Nature Reviews Microbiology | AOP, published online 3 August 2015; doi:10.1038/nrmicro3535

BIOFILMS

Oscillations relieve the siege

> a social interaction in which the periphery obtains ammonium from the interior of the biofilm resolves the metabolic conflict between the two populations

Biofilm growth is dependent on the supply of nutrients to all cells within the microbial community; however, the consumption of nutrients by cells on the periphery is expected to deplete the nutrient supply to cells in the interior, giving rise to a siege-like metabolic conflict between these two populations. A new study by Liu *et al.* now shows that a *Bacillus subtilis* biofilm community uses metabolic co-dependency to produce growth oscillations that resolve this conflict.

The authors used microfluidics and single-cell resolution analysis to study the patterns of growth in the periphery and interior of a *B. subtilis* biofilm. The expansion of the biofilm as a whole oscillated with an average period of 2.5 hours. Importantly, the oscillation was only evident once the biofilm had reached a threshold size. As all growth was restricted to cells in the periphery of the biofilm, this pattern suggested that the periodic pauses in the expansion of the biofilm were caused by excessive growth of peripheral cells. The authors propose that these pauses reduced nutrient consumption and thus relieved the 'siege' on the interior. As such, they may represent a social interaction in which different regions of the biofilm cooperate to promote community survival.

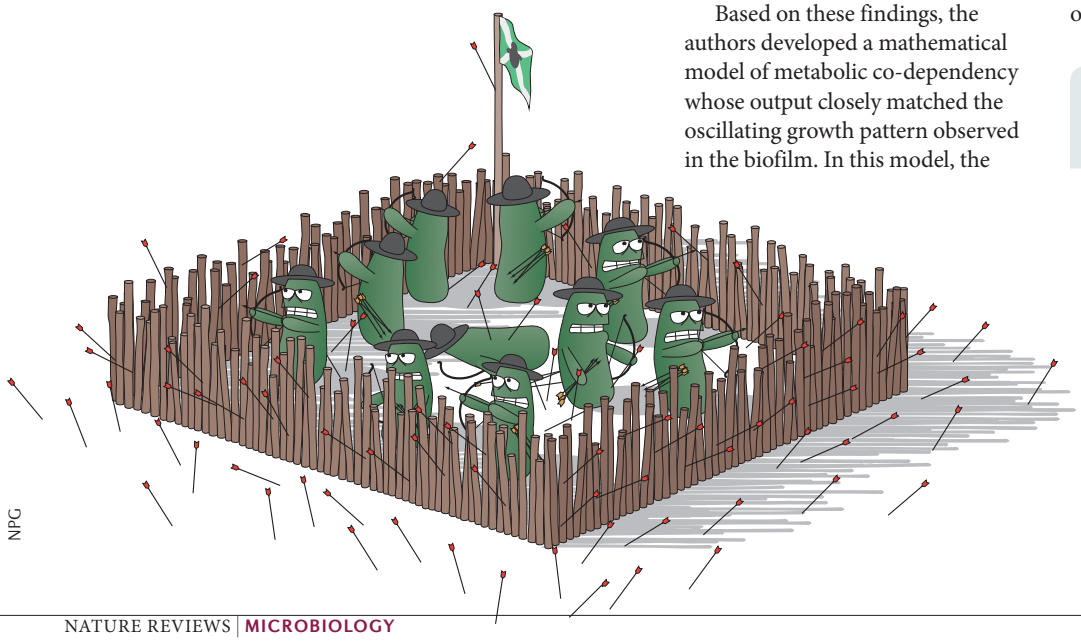
To identify which nutrients were regulating biofilm growth, the authors assessed the contribution of carbon and nitrogen sources to growth oscillations. Addition of glycerol as a carbon source had no effect on growth, whereas addition of the nitrogen source glutamine eliminated the growth oscillations. Under the growth conditions used in these experiments, glutamine synthesis required both glutamate from the medium and ammonium produced by the biofilm. Therefore, the authors next investigated the roles of these two substrates in biofilm growth. They found that increasing the availability of ammonium, but not glutamate, eliminated the growth oscillations, suggesting that ammonium was the limiting factor regulating glutamine synthesis in the peripheral cells of the biofilm.

Based on these findings, the authors developed a mathematical model of metabolic co-dependency whose output closely matched the oscillating growth pattern observed in the biofilm. In this model, the availability of glutamate to the interior of the biofilm reduces as it is consumed in increasing quantities by the growing cells in the periphery of the biofilm. In response to this starvation risk, the cells in the interior reduce their production of ammonium; this reduction decreases the availability of ammonium in the biofilm, which inhibits glutamine synthesis in the peripheral bacteria, and thus reduces their growth, allowing glutamate to reach the bacteria in the interior of the biofilm. A key assumption of the model is that the interior of the biofilm is the most important region for ammonium production, which is based on the expectation that this ambient molecule is poorly retained by peripheral cells owing to the exposure of these cells to media flow.

Collectively, these data suggest that a social interaction in which the periphery obtains ammonium from the interior of the biofilm resolves the metabolic conflict between the two populations and produces growth oscillations that ensure the survival of the biofilm community.

Naomi Attar

ORIGINAL RESEARCH PAPER Liu, J. *et al.* Metabolic co-dependence gives rise to collective oscillations within biofilms. *Nature* http://dx.doi.org/10.1038/nature14660 (2015)



NPG