A Novel Approach to Sustainable Ice Skating Surfaces Utilizing Recombinant Ice–Nucleating Proteins

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Abstract:

Ice rinks are a cultural cornerstone in Canada, with over 800,000 registered skaters, hockey players, and curlers using nearly 3,000 indoor facilities nationwide. Maintaining surface temperatures between -4 and -8°C for ice crystallization relies on energy-intensive refrigeration systems, contributing to 40% of corporate building emissions and making ice rinks major municipal energy consumers. Current polymer-based synthetic ice alternatives are expensive and compromise skating quality due to excessive blade dulling, limiting their viability at a commercial scale. As such, this paper proposes the use of ice–nucleating proteins (INPs), naturally produced by *Pseudomonas syringae*, to catalyze ice nucleation at higher temperatures, thereby reducing dependence on refrigeration systems. *Escherichia coli* will be genetically engineered to excrete these proteins, which will be applied to rink surfaces to facilitate ice formation.

Introduction

Maintaining ice rinks requires substantial energy to sustain surface temperatures between -8 and -4°C. On average, a single rink consumes approximately 520kWh per day, equivalent to the monthly energy usage for an entire household[1]. With nearly 3, 000 indoor rinks operating year-round in Canada, these facilities remain the largest energy consumers in municipal regions, contributing to approximately 40% of corporate building emissions[2][3]. The majority of this demand— nearly 50% — goes into refrigeration, which is essential for allowing ice surfaces to freeze. The energy consumed in this process directly contributes to high carbon emissions, leaving municipalities struggling to balance environmental concerns with the need for recreational activities. Beyond environmental impacts, the energy demand of ice rinks places a heavy financial burden on rink operators, as rising costs make it increasingly challenging to maintain these facilities. Without intervention, the dual pressure of environmental degradation and financial strain may erode the legacy of Canadian ice skating, necessitating innovative solutions that balance both sustainability and quality.

Several alternative approaches have been explored, yet none fully meet the dual requirements for energy efficiency and performance. Synthetic ice, made of polymers, reduces the need for refrigeration but significantly alters the skating experience due to increased friction and blade dulling. As a result, it remains unsuitable for high–performance sports such as hockey and figure skating. Alternatively, ammonia-based refrigeration systems provide a biodegradable alternative to traditional refrigerants but introduce safety concerns due to ammonia's toxicity and flammability[4]. Both these systems are costly to maintain, limiting their viability in the long term.

To address these challenges, this paper explored a novel alternative to conventional ice rink surfaces. We propose the genetic modification of *E. coli* with genes derived from *Pseudomonas syringae* to secrete

ice–nucleating proteins (INPs). These proteins catalyze the crystallization of ice at higher temperatures, reducing the energy required for refrigeration while maintaining the integrity of traditional ice.

Solution & Implementation

Objectives

This proposal entails three primary objectives; (1) to design a plasmid with genes derived from *Pseudomonas Syringae* to be transformed into *E. coli* cells for the secretion of ice-nucleating proteins (INPs).; (2) to model interactions between INPs and water to analyze the mechanisms for ice nucleation; and (3) to purify proteins from cell cultures and evaluate their nucleating capacity under different conditions to optimize their application.

Proof of Concept

Ice-nucleating proteins were first identified as naturally produced by *P. syringae*, a plant pathogen that induces frost damage to infect host plants. These proteins facilitate ice crystallization at temperatures as high as 2°C, significantly higher than the freezing point of pure water, which is as low as -38°C [5]. This unique property has been observed in atmospheric processes, where *P. syringae* contributes to cloud formations at low altitudes. Notably, the temperature required for ice nucleation in ice rinks, which is generally between -4°C and -8°C, is still lower than the threshold that INPs can induce, making them ideal for reducing energy demands in refrigeration systems.

