CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
Christopher L. Chau

Project Number
S0403

Project Title
How Bacteria Can Keep Us Cool

Abstract

Objectives/Goals

The ice nucleating activity of the proteins coded for by the inaZ gene of Pseudomonas syringae was determined by isolating the plasmid DNA, transforming BL21 E.coli cultures, and designing a variety of primers to cut the protein into different segments to establish the minimum composition needed to assure ice-nucleating activity. The original cultures of Pseudomonas syringae obtained from Greece were grown on plates of LB agar. Freezing assays and SDS-Page were run on the transformed protein-producing BL21 E.coli cultures in order to test for the presence of the inaZ gene. These were grown on LB plates with ampicillin, as the isolated plasmid consisted of both the inaZ gene and ampicillin resistance. 1-D protein gel electrophoresis was run to compare the genetic makeup of the transformants with that of the original inaZ culture. A time trial was run on cell cultures that were incubated in LB broth overnight, in order to determine the OD600 concentration at which ice-nucleating activity is optimal. The innoculations were grown to an optical density of 0.3-0.4, the point at which cells are in late log phase and protein expression begins, before IPTG was added to activate mass protein expression. Samples were taken every 30 minutes until an OD600 of 1.1. This variety of samples was then tested in a freezing assay. There was found to be a correlation between the ice-nucleating efficiency and the amount of protein on the membrane before the point at which the cell ceases to make the protein because it is harmful to itself. The cells also proved to grow better under harsh conditions with low nutrition, while greater amounts of PI (phosphatidylinositol), which the N-terminal of inaZ binds with, is directly related to the temperature at which it actively ice-nucleates. In the freezing assay, three of the five transformed cultures ice-nucleated at -6°C, while past research only showed ice-nucleating activity at a temperature of -9°C. A mid-range OD600 of about 0.6-1.1 was found to yield optimal protein expression. Finally, agar beads were designed as a synthetic matrix upon which to anchor the proteins, thereby eliminating the need for the bacteria itself. Six pairs of primers have been designed to cut the protein into different segments to determine minimum composition to assure the most efficient ice-nucleating activity. At this point further research is required.

Summary Statement

To test the freezing capabilities of ice nucleating bacteria and the inaZ gene.

Help Received

Used lab at Cate School and Montana State University under supervision of Professor Cindy Morris, Tami Goetz, and Cheryl Powers.