Psychrophiles and Psychrotrophs ☆

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Glossary

Barophiles (also known as piezophiles) Pressure-loving bacteria and archaea.
Cryobiosis A temporary state of reduced metabolism in which metabolic activity is absent or undetectable due to freezing. To initiate cryobiosis, the organism freezes all of the water within its cell(s). This allows the organism to endure the freezing temperatures until more hospitable conditions return. Studies have shown that the longer an organism remains in cryobiosis, the longer it takes for the organism to come out of cryobiosis. This is because the organism must use its own energy to come out of cryobiosis, and the longer it stays in cryobiosis the less energy it will retain.
Cryophiles Cold-loving eukaryotes.
Homeophasic adaptation The ability of the cell membrane to maintain a relatively constant viscosity (fluidity) throughout the growth temperature range.
Membrane fluidity The ability of the cell membrane to remain fluid in order to modulate the activity of the intrinsic proteins which perform functions such as electron transport, ion pumping, and nutrient uptake.
Psychrophiles Cold-loving bacteria and archaea.
Psychrotrophs Cold-tolerant bacteria and archaea.
Thermocline In the stratification of warm surface water over cold deeper water, the transition zone of rapid temperature decline between two layers.

Introduction

Psychrophiles are cold-loving bacteria or archaea, whereas cryophiles are cold-loving higher biological forms (e.g., polar fish). Owing to precedence, the term psychrophile has been retained. Morita (1975) defined psychrophiles as organisms having an optimal temperature for growth at about 15°C or lower, a maximal temperature for growth at about 20°C, and a minimal temperature for growth at 0°C or lower. The term, psychrotroph (also termed psychrotolerant), was retained to denote organisms that have the ability to grow at low temperatures, but have their optimal and maximal growth temperatures above 15 and 20°C, respectively. The reason why the maximal growth temperature was set at 20°C was simply because laboratory temperature in the United States is around 21–22°C, which is not considered cold. Although it is recognized that there is a continuum of cardinal temperatures among the various thermal groups, the above definition is a useful one because it has relevance in terms of their respective ecological distributions, as psychrophiles are limited to permanently cold environments (Baross and Morita, 1978). Most microbiologists have accepted the foregoing definitions, though there are differences in usage: food and dairy microbiologists prefer the adjective "psychrotrophic," whereas environmental microbiologists more commonly use "psychrotolerant."

Psychrophiles were first reported in 1884, but most of the early literature actually dealt with psychrotrophic bacteria and not with true psychrophiles. Since investigators were not working with extreme cold-loving bacteria, there was much debate and, as a result, many terms were coined to designate psychrophiles. These terms were cryophile, rhigophile, psychrorobe, thermophobic bacteria, Glaciale Bakterien, facultative psychrophile, psychrocartericus, psychrotrophic and psychrotolerant (Morita, 1975). This proliferation of terms also resulted from the fact that no true cold-loving bacteria existed in the various culture collections. Ingraham (1962) wrote, "Other authors have felt that the term psychrophile should be reserved for bacteria whose growth temperature optima are below 20°C if and when such organisms are found." Because of this situation the research on true psychrophiles was neglected, especially when compared to the research on thermophiles. The first true psychrophiles, employing the foregoing definition, to be described taxonomically in the literature were *Vibrio* (*Moritella* gen. nov.) *marinus* (*marina* comb. nov.) MP-1 and *Vibrio* (*Colwellia* gen. nov.) *psychroerythrus* (*psychrerythraea* comb. nov.) in 1964 and 1972, respectively. The first genome of a psychrophile was obtained for *Colwellia psychrerythraea* 34H, isolated from Arctic marine sediments (Methé *et al.*, 2005). Since then, many more have been sequenced and through both proteomic and genomic analyses have been shown to exhibit cold-specific adaptations (Lauro *et al.*, 2011; Collins, 2015).

Currently, the Arctic permafrost bacterium *Planococcus halocryophilus* has demonstrated the lowest growth temperature (− 15°C with a generation time of 50 days) of any organism authenticated by a growth curve (Mykytczuk *et al.*, 2013). Perhaps ironically, *P. halocryophilus* is merely psychrotolerant, having a maximum growth temperature of 37°C and an optimum growth temperature of 25°C. True psychrophiles growing at subfreezing temperatures have comparably long generation times, including 10 days at − 12°C for *Psychromonas ingrahamii* (Breezee *et al.*, 2004) and 39 days at − 10°C for *Psychrobacter arcticus* (Bakermans *et al.*, 2003).

