

# Dynamic interspecies interactions and robustness in a four-species model biofilm

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## Abstract

Interspecific interactions within biofilms determine relative species abundance, growth dynamics, community resilience, and success or failure of invasion by an extraneous organism. However, deciphering interspecific interactions and assessing their contribution to biofilm properties and function remain a challenge. Here, we describe the constitution of a model biofilm composed of four bacterial species belonging to four different genera (*Rhodocyclus* sp., *Pseudomonas fluorescens*, *Kocuria varians*, and *Bacillus cereus*), derived from a biofilm isolated from an industrial milk pasteurization unit. We demonstrate that the growth dynamics and equilibrium composition of this biofilm are highly reproducible. Based on its equilibrium composition, we show that the establishment of this 4-species biofilm is highly robust against initial, transient perturbations but less so towards continuous perturbations. By comparing biofilms formed from different numbers and combinations of the constituent species and by fitting a growth model to the experimental data, we reveal a network of dynamic, positive, and negative interactions that determine the final composition of the biofilm. Furthermore, we reveal that the molecular determinant of one negative interaction is the thiocillin I synthesized by the *B. cereus* strain, and demonstrate its importance for species distribution and its impact on robustness by mutational analysis of the biofilm ecosystem.

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