National Institute of Technology Rourkela

Synopsis Seminar

Seminar Title	: Role of functional amyloid and nucleotide second messenger in the marine bacterium Pseudomonas aeruginosa PFL-P1 for biodegradation of polycyclic aromatic hydrocarbon
Speaker	: Swetambari Kumari (Rollno : 518ls1002)
Supervisor	: Prof. Surajit Das
Venue	: Life Science Seminar Room
Date and Time	: 10 Jan 2025 (04:30 PM)

Abstract : Increasing levels of polycyclic aromatic hydrocarbons (PAHs) in the environment contribute significantly to pollution, posing a serious ecological challenge. Microbial biofilm-mediated bioremediation has proven to be a promising approach for mitigating PAH contamination in ecosystems. Among the components of the biofilm matrix, amyloid, a proteinaceous constituent of extracellular polymeric substances (EPS), plays a crucial role in enhancing the structural integrity and resilience of biofilms. The thesis illustrates the interaction of functional amyloid in *Pseudomonas* (Fap) with pyrene, a commonly encountered PAH. *Pseudomonas aeruginosa* PFL-P1, a marine biofilm-forming bacterium, showed strong tolerance (2200 ppm) and chemotaxis toward pyrene, indicating its potential for bioremediation in PAH-contaminated environments. The production of amyloid by *P. aeruginosa* PFL-P1 was confirmed through Congo red (CR) assay, thioflavin T (ThT) staining, and amplification of the *fapC* gene. The expression of *fapC* was upregulated six fold *(p<0.0001*) when pyrene was used as the sole carbon source. Molecular docking simulations revealed a strong binding affinity between FapC and pyrene, with a binding energy of -6.75 kcal/mol, indicating a robust interaction. Confocal laser scanning microscopy (CLSM) further demonstrated a significant increase in amyloid production during biofilm formation by *P. aeruginosa* PFL-P1 in the presence of pyrene (*p<0.0001*). The elevated expression of *fapC* and the hydrophobic interactions between FapC and PAH compounds indicate the critical role of Fap in PAH binding, facilitating their subsequent degradation. The study also explored the role of cyclic di-GMP (c-di-GMP), a bacterial secondary messenger that plays a central role in regulating biofilm formation and stability. *P. aeruginosa* PFL-P1 showed remarkable adaptability to varying environmental conditions, thriving under neutral to slightly acidic pH (5-7), low salinity (1-3%), and moderate temperatures (30-37°C). This adaptability is attributed to the presence of diguanylate cyclase (*dgc*) and phosphodiesterase (*pde)* genes, which regulate c-di-GMP synthesis and degradation. Gene expression analysis revealed a coordinated regulatory network of diverse genes during biofilm development. The genes responsible for c-di-GMP regulation (*dgc*, *pde*), functional amyloid synthesis (*fapC*), quorum sensing (*lasI* and *rhlI)*, and hydrocarbon degradation (*nahAc*) in *P. aeruginosa* PFL-P1 showed significant upregulation at 48 h, indicating this time point as the matured stage of biofilm development. At pH 4, the expression of the five genes except *pde* exhibited a strong adaptive response to acidic stress (*p<0.0001*). Gene expression stabilized from pH 5 to 9, reflecting optimal functional conditions. Low salinity (&le1%) triggered elevated expression of all six genes, supporting biofilm development, quorum sensing, and PAH degradation, whereas salinity above 5% caused a decline in expression due to osmotic stress (*p<0.0001*). At 40°C, upregulation of *dgc* (*p=0.0457*) and *fapC* (*p=0.0444*) indicated temperature-specific regulation favoring biofilm stability. Exposure to higher pyrene concentrations (100 ppm) induced significant upregulation of *dgc* (*p=0.0121*) , *lasI* (*p=0.0015*) , *rhlI* (*p=0.0005*), and *nahAc* (*p=0.0020*), promoting biofilm formation and hydrocarbon degradation while suppressing *pde* to enhance biofilm resilience. The addition of 1 µg/mL of C4-HSL and 3OC12-HSL upregulated *dgc*, *pde*, *fapC*, and *nahAc*, highlighting the role of quorum sensing role in biofilm formation and pyrene degradation (*p<0.0001*). Conversely, terrein, a quorum sensing and c-di-GMP inhibitor, downregulated *fapC* and *nahAc*, disrupting biofilm formation and PAH degradation (*p<0.0001*). The coordinated expression of *dgc* and *fapC* was strongly associated with intracellular c-di-GMP levels and amyloid production, demonstrating their critical roles in biofilm resilience under diverse environmental stressors. The positive correlation between c-di-GMP levels and amyloid percentages, along with the high binding energy of -11.8 kcal/mol between c-di-GMP and FapC, highlights a dual role for c-di-GMP as both a signaling molecule and a molecular chaperone facilitating amyloid fibril assembly. This study also reported the characterization of Fap fibrils from *P. aeruginosa* PFL-P1 and their interaction with pyrene, further assessing the impact on pyrene degradation. Overexpression of *fap* in *E. coli* BL21(DE3) cells enhanced biofilm formation (*p<0.0001*) and amyloid production (*p=0.0002*), particularly in the presence of pyrene. Defibrillated Fap analysis revealed FapC monomers, with increased fibrillation upon exposure to pyrene. Circular Dichroism (CD), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) confirmed characteristic amyloid peaks and structural changes in Fap fibrils upon pyrene exposure. Three-dimensional excitation-emission matrix (3D-EEM) analysis identified a protein-like fluorophore in Fap fibrils that exhibited fluorescence quenching upon pyrene binding. The binding constants ranged from 5.23 to 7.78 $M^{\&minus1}$, with a &DeltaG of -5.10 kJ/mol at 298K, indicating a spontaneous, exothermic interaction primarily driven by hydrophobic forces. Exogenous Fap fibrils significantly enhanced biofilm growth and pyrene degradation by *P. aeruginosa* PFL-P1, increasing the degradation rate from 46% to 64% within seven days (*p=0.0236*). Gas chromatography mass spectrometry (GC-MS) analysis identified various metabolites, suggesting the involvement of the phthalic acid pathway in pyrene degradation. This study provides a deeper understanding of the structural dynamics of Fap fibrils in response to pyrene exposure and offers potential applications in environmental bioremediation, particularly for the degradation of PAHs.