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The first and only truly psychrophilic archaeon to be isolated is *Methanogenium frigidum*, a methanogen from Ace Lake Antarctic (Franzmann *et al.*, 1997).

The Cold Environment

Most of the Earth's biosphere is cold. Approximately 14% of the Earth's surface is in the polar region, whereas 71% is marine. By volume, more than 90% of the ocean is 5°C or colder. Below the thermocline, the ocean maintains a constant temperature of at most 4–5°C, regardless of latitude. Therefore, pressure-loving marine microorganisms (i.e., barophiles or more correctly known as piezophiles) are also primarily either psychrophilic or psychrotrophic (Yayanos, 1986; Kato, 2012) and this is to be expected because the water below the thermocline of the ocean is under great hydrostatic pressure. In addition, the higher atmosphere is cold, hence the higher altitudes of mountain environments are also cold. The average temperature of the Earth is currently ~15°C. Although the Earth is predominantly cold, the amount of research on psychrophiles is very small compared to the research on other types of extremophiles. It is important to take environmental samples where the *in situ* temperature never exceeds the psychrophilic range and to ensure that the medium, pipettes, inoculating loops, etc. are kept cold before use. The lack of temperature controls was probably the main reason why early microbiologists, never realizing their abnormal temperature sensitivity, failed to isolate psychrophiles. With renewed interest in life in outer space, there is a renewed interest in microorganisms that live in extreme environments, especially the cold environment.

Psychrotrophs are found in the same cold environments as psychrophiles but in greater numbers. They can also be found in cold environments which fluctuate above the psychrophilic range, mainly due to the seasonal variation in the radiant energy from the sun. Thus, ice surfaces in either northern or southern polar regions attain temperatures as high as ~28°C. Psychrophiles are not present in these temperature-fluctuating environments. If the concept that either thermophiles or mesophiles were the first microorganisms to evolve on Earth, then it would follow that psychrophiles evolved from the psychrotrophs (Morita and Moyer, 2001). Since the volume of the sea below the thermocline is permanently cold, it is only logical that some of the psychrophiles are also piezophiles. These extremophiles are truly multifaceted, in that in addition to pressure, they are often also tolerant to other extreme environmental forcing functions such as high salt concentrations (i.e., halophiles), ultraviolet radiation, and can survive low nutrient and water availability.

The presence of psychrophilic and psychrotrophic bacteria in cold environments (including permafrost and sea ice) permits the essential process of nutrient regeneration to take place (Deming, 2002). For at least a million years, microbial communities have survived in permafrost (Vorobyova *et al.*, 1997) and at the base of the Antarctic ice sheet (Christner *et al.*, 2014). Microbial activity has been shown to occur down to −20°C in Arctic sea ice, adding to the concept that liquid inclusions provide an adequate habitat for microbial life (Junge *et al.*, 2004). There are no reports of growth below −15°C (Mykytczuk *et al.*, 2013); however, microorganisms have been shown to survive in situ at −30°C and have been predicted to metabolize at −40°C (Price and Sowers, 2004). It appears that the lower growth temperature is fixed by the freezing properties of the aqueous solutions outside and immediately adjacent to the cell.

Physiological Adaptations

The abnormal thermosensitivity of psychrophilic bacteria indicates the adaptation of cold-loving bacteria to their cold environment. Microbes do not have thermoregulatory mechanisms. When exposed to temperatures above their maximal growth temperatures, they expire; in some psychrophiles, this temperature can be between 10 and 20°C. When cells of psychrophiles are exposed to temperatures above their maximal growth temperatures, the ability to take up oxygen decreases. These thermally induced damages to the cell indicate that physiological adaptation to low temperature has evolved in the true psychrophiles. On the contrary, at optimal temperature, the oxygen uptake was minimal and increased substantially at suboptimal and supraoptimal growth temperatures (Herbert and Bhakoo, 1979). There are reports that, at suboptimal temperatures, psychrophiles produce more ribosomal ribonucleic acid (rRNA) and more protein (in terms of energy generating enzymes).

