CALIFORNIA STATE SCIENCE FAIR
2015 PROJECT SUMMARY

Name(s)

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Project Number

35723

Project Title

CryoSubtilis: A Synthetic Biological Approach to Engineering Cold Resistance in B. subtilis

Abstract

Objectives/Goals

B. subtilis is a popular host for the manufacture of enzymes, mainly due to its high competency and status as a GRAS organism. However, there are severe drawbacks to using it as a host for biomaterial production. The cost of maintaining bioreactors and incubators to grow B. subtilis is extremely high, and inadequate disposal of antibiotics used to select for transformed bacteria is one of the leading causes of pathogens gaining resistance to them. The objective of this project was to increase the cold resistance of B. subtilis through expression of CspC and ZeAFP proteins. By making B. subtilis cold resistant, both problems can be solved in one go.

Methods/Materials

The genes for the CspC and ZeAFP proteins were inserted into the plasmid vector pHT01 through a series of cloning steps, resulting in two plasmids: pHT01-CspC and pHT01-ZeAFP. Both plasmids were transformed into B. subtilis. Then, the OD (optical density), number of surviving cells, and survival rate was calculated in response to 4 cold shocks (10 minutes on ice for each cold shock).

Results

There is a clear difference between the survival rates of the transformed and untransformed bacteria. After the first cold shock, there was a 37.27% difference in the survival rates, and this trend continues. Over time, the difference in the percentages of living cells averages out to 40%. This means that after 4 cold shocks, the bacteria expressing both proteins are 12.86 times more likely to survive than bacteria without the proteins.

Conclusions/Discussion

The results presented support the hypothesis that the expression of these two proteins will increase the cold resistance of B. subtilis. These proteins can now be used to replace antibiotics as a selecting agent, developing a self-contained means of selection for transformation. They can eliminate the need for incubators by changing the optimal growth temperature of organisms, and can make organisms such as plants more cold resistant.

Summary Statement

This project aims to increase the cold resistance and tolerance of the bacterium B. subtilis through the expression of CspC protein from P. irgensii and ZeAFP protein from Z. elongatus.

Help Received

Parents drove me around from place to place; Mr. Lee helped throughout the project; Stephanie Yang of the Wyss Institute helped with questions about experiment design; Dr. Kenny Mok of UC Berkeley helped troubleshoot failed transformations.