Collapse of Genetic Div. of Labor and Evolution of Autonomy in Pellicle Biofilms

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Closely related microbes often cooperate, but prevalence and stability of cooperation between different genotypes remains debatable. Here, we explore the long term evolutionary dynamics of biofilms formed through genetic division of labor. Pellicle biofilms of Bacillus subtilis form at liquid-air interface where bacteria stick to each other encased in an extracellular matrix (ECM) composed of exopolysaccharide EPS and fiber protein TasA [1-3]. Failure to synthesize EPS or TasA prevents pellicle formation however Δeps and ΔtasA can complement each other and form robust pellicle [4]. We compared the evolution of these mutants under two alternative conditions: in mixed cultures with constrained division of labor and in monocultures where both strains perform poorly at the beginning due to the lack of either EPS or TasA. After over 200 generations of experimental evolution, both monoculture mutants evolved autonomous pellicles pursuing two distinct evolutionary trajectories. The molecular adaptation of Δtas took part through several alternative paths that all led to increased levels of EPS secretion and development of slimy pellicles that were less stiff and more viscous as compared to the wild type. On the contrary, two of six Δeps populations evolved interface colonization via distinct substitutions in TasA-encoding gene presumably altering the biochemical properties of TasA. Interestingly, while the ECM of evolved ΔtasA could be easily exploited by the non-evolved strain, the ECM of evolved Δeps was highly privatized. In co-cultures, the ΔtasA gradually outcompeted its partner in each parallel mixed population leading to rapid biofilm collapse and dramatic productivity loss, however, after the exclusion of Δeps the ΔtasA pursued a similar adaptive path as in monocultures. Despite the short-term success, genetic division of labor in biofilm formation collapsed and slowed down the evolution of autonomy of the winning partner. The loss of Δeps in the mix and its lower chance for autonomy evolution may indicate evolutionary trade-offs linked to privatization of public goods. Differences in dependency levels and availability of public goods exchanged by the cooperating partners likely contributed to cooperation collapse revealing a barrier against evolution of intraspecific cooperation in microbes. [1] Dragos & Kovacs 2017 The peculiar functions of bacterial extracellular matrix. Trends Microbiology [2] Martin et al 2017 De novo evolved interference competition promotes the spread of biofilm defectors. Nature Communications [3] Dragos et al 2018 Evolution of exploitative interactions during diversification in Bacillus subtilis biofilms. FEMS Microbiology Ecology [4] Dragos et al 2018 Division of labor during biofilm matrix production. Current Biology