Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms

Closely related microorganisms often cooperate, but the prevalence and stability of cooperation between different genotypes remain debatable. Here, we track the evolution of pellicle biofilms formed through genetic division of labour and ask whether partially deficient partners can evolve autonomy. Pellicles of Bacillus subtilis rely on an extracellular matrix composed of exopolysaccharide (EPS) and the fibre protein TasA. In monocultures, ∆eps and ∆tasA mutants fail to form pellicles, but, facilitated by cooperation, they succeed in co-culture. Interestingly, cooperation collapses on an evolutionary timescale and ∆tasA gradually outcompetes its partner ∆eps. Pellicle formation can evolve independently from division of labour in ∆eps and ∆tasA monocultures, by selection acting on the residual matrix component, TasA or EPS, respectively. Using a set of interdisciplinary tools, we unravel that the TasA producer (∆eps) evolves via an unconventional but reproducible substitution in TasA that modulates the biochemical properties of the protein. Conversely, the EPS producer (∆tasA) undergoes genetically variable adaptations, all leading to enhanced EPS secretion and biofilms with different biomechanical properties. Finally, we revisit the collapse of division of labour between Δeps and ΔtasA in light of a strong frequency versus exploitability trade-off that manifested in the solitarily evolving partners. We propose that such trade-off differences may represent an additional barrier to evolution of division of labour between genetically distinct microorganisms.

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