The Role of Exopolysaccharides in Resistance to Dessication in Agrobacterium tumefaciens

E. Dewars and A.G. Matthysse
Department of Biology, University of North Carolina at Chapel Hill

Abstract

Agrobacterium is a gram-negative bacterium that uses horizontal gene transfer to cause tumors in plants. Agrobacterium encounters many stressors in its soil environment as well as from injured plants it attempts to infect. Examples of these stressors include low water and nutrient content in the soil, which lead to starvation and desiccation. Exopolysaccharides (EPS) are often thought to be important in resistance to these stressors. Whether the following exopolysaccharides, beta-1,2-glucan, cellulose, succinoglycan, curdlan, and the unipolar polysaccharide play a role in protecting this bacterium against environmental stressors is unknown. The WT, C58 and its mutant derivatives were tested to measure the effects of desiccation on the survival of each strain. My data shows A1045 and CelA, which are unable to make beta-1,2-cyclic glucan and cellulose respectively, can tolerate desiccation slightly better than the parent strain, C58. These results suggest that beta-1,2-cyclic glucan and cellulose may not play a vital role in the survival of C58 under desiccated conditions. However, my data suggests that the mutant ExoF, which is unable to make succinoglycan cannot tolerate desiccation as well as the parent strain. This suggests that succinoglycan might play an important role in the survival of C58.

Methods

Starvation and Desiccation:
 Cultures of bacterial strains are grown overnight in 2ml of Luria Broth. Small pieces of filter paper are placed on Luria Agar with sterile non-serrated tweezers and 5 µl of the inoculated culture is pipetted onto each. The bacteria grow on the paper for two days and a dilution is performed on one piece to determine the initial cell count. Additionally, after letting the culture grow on the filter paper for two days, we transfer the remaining filter paper to a water agar plate and a dry plate. Every other day after the transfer, we perform dilutions of the culture and record viable cell counts.

Mutants of Interest

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Relevant Genotype</th>
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<tbody>
<tr>
<td>C58</td>
<td>Wild Type</td>
</tr>
<tr>
<td>A1045</td>
<td>chvB- mutant, no beta 1-2-glucan</td>
</tr>
<tr>
<td>CelA</td>
<td>CelA mutant, no cellulose</td>
</tr>
<tr>
<td>ExoF</td>
<td>ExoF mutant, no succinoglycan</td>
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Results

- The desiccation experiment shows that the mutant of A. tumefaciens strain C58, ExoF which is unable to make succinoglycan is more sensitive to desiccation than the parent strain C58.
- Mutant Cell A and A1045 both had a similar trend in the desiccation experiment and were both less sensitive to desiccation than the wild-type C58.

Conclusion

- Since mutants, A1045 and celA survive better than the parent strain, this suggests that they are not essential for survival under starved and desiccated conditions.
- ExoF does not survive as well as the WT C58, suggesting that succinoglycan may be important for survival under desiccated conditions.
- When one mutant does not produce an exopolysaccharide, this could result in another exopolysaccharide being produced to replace the missing exopolysaccharide.
- Future directions involve making three different double mutants, celA/exoF, exoF/A1045, and A1045/celA.

Figures

Figure 1: Starvation viable cell counts vs time on a semilog plot comparing C58 with A1045, CelA, and ExoF

Figure 2: Desiccation viable cell counts vs time on a semilog plot comparing C58 with A1045, CelA, and ExoF