Effects of the atmospheric environment on ice nucleating strains of *Pseudomonas syringae*

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Introduction

Biological ice nucleators are thought to play a role on cloud glaciation, a process important for precipitation formation. Cloud droplets may supercool to very low temperatures (down to -38 °C), only freezing upon interaction with a nucleating particle. Some of the most efficient ice nuclei (IN) known, active at the warmest temperatures (up to -2 °C), are produced by the bacterium *Pseudomonas syringae*, an epiphyte and a plant pathogen[1].

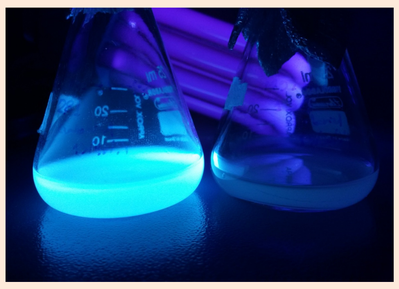
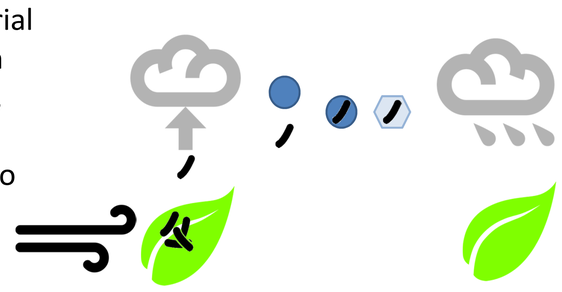


Figure 1: *Pseudomonas syringae* culture on King's B medium.

This organism's IN properties are due to a membrane-bound glycoprotein that allows the formation of ice crystals under low supercooling [2]. An environmental condition that may favor IN active strains is on aerosolized cells. Brought up to cloud heights after being swept by the wind, rainfall is the best escape for bacteria down to the ground. Being IN and contributing to precipitation, *P. syringae* survival may be favored. Indeed, this species presence has been reported on rain, snow, and cloud water samples [3].

As cruisers of the atmosphere, bacterial cells must endure harsh conditions. At high altitudes, pressure and temperature drops, leading to freezing and desiccation, while solar ultraviolet irradiance increases. Due to that, the Earth's upper atmosphere can be considered an extreme environment and a region of astrobiological interest [4].



