PSYCHROPHILY AND RESISTANCE TO LOW TEMPERATURE

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Summary

This article considers the phenomenon of psychrophily, the ability to grow down to or below 0 °C, as displayed by microorganisms. Being small, bacteria, yeasts, fungi, and microalgae are unable to insulate themselves against the effects of cold; therefore, they must adapt their structure if they are to survive and grow successfully in cold habitats. Following a discussion of the types of cold-adapted microorganisms, their growth characteristics, and the different cold environments they inhabit, the focus of this article is on cold-adaptation of cellular components.

In particular, the focus is on those adaptive changes in proteins and lipids that ensure the essential activity of enzymes and the functional integrity of membranes at low temperatures. The article also deals with the phenomenon of cold shock (the mechanism by which microorganisms cope with sudden decreases in temperature that are transient), and how this is related to more permanent adaptation to cold, the "secret of psychrophily," so important to life on earth since two-thirds of our planet is cold, that is, permanently below 5 °C.

1. Introduction

Psychrophily is the ability to live and reproduce at low temperatures, that are close to or below 0 °C. A wide range of organisms has this ability, but this article considers microorganisms, which in terms of species numbers and diversity dominate low temperature habitats. Thermally, humankind is a mesophile, living at what we consider moderate temperatures; we generally feel most comfortable within a rather narrow temperature range of about 10–30 °C. We regard temperatures outside this range as being extreme. In relation to cold, as soon as the temperature falls to near or below zero we must make adjustments by insulating ourselves with warm clothing or moving to a warmer environment to avoid compromising our internal body temperature of about 37 °C.

Microorganisms, on the other hand, are too small to be able either to insulate themselves from the cold or to move sufficient distances (even though they may be motile). Therefore, in order to survive, grow, and reproduce themselves, they must adapt. They do this by changing their chemical composition, that is by altering the makeup of their major macromolecules (lipids and proteins) and other small molecules within their cells. In fact, microorganisms have been remarkably successful at such adaptations and they are to be found colonizing every cold environmental niche on planet earth, whether it is soil, sediment, water, snow, or ice, as well as the plants, animals, or other organisms that inhabit these cold ecosystems.

Psychrophilic microorganisms that are able to grow in natural cold environments at or near zero temperatures have been known for nearly 200 years. The first record, made in 1840 by the botanist Hooker during the first naval Discovery explorations of Antarctica, was the observation that algae were associated with and colored sea-ice; this was followed in 1884 by Certes, who reported the existence of bacteria growing in sediments at low temperatures. In 1887, Forster was the first to measure the growth and reproduction at low temperatures of isolated bacteria that he had obtained from fish, and the term “psychrophilic” was first used in 1902 by Schmidt-Nielsen to describe such cold-adapted microorganisms. In the following decades there were sporadic publications of cold-adapted bacteria and yeasts, including the demonstration in 1960 that bacteria from Antarctic snow could grow at –7 °C, and there was a vigorous debate about terminology and of what constituted a psychrophile (see Section 2). Many of the physiological studies of growth were concerned with food-associated microorganisms (mainly bacteria) in relation to food preservation by refrigeration, while ecological studies were often focused on the alpine and polar regions, particularly Antarctica.

A renewed general scientific interest in membranes during the 1970s and 1980s and the application of sophisticated biophysical and spectroscopic techniques led to a surge of publications on the physiological and biochemical adaptation of membrane lipid composition in cold-adapted microorganisms. Other aspects of the molecular basis of cold adaptation have, until recently, been poorly investigated, often because of the lack of appropriate investigative methodologies. In addition, many investigations have been of the heat sensitivity of cold-adapted microorganisms, which (although of interest) is a consequence, not the cause, of cold adaptation. Two factors have stimulated recent interest in psychrophiles. First, in the context of global biodiversity there has been a massive increase in research on "extremophiles," (micro)organisms that live in extreme environments, and molecular studies of thermophiles that live at high temperatures have prompted comparisons and renewed interest in psychrophiles at the other end of the thermal spectrum. Second, the realization that cold-adapted microorganisms have huge biotechnological potential as a source of enzymes in cost-effective bioprocessing at low temperatures has driven fundamental studies of protein structure and cold adaptation in psychrophiles, utilizing the modern tools of molecular biology.

