

Extracellular matrix components are required to protect *Bacillus subtilis* from *Pseudomonas* invasion and co-colonization of plants.

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Plants are colonized by a vast variety of microbes. Among them, bacteria are the most predominant and are able to adapt to environmental changes and interact with other microorganisms using a wide array of molecules, metabolic plasticity and secretion systems. One way bacteria have evolved to succeed in this competitive scenario is the formation of biofilms which provides protection to the cells, modulates the flux of signals and controls cellular differentiation. Thus, efforts are encouraged to really determine the functionality of the bacterial extracellular matrix.

In this study, we have employed microbiological and microscopic techniques to study the interaction between *Bacillus subtilis* 3610 and *Pseudomonas chlororaphis* PCL1606. We demonstrate the important role of the extracellular matrix in protecting *B. subtilis* colonies from infiltration by *Pseudomonas*. Furthermore, time-lapse confocal laser scanning microscopy (CLSM) analyses of the bacterial interactions have permitted to complete the study of the bacterial behaviors and to measure bacterial expansion rates. Surprising, we find that the *Pseudomonas* type VI secretion system (T6SS) is required in the cell-to-cell contact with matrix-impaired *B. subtilis* cells, revealing a novel role for T6SS against Gram-positive bacteria. In response to *P. chlororaphis* infiltration, we find that *B. subtilis* activates sporulation and expresses motility-related genes. Confocal microscopy of the bacterial interactions using plant organs highlights the functional importance of these different bacterial strategies in their coexistence as stable bacterial communities. The findings further our understanding of the functional role played by biofilms in mediating bacterial social interactions.