Session: Emerging technologies

Are surface (S-) layer proteins public good biofilm matrix exopolymers?

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Extracellular polymeric substances (EPS) are the essence of biofilms, yet the identity and role of specific EPS in biofilm formation, particularly for environmental biofilms that are non-culturable, are largely unknown. Using an ionic liquid, we demonstrate the value of extracting and isolating the most abundant extracellular protein from an anaerobic ammonium oxidation (anammox) biofilm. This enabled single-molecule and immunological approaches to resolve structure-function relationships, and address questions regarding the role in biofilm ecology of an intractable exoprotein sequentially similar to an surface (S-) layer protein with intrinsically disordered repeats that enable it to phase separate into liquid condensates. We demonstrate that these condensates can support cellular adhesion. By immunofluorescence, we observed the anammox extracellular protein to form envelopes around anammox bacteria, confirming it as an S-layer protein. However, the protein was also localized at the edge of biofilm and away from anammox bacterial cells, providing the strongest evidence yet that S-layer proteins can also contribute to biofilm extracellular matrix formation. A polygonal scaffold of a filamentous Chloroflexi (i.e. a heterotroph) enveloped and provided structural support for the anammox bacteria. There was a strong co-association between the anammox S-layer protein and the Chloroflexi, particularly towards the biofilm surface. These Chloroflexi were coated in sugars, with S-layer protein the likely substrate for gluconeogenesis. One possible explanation is that the S-layer protein is transported through the matrix as an attractant for the Chloroflexi, which it relies on to build the biofilm scaffold. This is the first study, to the authors' best knowledge, to characterize function and spatial distribution of an extracellular (i.e. S-layer) protein within a mixed species environmental biofilm. We submit that the S-layer protein can moonlight as an EPS and contribute to coordinating the assembly of a bacterial biofilm framework that supports key syntrophic relationships among an anammox microbial community.