2. Cold-Adapted Microorganisms

2.1. Terminology

Whilst the term "psychrophily" is used universally to describe the ability of an organism to grow at or near 0 °C, the noun derived from it ("psychrophile") is not the only term used to describe the organisms themselves. The reason is that two broad groups of cold-adapted organisms can be distinguished, known as "psychrophiles" and "psychrotrophs" ("psychrotolerants"). The term "psychrotroph" is commonly used in the food industry to describe microorganisms that can spoil or poison refrigerated foods; the alternative term "psychrotolerant" is used in ecology and perhaps more clearly describes the thermal ability of this group. The word "psychrotroph," first introduced by Eddy in 1960, means "cold (literally frost)-eating" whilst "psychrophile" means "cold-loving": for this reason some researchers prefer the terminology of psychrophiles and psychrotolerants, because it is the former, not the latter, which are truly adapted to grow only at low temperatures. In the past, psychrophiles and psychrotolerants have also been distinguished as "obligate" and "facultative" psychrophiles, but these terms have the disadvantage that they imply (incorrectly) that it is possible to interconvert an organism between the two types by modifying growth conditions. Thomas Brock, author of the best-known microbiology textbook and discoverer of (hyper) thermophiles, has suggested the use of the terms "eurythermal" and "stenothermal" for organisms growing over wide and narrow growth temperature ranges, respectively. Thus, psychrotolerants would become eurythermal mesophiles (the use of psychrophile would remain). However, such terminology does not discriminate between extensions of the mesophilic growth temperature range to lower or higher temperatures.

Historically, use of the term psychrophile has been particularly hotly argued. The debate about terminology was linked to laboratory observations, often in relation to the cold storage of food, and definitions such as "visible growth on laboratory media within one or two weeks at 0 °C" or "grow well at 0 °C in two weeks" were suggested as working, but not always very precise, definitions of psychrophiles. Other researchers attempted to distinguish psychrophiles from mesophiles and even psychrotolerants on the basis of the temperature characteristic (μ) for growth (μ is derived by substituting growth rate for reaction rate in the Arrhenius equation, see below). However, despite some reports to the contrary, it was not generally found that μ values correlated with either the growth temperature range or the ability to grow with minimal or no lag following shift-down from warm to cold temperatures.

Inevitably with such pragmatic descriptions, different laboratories evolved their own versions and it was not until the mid-1970s when Morita introduced definitions that were based on cardinal growth temperatures (that is, the lower and upper growth temperature limits and the optimal growth temperature), that this problem was resolved. The cardinal growth temperatures may vary slightly with the composition of the growth medium or other growth parameters (particularly oxygen tension for facultative anaerobes), but not enough to invalidate their use as defining the type of organism, and Morita's definitions have now been generally accepted as a way of distinguishing the two groups of organisms that can grow at or close to zero. Psychrotolerants usually only grow at temperatures approaching 0 °C and grow optimally at temperatures around 20–25 °C and may have upper limits as high as 40 °C. In contrast, psychrophiles are specifically adapted to low-temperature growth, not only at 0 °C but below zero and have optimal growth temperatures below 15 °C and upper limits below 20 °C. Species of snow algae are among the microorganisms reported to have the lowest optimum temperatures for growth; although their growth rates are relatively slow compared with cold-adapted bacteria, some snow algae have apparent optima of 1–10 °C and upper limits of 10 °C. Among bacteria, several gram negatives found in sea-ice are particularly psychrophilic with optimum growth temperatures that are below 10 °C and *Polaromonas vacuolata* has an optimum of only 4 °C.

Despite these apparent distinctions between psychrophiles and psychrotolerants, some organisms have cardinal growth temperatures that are a hybrid of those suggested by Morita—confirming the acknowledged view that nature is a continuous spectrum of phenotypes. Nonetheless, the synthetic distinction between psychrophile and psychrotolerant organisms is a useful one since it guides and informs research efforts. Moreover, no organism has been found which has an optimum growth temperature in the psychrophilic range (i.e. <15 °C) and yet is capable of growing above 40 °C.

Special attention has focused on the lower limit of growth for psychrophiles. Growth rates of even very cold-adapted microorganisms may be very slow at zero and below, so that they become very difficult to measure in practice, while theoretical plots of growth rate versus temperature using a number of algorithms give values for the lower limit that may be 20 or 30 centigrade degrees below zero. Therefore, reports of minima that are below –10 to –12 °C (the reasons for these values are given in Section 3.3) are generally unconfirmed and must be treated with caution. Growth of yeast on peas that were frozen has been reported at sub-zero temperatures as low as –18 °C. Other reports of the growth of fungi at –20 °C and yeast at –34 °C have not been substantiated.

