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MINI-REVIEW

Bioprospecting for microbial products that affect ice crystal formation and growth

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Abstract At low temperatures, some organisms produce proteins that affect ice nucleation, ice crystal structure, and/or the process of recrystallization. Based on their ice-interacting properties, these proteins provide an advantage to species that commonly experience the phase change from water to ice or rarely experience temperatures above the melting point. Substances that bind, inhibit or enhance, and control the size, shape, and growth of ice crystals could offer new possibilities for a number of agricultural, biomedical, and industrial applications. Since their discovery more than 40 years ago, ice nucleating and structuring proteins have been used in cryopreservation, frozen food preparation, transgenic crops, and even weather modification. Ice-interacting proteins have demonstrated commercial value in industrial applications; however, the full biotechnological potential of these products has yet to be fully realized. The Earth's cold biosphere contains an almost endless diversity of microorganisms to bioprospect for microbial compounds with novel ice-interacting properties. Microorganisms are the most appropriate biochemical factories to cost effectively produce ice nucleating and structuring proteins on large commercial scales.

Keywords Ice-nucleating proteins · Antifreeze proteins · Ice-structuring proteins · Cryopreservation · Bioprospecting · Cryosphere

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Introduction

Cold-adapted organisms are prevalent in the three domains of life and our planet. More than 80% of the biosphere (by volume) is deep ocean at <5°C, and the cryosphere (i.e., frozen environments including sea ice, freshwater ice, snow, glaciers, ice sheets, ice shelves, icebergs, and permafrost) presently encompasses ~15% of the Earth's surface (Priscu and Christner 2004). Despite their widespread distribution (Deming 2002), contribution to global cell and carbon reservoirs (Priscu et al. 2008) and significance in biogeochemical cycling (e.g., Cavicchioli 2006), basic research on psychrophiles and cold-adaptation have lagged far behind that of thermophilic microorganisms. The heat-stable enzymes of thermophilic and hyperthermophilic bacteria and archaea have proved to be useful as low-cost alternatives to conventional industrial processes (e.g., Turner et al. 2007; Unsworth et al. 2007; Blumer-Schuette et al. 2008) and vital to the development of molecular biology (e.g., Saiki et al. 1988). In a similar way, the natural products of cold-adapted organisms have enormous biotechnological, biomedical, and industrial potential (Marx et al. 2007; Huston 2008; Venketesh and Dayananda 2008) and efforts to bioprospect for novel psychrophiles and cold temperature catalysts have increased significantly in recent years.

At temperatures below the freezing point of water, the phase transition to ice is directly associated with a variety of physical, osmotic, and metabolic cellular stresses (Sead and Park 2000; Tanghe et al. 2003; Kawahara 2008). It is clear that some microorganisms are inherently resistant to the stress of freezing and thawing (e.g., Walker et al. 2006) and capable of surviving while frozen for extended time frames (Christner et al., 2003; Miteva and Brenchley 2005; Bidle et al., 2007). However, the underlying molecular, biochemical, and structural adaptations responsible for freeze tolerance

and avoidance in prokaryotes have not been well-characterized. The intracellular accumulation of sugars, polyhydric alcohols, and amino acids is a common biological strategy for depressing the cytoplasmic freezing point (i.e., colligative properties) and/or preventing protein and membrane denaturation (Tanghe et al. 2003). Some microorganisms, polar fish, insects, plants, and fungi also produce proteins, often referred to as ice-binding or antifreeze proteins (IBPs or AFPs), which bind and inhibit the growth of ice crystals (Hoshino et al. 2003; Marshall et al. 2004; Liu et al. 2005; Raymond et al. 2008; Middleton et al. 2009). The ability to control ice crystal formation and growth would provide an obvious survival advantage to an organism that is frequently challenged with freezing or rarely experiences temperatures above the melting point of water.

