Extracellular Polymeric Substances Enhance Cross-Feeder Resilience to Cheaters
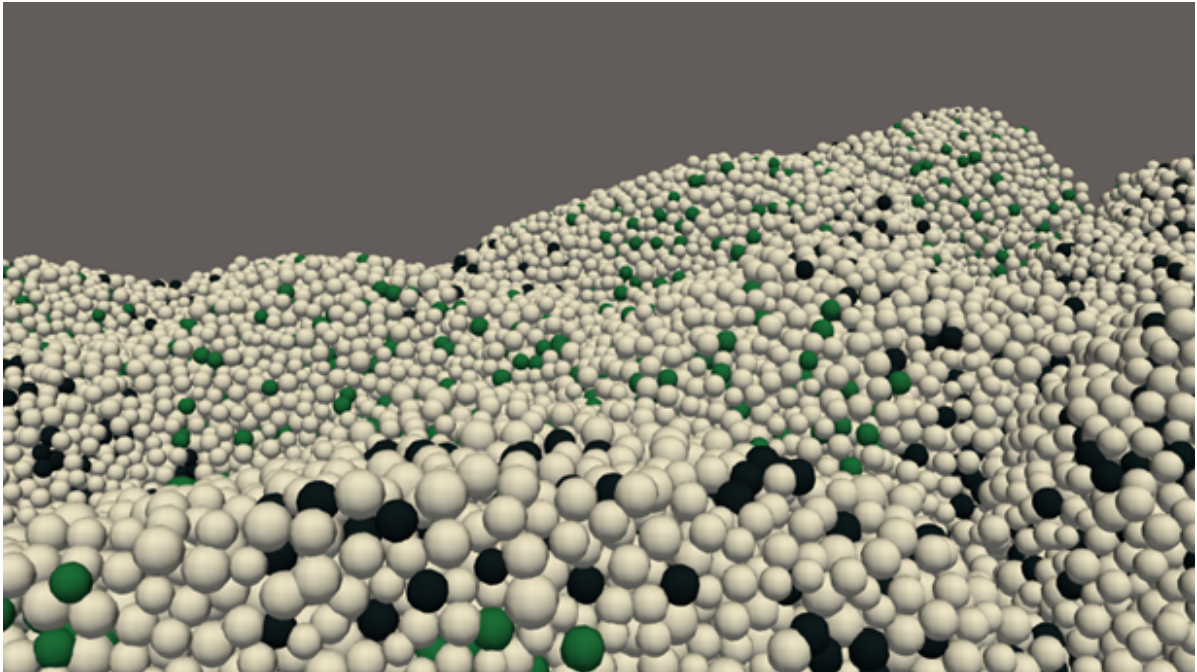
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Contents

1 Abstract

A central question in microbial ecology is how cooperation persists. Biofilms, the predominant form of microbial life, are tightly packed. In theory, this proximity makes it easier for cheaters to enter, thereby increasing the risk of community collapse. Cross-feeding is a kind of cooperation that is pervasive in nature, which indicates that cross-feeders are capable to stay stable in the face of cheaters. In this study, we use agent-based models to examine if extracellular polymeric substances (EPS), a major material of biofilm secreted by microbes, help cross-feeders withstand cheater invasion. We found that the existence of EPS in the system can elongate the survival time of cross-feeders cocultured with cheaters with higher fitness.

2   Introduction

Different bacterial species often share habitats, giving rise to interspecific interactions ranging from antagonism to cooperation. Because self production of essential nutrients is costly, some species reduce or even lose the pathways for biosynthesis. As these functions decline, they rely on partners that supply the missing nutrients and offer other benefits in return. This, in turn, leads to cross-feeding.

Cross-feeding, a prevalent form of cooperation within microbial communities, takes place in the surface waters of lakes and oceans, submarine volcano, soil, human intestine, etc. Generally, two species form a cross-feeding pair; each secretes an essential nutrient (e.g. metabolite) that the partner cannot synthesize. (Figure 1)
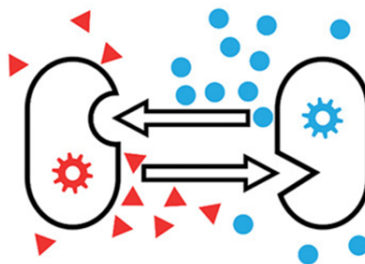

Figure 1: Schematic of Cross-feeding.