Methods

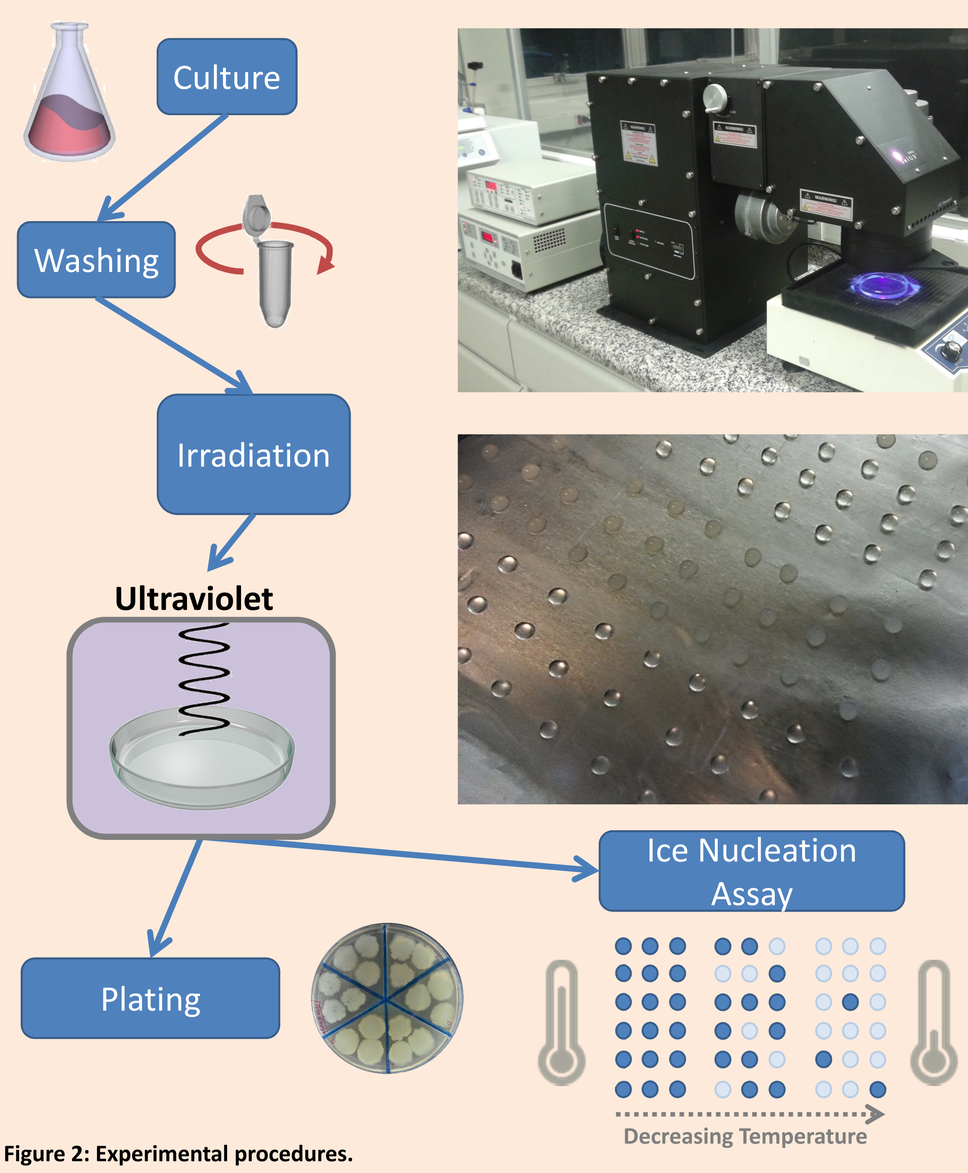


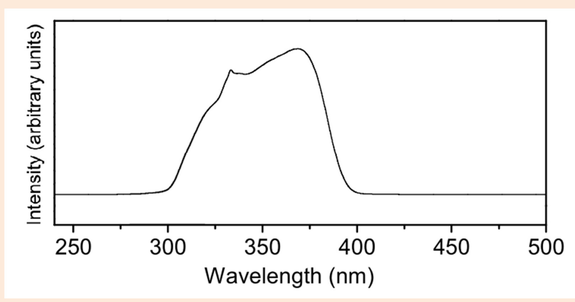
Figure 2: Experimental procedures.

Ultraviolet tolerance was tested for a cell suspension on a glass petri dish (without the lid). The plate was stirred during irradiation with "environmental UV" or UV-C. At each cumulative dose, aliquots were taken, diluted and plated for survival analysis by CFU. Also, the unirradiated control and the largest doses of UV tested (309 kJ/m² of UVA and 179 kJ/m² of UVB, 60 minutes of exposure, or 0,01 kJ/m² of UV-C, about a minute of exposure) were taken to IN activity measurement by the droplet freezing assay.

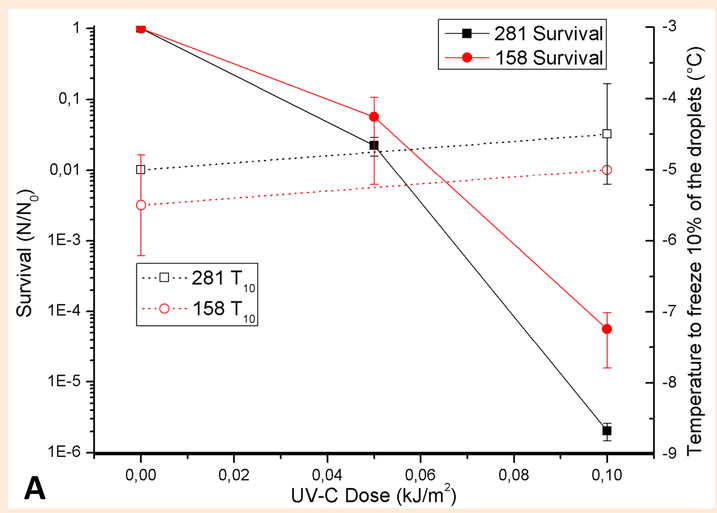
P. syringae was routinely grown on King's B medium. Two strains were used, 281 (subspecies pv. *syringae*) and 158 (subspecies pv. *garceae*). Cultures were washed and diluted before each experiment. IN activity was assayed by the drop freezing test. An array of droplets was placed on a paraffin-coated aluminum surface floating on a cold bath. Temperature was decreased gradually and the number of frozen droplets was counted at 1 °C intervals. Results were expressed as T_{10}, the temperature at which 10% of the droplets froze.