The polar regions are not the only endemic locales for microorganisms possessing proteins with ice-altering properties. Seasonal temperature changes in mid- and low-latitude biomes require the winter-dormant fraction of the community to be freeze-tolerant (Zhu et al. 2007; Philip et al. 2008) and can trigger successional events that select for cold-adapted organisms (e.g., Shatwell et al. 2008). A variety of temperate bacterial, fungal, plant, lichen, and invertebrate species produce proteinaceous compounds, which serve as effective ice nucleators (Lundheim 2002), and some plant-associated and/or phytopathogenic bacteria catalyze freezing at temperatures near −2°C (Maki et al. 1974). Thus, in contrast to AFPs, ice-nucleating proteins (INPs) induce the cascade of ice crystal formation at temperatures much warmer than what would occur spontaneously. The ability to catalyze freezing at elevated temperatures could benefit a plant-associated microorganism by providing a strategy for accessing nutrients leaked from freeze-damaged plant tissue and aiding in infection (Hirano and Upper 1995). Paradoxically, the INPs possessed by some animal species have been shown to be important in conferring freeze tolerance (Lundheim 2002). Recent data suggest that the ice-nucleating properties of some airborne microbiota may enhance their removal from the atmosphere, facilitate widespread dissemination, and perhaps, influence precipitation generation (Morris et al. 2004; Christner et al. 2008a, b; Pratt et al. 2009).

The present review will focus specifically on highlighting microbial adaptations, which provide some species with a phenotypic advantage at subzero temperatures. More precisely, we shall consider their unique proteinaceous cryoprotectants and freeze-inducing proteins in the context of their potential in industrial, agricultural, biomedical, and food-related applications. Whether functioning to alleviate the stress associated with ice formation or serving as potent freeze catalysts, ice-interacting proteins have great promise for applications in which it is important to control freezing and the ice crystal structure. The biotechnological potential of low- and high-temperature extremophiles have been shown to be numerous and laudable; one additional byproduct of these efforts is that we have gained insight into nature's solutions for thermal tolerance. Despite an emerging interest to understand microorganisms that live in extremely cold environments, very little is known about the mechanisms that combat the stresses of ice crystal formation and freezing. In this vein, the overarching goal of this report is to provide a brief review on the topic of subzero microbiology and motivate interest to biosprospect for novel ice-active proteins. Elucidating and characterizing the interesting biological properties of these natural products will have long-term benefits for a variety of fields in the basic and applied sciences.

Survival in icy environments

Food scientists who were attempting to understand how microorganisms alter frozen foods and impact public health were the first to make significant strides in the area of subzero microbiology. Eighty-years ago, Wienzirl and Gerdeman (1929) presented compelling evidence for bacterial replication in ice cream at −10°C, which emphasized the need for stringent sanitary measures during frozen food preparation and storage. An increased awareness of the tenacity of life has made it apparent that the biosphere contains a diverse array of biogeochemically important ecosystems that either freeze seasonally or rarely experience temperatures above 0°C. Snow cover can seasonally blanket up to 33% of Earth's terrestrial surface, glaciers and ice sheets currently cover ~10% of all land area, ~25% of soils are permafrost, and up to 1.6 and 2.0×10^7 km^2 of sea ice forms in the Artic and Southern Ocean each winter (http://nsidc.org/cryosphere). Microorganisms have been documented in snow (Carpenter et al. 2000), ancient glacier ice (Abyzov et al. 1998; Christner et al. 2003, 2006; Miteva and Brenchley 2005; Bidle et al. 2007) and permafrost (Vishnivetskaya et al. 2006; Johnson et al. 2007), subglacial waters (Sharp et al. 1999; Mikucki et al. 2009), basal ice (Skidmore et al. 2000; Sheridan et al. 2003; Miteva et al. 2004; Foght et al. 2004), subglacial sediments (Skidmore et al. 2005), subglacial lakes and accreted ice (Karl et al. 1999; Priscu et al. 1999; Christner et al. 2001, 2006; Gaidos et al. 2004), and sea ice (Bowman et al. 1997; Junge et al. 2002, 2004; Brown and Bowman 2006).

