Geophysical Research Abstracts
Vol. 18, EGU2016-15510, 2016
EGU General Assembly 2016

The presence of INA proteins on the surface of single cells of Pseudomonas syringae R10.79 isolated from rain

Tina Šantl-Temkiv (1), Meilee Ling (1), Stine Holm (2), Kai Finster (2), and Thomas Boesen (3)

(1) Aarhus University, Stellar Astrophysics Centre, Department of Physics and Astronomy, (2) Aarhus University, Department of Bioscience, Microbiology Section, (3) Aarhus University, Department of Molecular Biology and Genetics

One of the important open questions in atmospheric ice nucleation is the impact of bioaerosols on the ice content of mix phase clouds (DeMott and Prenni 2010). Biogenic ice nuclei have a unique capacity of facilitating ice formation at temperatures between –1 and –10 °C. The model biogenic ice nuclei are produced by a few species of plant-surface bacteria, such as Pseudomonas syringae, that are commonly transported through the atmosphere. These bacterial species have highly specialized proteins, the so-called ice nucleation active (INA) proteins, which are exposed at the outer membrane surface of the cell where they promote ice particle formation. The mechanisms behind the onset of INA protein synthesis in single bacterial cells are not well understood. We performed a laboratory study in order to (i) investigate the presence of INA proteins on single bacterial cells and (ii) understand the conditions that induce INA protein production.
We previously isolated an INA-positive strain of Pseudomonas syringae from rain samples collected in Denmark. Bacterial cells initiated ice nucleation activity at temperatures $\leq$–2°C and the cell fragments at temperatures $\leq$–8°C (Šantl-Temkiv et al 2015). We determined the amino-acid sequence of the INA protein and used the sequence to produce custom-made antibodies (GenScript, Germany). These antibodies were used to specifically stain and visualize the INA protein on the surfaces of single cells, which can then be quantified by a technique called flow cytometry. The synthesis of INA proteins by individual cells was followed during a batch growth experiment. An unusually high proportion of cells that were adapting to the new conditions prior to growth produced INA proteins ($\sim$4.4% of all cells). A smaller fraction of actively growing cells was carrying INA proteins ($\sim$1.2 % of all cells). The cells that stopped growing due to unfavorable conditions had the lowest fraction of cells carrying INA proteins ($\sim$0.5 % of all cells). To our surprise, exposure of cells to low temperatures, which is normally considered to be the main driver of the INA protein synthesis, had no or even a negative effect on the proportion of cells with INA proteins. Our results suggest that at certain conditions a high proportion of Pseudomonas syringae cells produces INA proteins and that this proportion depends on the physiological state of the cells. This and similar studies will ultimately improve our understanding of the quantitative role bacterial INA proteins play in atmospheric processes.

References

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