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37 **Psychrophilic Lifestyles: Mechanisms of Adaptation and Biotechnological**
38 **Tools**

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51 **Abstract**

52 Cold-adapted microorganisms inhabiting permanently low temperature environments were
53 initially just a biological curiosity but have emerged as rich sources of numerous valuable
54 tools for application in a broad spectrum of innovative technologies. To overcome the
55 multiple challenges inherent to life in their cold habitats, these microorganisms have
56 developed a diverse array of highly sophisticated synergistic adaptations at all levels within
57 their cells; from cell envelope and enzyme adaptation, to cryoprotectant and chaperone
58 production, and novel metabolic capabilities. Basic research has provided valuable insights
59 into how these microorganisms can thrive in their challenging habitat conditions and into the
60 mechanisms of action of the various adaptive features employed, and such insights have
61 served as a foundation for the knowledge-based development of numerous novel
62 biotechnological tools. In this review, we describe the current knowledge of the adaptation
63 strategies of cold-adapted organisms and the biotechnological perspectives and commercial
64 tools emerging from this knowledge. Adaptive features and, where possible, applications, in
65 relation to membrane fatty acids, membrane pigments, the cell wall peptidoglycan layer, the
66 lipopolysaccharide component of the outer cell membrane, compatible solutes, anti-freeze and
67 ice-nucleating proteins, extracellular polymeric substances, biosurfactants, chaperones,
68 storage materials such as polyhydroxyalkanoates and cyanophycins, and metabolic
69 adjustments are presented and discussed.

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71 **Keywords:** Psychrophiles • Cell Envelope • Cryoprotection • Enzymes • Chaperones •
72 Metabolic Adjustments

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82 Introduction

83 Habitats of permanently low temperature dominate the Earth's biosphere and have been
84 successfully colonised by a wide variety of organisms collectively termed psychrophiles, or
85 cold-adapted organisms. Microorganisms prevail in these cold habitats, especially bacteria,
86 archaea, yeasts, cyanobacteria and protists, but microalgae and viruses are also common, and
87 the ability of psychrophilic microorganisms to thrive in such environments reveals an
88 adaptation to their habitat (Margesin and Collins 2019). It demonstrates a capacity to
89 surmount the constraints inherent to life at low temperatures, which can only be achieved via
90 a complex range of structural and functional adaptations of all cellular components, from the
91 level of single molecules up to whole cells and even complete ecosystems (D'Amico et al.
92 2006; De Maayer et al. 2014; Morgan-Kiss et al. 2006; Siddiqui et al. 2013).

93 Since the first reports of cold dwelling organisms (Forster 1887), various comparative
94 physiology, microbiology, biochemistry, biophysics and molecular-based approaches have
95 been used in identifying the physiological adaptations, biogeographical distribution, diversity
96 and ecological roles of cold adapted organisms. More recently, various new emerging
97 technologies, namely various 'omics' approaches including genomics, transcriptomics,
98 proteomics and metagenomics, have broadened our understanding of these; enabling
99 identification of novel adaptation mechanisms and biotechnological tools, detection of key
100 functions and acquisition of a more global view of the structures and roles of microbial
101 communities in cold ecosystems (Barauna et al. 2017; Bowman 2017; Koh et al. 2017;
102 Raymond-Bouchard et al. 2018; Singh et al. 2014; Tribelli and Lopez 2018).

103 Life in the cold is characterised by a multitude of stresses in addition to low temperatures.
104 Indeed, besides a reduced thermal energy, low temperatures also provoke further
105 physicochemical constraints such as an increase in solvent viscosity and solubility of gasses,
106 namely an increased solubility of oxygen and reactive oxygen species (ROS), a decrease in
107 the solubility of solutes and nutrients, reduced diffusion, increased osmotic stress, desiccation
108 and ice formation. Furthermore, many cold ecosystems are often also characterised by
109 fluctuating environmental conditions and/or multiple additional ecological limiting factors,
110 including low nutrient levels, high salinity, oxidative stress, freeze-thaw cycles and low water
111 activity. In the deep sea and sub-glacial environments for example, microbial populations are
112 additionally subjected to high pressure stress. Extremes of light exposure are also common in
113 many cold ecosystems, from high light and UV irradiation in high altitude cold environments,
114 to low light exposure in ice-covered lakes and deep within ice layers and permafrost. Thus,

115 life in the cold biosphere requires a multitude of synergistic adaptations, not only to respond
116 to the low temperature challenge but also to the multitude of other interacting stresses
117 imposed by the particular environmental conditions.

118 Cold-adapted microorganisms have responded to the low temperature challenge via the
119 development of a cold-adaptation toolkit constituted by a number of elegant physiological and
120 structural adaptations, many of which are only beginning to be understood. Importantly, many
121 of these tools serve overlapping functions and may be used to respond to the various different
122 challenges or combinations of challenges encountered in a specific cold habitat. In fact, a
123 common problem encountered in characterising cold-adapted microorganisms is in
124 unravelling the different interacting parameters and deciphering the precise function of a
125 specific trait, whether it is a specific response to low temperatures or to another (or other)
126 environmental stressor(s) common in the particular habitat. Importantly also, microorganisms
127 do not always make use of all tools in their ‘cold-adaptation’ toolbox, and in fact, each
128 specific organism will use its own strategy, or combination of strategies, depending on its
129 own specific requirements and on the environmental parameters and microbial community
130 structure.

131 In the present review, we will discuss various adaptation strategies used by cold-adapted
132 microorganisms to enable life in the harsh environmental conditions of their habitat, and show
133 how an understanding of these different strategies is leading to the development of various
134 novel tools of commercial interest. See Table 1 for an overview of the cold-adaptation tools
135 with commercial potential. In this review, the various stresses to which microorganisms in
136 cold habitats are exposed will be presented, the adaptation strategies developed by
137 psychrophiles to cope with the challenges and their underlying mechanism of action
138 discussed, and examples of the biotechnological tools being developed from these will be
139 given.

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141 **Cell Envelope**

142 The cell envelope and its various components serve the critical functions of providing shape,
143 support and protection as well as regulating the movement of substances into and out of cells.
144 It protects cells from the surroundings and against turgor pressure, acts as a semipermeable
145 membrane functioning in nutrient uptake, product export and solute transport, and participates
146 in cell division, sensing, signaling and adhesion. Low temperatures adversely affect cell
147 envelope properties and functions by leading to reduced membrane fluidity, permeability and
148 diffusion rates, in addition to decreasing mobility and function of embedded proteins,

149 increasing turgor pressure and even leading to physical cell rupture by ice formation and/or
150 freeze-thaw cycles. While the cold adaptation traits of the cell membrane have been known
151 for some time, the adaptation strategies of the other envelope components; the outer
152 membrane, peptidoglycan layer and even exterior cell coatings; are now also beginning to be
153 understood.

