The Disruption and Control of Microbial Biofilms

BIOL

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Abstract:

Microbial biofilms of the species *Bacillus Subtilis* were grown so that the changes in the expression of several important phenotypes could be analyzed using fluorescence microscopy. Biofilms exist in all kinds of environments; instead of acting as independent swimmers, the cells work as a community, which in turn results in a number of benefits for the colony, making it the preferred living condition for bacteria. However, the ability of biofilms to survive in harsh environments can cause serious problems in the medical and industrial fields where they lead to the spread of infection and degradation of components. Understanding the factors that lead the bacteria to change from one phenotype to another can provide insight to the best approach in solving these issues. We performed a set of novel experiments where the bacteria were presented with physical barriers that interrupted the normal expansion of the colony across the surface of the agar substrate. The barriers led to a unique response from the bacteria in respect to the growth rate along certain areas as well as the expression of a certain phenotype in a specific location.

Introduction:

Microbial biofilms were grown on a 9 mm thick piece of agar substrate in a Petri® dish with laser-cut acrylic barriers that formed channels for the biofilm to grow through. The barriers acted as walls, and as a result the bacteria could not receive nutrients from one side of barrier. The width of the channels was varied between 2 mm, 5 mm, and 10 mm, while the length of the channel was kept at a constant 3.175 mm. One half of the biofilm grew towards the barrier, and the other half grew over a flat agar substrate, serving as the control of the experiment.

Throughout the experiments, there was a noticeable correlation between the width of the channel and growth pattern of the biofilm as well as the intensity of the matrix phenotype while and after the biofilm grew through the length of the channel.

Methods:

The laser-cut barriers were put in the agar substrate by pouring agar into the Petri dish to a height of 1-2 mm and letting the substrate cool down to the point where it was no longer liquid. The acrylic barrier was then placed in the agar at 90° relative to the bottom of the Petri dish. Another amount of agar was then poured into the Petri dish, around the barrier, up to a height of 7 mm.

Throughout the whole experimental process, we inoculated the bacteria 5 mm away from the entrance to the channel.

Results and Conclusions:

The biofilms that grew through the 10 mm wide channels barely changed their regular growth while and after they grew through the length of the channel. The biofilms kept a regular gene expression and followed a fairly regular radial growth pattern, almost as if there was no barrier in place.

Figure 1 shows a triple-reporter fluorescent image of a colony that was inoculated 5 mm away from the entrance to a 2 mm wide channel, nine days after its inoculation. Although most of the biofilm had already sporulated, this image shows the growth pattern and greater matrix intensity after the biofilm had passed through the channel.

We believe that the higher raw intensity for the matrix phenotype after the biofilm had grown through the channel, shown in Figure 2a, was due to when the biofilm grew into the channel. The matrix cells at the edge of the colony could have formed higher stack of cells in the smaller surface area, thus creating a higher intensity and a higher percentage of matrix cells at the exit of the channel. The condensation along the edge of the walls could have led to the rapid spread of the cells in this area, leading to the matrix cells growing away from the lining of the wall on the second side of the barrier.

We observed a tendency for the bacteria to sporulate as the biofilm grew towards the left and right edges of the channel on the inoculation side. Unlike the second side of the barrier, the biofilm did not grow along the wall on the inoculation side.

The percentage of the peak of the sporulation intensity increased by 10% between these two instances, shown in Figures 2b and 2c. However, there was only an increase of 5% when the variation of intensities was measured from the inoculation point to the control side.

The difference in the increase of percentages of sporulation leads us to believe that the presence of the barrier increased the amount of sporulation on the inoculation side of the experiment. Unlike the second side of the barrier, where the biofilm rapidly grew along the lining of the wall and agar, the biofilm sporulated and stopped expanding along the first side of the barrier. As the biofilm expanded towards the control side, it received many nutrients that allowed the cells to continue dividing and switch between the matrix and motility phenotypes.

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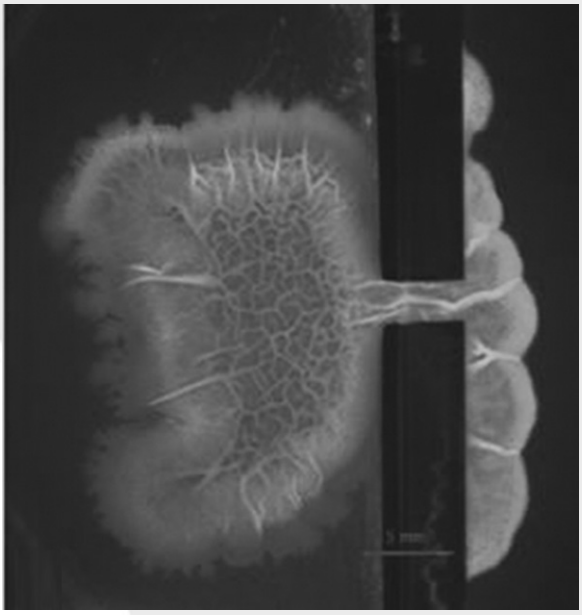

Figure 1: Fluorescent image of the biofilm nine days after inoculation. (See full color version on page xxxvi.)

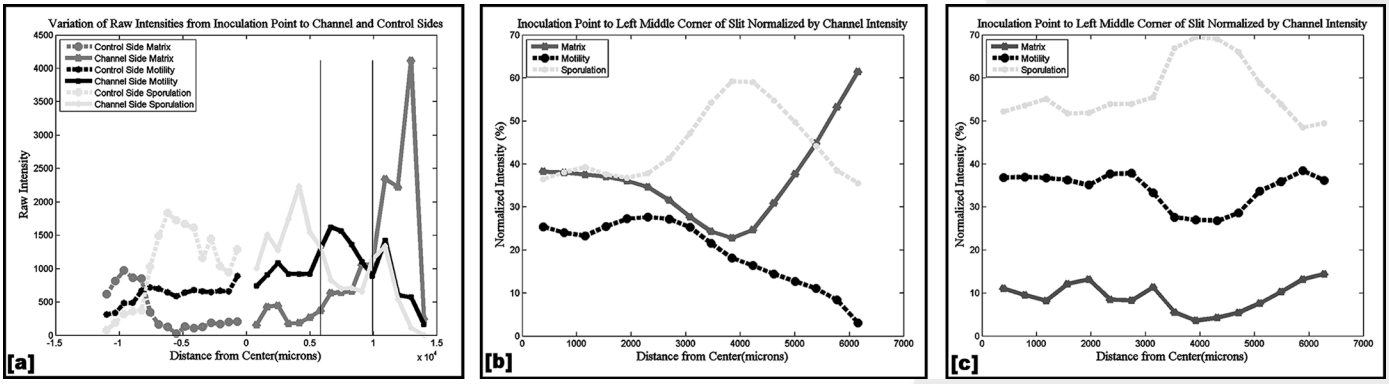

Figure 2: [a] Variation of the raw fluorescent intensities from the inoculation point to the edge of the biofilm on the control and channel sides. [b] Variation of phenotype intensities from the inoculation point to the left middle corner of the channel six days after inoculation. [c] Variation of phenotype intensities from the inoculation point to the left middle corner of the channel nine days after inoculation.