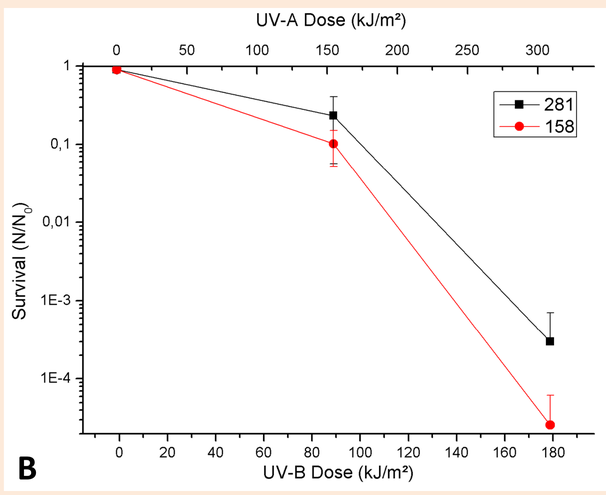
Figure 3: Simulated solar spectrum (~300-400 nm).

The Oriel Sol-UV-2 solar simulator, emits on UV-A and UV-B with the spectrum that reaches Earth's surface (with higher intensity), here called "environmental UV". Visible wavelengths are mostly cut off by a filter. For the UV-C test, a Philips 8 TUV-20W low-pressure Hg lamp (line emission at 253.7 nm) was used.



Results





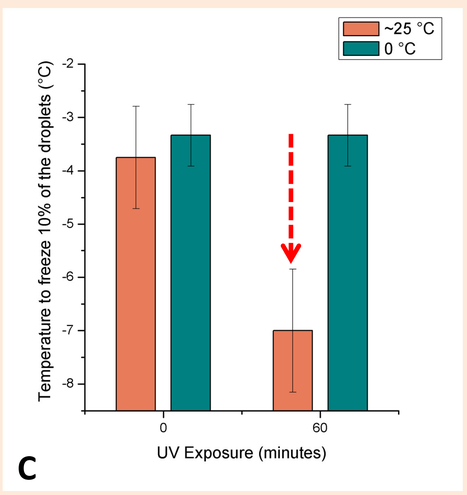


Figure 4: Survival and IN activity after irradiation.

A - UV-C tolerance assay: The strains were very sensitive to UV-C. This wavelength range does not reach Earth's surface, but can be present on very high altitudes at the stratosphere. Meanwhile, the cell's IN activity was unaffected by the treatment, even at higher doses (1 kJ/m², not shown on graph).

B - Environmental UV tolerance assay: *P. syringae* endured higher doses of UV-A and UV-B, more relevant to tropospheric conditions than UV-C.

C - Effect of temperature during irradiation on IN activity (strain 281): Initial tests, performed at room temperature, showed a decrease in IN activity following the higher doses of environmental UV. Follow up assays demonstrated that the relatively high temperature during the 60 minute experiment was the reason for that. Irradiation performed on plates over an ice bath exhibited the same T_{10} values as the control, similar to what happened on UV-C.

Conclusion

P. syringae cells may suffer serious damage while exposed to solar radiation, depending on weather conditions. Its IN activity though, could remain unaffected for longer periods, especially in low temperatures, still possibly having an impact on cloud dynamics. Ongoing experiments are trying to assess other factors, such as desiccation and freezing, and its interaction with radiation on cell survival.

References

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