofilm formation in urinary tract infection. *Iranian journal of public health*, 45(4), p.485.

- [27] Karigoudar, R.M., Karigoudar, M.H., Wavare, S.M. and Mangalgi, S.S., 2019. Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. *Journal of laboratory physicians*, 11(01), pp.017-022.
- [28] Brown, L.M., 2000. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiologic reviews*, 22(2), pp.283-297.
- [29] Brandwein, M., Steinberg, D. and Meshner, S., 2016. Microbial biofilms and the human skin microbiome. *NPJ biofilms and microbiomes*, 2(1), p.3.
- [30] Ioannidis, A., Chatzipanagiotou, S., Vassilaki, N., Giannakopoulos, P., Hatzaki, D., Magana, M., Sachlas, A., Mpekoulis, G., Radiotis, A., Tsakanikos, M. and Tzanakaki, G., 2023. Biofilm-Forming Bacteria Implicated in Complex Otitis Media in Children in the Post-Heptavalent Pneumococcal Conjugate Vaccine (PCV7) Era. *Microorganisms*, 11(3), p.545.
- [31] Vanderpool, E.J. and Rumbaugh, K.P., 2023. Host-microbe interactions in chronic rhinosinusitis biofilms and models for investigation. *Biofilm*, p.100160.
- [32] Rodriguez-Nava, G., Mohamed, A., Yanez-Bello, M.A. and Trelles-Garcia, D.P., 2020. Advances in medicine and positive natural selection: Prosthetic valve endocarditis due to biofilm producer *Micrococcus luteus*. *IDCases*, 20, p.e00743.
- [33] Xiong, S., Liu, X., Deng, W., Zhou, Z., Li, Y., Tu, Y., Chen, L., Wang, G. and Fu, B., 2020. Pharmacological interventions for bacterial prostatitis. *Frontiers in pharmacology*, 11, p.504.
- [34] Hardy, L., Jaspers, V., Abdellati, S., De Baetselier, I., Mwambarangwe, L., Musengamana, V., Van De Wijgert, J., Vanechoutte, M. and Crucitti, T., 2016. A fruitful alliance: the synergy between *Atopobium vaginae* and *Gardnerella vaginalis* in bacterial vaginosis-associated biofilm. *Sexually transmitted infections*, 92(7), pp.487-491.
- [35] Mehta, D. and Satyanarayana, T., 2017. Functional genomics of the extremophilic bacteria and archaea. In *Current Developments in Biotechnology and Bioengineering* (pp. 45-78). Elsevier.
- [36] Luke, K.A., Higgins, C.L. and Wittung-Stafshede, P., 2007. Thermodynamic stability and folding of proteins from hyperthermophilic organisms. *The FEBS journal*, 274(16), pp.4023-4033.
- [37] Chaudhari, R., Hajoori, M., Suthar, M., Desai, S. and Bharthana-Vesu, S., 2017. Isolation, screening and characterization of marine bacteria for exopolysaccharide production. *Biosci Discov*, 8, pp.643-9.
- [38] Kambourova, M., Mandeva, R., Dimova, D., Poli, A., Nicolaus, B. and Tommonaro, G., 2009. Production and characterization of a microbial glucan, synthesized by *Geobacillus tepidamans* V264 isolated from Bulgarian hot spring. *Carbohydrate Polymers*, 77(2), pp.338-343.
- [39] Zykwiniska, A., Marchand, L., Bonnetot, S., Sinquin, C., Collic-Jouault, S. and Delbarre-Ladrat, C., 2019. Deep-sea hydrothermal vent bacteria as a source of glycosaminoglycan-mimetic exopolysaccharides. *Molecules*, 24(9), p.1703.
- [40] Chalkiadakis, E., Dufourcq, R., Schmitt, S., Brandily, C., Kervarec, N., Coatanea, D., Amir, H., Loubersac, L., Chanteau, S., Guezennec, J. and Dupont-Rouzeyrol, M., 2013. Partial characterization of an exopolysaccharide secreted by a marine bacterium, *Vibrio neocaledonicus* sp. nov., from New Caledonia. *Journal of Applied Microbiology*, 114(6), pp.1702-1712.

