Antifreeze Proteins: Busting Biofilms and Crushing Ice Crystals

Kayla DeSantry, Eddie Dring, Chloe LaJeunesse, Julie Mazza, Dave Morgan, Charlotte Reames
Advisors: Professor Natalie Farny, Professor Joseph Duffy

Abstract

Antifreeze proteins (AFPs) have evolved in numerous cold-dwelling species to protect against the formation of cell-lyzing ice crystals in subzero temperatures. AFPs have many applications from tissue preservation to food security and more, but recently a novel application has emerged: inhibiting biofilms. Biofilms are problematic in many settings including healthcare, manufacturing, and the environment. The use of AFPs as an anti-biofilm factor is intriguing, however only a few studies have yet been shown to inhibit biofilms. We built a diverse library of AFPs and characterized both their antifreeze and biofilm-inhibiting properties in E. coli. Our results demonstrate novel biofilm-inhibiting properties for some AFPs and further characterize the freeze protection properties of many AFPs at low subzero temperatures. Our results will inform the design of novel synthetic AFPs optimized for use in E. coli, and provide a valuable new resource for the integration of AFPs into synthetic biology and biotechnology applications.

Background

Antifreeze proteins (AFPs) are a structurally diverse class of proteins that alter the dynamics of ice crystallization and thereby permit organisms to survive in temperatures below the freezing point of water. To do so, AFPs bind water molecules across their relatively flat ice-binding surfaces, maintaining those bonds primarily through van der Waals interactions. AFPs have tremendous promise in biotechnology applications including cryogenic tissue preservation, freeze tolerance in crops, and improvement in the quality of frozen foods. AFPs have been utilized in a limited way by a number of previous iGEM teams, though very few AFPs have been analyzed thoroughly for use in E. coli, and rarely have they been explored comparatively. One of the most exciting new applications for AFPs has only recently been discovered: Heisig et al. (2014) showed that a tick antifreeze glycoprotein, IAPGF, was disruptive to the formation of biofilms—cooperative structures formed by bacteria that contribute to serious problems in manufacturing, food processing, and human health. The current research suggests that the anti-virulent properties of IAPGF are based on structural elements of the protein that allow it to bind to microbes and disrupt biofilm formation (Heisig et al., 2014). However, very little is known about this, and only one study has been completed. More research is needed to determine whether other antifreeze proteins exhibit the same effect.

Our AFP Library

We constructed a library of 39 parts for the iGEM registry consisting of 16 AFP protein coding region BioBricks, 12 untagged AFP BioBricks, and 11 BclA tagged AFP BioBricks. BclA tagging resulted in cell surface expression of AFPs, whereas no tagging resulted in secretion or internal expression of AFPs.

Methods

Antifreeze activity of AFPs: E. coli DH5α transformed with plasmids were grown in LB minimal media to an OD600 of 1.5, then thawed and assayed for cell survival using a colorimetric MIT assay.

Biofilm formation assay: E. coli EMG2KX cells were transformed with plasmids from the library and grown in LB minimal media in a 96-well plate at 37°C for 48 hours, then stained with crystal violet to visualize biofilm formation.

Biofilm Results

Figure 1. Freeze Survival Results

Freeze Survival Results

Effects of AFP expression on freeze survival at -20°C

When expressed in E. coli, BclA-tagged GfAFP, BclA-tagged RifAFP, BclA-tagged EpAFP, IAfAFP, BclA-tagged IAFGP, TIAf, and BclA-tagged IAFGP appeared to confer increased freeze survival at -20°C. RifAFP, TIAf, IAf, BclA-tagged MaffAFP, EpAFP, and ApAFP, on the other hand, seemed to be detrimental to the freeze survival of E. coli.

Improved Characterization of ZeAFP and BclA

Enhancement of biofilm formation by ZeAFP

In our improved characterization of BBa_652004, the ZeAFP BioBrick designed by the Yale 2011 team, we found that ZeAFP increases biofilm formation in EMG2KX. and does not confer freeze survival in E. coli at -20°C.

Decreased freeze survival at -20°C for E. coli expressing ZeAFP

In our improved characterization of BBa_142000, the BclA BioBrick designed by the WPI 2014 team, cell surface expression of AFPs through the BclA generally resulted in increased biofilm formation and freeze survival.

Future Directions

The selective inhibition or promotion of biofilms has a variety of applications in synthetic biology. Inhibition in particular is useful for applications in medicine, and a therapy using non-lytic phases to incorporate AFP genes into bacterial genomes could be explored. Additionally, for applications in which “tuning” biofilm levels would be useful, a two-way genetic circuit could be devised.

Human Practices and Public Engagement

As part of our research into implementations of biotech innovation, we conducted an analysis of the safety of using non-lytic phases as a therapy for infection by endotoxins, biofilm-forming bacteria, finding that much more research is needed on the safety of such a therapy before implementation would be viable. We also participated in education and outreach programs that reached hundreds of people: we created activities for Touch Tomorrow, a family-oriented engineering and science festival, and held a synthetic biology workshop for the Women in Science camps for middle school students. We assessed the value of our educational activities by distributing a 5 question survey to Touch Tomorrow participants. A majority of the opinions on synthetic biology after participating in our activities were positive, so we believe our public engagement was successful.

Sponsors

Contact us at igem2015@wpi.edu