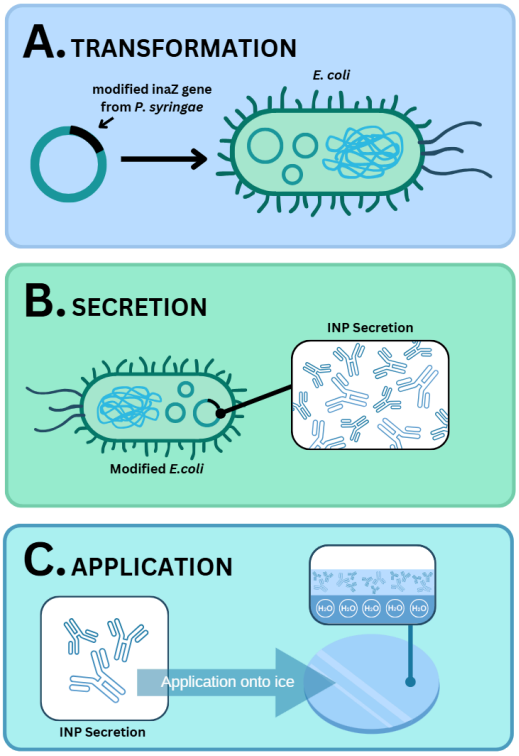

Figure 1. (A) Transformation of *E.coli* plasmid to produce INPs (B) Secretion of INPs by *E.coli* (C) Application of proteins

In this proposed system, *E. coli* will be engineered to secrete INPs through the insertion of inaZ genes from *P. syringae*. INPs are naturally bound to the surface of the cell—in this system, we aim to modify the plasmid to eliminate the membrane-bound structures, allowing the proteins to be secreted into the culture medium (*refer to 'Genetic Engineering')*. Once secreted, these proteins will be purified from the cell mass using standard protein purification methods. In the long term, we propose that the purified proteins be shipped to ice rink facilities, where they may be applied using an ice resurfacer, such as a Zamboni. Further details of the delivery mechanism will be explored further in the project.

The application of this project is further supported by the current success of INPs in ski resorts. These proteins are extracted from the microbial membrane, then concentrated and packaged for use in snowmaking systems[6][7]. By increasing the temperature required for snow formation, INPs substantially increase the efficiency of snowmaking in these areas, reducing water and energy usage while ensuring

consistent snow quality even under fluctuating temperatures. The successful implementation of this technology in snowmaking systems establishes a strong foundation for INPs to similarly optimize ice rink operations, offering a sustainable solution for energy-efficient ice maintenance.

Ice Nucleation in Nature

The formation of ice commonly occurs through heterogeneous nucleation, a process by which solid crystals are formed around a nucleating particle[8].In the absence of nucleating particles, pure distilled water begins to freeze at -40℃ in a phenomenon known as 'subcooling' [9]. While non-biological nucleators exist, the most efficient nucleating agents are proteins derived from biological systems. Non–biological nucleations are capable of inducing ice formation at temperatures below -15℃, whereas ice–nucleating proteins can facilitate nucleation at much higher temperatures, ranging from -15℃ to -2℃[10]. Numerous organisms, including algae, fungi, bacteria, fish, and plants, have been shown to produce INPs.

Ice Nucleating Proteins: Structure and Function

INPs' molecular structure consists of several small Ice Nucleating Protein units that can bind to each other to form large aggregates, also known as multimers. The INPs produced by *Pseudomonas syringae* consist of three primary structures; the central repeating domain (CRD), the N-terminal domain, and the C-terminal domain. The CRD comprises two structures; WO and R coils. WO coils form the majority of the protein and contain two beta strands that are folded in a β-helical conformation. The motifs are formed of three amino acids, TxT and SxL[T/I], where x is any amino acid[11]. The motifs are composed of both hydrophilic and hydrophobic groups, which play essential roles in nucleation. Hydrophilic groups, represented by the flow arrows, attract water molecules, forming active sites for nucleation. Hydrophobic groups, shown in white, stabilize trapped water within the helical loops. The overall structure of the protein resembles that of ice crystals, which is key to ensuring water nucleates around the protein[12].