154 *Cell Membrane*

155 Homeoviscous adaptation of cell membranes to low temperatures is accomplished in cold-
156 adapted microorganisms via modification of the fatty acid composition of the lipid bilayer
157 (D'Amico et al. 2001; Siddiqui et al. 2013). Cells mainly increase the content of unsaturated
158 fatty acids, but increases in the content of short chain, methyl branched and/or cis-isomeric
159 fatty acids are also common (Chintalapati et al. 2004; Russell 1997; Russell 2008). These
160 fatty acids disrupt the packing order and reduce the packing density of the phospholipid
161 bilayer, leading to a lowering of the liquid phase to gel phase transition temperature and
162 maintenance of functional fluid bilayers even at low temperatures. Indeed, in agreement with
163 this cold adaptation strategy, an overrepresentation and upregulation of genes encoding
164 various proteins involved in membrane biogenesis and fatty acid synthesis as well as in fatty
165 acid desaturation (desaturases, which simultaneously also protect against ROS), production of
166 branched chain fatty acids (KAS-II, KAS-III) and cis-isomerisation (fatty acid cis/trans
167 isomerases) have been reported for numerous cold-adapted organisms (De Maayer et al. 2014;
168 Goordial et al. 2016; He et al. 2015; Medigue et al. 2005; Methé et al. 2005). In addition, an
169 increased genome content of genes for proteins involved in the degradation of membrane
170 rigidifying molecules naturally present in the environment has also been observed (Medigue
171 et al. 2005) and may serve as a further means for reducing membrane rigidity at low
172 temperatures.

173 Finally, in relation to the other major components of the cell membrane, i.e. the proteins
174 embedded in the lipid bilayer, an upregulation of membrane transport proteins has been
175 observed in some psychrophiles and is believed to act in counteracting the reduced diffusion
176 rates and transport inherent to low temperatures (Bakermans et al. 2007; De Maayer et al.
177 2014).

178 *Cell Membrane LC-PUFAs*

179 While the role of unsaturated fatty acids with low numbers of double bonds in membrane cold
180 adaptation is well understood, the precise role of long chain polyunsaturated fatty acids (LC-
181 PUFAs) is still under discussion. These have been considered part of the cold adaptation
182 tactic for maintaining membrane fluidity and indeed some LC-PUFAs, such as

183 eicosapentaenoic (EPA, 20:5 ω 3), docosahexaenoic (DHA, 22:6 ω 3) and arachidonic (ARA,
184 20:4 ω 6c) acid, are preferentially distributed in marine organisms, with higher levels being
185 often produced at lower temperatures (Feng et al. 2014; Okuyama et al. 2008; Yoshida et al.
186 2016). In line with this, comparative genomics has indicated that the polyketide synthase gene
187 cluster (*pfaA,B,C,D* and *E*), responsible for the synthesis of these, is largely restricted to
188 marine organisms (Shulse and Allen 2011). Nevertheless, recent studies have indicated that,
189 rather than serving in cold adaptation, LC-PUFAs may primarily serve antioxidative
190 functions, protecting against reactive oxygen species naturally present at high levels in the
191 marine environment and augmented at low temperatures (Nishida et al. 2007; Okuyama et al.
192 2008). It is believed that LC-PUFAs act as membrane shields, forming more hydrophobic
193 interfaces between the lipid bilayers and thereby preventing entry of ROS such as H₂O₂ into
194 the cells. Therefore, their presence in low temperature environments may not be a direct
195 response to the cold, *per se*, but a response to other stress(es) inherent to low temperatures, in
196 this case, oxidative stress. Interestingly, in addition to their antioxidative function, functions
197 as chaperones for membrane proteins, in efflux processes and in cell division have also been
198 proposed (Okuyama et al. 2008; Yoshida et al. 2016).

199 PUFAs have considerable nutritional and pharmaceutical value (Yoshida et al. 2016). They
200 are components of neuronal and thrombocytes cells, neutrophils and monocytes and are found
201 at high concentrations in the brain and retina. Importantly also, they are precursors of
202 eicosanoid signaling molecules and endocannabinoid neurotransmitters (Ochsenreither et al.
203 2016). As such they have a wide array of functions in human health; from regulating the
204 cardiovascular system, immune system and inflammation, to participating in the development
205 and proper functioning of the brain, eyes and central nervous system. Some PUFAs such a
206 linoleic acid (LA) and α -linolenic acid (ALA) are essential fatty acids which have to be
207 obtained from the diet while LC-PUFAs such as DHA and ARA are recommended in infant
208 diets. Indeed, a balanced intake of various ω -3 (ALA, EPA and DHA) and ω -6 PUFAs (LA,
209 ARA) is recommended as part of a normal healthy diet. Fish oils are the main source of
210 PUFAs, especially LC-PUFAs, but problems associated with flavor, allergies, effects on
211 global fish stocks and presence of various environmental pollutants has resulted in the search
212 for alternative sources, including microorganisms. However, membrane levels of
213 phospholipid PUFAs in microorganisms are in general too low for commercial production.
214 On the other hand, oleaginous microorganisms, which produce PUFAs as components of
215 triacylglycerol in stored oils, and which can accumulate lipids at concentrations of up to 80%
216 of dry biomass weight, may prove suitable sources. In this sense, cold-adapted organisms,

217 with increased levels and/or varied distributions of PUFAs may be of interest, especially for
218 animal, and in particular aquaculture feed. Indeed, marine microalgae are potent producers of
219 various LC-PUFA and the marine dinoflagellate *Cryptothecodinium cohnii* is used in
220 commercial production of DHA as an infant formula additive. In addition, filamentous fungi
221 of the genus *Mortierella*, many of which are cold adapted, and various cold-adapted yeasts are
222 also reported to accumulate high levels of PUFAs (Amaretti et al. 2010; Ochsenreither et al.
223 2016) and may prove potent commercial sources.