Some mutated members of cross-feeding communities become cheaters that only consume and do not secrete. In classical ecology theory, these cheaters are expected to have higher fitness because they pay no secretion cost. As they spread, they can eliminate cross-feeders and collapse the community. For this reason, people study how cross-feeders persist in the face of cheaters.

As a widespread form of microbial life, biofilms embed cells in an extracellular matrix rich in extracellular polymeric substance (EPS). Yet most work on population dynamics has not examined the specific role of EPS. Here, we test whether EPS secretion helps cross-feeders resist cheaters using agent-based models.
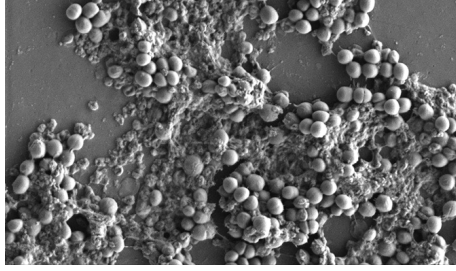
Figure 2: SEM image of biofilm.

We used NUFEB to perform agent-based simulation. Built on top of the molecular dynamics software LAMMPS, NUFEB is an open-source engineering simulator for the dynamics of biofilm. NUFEB allows biological, physical, and chemical processes to be implemented to simulate real-world systems. To simulate cross-feeders and cheaters dynamics in this study, we've developed new growth models. Then we compared the results of simulations with and without EPS secretion to see its effect on the resilience of cross-feeders.

3   Methods

NUFEB allows multiscale agebt-based simulation. There are models for physical forces, biological processes, chemical reaction and diffusion. Microbes and EPS are simulated as particles (Figure 4)

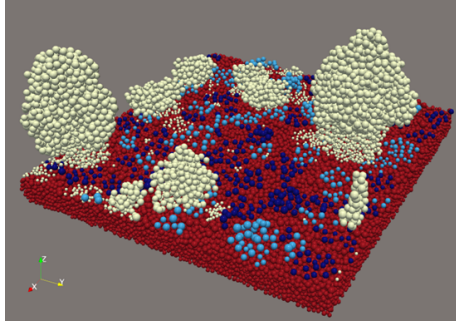Figure 3: Visualization of NUFEB simulation.

3.1   Physical Forces

Equation of motion of a particle (microbe or EPS):

$$m_i \frac{\mathrm{d}\vec{v}_i}{\mathrm{d}t} = \mathbf{F}_{c,i} + \mathbf{F}_{a,i} + \mathbf{F}_{d,i} + \dots \tag{1}$$

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where m_i is the mass, and $\vec{v}_i$ is the velocity. The contact force $\mathbf{F_c}$,is a pair-wise force exerted on the particles:

$$\mathbf{F}_{c,i} = \sum_{j=1}^{N_i} \left(K_n \, \boldsymbol{\delta n}_{i,j} - m_{i,j} \, \gamma_n \, \mathbf{v}_{i,j} \right) \tag{2}$$

where N_i is the total number of neighbouring particles of i, K_n is the elastic constant for normal contact, $\boldsymbol{\delta n}_{i,j}$ are overlap distance between the center of particles i and its neighbour particles j, $m_{i,j}$ is the effective mass of particles i and j, γ_n is the viscoelastic damping constant for normal contact, and $\mathbf{v}_{i,j}$ is the relative velocity of the two particles. The first term in the summation describes the repulsive force that arises when two cells overlap. The second term describes the damping force that opposes their relative motion, whether they are approaching or moving apart. The EPS adhesive force $\mathbf{F}_{a,i}$ is modelled as a van der Waals force:

$$\mathbf{F}_{a,i} = \sum_{j=1}^{N_i} \frac{H_a \, r_{i,j}}{12 \, h_{\min,i,j}^2} \, \mathbf{n}_{i,j} \tag{3}$$

where H_a is the Hamaker coefficient which describes the intrinsic attraction strength of the materials (EPS),$r_{i,j}$ is the effective outer-radius of particles i and j ($\frac{R_i R_j}{R_i + R_j}$), $h_{\min,i,j}^2$ is the minimum separation distance of the two particles, and $\mathbf{n}_{i,j}$ is the unit vector from particle i to j.