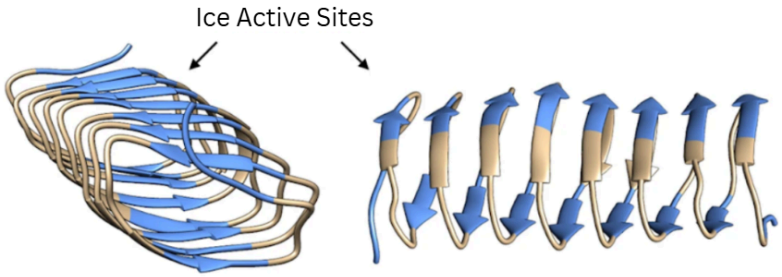

Figure 2. Model of hydrophilic motifs in the Central Repeating Domain (CRD). From "*Ice-nucleating proteins are activated by low temperatures to control the structure of interfacial water". Nature Communications, 12(1), 1183. https://doi.org/10.1038/s41467-021-21349-3*

Similarly, the second component of the CRD, the R–coils, consists of repeating motifs. These structures are critical for both ice nucleation and overall protein stability. Studies have demonstrated that the removal of specific R-coil motifs reduces nucleation efficiency, whereas the addition of motifs enhances it[13]. At the end of the R-coils, the C-terminal domain regulates protein structure and modulates activity in response to environmental damage. Conversely, the N-terminal domain is essential for protein folding, maintaining tertiary structure, and facilitating ice nucleation. Notably, the N-terminal domain anchors the protein to the cell membrane through hydrophobic interactions. This domain enables the protein to form bonds with polysaccharides and lipids, as well as to bind with other INPs, forming aggregates within the membrane[14].

Due to their small size, individual INPs have limited capacity to nucleate large ice crystals. To enhance this, multiple INPs can chain together to create larger multimers through hydrophobic interactions at the N-terminal domains[14]. Studies indicate that larger multimers exhibit increased ice-nucleation efficiency, producing greater quantities of ice. Furthermore, multimers located outside the cell membrane are more resistant to pH and temperature fluctuation, a quality that can be critical in different commercial applications of the protein[15].

Advantages of Utilizing Pseudomonas syringae Genes in INP Synthesis

INPs are produced by various organisms, theorized to have occurred in order to avoid subcooling and improve heat retention. Of these, the INPs derived from *Pseudomonas Syringae* are among the most efficient and well-characterized. These INPs are encoded by the inaZ gene and are expressed on the outer cell membrane to maximize interactions with water molecules[16]. Notably, INPs from Pseudomonas syringae can initiate ice formation at temperatures as high as -2℃— the highest recorded nucleation temperature[17]. This efficiency makes *P. Syringae* an ideal candidate for reducing energy demands in ice rink maintenance.

Advantages of utilizing E. Coli in INP Synthesis

E. coli was chosen as the host organism for transformation with *P. syringae* INP genes due to its well-established use in plasmid design. In this project, *E. coli* DH5α will be initially transformed as it is optimal for plasmid uptake and amplification. Subsequently, the BL21 strain will be employed for protein expression and purification followed by functional tests.

Genetic Engineering

This project aims to optimize the system for protein extraction by designing a plasmid that incorporates the inaZ genes from *P. syringae*, which encode for ice nucleation proteins (INPs). We also plan to eliminate the genes responsible for anchoring INPs to the cell membrane, enabling *E. coli* to secrete them. Upon successful testing and prototype building, we additionally plan to incorporate further modifications, such as the integration of fluorescent proteins to increase consumer appeal. As this project is in its proposal stage, our immediate focus is research into the interactions of INPs and the role of the N-terminal domain in binding INPs to the cell membrane, with particular attention to the formation of INP multimers and aggregates.

Molecular Cloning (MoClo)

Our group intends to transform bacterial cells with a carefully designed plasmid that will contain modified inaZ genes from *P. syringae* efficient protein synthesis optimized for extraction. To achieve this, we will employ the Molecular Cloning (MoClo) technique, a genetic engineering method that utilizes Type IIS restriction enzymes. These enzymes bind to specific restriction sites and cut DNA downstream of their binding sites in a predictable manner that includes directionality[18][19]. This predictability of Type IIS restriction enzymes makes them particularly advantageous for precise and efficient gene insertion.

Additionally, we seek to isolate the functional motifs of the INP dimers, focusing on membrane binding properties, and optimizing INP expression for extraction, which will facilitate protein harvesting.