224 *Membrane Pigments*

225 Pigments, and especially carotenoids (pigmented polyisoprenoid hydrocarbons), have also
226 been suggested to play a role in the modulation of cell membrane fluidity. Pigment production
227 is common in psychrophilic microorganisms, being reported in isolates from ice cores and
228 glaciers (Shen et al. 2018), marine surface waters (Dieser et al. 2010) and high altitude soils
229 (Pandey et al. 2018). A few reports have shown an increased production of these at low
230 temperatures (Chattopadhyay et al. 1997; Pandey et al. 2018), with a preference for polar
231 carotenoids (Chattopadhyay et al. 1997; Jagannadham et al. 2000), yet a recent study
232 identified a decrease in pigmentation upon slowly reducing the temperature for a number of
233 Arctic bacteria (Singh et al. 2017). Interestingly, polar carotenoids are believed to enhance
234 membrane rigidity and hence increased concentrations should be counterproductive to cold-
235 adapted organisms at low temperatures. It has been suggested that these may serve in
236 homeoviscous adaptation by counterbalancing the fluidizing effects of the unsaturated fatty
237 acids and stabilizing the membrane (Jagannadham et al. 2000). Furthermore, these pigments
238 have been shown to also play a number of other important roles, including, in photoprotection
239 (acting, in conjunction with other molecules such as scytonemin and mycosporine-like amino
240 acids, as light screeners protecting against high light and UV radiation common to many cold
241 habitats,), as antioxidants (protecting against ROS, also commonly produced in low
242 temperature and/or high light environments), as light harvesters (in photosynthetic
243 microorganisms), and even as antimicrobials (Pandey et al. 2018). Importantly, a potential
244 role as cryoprotectants, imparting resistance to freeze-thaw cycles, has also been
245 demonstrated (Dieser et al. 2010). These various overlapping functions would variably
246 influence pigmentation levels and could lead to the conflicting pigmentation levels observed
247 at low temperatures. Further studies are therefore required to unravel the true role and specific
248 functions of membrane pigments in cold-adapted organisms and their relationship to pigment
249 type and specific structure, as well as the precise effects of the different pigments on
250 membrane properties.

251 Numerous carotenoids have various beneficial health effects (Kirti et al. 2014). They have
252 been shown to be effective antioxidants and pro-vitamins (vitamin A), and to be important for
253 healthy growth and development, in maintenance of the immune system, and in eye health. In
254 addition they can be used as natural food colorants, in skin care, in sunscreen products and as
255 precursors of chemicals for fragrance products, as well as in animal/aquaculture feed to
256 impact desired colours on products, e.g. egg yolks, salmon. Cold-adapted organisms with
257 enhanced levels of various different carotenoids may prove interesting microbial sources of
258 carotenoids for such commercial applications.

259 *Cell Wall: Peptidoglycan Layer*

260 Cold adaptation of other cell envelope components has been much less investigated than the
261 cell membrane but recent studies have begun to unveil possible strategies. In relation to
262 adaptation of the cell wall; an upregulation of peptidoglycan biosynthesis genes and a
263 thickened peptidoglycan layer at low temperatures has been reported in some cold-adapted
264 bacteria (Mykytczuk et al. 2013; Rodrigues et al. 2008). Similarly, *Planococcus*
265 *halocryophilus* Or1 also displays a thickened outer cell surface, but this is achieved by a
266 rather unique mechanism involving extracellular cell wall associated hydrophobic
267 encrustations composed of peptidoglycan, calcium carbonate and choline (Mykytczuk et al.
268 2013). Such strategies for thickening and strengthening the cell outer surfaces would
269 obviously lead to a reinforced physical barrier which could protect psychrophiles against cell
270 disruption by ice formation, freezing-thawing, and/or increased osmotic pressure at low
271 temperatures.

272 *Cell Wall: Outer Membrane*

273 Cold adaptation traits have also been observed in the outer membrane layer of the cell wall of
274 gram negative bacteria, and namely in the lipopolysaccharide (LPS) structure which accounts
275 for ~75% of this layer (Corsaro et al. 2017). Only a few cold adapted LPS structures have
276 been analyzed to date but it appears that most produce LPS solely as rough LPS (i.e. without
277 the specific O-chain component) of shortened length at low temperatures (Carillo et al. 2013;
278 Carillo et al. 2011; Corsaro et al. 2017; Corsaro et al. 2002). Many more cold-adapted LPS
279 structures need to be analyzed to determine the extent of these observations, but it has been
280 suggested that these alterations may increase outer membrane flexibility and stability at low
281 temperatures (Corsaro et al. 2017). As regards to the other LPS components, a similar strategy
282 to that used for the lipid bilayer of cell membranes is observed for the lipid A component of
283 LPS, i.e. increased content of short chain and/or unsaturated fatty acids to increase fluidity,
284 while a high negative charge of the core oligosaccharide component has been suggested to be

285 important in sequestration of divalent cations common in many cold environments (Casillo et
286 al. 2017b; Corsaro et al. 2017; Sweet et al. 2015). Finally, in common with that observed for
287 other cell envelope components, transcriptome analyses have shown an upregulation of genes
288 involved in biosynthesis of outer membrane components, with LPS biosynthetic genes
289 (mainly glycosyltransferases) and outer membrane proteins being upregulated at low
290 temperatures (De Maayer et al. 2014; Frank et al. 2011; Gao et al. 2006). In agreement with
291 this, a recent study showed how mutation of a core LPS glycosyltransferase gene (*wapH*)
292 impaired growth of an Antarctic bacterium at low temperatures (Benforte et al. 2018).

293

294 **Cryoprotection**

295 Subzero temperatures provoke ice formation which can lead to cryoinjury, osmotic stress,
296 dehydration and even cell rupture and death. In the natural environment, onset of ice
297 formation is usually delayed in the cell interior as compared to the cell exterior due to the
298 lower volume and densely packed, highly crowded nature of the former. Intracellular ice
299 crystal formation, which is normally lethal to cells, can indeed occur when temperatures
300 decrease at high rates, but in the natural environment cooling is usually relatively slow and ice
301 formation is thus mostly restricted to the extracellular space (Fonseca et al. 2016).
302 Extracellular ice formation, which can lead to physically damaging membrane fracturing,
303 occurs with exclusion of solutes and removal of available liquid water. This leads to elevated
304 extracellular solute concentrations and provokes intra/extracellular osmotic imbalances. In
305 turn, this leads to stresses related to osmotic shrinkage and dehydration of the cell interior,
306 negatively affecting cell function and survival, but also preventing intracellular ice formation
307 and instead leading to a non-crystalline amorphous (colloidal glassy) state with inhibition of
308 cell metabolism (Fonseca et al. 2016). In addition, at relatively high subzero temperatures, in
309 partially frozen environments subjected to freeze-thaw cycles and during temperature
310 fluctuations, cells can also be subjected to harmful ice recrystallization stress, a
311 thermodynamically driven process causing ice crystal coalescence and growth of large, fatally
312 damaging ice crystals at the expense of smaller crystals (Bar Dolev et al. 2016b). Cold-
313 adapted microorganism respond to these multiple freezing related detrimental challenges by
314 production of a variety of novel tools including, compatible solutes, ice-binding proteins
315 (antifreeze and ice-nucleating proteins), extracellular polymeric substances (EPS) and/or
316 biosurfactants.

