CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

S0534

Project Title

An Investigation of the Ice Nucleation Protein from Pseudomonas syringae

Abstract

Objectives/Goals

The purpose of this research project was to improve the ice nucleation protein of Pseudomonas syringae a plant pathogen. Pseudomonas syringae produces an ice nucleation activity protein Z [inaZ] that acts as a template for ice formation (crystals) at high temperatures such as -1.0 C. Although research has been done to remove this gene (it is a leader in frost damage), no one has improved the ice nucleation ability. To improve the ice nucleation ability of Pseudomonas syringae an analysis of the sequence was done to find 800 repeating amino acids which acts as the template in inaZ. The hypothesis is that if the repeated region was to be doubled there would be an increase in ice nucleation. To assay for improved ice nucleation a method has been devised involving crossed polarizers and the ice property of birefringence.

Methods/Materials

The materials used in this research project actually does not include Pseudomonas Syringae but rather the inaZ in a 29kb pVS1 vector called KI (KAN) [ice plus]. To repeat the amino acid sequence in inaZ it first had to be moved to another vector for easier manipulation. The vector of choice was pMUC-BS (3.5kb) a much more stable plasmid. To move the gene; a series of PCR engineering steps were used. This involved engineering in XhoI and NotI sites at the promoter and terminator. In addition two other unique sites were engineered at the start of the repeated region in [inaZ].

The ice nucleation assay method was done using cross polarizers and a ice+ and an ice- bacterium. Using a light box, the bacterium was checked from a freezer at constant time intervals.

Results

The results were not hopeful for the transfer of inaZ. DNA experiments from the PCR was run on a 1% agarose gel showing that the PCR was successful. After the second engineering stage, the band of desired PCR gene was very faint. However, when trying to purify this; the DNA was lost. The ice nucleation assay method was not useless, however, because pictures (and observation) showed that there was a significant difference in light between the ice+ and the ice-.

Conclusions/Discussion

In conclusion, (although the PCR did not work) the ice nucleation assay method showed a promising more reliable method to screen for ice nucleation in Pseudomonas syringae. Improving ice nucleation abilities is important to ski resorts that already rely heavily on Pseudomonas syringae for their ice machines.

Summary Statement

Improving the Ice Nucleation ability of Pseudomonas Syringae

Help Received

Used equipment from the University of California Davis under the supervision of Dr. Venkatesan Sundaresan