PSEUDOMONAS SYRINGAE
A MICROBIAL ICE MAKER

ANANDA WOLZ , LOLA BARDEL , TIMOTHY MANN , THOMAS CANELLAS & IZABELLA POMYKALSKA

P. SYRINGAE[2]

A gram negative and rod-shaped plant-pathogenic bacterium with polar flagella.

Having an ice nucleating activity, it causes water in plants to freeze which damages the plant cell wall and enables the bacterium to penetrate the cells and uptake nutrients .

This biological ice nucleator, when airborne in the atmosphere, promotes nucleation in the clouds, affecting global precipitations !

FUN FACT

The appearance of frost on plant leaves, under unusual conditions, led to the discovery of *P. syringae*

WHAT IS ICE NUCLEATION[1]

Nucleation is the first step in the creation of a new thermodynamic phase.

HOMOGENOUS NUCLEATION

Homogenous ice nucleation takes place below -40°C and only H_2O molecules participate in the reaction.

METASTABLE STATE
A particular excited state of a system with a lifetime longer than the usual excited state but shorter than the ground state

HETEROGENOUS NUCLEATION

A foreign material serves as a surface for H_2O molecules to form bigger clusters (embryos), of molecules in a metastable phase. The growth of ice embryos is governed by fluctuation dynamics of addition and detachment of H_2O molecules to the embryo. Once it reaches the size at which growth is more likely than decay, it becomes an ice nucleus, the first appearance of the new phase.

KEEP CALM AND MAKE ICE

P. syringae attack strategy evolved to take advantage of frost damage on plant leaves by nucleating ice, allowing it breach through the plant cell wall.

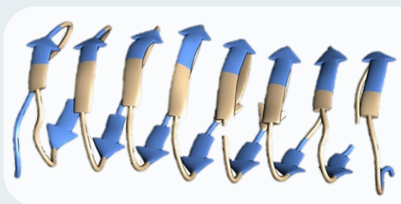
Ice nucleating activity is not only a useful tool to gain nutrients but it is also a mode of transport !
P. syringae has been isolated in hail stones suggesting that some of it can be found in clouds and carried by them.

THE ICE MAKING PROTEIN[2]

How cool is that?

The ice-nucleating activity of InaZ promotes ice growth at high sub-zero temperatures such as -2°C !
In comparison, pure supercooled water droplets remain liquid at –40°C in the atmosphere.

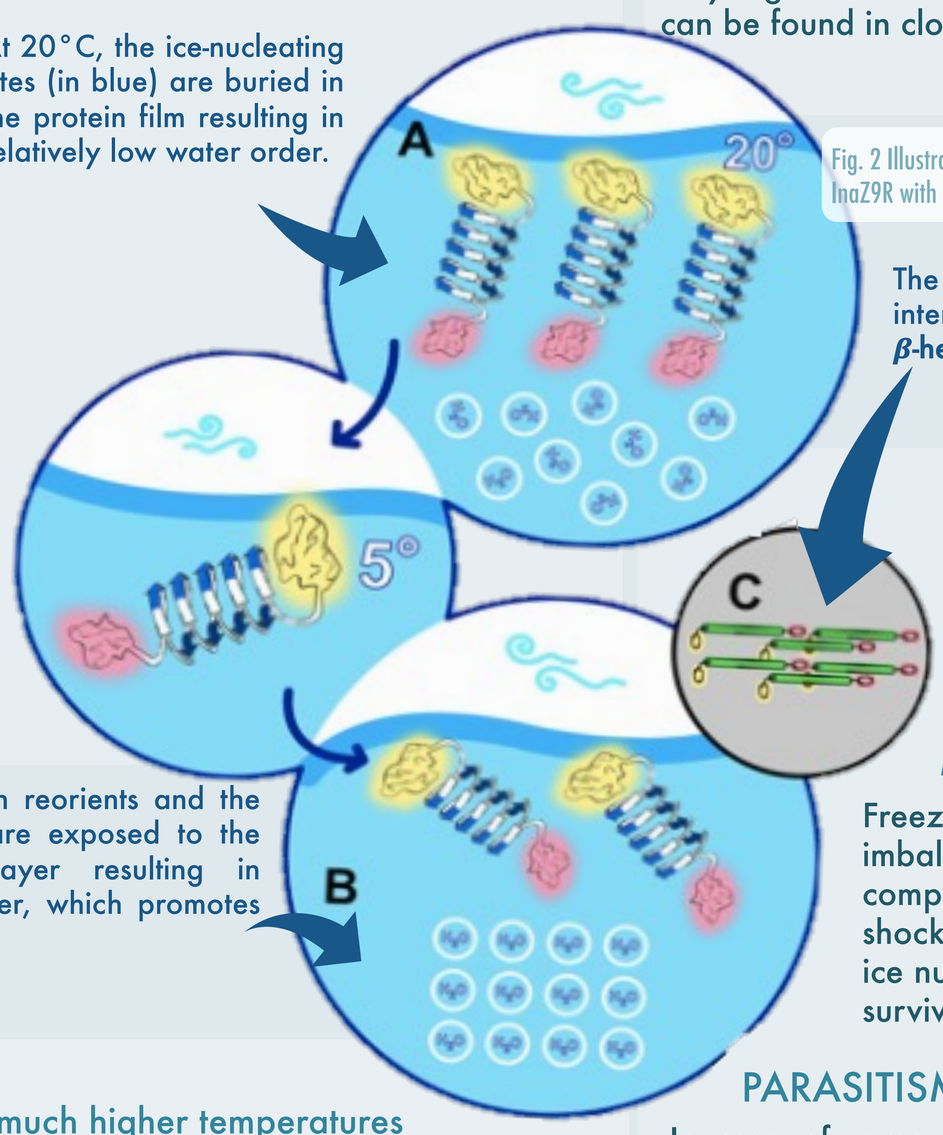
Thanks to vibrational sum-frequency generation (SFG) a model of the tertiary structure of ice-active repeats has been proposed:



β-helix

Fig.1 Repeat structure of the ice-nucleation active protein InaZ without the N- and C-terminal domains. In blue, ice-active repeats

The conformation of the ice-active imposes a structural ordering on the interacting H_2O molecules.



At 20°C, the ice-nucleating sites (in blue) are buried in the protein film resulting in relatively low water order.

Fig. 2 Illustration of the interaction of InaZ9R with water molecules.

The low-temperature protein pose parallel to the water interface.
β-helix (green), N- and C termini marked yellow and pink.

At 5 °C, the protein reorients and the ice-nucleating sites are exposed to the interfacial water layer resulting in increased water order, which promotes ice nucleation.

FUN FACT

Ice nucleating bacteria can promote the growth of ice better than any other material[1] !

SYMBIOSIS

Different cases of parasitism and mutualism have been spotted between *P. synrigae* and various eukaryotic hosts.

MUTUALISM

Freezing at higher temperatures lowers osmotic imbalances between intracellular and extracellular compartments, minimizing the potential for osmotic shock. In freeze tolerant vertebrates it is believed that ice nucleation at high temperature is essential for their survival[3]

PARASITISM

In case of some insects, many of them enhance the supercooling capacity of their body fluids, thereby avoiding the lethal effects of tissue freezing. The presence of nucleating agents that catalyse ice formation at high sub-zero temperatures is the primary factor limiting supercooling capacity.[4]

APPLICATIONS[5]

The ability to nucleate ice at much higher temperatures than usual opens the doors to many different applications...

FROZEN FOODS

The addition of ice nucleating gel, extracted from *P. syringae*, to liquid food such as egg whites and lemon juice makes them freeze before reaching the sub-zero temperatures.

SNOW MAKING

Ski resorts apply the ice making skills of *P. syringae* to create fake snow for the slopes.

CRYSTALLIZATION CONTROL

By controlling the number of *P. syringae* cells as well as other parameters, we can control fairly well the crystallization process which has potential for energy savings.

AND POSSIBLY ...

CLOUD MAKING

Send ice-nucleating cells in the sky to induce cloud condensation at higher temperatures than usual and to fight droughts

CROP MAKING

Reverse engineer the ice-nucleation properties of P. *Syringae* to protect the plants

CONCLUSION

Thanks to its ability of ice nucleation, *P. syringae* is among the most fascinating micro-organisms.

Although considered for long only as a plant-pathogen, it is believed now to participate in many important hydrological cycles.

Understanding its ice nucleating protein opens the door to many opportunities and applications going from food preservation to geo-engineering.

1 Lee, Richard E., Garreth Warren, et al. Biological Ice Nucleation and Its Application. 1994.
2 Roeters, Steven J., et al. “Ice-Nucleating Proteins Are Activated by Low Temperatures to Control the Structure of Interfacial Water.” Nature Communications, vol. 12, no. 1, 2021, p. 1183, https://doi.org/10.1038/s41467-021-21349-3.
3 Zachariassen, K. E. “Ice Nucleating Agents in Cold-Hardy Insects.” Water and Life, edited by George Nicholls Somero et al., Springer Berlin Heidelberg, 1992, pp. 261–81, https://doi.org/10.1007/978-3-642-76682-4_16.
4 Lee, Richard E., Janet M. Strong-Gunderson, et al. “Isolation of Ice Nucleating Active Bacteria from Insects.” Journal of Experimental Zoology, vol. 257, no. 1, 1991, pp. 124–27, https://doi.org/10.1002/jez.1402570116.
5 (Modern Language Association 9th edition) Cochet, N., and P. Widehem. “Ice Crystallization by Pseudomonas Syringae.” Applied Microbiology and Biotechnology, vol. 54, no. 2, Aug. 2000, pp. 153–61, https://doi.org/10.1007/s002530000377.

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