Due to the apparent absence of an exogenous supply of redox couples and nutrients, it has been generally assumed that cells surviving entrapment within a water ice matrix persist in a state of anabiosis (e.g., Abyzov et al. 1998). Therefore, long-term survival under frozen conditions was attributed to the ability of cells to persist by entering a state of metabolic arrest (Morita 1988), which is similar to that

of an endospore. However, recent investigations of icy environments and their microbial inhabitants have provided a new perspective on the potential for functional biological processes under frozen conditions (Carpenter et al., 2000; Rivkina et al. 2000; Christner 2002; Junge et al. 2004, 2006; Panikov et al. 2006; Amato et al. 2009). The three-dimensional network of brine inclusions in sea ice provides a habitat for photosynthetically driven microbial communities that remain active down to temperatures as low as −20°C (Junge et al. 2004). Permafrost substrate can be covered with nanometer-thick unfrozen water layers and contains a 1 to 2% unfrozen water content at temperatures approaching −20°C (Rivkina et al. 2000). Price (2000) hypothesized that cells may be metabolically active in the solute-rich interstitial liquid vein habitat that exists between three-grain crystal boundaries in glacier ice. Indeed, cells have been shown to be partitioned into the aqueous veins at ice crystal boundaries during freezing (Junge et al. 2004; Mader et al. 2006; Amato et al. 2009; Raymond et al. 2009).

Physiological studies have confirmed that bacteria and fungi remain metabolically active under frozen conditions to very low temperatures (Christner 2002, Junge et al. 2006, Panikov et al. 2006; Panikov and Sizova 2007; Amato and Christner 2009; Amato et al. 2009). Price and Sowers (2004) compiled measured and inferred (i.e., based on geochemical analysis) rates of metabolism from various temperatures, emphasizing that the rates of carbon consumption at low subzero temperatures were inadequate for cellular growth but sufficient to support maintenance or survival metabolism. Although survival metabolism has never been directly measured, it is defined as the minimal amount of energy required to repair damage to DNA and offset amino acid racemization (Morita 1997). Johnson et al. (2007) provided evidence for DNA repair in permafrost samples up to 600,000 years old and concluded that microbes conducting active DNA repair (c.f., dormant or spore-forming species) were the most likely to persevere over extended periods of frozen entrapment. If this emerging picture of microbial metabolism under frozen conditions is accurate and truly reflects the physiological capabilities of microbes inhabiting natural icy environments, slow rates of metabolism may offset cellular and macromolecular damage and allow survival over prolonged time frames. Panikov and Sizova (2007) determined rates of fungal respiration at various subzero temperatures and reported that metabolic rates decreased below the level of detection after 2 weeks at temperatures below −20°C. Based on these data, the authors concluded that a short burst of metabolism prepared the cells for long-term periods of nongrowth and argued against the idea of cells maintaining a low, but sustained level of metabolism over time. Short-term laboratory experiments have provided valuable data that has improved our understanding of microbial metabolic potential while frozen, but are difficult to extrapolate over geological time frames. Future investigations that directly analyze ice-entrapped microbial assemblages and aim to determine in situ turnover rates would be valuable.

Ice-structuring proteins that control freezing and ice crystallization

Extracellular ice formation establishes a solute gradient across the cell membrane, lowering the intracellular osmotic pressure, and resulting in cell dehydration. Slow cooling rates facilitate cell water outflow, equilibration of the solute imbalance, and supercooling of the cytoplasm; however, if the thermal outflow exceeds that of water (i.e., by increasing the cooling rate), intracellular crystallization may occur (Dumont et al. 2004). The prevention or limitation of internal ice crystal growth and the ability to effectively manage cellular water content during osmotic stress are, therefore, properties that are important for cellular freeze tolerance (Tanghe et al. 2003). Once formed, ice crystal boundaries remain dynamic and larger grains of ice grow at the expense of smaller grains (i.e., recrystallization). Conditions that favor recrystallization are particularly damaging to cell membranes and integrity, and cellular freeze-thaw tolerance increases significantly when recrystallization is prevented (e.g., Kang and Raymond 2004). Water that is frozen very rapidly and maintained below the glass transition temperature (130 K at 1 bar; Mishima and Stanley 1998) forms a vitrified solid-state molecular arrangement (i.e., a noncrystalline, amorphous ice) that has distinctly different physical properties than conventional ice. The vitreous form of water does not induce cell water outflow and osmotic stress, providing a viable cryopreservation strategy for many cells and tissues (e.g., Dumont et al. 2004; Dahl et al. 2006).