The drag force $\mathbf{F}_{d,i}$ is the fluid-particle interaction force acting opposite to the microbe motion:

$$\mathbf{F}_{d,i} = -\frac{V_{p,i}}{\varepsilon_{f,i} \, \varepsilon_{s,i}} \, \beta_i \, \mathbf{u}_{p,i} \tag{4}$$

where $\varepsilon_{s,i}$ is the fluid-particle volume fraction, $\varepsilon_{f,i}$ is the fluid volume fraction, $V_{f,i}$ and $\mathbf{u}_{p,i}$ are volume and velocity of particle i respectively, and β_i is the drag correction coefficient.

3.2 Growth Models

To simulate the growth dynamics of cross-feeders and cheater, we developed new growth models for each. The growth of the biomass of all species follows:

$$\frac{\mathrm{d}m_i}{\mathrm{d}t} = \mu_i m_i \tag{5}$$

where m is biomass, μ is the growth rate.

The growth rate of one of the group of cross-feeders depend on the concentration of substrate and metabolite secreted by their partner cross-feeders. For example:

$$\mu_i = \mu_{\max} \left(\frac{[\mathrm{metabolite_2}]}{K_{\mathrm{metabolite_2}} + [\mathrm{metabolite_2}]} \right) r - d \tag{6}$$

The growth rate of cheaters depend on the concentration of substrate and both of the metabolite secreted by two species of cross-feeders:

$$\mu_i = \mu_{max} \left(\frac{[\text{sub}]}{K_{\text{sub}} + [\text{sub}]} \right) \left(\frac{[\text{metabolite}_1]}{K_{\text{metabolite}_1} + [\text{metabolite}_1]} \right) \left(\frac{[\text{metabolite}_2]}{K_{\text{metabolite}_2} + [\text{metabolite}_2]} \right) - d \tag{7}$$

where K is the affinity of a chemical, and r is the reduction of growth due to metabolite secretion. Division and death are implemented in NUFEB as instantaneous events that occur when the radius exceeds or falls below a certain threshold.

3.3 Chemical Models

The rate of nutrient consumption (or reaction rate) is calculated at each voxel. The concentration of nutrients will vary due to diffusion and consumption or secretion by microbes. The concentration S can be described by the diffusion-reaction equation:

$$\frac{\partial S}{\partial t} = \nabla \cdot \left(D \nabla S \right) + R \tag{8}$$

where S is the concentration of a chemical, D is the diffusivity, and R stands for the reaction rate of the chemicals. For example, the reaction rates of all the chemicals for one species of cross-feeders in our simulation follow:

$$R_{sub} = \left(-\frac{1}{Y} \right) (\mu_i + \psi_i)\rho \tag{9}$$

$$R_{metabolite1} = \left(\frac{1}{Y} \right) \psi\rho \tag{10}$$

$$R_{metabolite2} = \left(-\frac{1}{Y} \right) \mu_i\rho \tag{11}$$

where $\psi_i = \alpha\mu_i$ is the additional substrate consumption due to metabolite secretion, Y is the yield, and ρ is the density of biomass within a grid of the simulation box.

3.4 Survival Analysis

We simulated communities with two cross feeders and one cheater. At t = 0, each species started at 50 cells, positioned uniformly at random on a plane (Fig. 4). We compared the group in witch cross-feeders secrete EPS and cheater do not with the group in witch all species do not secrete EPS, using 50 independent replicates per group generated by distinct random seeds. The primary outcome was time to collapse, defined as the first moment both cross feeders fell below 50 cells. Survival was estimated with Kaplan Meier curves, 95% confidence

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bands were shown, and groups were compared using the log rank test (p value reported).
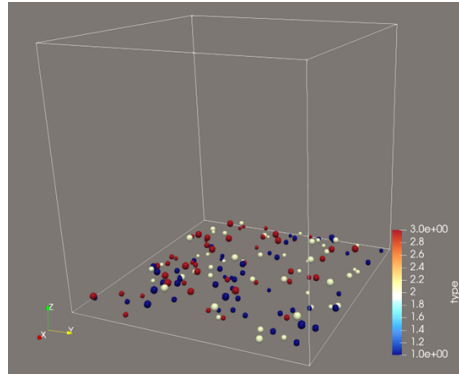