Modelling INP Interactions

Purpose of Protein Modelling

Molecular dynamics (MD) simulations provide a powerful tool for examining molecular interactions within proteins and revealing critical components of the ice nucleation process. Simulations enable predictions regarding how modifications to protein structures, such as the removal of membrane-binding regions, may enhance nucleation efficiency[20]. Additionally, MD simulations facilitate deeper investigations into the mechanisms of ice nucleation, contributing to the optimization of protein design[21]. To optimize the efficiency of INPs, we will conduct simulations for both unmodified and modified protein variants. This comparative analysis will provide insights into structural components that enhance the nucleation process, guiding future modifications and experimental validations.

Molecular Dynamic Simulation Software

To analyze these structures, MD simulation software such as GROMACS and CHARMM will be utilized to observe protein stability and interactions under various conditions. CHARMM provides comprehensive, widely used force fields that accurately model protein-water interactions and can be integrated with GROMACS. GROMACS is highly computationally efficient, optimized for modern hardware, and allows for large-scale simulations with reduced computational costs. GROMACS is also open-source and widely accessible, ensuring increased availability and flexibility with the software.

Variables for Simulation

The structure of the INPs will be obtained from Alpha Fold, and simulated using GROMACS with the following target conditions; an ideal temperature of 0 to -2 degrees, multiple thin layers of water to replicate ice rink conditions, and gradual cooling to observe nucleation events. During the simulation, interactions between water molecules and the protein will be monitored to track changes in their structure and dynamics. Our MD simulations will specifically examine the following aspects of ice nucleation; water structure and dynamics at the INP surface, surface and physical properties of INPs and supercooling effects. Hydrogen bond networks, water orders, and the structure of ice crystal lattices near the INP surface will be evaluated to determine structural dynamics at the surface of INPs[22]. Similarly, the surface and physical properties of INPs such as hydrophilicity, roughness, and functional protein structures will be assessed to determine their effects on ice nucleation. Finally, supercooling effects will be observed to determine how the presence of INPs influences the temperature

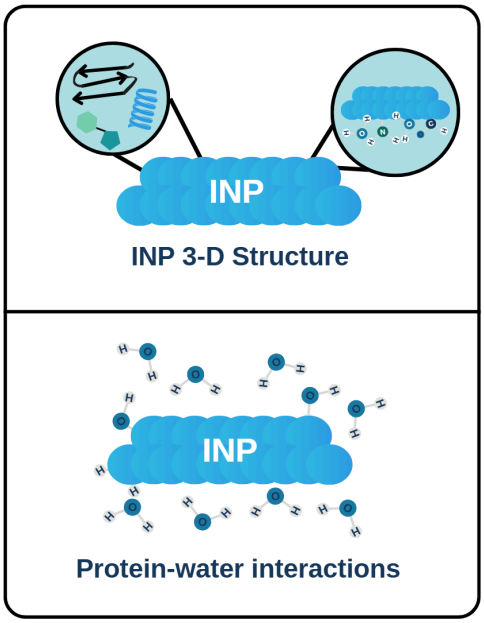

Figure 3. INP protein structure and water interactions

and degree of supercooling required for nucleation[23]. By simulating these parameters, we aim to develop a molecular-level understanding of how INPs enhance ice nucleation and identify modifications that aim to improve efficiency for practical applications.

Experimental Considerations

Experimental Variables in Functional Assays

To investigate the impact of ice nucleation proteins (INPs) on ice formation, three key variables will be examined: INP concentration, temperature, and time. These factors were selected based on their influence on the efficiency of ice nucleation. The concentration of INPs in the sample directly affects the efficiency of ice crystal lattice formation, thereby affecting the time required for the water to freeze. Previous studies have demonstrated that the probability of ice formation depends upon both temperature and the concentration of ice nucleators present. Current research has demonstrated optimal cellular concentrations for INP-producing bacterium such as *P. syringae*, with further research and experimentation needed to determine the ideal protein concentration[24].

