Localization and activity of a novel *Pseudomonas borealis* ice nucleating protein in *E. coli* and *P. syringae*

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An ice nucleating protein (INP) with 66% sequence identity to the better-known INP of *Pseudomonas syringae* has been described in an environmental isolate of *P. borealis* (*Pb*). Using the coding sequence and a green fluorescent protein tag (GFP), a *Pb*INP-GFP fusion protein construct was inserted into a pet24α vector in order to visualize the expression and localization of the protein in *E. coli*. The *Pb*INP-GFP protein appears to localize at the poles of *E. coli*, but the nucleation temperature for these cells was only marginally above -9°C, which indicated poor nucleation activity. We are now attempting to use a strain of *P. syringae* with no functional INP gene (Ice$^-$ strain) as a host for a construct of *Pb*INP-GFP in the vector pMEKm12 in an attempt to improve the nucleating activity and to localize the protein in the native genus. It is hoped that additional functional characterization of this *Pb*INP will lead to a better understanding of these proteins and their importance to the handful of bacteria that exhibit this activity.

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