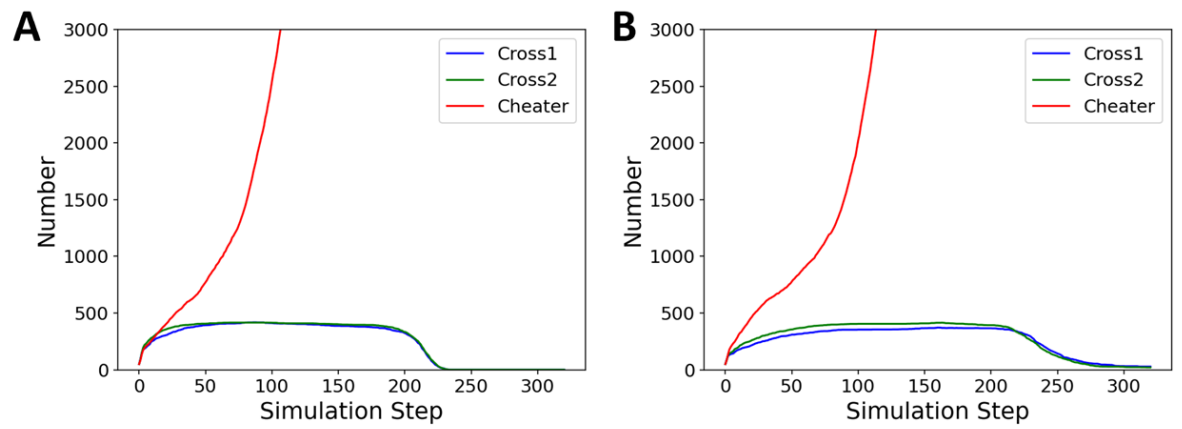Figure 4: Cells are uniformly distributed on the plane.

4 Results

Figure 5: Population dynamics of the three species.
A: without EPS, B: with EPS

Figure 5 shows the change of the population of three species overtime. We can see that when cross-feeders secrete EPS, the survival time is elongated (B). To justify this phenomenon, we simulated both situations for 50 times with different sets of random seeds.
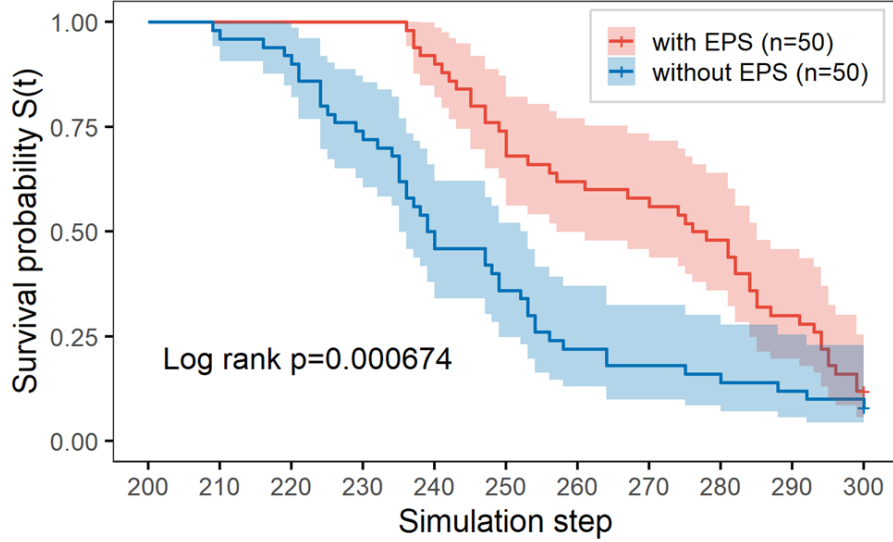

Figure 6: Survival curves of groups with and without EPS.

The survival curves of two groups were plotted in figure 6. The group with EPS shows significantly higher survival than the group without EPS (p = 0.000674).

5   Conclusion and Future Work

Qualitatively, we find that EPS secretion by cross-feeders prolongs survival under cheater pressure. This observation suggests that, in natural communities, elevated EPS production could serve as a defense that enables cross-feeders to resist exploitation, potentially by reinforcing matrix-mediated spatial structuring and modulating metabolite diffusion. We will next quantify the size and robustness of this effect across biologically plausible parameter ranges, delineate its mechanism through further simulations, and design complementary experiments to validate and refine these predictions.

6 References

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