Additionally, temperature is a critical determinant in ice formation and will be measured to evaluate the optimal environment for ice nucleation. A method for determining the temperature at which water transitions to ice was developed by Maria A. Majorina et al. in *The Influence of P. syringae on Water Freezing and Ice Melting*[24], where a solid-state thermostat was used to maintain a fixed temperature, with real-time data recorded. A similar experimental setup could be adapted and refined to more accurately measure the temperature at which ice nucleation occurs in the presence of INPs. Time also plays a crucial role in ice formation, particularly in applications such as ice rink surfaces, where ice is formed in thin sheets of water. Given that INPs may influence freezing kinetics, time can be monitored to assess their effect on freezing rates[25].

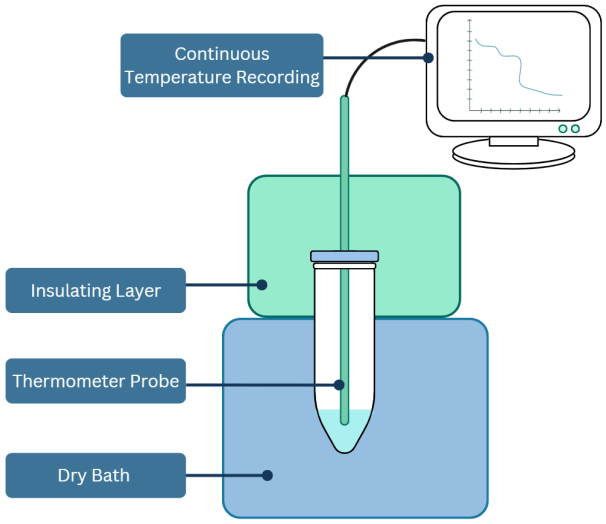

Figure 4. Proposed experimental setup

Experimental Constants

To ensure consistency across experiments, parameters such as ice thickness, freezing duration and water purity will be held constant. The standard ice thickness for professional rinks ranges from ¾ to 1 1⁄2 inches; as such, experiments will be conducted within this range to maintain real-world applicability[26]. Each sample will be frozen for a set period of time and ice formation will be periodically checked before being returned to the freezing environment. Deionized and filtered water is ideal to prevent impurities from being ejected to the surface, which could result in irregular ice texture and interfere with interactions between INPs and water molecules[27].

Social Impact

This project seeks to address the significant issue of energy consumption in ice rinks and the resulting challenges related to cost, sustainability, and efficiency. By developing a more sustainable solution, we aim to reduce environmental impact while preserving the cultural and recreational value of ice rinks. This will allow for the continued enjoyment and fulfillment that ice skating brings to communities.

To achieve these objectives, our Human Practices team will begin focusing on outreach and education—first, through assessing the general public's understanding of the issue and their connection to the problem. We aim to curate surveys targeting both smaller communities and larger populations to determine their awareness of the problem, as well as to identify key demographics and stakeholders concerned. From this, we plan to increase the engagement and understanding of the general public with this project through workshops and social media engagement. As the project progresses, we also plan to engage with stakeholders involved in ice rink maintenance, as well as experts in INPs, to gather feedback on our project and ensure it aligns with community values whilst contributing to the preservation of the Canadian ice skating tradition.

Conclusion

This paper outlines the energy-intensive nature of ice rinks due to the refrigeration process required to maintain freezing temperatures. As such, we propose the genetic modification of *E. coli* with modified inaZ genes derived from *Pseudomonas syringae* to secrete ice-nucleating proteins, which catalyze ice crystallization at higher temperatures. This paper also discusses methods for modelling the interactions between INPs and water molecules, as well as determining the efficiency of modified protein structures. Thus far, we have begun research into the structure of INPs and modelling systems, and plan to continue the research and development phase to a functioning prototype.

Biosafety

There is minimal biosafety concern regarding our project or its future applications. Ice nucleating proteins are naturally occurring and known for their role in cloud and frost formation. Our project focuses on optimizing the production of already existing INPs and as such, the risks of the proteins creating environmental harm are highly unlikely, as far as research suggests. Additionally, all modified biological components will be contained within a Risk Level 1 facility.

Conflict of Interest

We would like to acknowledge that we are not affiliated with or thereby funded by any external organizations dealing with areas of synthetic and microbiology, or that are focused on concepts relating to our project. All funds and support are received directly from Renert School.

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