317 *Compatible Solutes*

318 Compatible solutes are low molecular mass, non-toxic organic osmolytes. Many cold-adapted
319 microorganisms have an increased genome content of compatible solute biosynthesis, uptake
320 and degradation genes and often accumulate up to molar concentrations of various different
321 compatible solutes; with glycine betaine, trehalose, glycerol, sucrose, sarcosine, mannitol and
322 sorbitol being commonly reported (Ghobakhlou et al. 2015; Goordial et al. 2016; Mykytczuk
323 et al. 2013). Accumulation of these organic osmolytes contributes to restoring osmotic
324 balance and thereby counteracts water loss and cell shrinkage during freezing. In addition,
325 they depress the freezing point of solution and, importantly also, the intracellular colloidal
326 glass transition temperature (T_g) (Fonseca et al. 2016). In fact, a recent study showed
327 reductions of as much as 30 °C in the T_g of the cytoplasm of bacterial cells and improved post
328 thaw viability upon glycerol addition (Fonseca et al. 2016). Furthermore, compatible solutes
329 are also believed to be involved in scavenging free radicals and, due possibly to their
330 preferential exclusion from protein surfaces and/or water entrapment effects, compacting
331 effects on proteins and destabilizing effects on the unfolded state, also play roles in
332 preventing protein aggregation, enhancing protein folding and stabilising proteins and
333 membranes at low temperatures.

334 In relation to applied aspects, various compatible solutes are commonly used in the
335 stabilisation, preservation and cryopreservation of diverse biological materials, ranging from
336 enzymes to whole cells and tissues. In addition, their use in increasing the freshness and
337 stability of foods, in cosmetics and skin care products, as well as in extending the growth
338 performance of plants in saline, dry and low temperature environments has also been
339 investigated (Wani et al. 2013).

340 *Ice-Binding Proteins: Antifreeze Proteins*

341 Antifreeze proteins (AFPs), also known as ice structuring or thermal-hysteresis proteins, are
342 noncolligative biological antifreezes which can bind to ice and inhibit ice growth and
343 recrystallisation (for recent reviews on AFPs see e.g. Bar Dolev et al. (2016b); Lorv et al.
344 (2014) and Voets (2017)). They were first identified in the blood of Antarctic fish but have
345 since been reported in various bacteria, fungi, diatoms, plants, insects and crustaceans.
346 Various types of AFPs of diverse structure exist in nature, being frequently glycosylated
347 and/or lipidated and varying in size from ~2kDa to ~50 kDa, with a 1.5 MDa multi-domain
348 ice adhesion AFP being reported for the Antarctic bacterium *Marinomonas primoryensis* (Bar
349 Dolev et al. 2016a). AFPs are believed to function by irreversibly binding to specific ice
350 crystal planes, thereby blocking secondary nucleation events and shaping a unique ice
351 morphology. Due to the Kelvin effect, ice surface expansion between the adsorbed AFPs

352 leads to local curvature of the ice face which is energetically unfavourable for further ice
353 growth. This leads to thermal hysteresis (TH) in which the freezing point is depressed below
354 the equilibrium melting point to create a thermal-hysteresis gap in which ice crystal growth is
355 halted. TH activities from ~ 0.1 °C to as high as ~ 13 °C have been measured (Duman et al.
356 2004; Voets 2017) and these activities can be increased in the presence of other AFPs, solutes
357 and ions. AFPs are also effective ice recrystallisation inhibitors (IRI), being frequently more
358 effective in IRI (often requiring only sub- μ M concentrations) than in TH (often requiring mM
359 concentrations). Indeed, IRI is believed to be the primary function of many secreted AFPs
360 from cold-adapted organisms and AFPs from Antarctic algae and glacier ice bacteria were
361 shown to effectively stabilise brine pockets and contribute to the preservation of a liquid
362 environment in the cell vicinity (Raymond et al. 2008). Finally, in addition to their TH and
363 IRI activities, AFPs are also believed to help stabilize cell membranes and protect structural
364 integrity, and, in some cases, via their adhesion domains and ice binding function, are thought
365 to play a role in positioning cells on ice so as to enhance access to oxygen and nutrients in the
366 phototrophic zone (Bar Dolev et al. 2016a; Lorv et al. 2014; Voets 2017).

367 AFPs are already being applied in the food industry for improved food preservation during
368 freezing. They are used to preserve a smooth texture in ice creams and frozen yoghurts and
369 can reduce drip loss in frozen meats and fish, enhance frozen dough bread quality, and
370 improve post-freezing quality of currently difficult to freeze foods such as fruit and
371 vegetables (Muñoz et al. 2017; Regand and Goff 2006; Voets 2017). They also have obvious
372 potential as cryoprotective agents and have been shown to improve cryopreservation
373 efficiency and post thaw viability of various types of biological materials. Furthermore, the
374 ability of certain AFPs at high concentrations to induce formation of needle-like structures
375 which penetrate and destroy cells is being investigated for use in cryosurgery, e.g. in tumor
376 ablation. They are also being developed for use in improving freeze tolerance of crops and
377 aquaculture fish, in gas hydrate inhibition in the petroleum industry and in anti-freeze/de-
378 icing materials via surface coating of materials for prevention/decreasing of ice formation,
379 e.g. on aircrafts, power lines, roads (Bar Dolev et al. 2016b; Lorv et al. 2014; Voets 2017).

