



## **Genome-based identification and characterization of a Gram-positive precipitation-associated ice nucleation-active bacterium**

Boris Vinatzer (1), Kevin Failor (1), Long Tian (1), Sophie LeBlanc (1), Haijie Liu (1), Noam Eckshtain-Levi (1), Christopher Lefevre (2), and Caroline Monteil (2)

(1) School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, United States (vinatzer@vt.edu), (2) CNRS/CEA/Aix-Marseille Université, UMR7265 Biosciences and Biotechnologies Institute of Aix-Marseille, Saint-Paul-lés-Durance, France

Two ice nucleation-active bacterial isolates were previously cultured from a sample of freezing rain in Virginia, USA, and identified as members of the Gram-positive genus *Lysinibacillus* based on 16S rRNA sequencing. Ice nucleation activity (INA) of the two isolates was found to be associated with submicron particles secreted from bacterial cells. The activity was determined to be heat-resistant and proteinase-resistant. These characteristics point to non-proteinaceous ice nuclei with very different characteristics compared to the long-known INA protein produced by the Gammaproteobacteria. To identify the *Lysinibacillus* isolates to the species level and to get insight into the genetic basis of their INA, the genomes of the two isolates were sequenced and compared to all publicly available genome sequences of *Lysinibacillus* species. Based on this comparison, the two *Lysinibacillus* isolates were identified as members of the *Lysinibacillus parvivoronicapiens* species. Interestingly, the type strain of *L. parvivoronicapiens* was found to have INA while all other *Lysinibacillus* species were found to be negative for INA. This suggests that within the *Lysinibacillus* genus, INA is an exclusive characteristic of *L. parvivoronicapiens*. The genome sequences of the *L. parvivoronicapiens* strains with INA were then compared to the genome sequences of all other *Lysinibacillus* strains that do not display INA. 238 genes were found to be exclusively present in the *L. parvivoronicapiens* strains. Of these, 131 genes were found to be expressed. Therefore, the genes responsible for INA in *L. parvivoronicapiens* can be expected to be among these 131 genes. These genes are being characterized further.