- [41] Marin-Sanhueza, C., Echeverria-Vega, A., Gomez, A., Cabrera-Barjas, G., Romero, R. and Banerjee, A., 2022. Stress dependent biofilm formation and bioactive melanin pigment production by a thermophilic *Bacillus* species from Chilean hot spring. *Polymers*, 14(4), p.680.
- [42] Wang, J., Goh, K.M., Salem, D.R. and Sani, R.K., 2019. Genome analysis of a thermophilic exopolysaccharide-producing bacterium-*Geobacillus* sp. WSUCF1. *Scientific reports*, 9(1), p.1608.
- [43] Banerjee, S., Cabrera-Barjas, G., Tapia, J., Fabi, J.P., Delattre, C. and Banerjee, A., 2024. Characterization of Chilean hot spring-origin *Staphylococcus* sp. BSP3 produced exopolysaccharide as biological additive. *Natural Products and Bioprospecting*, 14(1), pp.1-16.
- [44] Schiano Moriello, V., Lama, L., Poli, A., Gugliandolo, C., Maugeri, T.L., Gambacorta, A. and Nicolaus, B., 2003. Production of exopolysaccharides from a thermophilic microorganism isolated from a marine hot spring in flegrean areas. *Journal of Industrial Microbiology and Biotechnology*, 30(2), pp.95-101.
- [45] Kunte, H.J., 2006. Osmoregulation in bacteria: compatible solute accumulation and osmosensing. *Environmental Chemistry*, 3(2), pp.94-99.
- [46] Llamas, I., Sánchez, M.J., Argandona, M., Béjar, V., Quesada, E. and Moral, A.D., 2002. Analysis of the genome of the moderate halophile *Halomonas eurihalina*. *Current microbiology*, 45, pp.233-239.
- [47] Bejar, V., Calvo, C., Moliz, J., Diaz-Martinez, F. and Quesada, E., 1996. Effect of growth conditions on the rheological properties and chemical composition of *Volcaniella eurihalina* exopolysaccharide. *Applied biochemistry and biotechnology*, 59, pp.77-86.
- [48] Mabinya, L.V., Cosa, S., Mkwetshana, N. and Okoh, A.I., 2011. *Halomonas* sp. OKOH—A marine bacterium isolated from the bottom sediment of Algoa Bay—Produces a polysaccharide bioflocculant: Partial characterization and biochemical analysis of its properties. *Molecules*, 16(6), pp.4358-4370.
- [49] Poli, A., Kazak, H., Gürleyendağ, B., Tommonaro, G., Pieretti, G., Öner, E.T. and Nicolaus, B., 2009. High level synthesis of levan by a novel *Halomonas* species growing on defined media. *Carbohydrate polymers*, 78(4), pp.651-657.
- [50] Byun, B.Y., Lee, S.J. and Mah, J.H., 2014. Antipathogenic activity and preservative effect of levan (β -2, 6-fructan), a multifunctional polysaccharide. *International journal of food science & technology*, 49(1), pp.238-245.
- [51] Poli, A., Moriello, V.S., Esposito, E., Lama, L., Gambacorta, A. and Nicolaus, B., 2004. Exopolysaccharide production by a new *Halomonas* strain CRSS isolated from saline lake Cape Russell in Antarctica growing on complex and defined media. *Biotechnology letters*, 26, pp.1635-1638.
- [52] Yuan, L., Burmølle, M., Sadiq, F.A., Wang, N. and He, G., 2018. Interspecies variation in biofilm-forming capacity of psychrotrophic bacterial isolates from Chinese raw milk. *Food Control*, 91, pp.47-57.
- [53] Ali, P., Shah, A.A., Hasan, F., Hertkorn, N., Gonsior, M., Sajjad, W. and Chen, F., 2020. A glacier bacterium produces high yield of cryoprotective exopolysaccharide. *Frontiers in Microbiology*, 10, p.466434.

- [54] Carrión, O., Delgado, L. and Mercade, E., 2015. New emulsifying and cryoprotective exopolysaccharide from Antarctic *Pseudomonas* sp. ID1. *Carbohydrate polymers*, 117, pp.1028-1034.
- [55] Mancuso Nichols, C.A., Garon, S., Bowman, J.P., Raguénès, G. and Guezennec, J., 2004. Production of exopolysaccharides by Antarctic marine bacterial isolates. *Journal of Applied Microbiology*, 96(5), pp.1057-1066.
- [56] Qin, G., Zhu, L., Chen, X., Wang, P.G. and Zhang, Y., 2007. Structural characterization and ecological roles of a novel exopolysaccharide from the deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913. *Microbiology*, 153(5), pp.1566-1572.
- [57] Caruso, C., Rizzo, C., Mangano, S., Poli, A., Di Donato, P., Nicolaus, B., Di Marco, G., Michaud, L. and Lo Giudice, A., 2018. Extracellular polymeric substances with metal adsorption capacity produced by *Pseudoalteromonas* sp. MER144 from Antarctic seawater. *Environmental Science and Pollution Research*, 25, pp.4667-4677.
- [58] Ferheen, I., Ahmed, Z., Alonazi, W.B., Pessina, A., Ibrahim, M., Pucciarelli, S. and Bokhari, H., 2023. Diverse Repertoire and Relationship of Exopolysaccharide Genes in Cold-Adapted *Acinetobacter* sp. CUI-P1 Revealed by Comparative Genome Analysis. *Microorganisms*, 11(4), p.885.