380 *Ice-Binding Proteins: Ice-Nucleating Proteins*

381 Ice-nucleating proteins (INPs) are large membrane bound proteins that facilitate ice
382 formation. They initiate heterogeneous ice crystallisation at high subzero temperatures and are
383 proposed to act by providing a template for the ordering and stabilisation of water molecules
384 in an ice-like structure (Lorv et al. 2014; Pandey et al. 2016; Pummer et al. 2015). This lowers
385 the activation energy barrier for freezing and nucleates ice growth at temperatures as high as -

386 2 °C (Li et al. 1997). INPs are produced by diverse organisms, most commonly as large,
387 extracellular, repetitive multimeric aggregates, with larger complexes enabling higher activity
388 (Bar Dolev et al. 2016a). In cold-adapted microorganisms, INPs are believed to counteract
389 low temperature damage by directing ice nucleation to the extracellular space (Lorv et al.
390 2014) which can prevent formation of lethally damaging intracellular ice via removal of the
391 available intracellular liquid water as discussed above. In addition, INPs favour development
392 of small extracellular crystals which are less damaging to the cell than large crystals while the
393 release of latent heat of crystallization during the freezing process could also be beneficial in
394 preventing further temperature decreases (Pummer et al. 2015). In addition to their role in
395 cold adaptation, INPs also play a role in nutrient mining by many plant pathogenic organisms
396 where they are used to induce frost damage in plants and enable access to nutrients.

397 INPs are presently being commercialised for artificial snow production (Cochet and Widehem
398 2000) but are believed to have a number of other potential applications. These include,
399 reducing freezing energy costs in the food industry (Li et al. 1997), freeze-concentrating
400 beverages, in freeze–thaw valves of microfluidic devices (Gaiteri et al. 2017), as anchoring
401 motifs for cell surface display applications (Jung et al. 1998), as well as in cloud seeding for
402 climate control (Pummer et al. 2015).

403 *Extracellular Polymeric Substances*

404 Extracellular polymeric substances (EPS) are multifunctional, high molecular weight
405 biopolymer complexes secreted by various organisms into their local environment. They are
406 large, complex, highly diverse architectural structures composed principally of carbohydrates
407 (homo- or hetero-polysaccharides), but also proteins and lower concentrations of nucleic
408 acids, lipids, phenols and humic substances. EPS are produced by a wide variety of organisms
409 and are found either attached to the cells outer surface or released into the surrounding
410 environment. They form hydrated gels that play an important role in the formation of biofilms
411 and in the modification of the physical, chemical and biological characteristics of the cell
412 environment. Indeed they are believed to have multiple functions, including in cell adhesion
413 and nutrient scavenging, but are also thought to be important in protective functions such as
414 osmoprotection, ROS scavenging, extracellular protein protection and even in cryoprotection.
415 For reviews of the subject see e.g. Deming and Young (2017) and Ewert and Deming (2013),
416 and references therein.

417 In relation to cold-adaptation, metagenomics studies have identified numerous genes for EPS
418 biosynthesis in both Antarctic and Arctic ice shelf ponds (Varin et al. 2012) and cold-adapted
419 bacteria have been found to produce high concentrations of EPS at low and especially

420 subfreezing temperatures (Caruso et al. 2018; Feng et al. 2014; Marx et al. 2009; Mykytczuk
421 et al. 2013). The hydrated EPS gel matrix is believed to protect against low temperatures by
422 forming a protective shell around cells which acts as a diffusion barrier to solutes and a
423 physical-like barrier to ice formation (Caruso et al. 2018; Deming and Young 2017; Ewert
424 and Deming 2013; Krembs et al. 2011). This diminished solute diffusion limits freezing
425 induced osmotic stress and desiccation damage. Furthermore, the gel like state of EPS reduces
426 the available free energy for ice nucleation while solute exclusion to the surrounding liquid
427 phase impedes ice crystal growth in this, thereby protecting cells from ice damage and
428 increasing the available habitable liquid space. Interesting also, recent studies have indicated
429 ice binding functions and IRI activity similar to AFPs (described above) for EPSs produced
430 by cold adapted organisms (Casillo et al. 2017a). As compared to other cold-adaptation traits,
431 EPS are much less well studied and the important role of these in coping with low
432 temperatures is only beginning to be unraveled. Further studies are warranted to gain a better
433 understanding of the different interrelated cold adaptation roles and physiological and
434 ecological functions of EPS and their relationship to composition and structure.

435 Microbial EPS and their various components, as biodegradable, biosustainable, non-toxic and
436 biocompatible biopolymers, have been recognized as potential alternatives to chemical
437 polymers in a number of applications, ranging from the pharmaceutical to the cosmetics and
438 food industry and environmental biotechnology. Their capacity to adsorb heavy metals and
439 organic pollutants combined with their flocculation properties for removal of suspended
440 solids, organic matter etc. are advantageous for use as bioflocculants and bioabsorbants in soil
441 and water bioremediation and decontamination as well as in wastewater and sludge treatment
442 (More et al. 2014). Their use, especially the polysaccharide component, as cryoprotectants is
443 also under study and an ability to improve the freeze-thaw survival of various Antarctic
444 bacteria has already been shown (Caruso et al. 2018). The high emulsifying activity of some
445 EPS indicates potential as bioemulsifiers (Caruso et al. 2018) while their role as bioadhesives
446 has also been indicated (Muralidharan and Jayachandran 2003). In the food industry, EPS
447 polysaccharides can be used as thickening agents and emulsifiers, conferring improved
448 texture and stability to foods and beverages while, more recently, beneficial effects of EPS on
449 human health via potential immunomodulatory, anticoagulant, anti-inflammatory, and
450 antioxidant capacities have been suggested (Collic Jouault et al. 2001; Leroy and De Vuyst
451 2016). Furthermore, their use in tissue engineering has even been suggested (Kumar et al.
452 2018). A number of microbial EPS carbohydrates, e.g. xanthan gum, gellan gum and dextran,
453 have already been commercialised for a number of applications, but studies of cold-adapted

454 EPS components with high potential for identification of unique structures and functions
455 could be expected to reveal novel products with novel applications.

