Chill Out

Ice nucleating bacteria: How recombinant DNA technology can protect plants from frost damage…

with the added bonus of revolutionizing the production of artificial snow!

Introduction

Ice nucleators: Did you think that water freezes at 32°F? In reality, pure water can be "super-cooled" to temperatures as low as -40°F before freezing occurs. Ice crystals are formed in cold temperatures, in the presence of moisture, and with the help of a "seed" to get the process started. This seed is known as an ice nucleator. The addition of an ice nucleator slows down the motion of water molecules, removing enough energy from the water to allow the molecules to align in a crystalline lattice and enter the solid state. An ice nucleator is any foreign particle, such as a piece of dust, which incites the freezing process.

Frost damage: Frost is responsible for approximately $1 billion in crop losses each year in the United States. Bacteria living on plant surfaces are partly responsible for frost damage by serving as biological ice nuclei. *Pseudomonas syringae*, a common bacterium found on plants, in soil, and in the air, produces an ice-nucleation protein in the outer-membrane of its cells. With *Pseudomonas syringae* inhabiting a citrus blossom, water can freeze at higher temperatures and effectively kill the buds! Many of us have heard about frost damage to Florida's citrus crop on the news!

Historical Perspective: What was the first recombinant microbe released into the environment? *Pseudomonas syringae*.

This all started when scientists envisioned creating a strain of the bacterium which does not produce the ice nucleation protein. The idea was that an 'ice minus' mutant could be sprayed on crops in hopes that it would outcompete the naturally occurring ice positive strains. This is the first use of a genetically modified organism to biologically control another organism.

The Controversy: The genetic modification of *P. syringae* did not involve the addition of any genetic material, but rather, the deletion of a gene. Regardless, at the time of this work (early 1980s), any genetic modification of an organism was controversial. A company, Advanced Genetic Sciences, applied to the government to get permission to conduct field trials with the ice minus bacterium. In 1983, the company gained permission from the National Institute of Health (NIH) and the Environmental Protection Agency (EPA) to conduct field experiments with the ice minus strain, but environmental groups legally challenged these experiments. It wasn't until 1987 that the first field trials could be completed. The result: spraying a potato crop with the ice minus bacterium offered protection from frost. A success! (Diagram 1)

Production of 'Ice Minus' Bacteria: To delete the ice gene from *P. syringae*, researchers first had to find and clone the gene. This was accomplished by cutting *P. syringae*'s DNA into pieces using restriction enzymes. The pieces of DNA were then inserted into a plasmid of *E. coli*. Because many different recombinants were produced, the *E. coli* containing the ice gene had to be selected. This is relatively simple since the *E. coli* with the ice gene will have the phenotype of an ice nucleator. Diagram 2 illustrates the process of cloning the ice gene. Once researchers found and cloned the ice gene, they were able to make recombinant, 'ice minus' mutants of *P. syringae* with deletion of the ice gene.

Purpose

To demonstrate that some bacteria can serve as ice nucleii, and to observe the phenotypic difference between the 'ice minus' and 'ice positive' forms of *P. syringae.*

Materials

Pseudomonas syringae 'ice positive' culture (5 plates, 2 days old)
Pseudomonas syringae 'ice negative' culture (5 plates, 2 days old)
2 rubber policemen
bucket containing 50% crushed ice and 50% ethanol
2 large plastic test tubes containing 5 ml of 0.01M KPO_4 (pH 7)
15 glass test tubes containing 0.1M KPO4 (pH 7)
2 Pasteur pipettes

Treatments

This experiment requires three treatments.

Treatment 1—Control, 0.1M KPO_4 (pH 7) buffer
Treatment 2—Ice positive bacterium
Treatment 3—Ice minus bacterium

Methods
1. Label large plastic test tubes, "Ice positive" and "Ice negative".
2. Using rubber policemen, scrape bacteria off the plates and into respective large plastic test tubes to make two cloudy bacterial suspensions.
3. Label 5 replicate glass test tubes for each treatment.
4. Place glass test tubes in the ice/ethanol slurry to supercool. The temperature will be between -5C and -10C. Note that the buffer (treatment 1) does not freeze.
5. Pipette one drop of the ice positive bacterial suspension to each of the five test tubes labeled for treatment 2, and one drop of the ice negative bacterial suspension to each of the five test tubes labeled for treatment 3.

6. Observe.

Questions

1. Why do we set up 5 replicates for each treatment?
2. How did the test tubes containing bacteria compare to the control treatment?
3. Which bacterium could be used as a biological control agent to protect crops from frost damage? Why?
4. Can you think of any other applications for either the ice minus or ice positive bacterium?

Check out these websites and resources:

Lindow, S.E. 1990. Use of genetically altered bacteria to achieve plant frost control. Biotechnology of Plant-Microbe Interactions. Editors: P. Nakas and C. Hagedorn. McGraw-Hill, New York, NY, pp 85-110.

International Cryobiology Young Researchers Group
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