Microbiologists have substituted bacteria growth rate for reaction rate in the van't Hoff–Arrhenius equation to obtain the temperature characteristic of growth (μ); μ is then analogous to activation energy. This concept does not appear to be valid for psychrophiles nor psychrotrophs. The minimum temperature for growth of mesophiles is considered by many investigators to be low-temperature inhibition of substrate uptake. These adaptations are reflected mainly in their enzymes, membranes and posttranscriptional modification of transfer RNA. In the latter situation, unprecedented low amounts of modification were found in psychrophiles, especially from the standpoint of structural diversity of modification observed (Dalluge *et al.*, 1997) and these findings support the concept that a functional role for dihydrouridine is in the maintenance of conformational flexibility of RNA, which can be contrasted with the role of modification contained in RNA from thermophiles. In thermophiles, it is to reduce regional RNA flexibility and provide structural stability to RNA for adaptation to high temperature. Conversely, in psychrophiles it is their ability to retain their membrane fluidity at low temperatures (homeophasic adaptation), so that nutrient transport can take place, and this appears to be the primary adaptation to life at cold temperatures.

Still, adaptation of psychrophiles at low temperature permits the organisms to grow rapidly. This is especially true when optimal conditions, mainly the energy source, are available to the cells. Obligate psychrophiles have even been shown to grow

faster and thereby out-compete psychrotrophs, indicating that they may have a greater impact upon mineralization processes in cold environments (Harder and Veldkamp, 1971). In addition, antifreeze proteins and cryoprotectants aid in lowering the temperature at which an organism can grow by lessening the effects of ice crystalization. Antifreeze proteins have been shown in a diverse group of bacterial isolates from Antarctic lakes (Gilbert *et al.*, 2004) and a hyperactive, Ca^{2+}-dependent antifreeze protein has been described showing over a 2°C freezing point depression (Gilbert *et al.*, 2005). The production of exopolysaccharides also seems to yield an important role in the cryoprotection of psychrophiles and psychrotrophs. High concentrations, especially at lower temperatures, of exopolysaccharides have been detected in Arctic winter sea ice (Krembs *et al.*, 2002) and in conjunction with Antarctic marine isolates (Nichols *et al.*, 2005).

Enzymes

Cytoplasmic proteins from psychrophiles are more heat labile (i.e., low thermal stability) than those from their mesophilic counterparts. Early studies indicated that Malic dehydrogenase (MDH) from washed cells of *Moritella marina* was found to be stable between 0 and 15°C (organism's optimal temperature) and that inactivation takes place between 15 and 20°C. Inactivation of partially purified MDH became very pronounced when exposed to temperatures above 20°C. Yet other enzymes from the same organism are stable at 35°C or beyond. It appears that most enzymes from psychrophiles will operate above the maximal temperature for psychrophiles, but they are often more sensitive to warmer temperatures than their counterparts from mesophiles and thermophiles, losing their activity at approximately 30°C (see Morita, 1975).

Molecular enzymology using psychrophiles has only recently been investigated to any great degree. Cold-adapted enzymes often have high specific activities, up to an order of magnitude greater, at low temperature than those from mesophiles (Feller and Gerday, 2003). Recently, psychrophilic enzymes have been crystalized, which has facilitated three-dimensional structure modeling. This together with gene homology studies have demonstrated how multiple strategies for adaptation are used by different enzymes to achieve conformational flexibility at low temperatures. Common themes to enhance flexibility include: the reduction of the number of ion pairs, hydrogen bonds and hydrophobic interactions; decreased intersubunit interactions; increased interaction with the solvent; a reduced nonpolar fraction in the core; higher accessibility to the active site; increased exposure of nonpolar residues to the solvent; decreased cofactor binding; clustering of glycine residues; and a lower proline and arginine content (D'Amico *et al.*, 2006). The overarching theme suggests that psychrophilic enzymes have an improved flexibility of the catalytic region, whereas other non-catalytic regions may be even more rigid than their mesophilic and thermophilic counterparts. From the data, it appears that "psychrophilic" enzymes have not evolved to the same low temperature level as the membranes of psychrophiles. Nevertheless, the foregoing implies that a molecular volume decrease of the psychrophilic enzymes has evolved through natural selection (as opposed simply to genetic drift via a lack of selection).