456 *Biosurfactants*

457 Biosurfactants are surface active amphiphilic compounds of microbial origin which reduce
458 surface and interfacial tension between liquids, solids and gases. Generally they are of low
459 molecular weight, in contrast to bioemulsifiers, such as for example EPS, which tend to be of
460 high molecular weight, and can be composed of sugars, amino acids, fatty acids and/or
461 functional groups such as carboxylic acids. They are structurally diverse compounds but the
462 most commonly reported are glycolipids (rhamnolipids, sophorolipids, trehalose lipids and
463 mannosylerythritol lipids etc.), lipopeptides (surfactin, iturin, fengycin, viscosin etc.),
464 phospholipids, fatty acids and neutral lipids. In nature, they are believed to play roles in
465 enhancing the bioavailability of poorly soluble hydrophobic substrates, in regulating biofilm
466 structure and surface attachment/detachment, in bacterial pathogenesis and quorum sensing,
467 as well as acting as antibacterial and antifungal agents. In relation to cold adaptation, a
468 glycolipid biosurfactant isolated from an Antarctic yeast was shown to have IRI activity
469 (Kitamoto et al. 2001), while a role for biosurfactants as osmolytes has also been suggested
470 (Perfumo et al. 2018). Interestingly, even though biosurfactant production appears to be
471 widespread among cold adapted organisms (Gesheva et al. 2010; Malavenda et al. 2015;
472 Perfumo et al. 2018; Vollú et al. 2014), studies investigating their potential role in cold
473 adaptation are scant. Further studies are obviously required to clarify whether these
474 compounds constitute part of the cells toolkit for adapting to cold and to elucidate their
475 precise role and mechanism of action in this.

476 Biosurfactants offer non-toxic, sustainable, biodegradable, and ecofriendly alternatives to the
477 chemical surfactants currently used in a huge variety of applications as diverse as
478 bioremediation, in cleaning agents, cosmetics, pharmaceuticals, agriculture and the petroleum
479 industry. Interest in ‘cold’ biosurfactants has only recently been initiated, but already potential
480 in a number of applications has been demonstrated; they have been shown to have potential
481 for use as antiagglomerants in ice–water slurry technologies (Kitamoto et al. 2001), as low
482 temperature detergents (Perfumo et al. 2018), as fuel additives to improve the flow properties
483 of biodiesel and diesel at low temperatures (Madihalli et al. 2016), and for recovery of natural
484 gas hydrates in gas hydrate technologies (Arora et al. 2016; Perfumo et al. 2018).

485

486 **Enzymes**

487 One of the principal challenges that cold-adapted microorganisms have to contend with is the
488 negative effect of low temperatures on reaction rates. All reactions, including enzyme
489 catalysed reactions, are influenced by temperature according to the Arrhenius Law wherein
490 any decrease in temperature induces an exponential decrease in the reaction rate (Arrhenius
491 1889). Indeed, with most non-adapted enzymes, a reduction in temperature from 37 °C to
492 0 °C results in a 16-80 fold reduction in activity. In contrast, most enzymes produced by
493 psychrophilic organisms are adapted to their environment and maintain high specific activities
494 enabling appropriate metabolic rates in their cold habitats (for recent reviews see e.g. Collins
495 et al. (2002a); Collins and Gerday (2017); Fields et al. (2015); Gerday (2013); Santiago et al.
496 (2016) and Siddiqui (2015)).

497 With few exceptions, e.g. Oswald et al. (2014); Roulling et al. (2016), most cold-adapted
498 enzymes studied to date are characterised by a higher catalytic activity at low to moderate
499 temperatures, a shift in the optimum temperature for activity towards lower temperatures and
500 a decreased stability as compared to mesophilic and thermophilic homologous enzymes.
501 These 'cold enzymes' successfully reduce the activation energy, ΔG^* , and temperature
502 dependence of reactions through a reduced activation enthalpy, ΔH^* , thereby implying a
503 decreased number and/or strength of enthalpic interactions that are broken during the catalytic
504 cycle (Lonhienne et al. 2000). This is accompanied by a lower, more negative activation
505 entropy value, ΔS^* , suggestive of greater re-ordering during activation, which, in conjunction
506 with a more negative heat capacity change during activation, suggestive of a higher heat
507 capacity and thus increased vibrational modes for the ground state (Arcus et al. 2016), and a
508 lower substrate affinity (higher K_m) (Collins et al. 2002a), as compared to mesophilic and
509 thermophilic homologs, implies a more disordered enzyme-substrate (ES) ground state for
510 psychrophilic enzymes. Such a disordered state could give rise to an ES complex of reduced
511 stability and higher free energy and hence lead to the observed reduced ΔG^* and enhanced
512 catalytic rates for these enzymes.

513 A number of different studies using a range of techniques have investigated this disorder, or
514 structural flexibility, in various psychrophilic enzymes. It is important to note here that
515 protein flexibility is a complex parameter which is difficult to measure and further in-depth
516 comparative studies characterising the specific amplitudes, time frames, regions involved and
517 temperature dependence of protein motions are called for, with use of powerful techniques
518 such as NMR being key to better understanding this parameter. Nonetheless, notwithstanding
519 current limitations, it is now generally accepted that psychrophilic enzymes do indeed display
520 an increased structural flexibility and that they have overcome the low temperature challenge

521 by increasing the plasticity or flexibility of specific regions (at or near the catalytic site) or of
522 the whole protein. This flexibility enables the conformational changes necessary for activity
523 at a low energy cost, but also leads to a decreased stability. At the structural level, this
524 increased flexibility is mainly achieved by a reduction in the number and/or strength of
525 stabilising interactions such as H-bonds, salt bridges, aromatic interactions, disulphide bonds,
526 ion binding sites and/or a weakening of the hydrophobic core. In addition, a reduction in the
527 proline content and increase in glycine content, especially in loop regions, and a greater
528 exposure of hydrophobic residues have also been reported. Such modifications would allow
529 for the increased flexibility necessary for increased low temperature activity but would also
530 lead to the reduced stability inherent to most psychrophilic enzymes (Collins and Gerday
531 2017). Importantly, it has been found that different enzymes can use different structural
532 adaptation strategies, with each enzyme using its own specific strategy, employing any one or
533 combination of these modifications depending on its own specific characteristics, its
534 environment and its requirements.

535 The inherent characteristics of a high activity at low to moderate temperatures and a reduced
536 stability of psychrophilic enzymes offers many advantages in a variety of applications. They
537 can be used to enhance the efficiency and economics of low to moderate temperature
538 processes, reduce the process temperature and hence improve process economics and
539 environmental impact, and enable more simplified enzyme inactivation. Cold enzymes are
540 already being employed in all three sectors of the industrial enzymes markets; food, feed and
541 technical, and are expanding into new areas in pharmaceutical and fine chemical synthesis.
542 For a recent in-depth review see Barroca et al. (2017a). Some of the better known commercial
543 successes include a cold-adapted lipase used in the organic synthesis of various
544 pharmaceutical, cosmetic and flavor compounds (Kirk and Christensen 2002); a xylanase for
545 enhancing bread quality (Barroca et al. 2017b; Collins et al. 2012; Collins et al. 2006; Collins
546 et al. 2002b; Dutron et al. 2010-2012); various hydrolases in detergents for low temperature
547 cleaning (Sarmiento et al. 2015), and various enzymes (alkaline phosphate, nuclease and
548 uracil-DNA N-glycosylase) used in molecular biology for their high activity and ease of
549 inactivation (Barroca et al. 2017a).