In the late 1960s, DeVries and Wohlschlag (1969) reported that a carbohydrate-containing protein (antifreeze glycoprotein) isolated from the blood plasma of an Antarctic notothenioid fish accounted for a freeze point depression of −1.31°C. This discovery provided a biophysical explanation for how such organisms escape lethal freezing events despite continual contact with −1.9°C sea water. The AFP identified was orders of magnitude more active than that which could be explained by colligative properties, had no effect on the melting point (i.e., thermal hysteresis (TH)), and had recrystallization inhibition (RI) activity (Knight et al. 1984). The flat AFP peptide contact sites bind to the ice lattice and interfere with crystal growth along the *a*-axis by making it thermodynamically unfavorable for water molecules to join the ice surface (Raymond

and DeVries 1977). Several models have been proposed that describe how molecular binding between the peptide and ice occurs (e.g., Davies et al. 2002), but the specific nature of this interaction is still not well-understood. The noncongruent gene phylogenies of AFPs from polar fish, cold-adapted insects, and bacteria (Knight et al. 1991; Graham et al. 1997; Kawahara 2008) provide strong evidence for convergent evolution. The AFPs, thus, far described, share many similar ice-structuring properties and reduce the growth rates of ice crystal facets, and the crystals grown are typically needle-like and form along the *c*-axis (Fig. 1). Not all AFPs have an effect on freezing point depression (i.e., RI activity only; e.g., Wharton et al. 2005), and such IBPs are often referred to as ice-active or ice-structuring proteins (Clarke et al. 2002).

Microbial IBPs have recently been identified in the bacterial phyla Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria (Walker et al. 2006; Kawahara 2008; Raymond et al. 2007, 2008) and also in snow molds (Hoshino et al. 2003) and sea ice diatoms (Janech et al. 2006). Within the bacteria, three nonhomologous forms of IBP have been identified in *Pseudomonas putida* (Muryoi et al. 2004), *Marinomonas primoryensis* (Gilbert et al. 2005), and *Colwelia* sp. SLW05 (Raymond et al. 2007). The IBP of