Membrane Structure and Function

Homeophasic adaptation has been proposed to emphasize the necessity to maintain the membrane lipids in a bilayer phase so that the membrane can carry on the important functions of nutrient uptake and the regulation of intracellular ionic composition, which are performed by carrier systems and ion pumps. For instance, uptake of substrate is minimal when *Escherichia coli* is exposed to its minimum temperature of growth.

Thermally Induced Leakage

When cells of *M. marina* are exposed to a few degrees above their maximal growth temperature, leakage of cellular proteins (including several enzymes) as well as DNA, RNA and amino acids occurs. Thermally induced lysis and leakage of the psychrophile's membrane are probably the reasons why psychrophiles are not found in environments where the temperature fluctuates above 20°C. Thermally induced lysis has been shown to take place in both *M. marina* and *C. psychrerythraea*. Fluidity of the membrane must also be maintained at low temperatures. Thus, membranes play an important role in the thermal stability of psychrophiles at low temperature. The abnormal thermolability of the membrane of psychrophiles compared to their enzymes indicates that the membrane is probably the primary site of thermal damage.

Membrane Lipids

It has been known for many years that the lipid composition of the membrane changes in response to temperature. Depending on the bacterium in question, the fatty acid changes in the membrane can be in (poly)unsaturation, chain length, branching or cyclization, often in combination (Russell, 1992). Psychrophiles and psychrotrophs are known to contain unsaturated, polyunsaturated, short chain, branched and/or cyclic fatty acids; this occurs more than in their other thermal counterparts. In general, decreasing the culture temperature of a psychrophile increases levels of (poly)unsaturated phospholipids and neutral lipids in order to maintain membrane fluidity at low temperatures. There are also data that indicate that the acyl chain length changes in the phospholipids, where the acyl chain length shortens when the temperature is lowered. However, this latter process is slow, taking place over several generations. The following data depend on the isolate of psychrophile in question. Psychrophiles are endowed

with a higher proportion of unsaturated fatty acids, especially hexadecenoic (16:1) and octadecenoic (18:1) acids than are mesophiles. The amounts of 14:0 (myristic acid) and 16:0 (palmitic acid) are higher in cells that are grown at higher temperatures, but when grown at 0°C there is an increase in 22:6 (docosahexaenoic acid) content in some strains, while other psychrophiles were shown to contain 20:5 (eicosapentaenoic acid) (Hamamoto *et al.*, 1995). Membrane fluidity was found to be partly affected by *cis–trans* isomerization of the double bonds of fatty acids. When the psychrophilic *Moritella* ANT-300 was starved, qualitative and quantitative changes in fatty acids were induced. The major fatty acid palmitoleate (16:1) increased from 46 to 62.5% at the expense of myristate (14:0), which decreased from 26 to 13% in membrane lipids (Oliver and Stringer, 1984). A study of Antarctic sea-ice bacteria suggested that psychrotrophs, but not psychrophiles, are able to alter their fatty acid composition (Rotert *et al.*, 1993). Since psychrotrophs are found in fluctuating environments, this could have validity. Other potential adaptations for increased membrane fluidity include an increase in large lipid head groups, proteins and nonpolar carotenoids (Chintalapati *et al.*, 2004). However, it should be mentioned that the effects of temperature on phospholipid composition are variable and often species-specific.

Since lipid desaturation is the most commonly observed change in the membrane when the temperature is decreased, the desaturase acting on the acyl chyl chains of the membrane lipids comes into play, thereby increasing the amount of unsaturated lipid (Russell, 1992). This desaturase activity occurs first and may be followed by temperature-dependent changes in fatty acid chain length and branching mediated by additional synthesis. Thus, all the above help to maintain the membrane's ability to function properly at low temperatures. Nevertheless, Russell (1992) points out that "much more detailed studies are needed to resolve the question of whether solute uptake ability at low temperature is a feature that truly distinguishes psychrophiles and mesophiles, i.e., a determinant of psychrophily."

