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Desiccation Survival/Resiliency of Natural Biofilms Enhanced by EPS Matrix-evidence of Organic Glass Formation

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Natural microbial communities across many Earth environments are faced with transient desiccation (*i.e*., water limitation). Desiccation compromises the functioning of biomolecules and can be lethal to cells. It is intriguing, therefore, that complex biofilm communities in terrestrial soils, fringes of aquatic environments, desert crusts and extreme environments such as the microbial mats of hypersaline ponds, survive desiccation. Also, upon rehydration, they can quickly (min-hrs) revive cellular activities and even complex community interactions.

Microbial mats are thick (cm) biofilms having complex community interactions. They possess costly reservoirs of extracellular biomolecules such as *e*-enzymes, signaling molecules, antibiotics, vesicles and plasmids, that are localized in the EPS at any given time. To resume community activities, following desiccation, cells would therefore need to resynthesize these extracellular biomolecules- a costly metabolic process. Alternatively, these extracellular biomolecules could be preserved localized in EPS during dessication, then released in an active state upon rehydration. Some biofilm systems (*e.g*, in desert crusts) face desiccation/rehydration on a diel (day/night) cycle. Others, such as hypersaline mats can remain desiccated for months and are then rehydrated with a change of seasons.

Examinations of hypersaline mat communities are lending insight into how complex communities and their (extracellular-mediated) interactions can survive relatively intact during prolonged desiccation. Since EPS are abundant and protective components of natural biofilms (mats), it follows that certain of these components change in response to stressful conditions. Typical EPS components are carbohydrates but also proteins such as amyloids, nucleic acids, which can be structural in their roles, and lipids. Following desiccation and rehydration, approx. 80-85% of cells in our mats were found to have intact cell membranes, and most were encapsulated, as determined by TEM. Information signaling cues, called acyl-homoserine lactones (AHLs), were shown to be active and extractable, immediately following rehydration, suggesting they remained intact during the previous dehydration period. AHLs help to coordinate group activities within/between species. We initially focused on Trehalose (Tre), a Glu-Glu disaccharide that was found to be a common component in mat EPS. Natural mats exhibited glass (Tg) and thermogravitometric (TGA) phase-transitions consistent with α,α-Tre-glass. Tre is a molecule that attaches to other biomolecules during desiccation to maintain their intact steric conformations and prevent denaturation. Tre production and cycling genes were also commonly found in the community metagenome. Tre was shown to form an amorphous glass-state (via DSC, TGA), which protected AHLs (in the lab). Solid-state NMR indicated Tre prevented AHLs from denaturation by keeping AHLs in a near-‘hydrated’ conformation (without water being present). This allows a desiccated community to rapidly resume activities following rehydration.

The work that community information in the extracellular EPS matrix can be preserved during desiccation and begins to address a mechanistic understanding of the survival of complex biofilm communities and may even provide a looking glass into microbial resiliency during climate change and the possibility of life elsewhere.