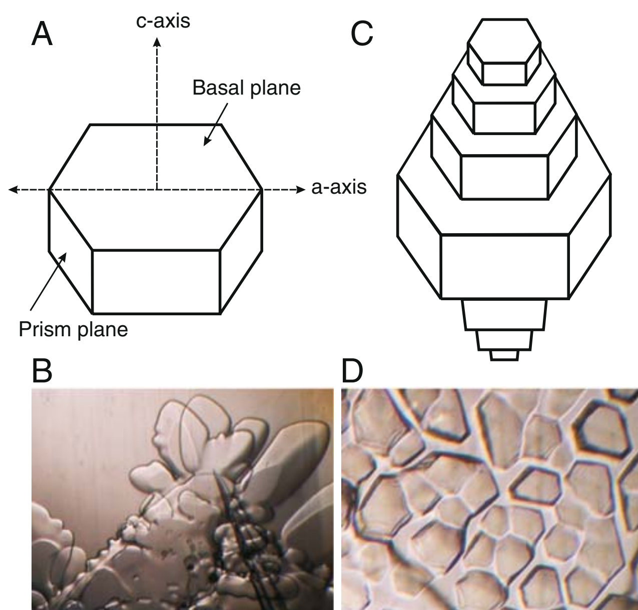

Fig. 1 Affect on IBPs on the ice crystal structure. **a** In the absence of an IBP, growth occurs on the *prism faces* (the edges of a flat ice crystal) and the ice structures formed are **b** round and irregular. **c** IBPs bind to the *prism face* and interfere with normal crystal growth, producing ice morphologies **d** that are highly pitted. Ice crystal schematics adapted from Davies and Hew (1990) and Kawahara (2008). The ice morphology micrographs are courtesy of James Raymond (see also Raymond and Fritsen 2001; Raymond et al. 2007, 2008) and the *c*-axis of the crystals shown are *perpendicular* to the page. Scale for micrographs—image width is ~0.5 mm

M. primoryensis is a bona fide AFP and one of the largest bacterial proteins known (>1 MDa). TH activity is conferred by a 322-amino acid region that consists of a tandemly repeated 19-amino acid motif that requires bound Ca^{2+} for activity (Garnham et al. 2008). The AFP of *M. primoryensis* is the only known bacterial IBP that can depress the freezing point by more than 2°C, which is a TH activity higher than most fish and insect AFPs. The low TH activity of most bacterial and plant IBPs may reflect an adaptation for freeze-tolerance (c.f., freeze-avoidance), as high supercooling eventually leads to uncontrolled ice growth, and terrestrial organisms are frequently exposed to temperatures too low for noncolligative freeze point depression (Zhang et al. 2004; Garnham et al. 2008). IBPs that inhibit recrystallization but lack a significant TH activity have been identified in bacteria isolated from winter soils (Walker et al. 2006) and Antarctic sea ice (Raymond et al. 2007), glacier ice (Raymond et al. 2008), lakes (Gilbert et al. 2004), and cyanobacterial mats (Raymond and Fritsen 2001).

Microbial proteins that limit supercooling

Bacteria with the ability to efficiently catalyze freezing at temperatures near −2°C were first described in the early 1970s by two research groups who demonstrated that strains of *Pseudomonas syringae* were potent ice nuclei (Maki et al. 1974) and responsible for inciting frost damage to corn (Arny et al. 1976). Subsequent studies revealed that the ability to nucleate ice is unique to a few culturable bacteria (i.e., *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Pantoea agglomerans* [formerly *Erwinia herbicola*], and *Xanthomonas campestris*), as well as some fungi (e.g., *Fusarium avenaceum*), algae, plants, and insects (Table 1). A common theme among many of the known ice-nucleating bacteria is their association with plants, either in the phyllosphere as plant pathogens or antagonists of plant pathogens (Lindow and Brandl 2003) or in association with decomposing leaf litter (Schnell and Vali 1973). Ice-nucleating activity in *P. syringae*, the most widely known and well-studied ice-nucleating bacterium, is specifically associated with intact cells and decreases significantly when the cell is disrupted by physical or chemical treatment (Maki et al. 1974). The subsequent loss of ice nucleation activity upon cellular disruption, coupled with low INP abundance (~10^4 protein molecules $cell^{-1}$) and ice nucleating frequency in a population, complicated initial attempts to describe the nature of bacterial ice nuclei (Fall and Wolber 1995). Using expression cloning, the molecular basis for bacterial ice nucleation was revealed when a ~4 kb genomic DNA fragment from *P. syringae* and *P. agglomerans* was shown to convey the ice nucleation phenotype in *E. coli* (Orser et al. 1985).

Table 1 Approximate temperature of activity for some ice-nucleation active materials and organisms. Unless otherwise noted, data are from Ariya et al. (2009)

Ice-nucleating material	Temperature of ice nucleation (°C)[a]
Kaolinite	−22
Montmorillonite	−14
Birch pollen[b]	−12
Snowmax™[c]	−8
Melampus bidentatus (intertidal gastropod)[d]	−8 to −11
Ice^{+} insects[d]	−6 to −10
AgI	−4.5
Pantoea agglomerans	−3
Pseudomonas syringae[e]	−2 to −4
Metaldehyde[f]	−1

[a] All temperatures are for the immersion mode of ice nucleation unless indicated

[b] IN activity for the contact mode of freezing

[c] Commercial preparation of *P. syringae* (Johnson Controls Inc.)