550

551 **Chaperones**

552 Protein and RNA/DNA chaperones, which facilitate efficient protein and RNA/DNA folding,
553 respectively, play important roles in counteracting low temperature stabilisation of RNA and
554 DNA secondary structures as well as protein misfolding and aggregation. DNA and RNA

555 chaperones are important in maintaining efficient transcription, translation and DNA
556 replication. They are transiently produced as part of the cold-shock response in mesophilic
557 and thermophilic microorganisms but are often continuously overexpressed as cold-
558 acclimation proteins or up-regulated at low temperatures in psychrophiles (Lim et al. 2000).
559 In relation to protein chaperones in cold-adapted microorganisms, continuous overexpression,
560 upregulation of production, and production of cold adapted variants, but also no
561 overexpression and cold repression, have been reported (Ferrer et al. 2003; Godin-Roulling et
562 al. 2015). Importantly, while protein misfolding and precipitation are believed to be strongly
563 reduced at low temperatures, due mainly to a weakening of hydrophobic interactions, proteins
564 at the low range of the temperature spectrum are faced with another phenomenon; cold
565 denaturation (Collins and Gerday 2017; Romero-Romero et al. 2011). Cold denaturation is
566 thought to be due to a preferential hydration and weakening of hydrophobic and ionic
567 interactions at low temperatures and intriguingly it appears that psychrophilic enzyme may be
568 more susceptible to this than their higher temperature adapted homologs (D'Amico et al.
569 2003). Nevertheless, the underlying basis for this is still not fully understood and future
570 studies should address this.

571 Protein and RNA/DNA chaperones have potential for use in recombinant protein production,
572 in the low temperature production of proteins prone to aggregation/misfolding and an *E. coli*
573 host producing protein chaperones from an Antarctic bacterium has already been
574 commercialised for such a use (Ferrer et al. 2003).

575

576 **Metabolic Adjustments**

577 Recent studies making use of modern 'omics' approaches such as genomics, transcriptomics
578 and proteomics have revealed a number of additional traits common to various cold adapted
579 microorganisms (see e.g. Tribelli and Lopez (2018) for a recent review. In particular, studies
580 have indicated various metabolic adjustments at low temperatures, including a down
581 regulation of primary metabolism pathways and substitution with abridged or alternative
582 secondary pathways, as well as accumulation and metabolism of reserve compounds.
583 Oxidative metabolic processes, namely, glycolysis, the pentose phosphate pathway, the TCA
584 cycle, and/or the electron transport chain, but also pathways involving metal ions and
585 molybdopterin metabolism are reported to be downregulated at low temperatures in some
586 psychrophiles (Medigue et al. 2005; Piette et al. 2011; Tribelli et al. 2015). While still poorly
587 understood, studies have indicated their substitution with alternative/shortened pathways such
588 as the glyoxylate, methylglyoxal and 2-methylcitrate cycles, the ethanol oxidation pathway,

589 acetate metabolism and propionyl-CoA catabolism (Ayala-del-Río et al. 2010; Tribelli and
590 Lopez 2018). The actual alternative pathway(s) used being dependent on the organism and its
591 ecological niche. Such a strategy of metabolic reprogramming may alleviate oxidative stress
592 inherent to low temperatures by avoiding ROS producing pathways but may also be important
593 in energy conservation and long term survival.

594 Screening studies have indicated a high content of polyhydroxyalkanoate (PHA) producers in
595 cold habitats (Ciesielski et al. 2014; Goh and Tan 2012; Pärnänen et al. 2015) and many
596 psychrophiles can accumulate and degrade PHA (López et al. 2009; Methé et al. 2005; Ting
597 et al. 2010) and/or cyanophycin-like (Duchaud et al. 2007; Methé et al. 2005; Vollmers et al.
598 2013) compounds. These compounds can act as dynamic reserves of carbon, nitrogen,
599 reducing equivalents and energy in cells and are thought to be important in overcoming low
600 temperature challenges to carbon and nitrogen uptake. Nevertheless, functions in
601 cryoprotection, oxidative stress resistance, maintenance of cellular redox balance and cell
602 motility have also been suggested for PHAs (Méthé et al. 2005; Tribelli and Lopez 2018). In
603 relation to applied aspects, these compounds, or derivatives, are suggested as non-toxic,
604 biodegradable, biocompatible biopolymers to replace petrobased polymers. PHAs are a family
605 of microbial polyesters with interesting thermoplastic and elastomeric properties and potential
606 for application in almost all areas of the conventional plastics industry. For reviews on PHA
607 applications see e.g. Chen (2009) and Singh et al. (2019). A specific focus of application has
608 been in the medical (tissue engineering, bio-implants, drug delivery, sutures etc.), and fine
609 chemical synthesis fields (chiral starting materials for the synthesis of antimicrobials,
610 vitamins, fragrances etc.), but also in materials (packaging, smart materials etc.) and biofuels.
611 Recently novel applications such as in enhancing stress tolerance in plants has even been
612 suggested (Stritzler et al. 2018). Cyanophycin-like compounds are polyamides composed
613 mainly of aspartic acid and arginine. Derivatives of this have been suggested for use in
614 nutrition (sources of highly bioavailable amino acids/peptides), biomedicine (as polyaspartic
615 acid hydrogels), laundry detergents (polyaspartic acid), anti-scalants (polyaspartic acid) and in
616 bioflocculation (polyaspartic acid). For a recent review on cyanophycins and their
617 applications see e.g. Frommeyer et al. (2016).

