Detecting ice nucleating bacteria in environmental samples using PCR of the gene conferring ice nucleation activity. Abstract

Cindy E. Morris, Caroline Guilbaud, Helen Ahern, Hélène Dominguez, Catherine Glaux, Bruce Moffett, Tom Hill

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Detecting ice nucleating bacteria in environmental samples using PCR of the gene conferring ice nucleation activity

Dr. Cindy Morris
Plant Health and Environment Inst. Nat'l de la Recherche Agronomique - Franc

Caroline Guilbaud, Helen Ahern, Hlne Dominguez, Catherine Glaux, Bruce Moffett, Tom Hill

Fewer than 10 species of bacteria are known to be ice nucleation-active. Furthermore, this property is conferred by a single gene and the overall gene sequence is rather well conserved among the 5 or 6 variants of the gene whose sequences have been reported. Nevertheless, there have been no methods reported for detecting ice nucleation-active bacteria in environmental samples via PCR (Polymerase Chain Reaction for amplification of the nucleic acid sequence) that take into account the full range of known variability of the bacterial gene. We have designed sets of primers for PCR based on reported sequences of the core and C-terminal regions of the inaW, inaY and inaZ, inaK and inaV alleles and sequences of strains from our culture collections. The sensitivity and specificity of these primers were determined in PCR conducted on about 100 pure cultures of ice nucleation active (and some inactive) strains of Pseudomonas syringae, P. viridiflava, P. fluorescens, P. putida, Panteoa agglomerans and Xanthomonas campestris collected from plants, rain, snow, clouds, aquatic habitats, frogs and insects in North American, Europe, China and Antarctica. The sensitivity of these primers was tested with environmental samples (rain, snow, aquatic habitats) from which previous isolations revealed the presence of ice nucleation active bacteria, and in environmental samples (rain, snow, aerosol samples) seeded with the test strains from our collection. This tool will be used to detect and quantify ice nucleation active bacteria in environmental samples and can be a means of collecting corroborative evidence of the role of these bacteria in atmospheric processes.

Keywords: ice nucleation gene, pcr