[d] Lundheim (2002)

[e] Maki et al. (1974)

[f] Pruppacher and Klett (1997)

The INP of *P. syringae* is a 120–180 kDa protein that is comprised of a highly repetitive central domain flanked by nonrepetitive N- and C-terminal domains and forms large homoaggregates on the surface of the outer membrane (Kajava 1995). The INP requires a "lipid environment" for optimal activity, and the tandem octapeptide repeats of the central domain are hypothesized to form a β-helical fold secondary structure that orderly bind water molecules in a configuration similar to the ice lattice (Gurian-Sherman and Lindow 1993). Nucleic acid sequencing of the *ina* genes from various ice-nucleating bacteria revealed that coding portions of the N- and C-terminal gene product ends are homologous, but the number of repetitive sequences present in the central domain varies widely (832 to 1,280 residues; Kawahara 2008). All INPs and some AFPs contain a threonine motif (TXT, where X is any amino acid) in their repeated regions, and mutational studies have implicated these residues as important to ice-binding in an insect AFP (Graether et al. 2000). Graether and Jia (2001) noted the structural similarity between INPs and AFPs and used theoretical considerations to argue that the opposing functions of these proteins may be explained by the "size of the ice-interacting surface." At temperatures near −2°C, INPs are capable of binding water to form an ice embryo spherical surface of the critical size to induce nucleation. In contrast, AFPs contain up to ten times fewer TXT motifs and are predicted to form ice embryos below the critical size, which are subsequently unstable and decay (Graether and Jia 2001).

The ability to catalyze freezing could benefit a plant-associated microorganism in several possible ways by providing access to nutrients leaked from freeze-damaged plant tissue, aiding in infection, increasing plant host survival by preventing excessive supercooling, protecting cells during freeze-thaw stress, and enhancing the accumulation of water from the atmosphere (Hirano and Upper 1995). However, the protein may have another adaptive advantage that has not been fully realized. The ability of *P. syringae* and other airborne microorganisms to efficiently catalyze ice formation may represent an important component of their life cycle, providing a way for the cells to induce precipitation and/or their own precipitation from the atmosphere. Distribution through precipitation could provide a dispersal strategy for certain plant-associated bacteria, allowing aerosolized cells to disseminate to new plant hosts via snow and rain (Sands et al. 1982; Morris et al. 2004). In a specific study that targeted *P. syringae*, all strains isolated from snow and rain were ice nucleation active, whereas, those from other substrates that it inhabits (lakes and rivers, plants, and rock-surface biofilms) were less frequently ice-nucleation active (Morris et al. 2008). This observation clearly supports the hypothesis that ice-nucleation activity facilitates dissemination of *P. syringae* via precipitation.

Exploiting natural products that control ice formation and crystallization

Substances that bind, inhibit or enhance, and control the size, shape, and growth of ice crystals could be useful to a number of biomedical and commercial applications. INPs are or have been used in snow making (Margaritis and Bassi 1991), gene reporting (Gurian-Sherman and Lindow 1993), freezing of food (Hwang et al. 2001), enhancing frost tolerance in plants (van Zee et al. 1996), spray-ice technology (LaDuca et al. 1995), and attempts at weather modification (Ward and DeMott 1989). AFPs and cryoprotective proteins with RI activity have been exploited to a lesser degree but have shown potential in improving the texture of ice cream (Regand and Goff 2006), cold tolerance in plants (Wen-li et al. 2005), and the cryopreservation of mammalian tissues (Bagis et al. 2006). It may be possible to use AFPs and INPs in tandem to individually prevent excessive intracellular supercooling and recrystallization, respectively, increasing the viability of various tissues, embryos, and oocytes during cryopreservation (Fahy 1995). AFPs have been shown to increase the viability of frozen zebrafish embryos (Martínez-Páramo et al. 2008) and could aid in reducing the level of mechanical, thermal, and chemical damage incurred during the cryopreservation of mammalian tissues and embryos.