618

619 **Other Applications**

620 In addition to the numerous biotechnological tools related to the cells response to low
621 temperatures presented above, an array of other bioactive metabolites and bioproducts,
622 including novel antimicrobials, anti-fungal agents, anti-cancer drugs, anti-tumor and anti-

623 inflammatory agents, antioxidants, alkaloids, organic acids etc. have also been identified in
624 psychrophilic microorganisms. For reviews on these see e.g. Avila (2016); Borchert et al.
625 (2017) and Soldatou and Baker (2017). The majority of these bioproducts used today
626 originate from moderate temperature or warm environments whereas cold environments have
627 been much less investigated and hence offer an extraordinary opportunity as an underexplored
628 source of potential novelty. Indeed, the abundance and diversity of psychrophiles, combined
629 with the vastness, enormous diversity and severity of their habitats points to their tremendous
630 potential as rich reservoirs of novel biomolecules and metabolites of applied interest. Recent
631 growing interest in cold environments has led to identification of numerous new products,
632 mainly from microbes, (Soldatou and Baker 2017), and further bioprospection of these
633 environments using modern high throughput techniques such as metagenomics-based
634 approaches will surely lead to discovery of further novel tools with diverse bioactivities and
635 applications (Borchert et al. 2016).

636 Cold-adapted microorganisms have also been shown to have potential for use in
637 bioremediation, as probiotics and as cell factories. Their potential for degrading a wide range
638 of organic compounds of environmental concern, including mineral oil hydrocarbons,
639 phenolic compounds, polyaromatic hydrocarbons, pesticides and persistent pollutants, but
640 also proteins, carbohydrates and lipids, has found broad application in bioremediation of
641 polluted cold soils and waters, and their use in wastewater and groundwater treatment has also
642 been suggested (reviewed by e.g. Bajaj and Singh (2015) and Margesin (2017)). As
643 probiotics, psychrophiles are believed to have potential for use as dietary supplements in
644 aquaculture to improve health and nutrition of livestock (Makled et al. 2017; Sun et al. 2011;
645 Wanka et al. 2018). While studies in this are scarce, their adaptation to low temperatures is
646 suggested to be beneficial for a more efficient utilisation in the marine habitat as compared to
647 currently available terrestrial and/or moderate temperature adapted probiotic organisms.
648 Finally, in relation to their utilisation as cell factories, cold-adapted microorganisms can be
649 used for the production of heat-sensitive compounds and difficult to express or aggregation-
650 prone proteins at low temperatures with reduced environmental and economic impact due to
651 the absence of heating requirements (Miyake et al. 2007; Parrilli and Tutino 2017).

652

653 **Conclusions**

654 Cold adapted microorganisms inhabiting permanently low temperature environments have
655 evolved a suite of highly sophisticated adaptations at all levels of their cells to cope with their
656 challenging habitat conditions. Many of these adaptive features and their underlying

657 mechanism of action are beginning to be understood but questions remain in a number of
658 areas. These include, among others, the structure-function relationships and precise role(s) of
659 EPS, PUFA, LPS, biosurfactants, membrane pigments and accumulated compounds such as
660 PHA in cold adaptation; the effects of cold denaturation on proteins adapted to different
661 temperatures and the protective measures employed by psychrophiles to counteract this; a
662 better understanding of the dynamic motions, amplitudes and time frames of protein
663 structures and their role in temperature adaptation and function; and a better understanding of
664 the various metabolic adjustments employed by psychrophiles. Further studies making use of
665 modern technologies will advance our understanding in these areas and will undoubtedly
666 unveil further adaptive traits and novel metabolic peculiarities employed by these
667 microorganisms, leading to identification of further novel biomolecules with novel properties
668 for use as innovative biotechnological tools. Indeed, while a number of psychrophile derived
669 commercial tools are already being commercialised, the adaptive features, abundance and
670 diversity of psychrophiles and their habitats points to their high potential as rich sources of
671 further novel biomolecules and compounds of applied interest, and further bioprospection of
672 these organisms and their environments should lead to an improved valorisation of their
673 commercial potential.

674

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685

686 **Compliance with ethical standards**

687 **Ethical statement**

688 This article does not contain any studies with human participants or animals performed by any
689 of the authors.

690

691 **Conflict of interest**

692 The authors declare that they have no conflict of interest.

693

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Table 1. The toolkit for a psychrophilic lifestyle. Bioproducts believed to play a role in cold adaptation of micro-organisms, their proposed function in cold-adaptation and their potential biotechnological applications are listed. Only those tools with a commercial potential and described potential role in cold adaptation are given. ‘?’ indicates that questions still remain as to the true function in cold adaptation.

Bioproduct	Proposed Cold-adaptation Functions	Potential Applications
Membrane fatty acids: unsaturated fatty acids, long chain polyunsaturated fatty acids (LC-PUFA)	Unsaturated fatty acids: maintenance of membrane fluidity LC-PUFA: maintenance of membrane fluidity?	Nutrition and health
Membrane pigments: carotenoids	Maintenance of membrane fluidity? Cryoprotection?	Nutrition and health
Compatible solutes	Osmoprotection: against freezing induced osmotic stress Desiccation protection: against freezing induced desiccation Freezing point depression Colloidal glass transition temperature depression Protein and membrane stabilisation	Cryopreservation Stabilisation/preservation of biological materials Food stability and freshness Cosmetics and skin care Plant resistance enhancement
Antifreeze proteins	Ice growth inhibition (thermal hysteresis) Ice recrystallisation inhibition Membrane stabilisation? Ice adhesion?	Frozen foods preservation and quality enhancement Cryopreservation Cryosurgery Freeze tolerance enhancement, e.g. crops, fish Gas hydrate inhibition ‘Ice-prevention’ materials
Ice-nucleating proteins	Extracellular ice crystal nucleation - prevention/reduction of damaging intracellular ice formation - small ice crystals? - release of latent heat of crystallisation?	Artificial snow production Frozen foods and beverages industries Microfluidic devices: freeze–thaw valves Cell surface display Climate control
Extracellular polymeric substances	Ice growth inhibition Osmoprotection: against freezing induced osmotic stress Desiccation protection: against freezing induced desiccation Ice-recrystallisation inhibition	Biopolymers Biofloculants Bioabsorbants Bioemulsifiers Bioadhesives Thickening agents Cryopreservation
Biosurfactants	Ice-recrystallisation inhibition?	Biosurfactants

	Osmoprotection?	
Cold-adapted enzymes	Maintenance of adequate metabolic flux	Enzymes markets: low to moderate temperature processes
Chaperones	Promotion of protein folding and stability Destabilisation of RNA/ DNA secondary structures	Low temperature recombinant protein production
Storage compounds: polyhydroxyalkanoates, cyanophycins	Overcoming carbon and nitrogen uptake deficiencies	Biopolymers Fine chemical synthesis Biofuels Stress tolerance enhancement: plants