EFFECTS OF THE ATMOSPHERIC ENVIRONMENT ON ICE NUCLEATING STRAINS OF PSEUDOMONAS SYRINGAE. G. G. Araujo[1], F. L. T. Gonçalves[2], F. Rodrigues[3] and D. Galante[4], [1] Interunities Biotechnology Graduate Program - USP (Av. Prof. Lineu Prestes, 1374, São Paulo/SP, Brazil, 05508-900) gabriel.guarany@lnls.br, [2]Institute of Astronomy, Geophysics and Atmospheric Sciences – USP (Rua do Matão, 1226, São Paulo/SP, Brazil, 05508-090) fabio.goncalves@iag.usp.br, [3]Institute of Chemistry - USP (Av. Prof. Lineu Prestes, 748, São Paulo/SP, Brazil, 05508-000) farod@iq.usp.br, [4]Brazilian Synchrotron Light Laboratory (Av. Giuseppe Maximo Scolfaro, 10000, Campinas/SP, Brazil, 13083-100) douglas.galante@lnls.br

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 warmer temperatures (up to -2 °C), are produce by bacteria, surpassing commonly found mineral aerosols [1].

One of the best studied of those biological IN is *Pseudomonas syringae*, an epiphyte and a plant pathogen. 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 and snow samples, in addition to cloud water collected in high altitudes [3].

As cruisers of the atmosphere, bacterial cells must endure harsh conditions much different from their ground habitats. 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]. The present study aim to better understand the different impacts, mainly by irradiation, aerosolized *P. syringae* suffers.

Material and Methods: *P. syringae* cells were routinely grown on TSB medium. Two strains were used, 281 (subspecies pv. *syringae*) and 158 (subspecies pv. *garceae*). Stationary stage cultures were centrifuged and washed 3 times with Milli-Q ultrapure water and diluted to circa 10^6 CFU/ml before each experiment. IN activity was assayed by the drop freezing test. 32 droplets of 10 µl were placed on a paraffin-coated aluminum surface floating on an ethanol-water cold bath. Temperature was decreased gradually and the number of frozen droplets was counted at 1 °C intervals. Data was analyzed according to ref. [5].

Ultraviolet tolerance was tested for a cell suspension on a glass petri dish (without the lid). The plate was stirred during irradiation with an Oriel Sol-UV-2 solar simulator, emitting on UVA and UVB with the spectrum that reaches Earth's surface with higher intensity, here called "environmental UV". At each cumulative dose, aliquots were taken, diluted and plated to survival analysis by CFU. Also, the unirradiated control and the largest dose tested of UV (272 kJ/m² of UVA, 223 kJ/m² of UVB, 60 min of exposure) had their IN activity measured by the drop freezing test.

Results: Environmental UV was severely deleterious to *P. syringae*. 20 min of exposure reduced viability by nearly 100-fold, 281 faring slightly better than 158 (data not shown). After 60 min, no CFU were found, meaning a decrease of over 10^5 times in survival.

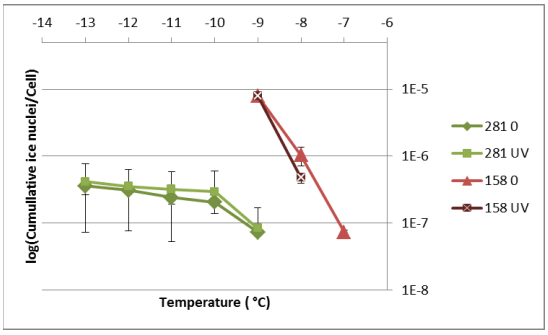

Fig. 1. Cumulative IN activity of the strains, before ("0") and after ("UV") irradiation. 158 was active at higher temperatures than 281, while 60 min of environmental UV seemingly did not affect ice nucleation on both strains.

Conclusion: *P. syringae* cells may suffer serious damage while exposed to solar radiation. Its IN activity though, could remain unaffected for longer periods, still possibly having an impact on cloud dynamics.

References: [1] Hoose, C. et al. (2010) *Environ. Res. Lett., 5*, 024009 (7pp). [2] R.M. Bowers et al. (2009) *Appl. Environ. Microbiol., 75*, 15, 5121-5130. [3] Morris, C.E. et al. (2008) *The ISME Journal, 2*, 321–334. [4] Smith, D.J. (2013) *Astrobiology, 13*, 10, 981-990. [5] Morris, C.E. et al. (2013) *Atmos. Chem. Phys., 13*, 4223–4233.