Psychrophiles and Psychrotrophs

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Psychrophiles are extremophilic bacteria or archaea which are cold-loving, 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. Psychrotrophs are cold-tolerant bacteria or archaea that have the ability to grow at low temperatures, but have optimal and maximal growth temperatures above 15 and 20°C, respectively. The Earth has extensive cold ecosystems that do not reach temperatures greater than 5°C (e.g. worldwide deep oceans, polar surface, polar ice-cap regions, permafrost). Psychrophiles and psychrotrophs are cold-loving extremophiles adroitly adapted to these environmental conditions, and are often capable of enduring extended periods of cryobiosis.

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 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 USA 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). The food and dairy microbiologists and, indeed, most microbiologists have accepted the foregoing definition.

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 amount of 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.

Currently, the Arctic sea ice bacterium *Psychromonas ingrahamii* has demonstrated the lowest growth temperature (−12°C with a generation time of 240 h) of any organism authenticated by a growth curve (Breezee *et al.*, 2004). The first and only truly psychrophilic archaeon to be isolated is *Methanogenium frigidum*, a methanogen from Ace Lake, Antarctica (Franzmann *et al.*, 1997). More recently, the genome of the *Colwellia psychrerythraea* 34 H, isolated from Arctic marine sediments, has been sequenced and through both proteomic and genomic analyses has been shown to exhibit several cold-specific adaptations (Methé *et al.*, 2005).

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, a maximum of 4–5°C, regardless of latitude. Therefore, all pressure-loving microorganisms (i.e. barophiles) are either psychrophilic or psychrotrophic (Yayanos, 1986) and this is to be expected because the water below the thermocline of the ocean is under 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 thermophiles. 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 barophiles. 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. **See also**: Barophiles and Piezophiles

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 some million years, microbial communities survived in permafrost in a state of cryobiosis (Vorobyova *et al.*, 1997). 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 −12°C (Breezee *et al.*, 2004), 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. **See also**: Arctic Ecosystems

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, 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. **See also**: Barophiles and Piezophiles

Microbiologists have substituted bacterial 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 post-transcriptional 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. **See also**: Bacterial Reproduction and Growth

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 crystallization. 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 *Mo. marina* was found to be stable between 0 and 15°C (organism's optimal temperature) and inactivation takes place between 15 and 20°C. Inactivation of partially purified MDH became very pronounced when exposed to temperatures above 20°C. In the same organism, the phosphofructokinase/glyceraldehyde 3-phosphate dehydrogenase complex, lactic dehydrogenase, hexokinase and aldolase lost nearly all their activity when incubated at 35–40°C for 1 h. Purified aldolase (similar in properties to type II aldolases) from *Mo. marina* had an optimum temperature for activity at 25°C and no activity was evident above 32°C, but activity was observed at 4°C (lowest temperature tested). Partially purified glucose 6-phosphate dehydrogenase (G6PD) from *Mo. marina* was found to be stable between 5 and 26°C, but when it was exposed to 36°C for 1 h, 90% of its activity was lost. On the contrary, glyceraldehyde 3-phosphate dehydrogenase was found to be quite thermostable. Caution should be used in dealing with the thermal inactivation temperatures of the above enzymes, mainly because ammonium sulfate, which is a neutral salt, was used in their preparation and it protects the enzymes from thermal denaturation. 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, the former losing their activity at approximately 30°C (see Morita, 1975). **See also**: Protein Denaturation and the Denatured State

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 crystallized, which has facilitated three-dimensional structure modelling. 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 noncatalytic 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 preformed by carrier systems and ion pumps. For instance, uptake of substrate is minimal when *Escherichia coli* is exposed to its minimum temperature of growth. **See also**: Bacterial Cytoplasmic Membrane

Thermally induced leakage

When cells of *Mo. marina* are exposed to a few degrees above their maximal growth temperature, leakage of cellular protein, MDH, G6PD and deoxyribonucleic, ribonucleic 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 *Mo. 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). Another study suggested, of Antarctic sea-ice bacteria, 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'. **See also**: Archaeal Membrane Lipids; Membrane Dynamics

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). Other genera that have been described more recently are: Agreia, Arthrobacter and Cryobacterium (Division: Actinobacteria); Phormidium (Division: Cyanobacteria); Algoriphagus, Bacteroides, Cytophaga, Gelidibacter, Polaribacter, Psychroflexus and Psychroserpens (Division: Bacteroidetes); Acetobacterium, Carnobacterium, Exiguobacterium, Planococcus and Planomicrobium (Division: Firmicutes); Octadecabacter, Aquaspirillum, Polaromonas, Desulfotalea, Alteromonas, Marinobacter, Marinomonas, Methylosphaera, Pseudoalteromonas, Psychrobacter, Colwellia*, Moritella*, Photobacterium*, Psychromonas* and Shewanella* (Division: Proteobacteria; those that also include barophilic isolates are designated with an asterisk). It therefore appears that the 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 some 30 psychrophile and psychrotrophic genome projects (including four 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.

Archaeoplankton have been reported by DeLong *et al.* (1994) to comprise up to 34% of the prokaryotic biomass in coastal Antarctic surface waters ($-1.5°C$), after employing a molecular phylogenetic survey (no isolates and no cardinal temperatures). This does constitute evidence that uncultured psychrophilic archaea do exist in nature. However, there is currently only one isolated archaeal psychrophile. *Me. frigidum* (Division: Euryarchaeota), isolated from Ace Lake, Antarctica, is a hydrogen-utilizing methanogen that has an optimal 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 recently 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. The reason why different psychrophiles have not been elucidated in the past is simply because no one bothered to look for them, especially when employing the right techniques. How many psychrophiles remain undiscovered? **See also**: Archaea; Extremophiles

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