Basic research and technical innovations are required to expand the use of ice nucleating and structuring proteins into new applications and fully optimize their ice-interacting properties. AFPs are readily isolated from cold-adapted fish, insects, and plants in quantities sufficient for biochemical characterization, but scaling up this process to meet commercial demands is not the most cost-effective approach. Instead, the use of proteins originating from and/or expressed in prokaryotic or eukaryotic microbial hosts provides a more financially and logistically feasible option for producing these products on a large scale. A wealth of information exists on the industrial production of bacterial INPs in high volume fermentation facilities (Woerpel 1980; Hendricks et al. 1992). All INPs described, thus, far require a lipid environment for maximum activity (Lundheim 2002), which appears to have a central role in anchoring the protein to the cell and providing the optimal confirmation for the protein to function as an IN. Introduction of phytopathogenic or enteric bacteria (i.e., ice^{+} species of *Pseudomonas* or *Erwinia*, respectively) to food products is clearly undesirable. In applications where viable microorganisms are a key component (e.g., the fermentation of bread dough), recombinant DNA technology provides a convenient way for in situ INP production (e.g., Hwang et al. 2001). Alternatively, nonpathogenic ice+ stains of *Xanthomonas campestris* or their cell-free ice-nucleating (IN) active membrane-bound aggregates could provide a hygienic source of INP (Wantanabe and Arai 1995). Heterologous expression of the *inaZ* gene of *P. syringae* in *Halomonas elongata* has been shown to produce cell-free IN that are free of lipid (Tegos et al. 2000), which may be ideally suited for biotechnological purposes.

Progress in the applied use of ice nucleating and structuring proteins will inevitably lead to a demand for products that affect ice formation and crystallization in a particular fashion. Fahy (1995) suggests that the ability to design specific ice-interacting molecules would “essentially change the physics of ice and thereby, change many of the basic phenomena of cryobiology.” Hence, chemical and genetic engineering could provide the means to improve and refine these proteins to perform a specific function. The microbial INPs and AFPs which have been described clearly effect ice nucleation, crystallization, and structure differently (Chao et al. 1995; Kawahara 2008). Within the bacteria, only a handful of culturable species possessing INPs or AFPs have been documented, implying that novel forms of these proteins remain to be discovered in the largely uncharted realms of the microbial biosphere. Efforts to selectively enrich, identify, and characterize novel ice-interacting proteins (e.g., Kuiper et al. 2003; Zhongqin et al. 2009) will benefit fields in basic and applied research, providing needed information on microbial adaptations to Earth’s icy environments and biotechnological fodder that can be exploited for various biomedical and commercial applications.

Closing remarks

Permanently cold environments are pervasive in the biosphere (Priscu and Christner 2004), the majority of microbes are inaccessible using conventional culturing procedures (e.g., Connon and Giovannoni 2002), and most of the biomass (Whitman et al., 1998) and genetic diversity (Pace 1997) is microbial. Considering this, it can be assumed with some confidence that we have only begun to scratch the proverbial surface of microbial diversity and are largely ignorant to the vast assortment of ice-interacting proteins that exist in nature. The unique ability of these proteins to affect ice crystallization and structure has high biotechnological potential. AFPs from fish are commercially available; however, their widespread usage is hampered by the high cost associated with production. Ice-interacting proteins that originate from and are produced in microorganisms provide a tenable solution for overcoming this financial barrier and extending the use of these products to a much broader market. Metagenomic approaches provide the means to elucidate genomic data from the culturably recalcitrant fraction of the microbial world. In the near future, we can expect this emerging technology to be at the forefront of efforts to discover and exploit ice nucleating and structuring proteins with novel functions and properties.

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