Biodiversity

Before molecular techniques were developed for microbial taxonomy, the psychrophiles were originally assigned to the following genera: *Brevibacterium*, *Microbacterium* and *Micrococcus* (Division: Actinobacteria); *Flavobacterium* (Division: Bacteroidetes); *Bacillus* and *Clostridium* (Division: Firmicutes); *Alcaligenes*, *Achromobacter*, *Pseudomonas* and *Vibrio* (Division: Proteobacteria). Today there are at least 69 bacterial genera containing reported psychrophiles, distributed amongst the following Orders: Micrococcales and Propionibacteriales (Division: Actinobacteria); Bacteroidales, Cytophagales, Flavobacteriales, Sphingobacteriales (Division: Bacteroidetes); Bacillales and Clostridiales (Division: Firmicutes); Oscillatoriales (Division: Cyanobacteria); Deinococcales (Division: Deinococcus-Thermus); and Alteromonadales, Burkholderiales, Desulfobacterales, Desulfuromonadales, Neisseriales, Oceanospirillales, Pseudomonadales, Rhizobiales, Rhodobacterales, Sphingomonadales, Vibrionales, Xanthomonadales (Division: Proteobacteria). Psychrophiles are widespread among the domain Bacteria with the majority of isolates coming from the Gram-negative divisions Bacteroidetes and Proteobacteria. Thus, psychrophiles are autotrophic or heterotrophic, aerobic or anaerobic, spore-formers and nonspore-formers, phototrophs and nonphototrophs. Interestingly, all of the currently known psychrophiles that are also pressure-requiring, within the five genera denoted, are contained within the class γ-Proteobacteria. The indication, based on phylogenetic reconstruction, is that the combined barophilic and psychrophilic phenotype evolved independently within these genera (DeLong *et al.*, 1997). Studies also indicate that members of the same genera of psychrophiles occur at both poles; however, cosmopolitan species have yet to be discovered. It has been concluded that many additional isolations would be necessary before endemic populations can reasonably be inferred (Staley and Gosink, 1999). It must also be recognized that many isolates have been reported as psychrophiles, but do not fit the definition used in this entry; others need to have their cardinal temperatures determined. Furthermore, many of the above-named genera contain isolates that have not yet undergone classification by molecular means. Still, there are well over 200 psychrophile and psychrotroph genome projects (including several cold-adapted Archaea) either complete or in progress. Investigations at the genome and proteome levels, including metabolic pathway reconstructions, are in part due to the increased interest in their potential for biotechnical applications.

Archaea are widespread in permanently cold environments, and are particularly prevalent in Arctic, Antarctic, and deep ocean waters. Archaeoplankton were initially reported by DeLong *et al.* (1994) to comprise up to 34% of the prokaryotic biomass in a coastal Antarctic surface waters (-1.5°C), after employing a molecular phylogenetic survey (no isolates and no cardinal temperatures). Archaeal assemblages in Arctic seawater are structured by depth and composed predominantly of a ubiquitous marine clade formerly known as Marine Group I Crenarchaeota (Galand *et al.*, 2009). A mesophilic representative, *Nitrosopumilus maritimus*, was isolated from temperate latitudes and eventually assigned to a new phylum, *Thaumarchaeota*, based on genomic evidence (Brochier-Armanet *et al.*, 2008); psychrophilic strains have not yet been isolated. *N. maritimus* is an ammonia oxidizing chemolithoautotroph, but cold-adapted methanogens have also been discovered. *M. frigidum* (Division: Euryarchaeota), isolated from Ace Lake, Antarctica, is a hydrogen-utilizing methanogen that has an optimum growth temperature at 15°C and a maximal growth at 18°C, with a minimal growth temperature at -2°C (Franzmann *et al.*, 1997). Several cold-adapted archaea have also been studied, as reviewed by Cavicchioli (2006). This opens up a new area of research, as well as suggesting that the psychrophiles are phylogenetically diverse in both the bacteria and the archaea. Metagenomic reconstruction of genomes from permanently cold habitats is enabling access to the "microbial dark matter" that is hidden by limitations in culturing techniques. A recent study used this technique on Arctic deep-sea sediments to determine 92% of the genome of a complex archaeon that "bridges the gap" between Archaea and Eukaryotes (Spang *et al.*, 2015). The reason why more psychrophiles have not been elucidated in the past is simply because no one bothered to look for them, especially employing the right techniques. How many undiscovered psychrophiles remain?

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