2.2. Habitats

Most of Earth is cold (that is, it has a temperature that is more or less permanently below 5 °C), and so it is not surprising that cold-adapted microorganisms are widely distributed in nature. About 70% of the earth is covered by saline oceans, which at depths below 1000 meters have a relatively constant temperature of below 5 °C irrespective of latitude, and 90% of the sea floor has a temperature that is below 4 °C. Despite the extent of this coverage (and as in most fields of biology), we know more about the microbiology of the terrestrial third than the aquatic two-thirds of earth's habitats. That fact is exemplified by the recent realization that sediments covering the deep oceanic floors are not sterile but house a varied and large population of cold-adapted microorganisms. Similarly, it is only the recent application of molecular biological techniques that has led to the discovery that psychrophilic and psychrotolerant planktonic archaea are abundant in oceanic waters where they may represent up to a third of the prokaryotic diversity, and they are also found in sea-ice. However, none of these archaea has been cultured as yet, although in cold soils and sediments archaeal types such as methanogens are common. The results of such molecular screening studies indicate that we still have much to learn about not just the extent of archaeal colonization of cold environments but also the prokaryotic diversity in general. The microbial diversity of low temperature environments is discussed in detail in *Ice Ecosystems and Biodiversity*. What now follows is a brief introduction to some important cold habitats that occupy large portions of the earth.

The polar regions constitute about 14% of earth's surface and are generally cold, apart from exposed soils that warm in the summer months. Cold-adapted microorganisms are particularly important in the ecology of cold habitats such as Antarctica, where they have key roles in primary biomass production and nutrient cycling. Sea-ice in the polar regions accounts for 10% of the oceanic surface on earth and harbors a unique ecosystem on a vast scale that is dominated by microorganisms. The sea-ice microbial communities (SIMCOs) are comprised of bacteria, algae, fungi, viruses, and protozoa living within the ice and brine channels that permeate the ice. Each year more than 20 billion cubic meters of sea-ice melts in the polar regions, a process that has global significance. For example, the release of viable microorganisms from sea ice around Antarctica during the summer melting fuels the Southern ocean food web, which has global influence because of its interactions with the major oceans of earth; and sea-ice microorganisms make major contributions on a global scale to such processes as the export and sequestration of biogenic carbon that is relevant to climate change.

The habitats that remain permanently very cold, such as deep-ocean waters, some polar regions, and some particular terrestrial habitats (for example, sediments in cold caves and on glaciers), are those where psychrophiles are most likely to be found since there they can compete successfully with psychrotolerants. Environments that experience periodic, diurnal, or seasonal fluctuations in temperature are favorable to psychrotolerants, which are able to grow over a wider temperature range and to restart their metabolic activity rapidly after thawing. However, this distinction may not be a good guide as to the type of cold-adapted microorganisms that predominate: for example, in some permanently cold freshwater lakes (maximum temperature 5 °C) and some polar seawater (maximum temperature –1 °C), it is psychrotolerants that predominate, and the same is true even in Arctic and Antarctic permafrost, that is, permanently-frozen soil. Remarkably, viable bacteria are present in permafrost at depths of many meters that are up to several million years old!

Another remarkable type of cold-adapted ecosytem is the range of endolithic microbial communities that are found a few millimeters beneath the surface of exposed sandstone rocks in Antarctic dry deserts. These communities are based on lichens (that is, algae plus fungi) or cyanobacteria and also contain heterotrophic bacteria, and they cope with extreme conditions of low temperature, water availability, and nutrient levels, plus high incident UV irradiation. The external air temperature seldom rises above zero, but the rocks warm in the sun and at the level of the endolithic communities under the surface temperatures may be 20 centigrade degrees higher. Therefore, despite the apparently extremely harsh nature of this cold niche habitat, it is not surprising that psychrotolerants dominate over psychrophiles. This gives the communities the flexibility to cope with sudden relatively large temperature rises and to increase metabolic activity and population size accordingly, whilst remaining semi-dormant or dormant for large periods of time.

An explanation for the general dominance of psychrotolerants may be that, despite the cyclical interruptions of ice ages, the overall evolutionary trend is that the earth is still cooling from the time about 3.4 billions years ago when the first microorganisms appeared on what was a hot planet. An alternative explanation relates more to present conditions in that the influence of nutrient or moisture availability overrides the effect of temperature on the distribution of psychrophiles and psychrotolerants. The latter are defined, of course, by their thermal growth characteristics in laboratory tests that may not reflect those in the natural habitat. The importance of nutrient supply is reflected in the observation that a higher proportion of psychrophiles is found in Antarctic compared with arctic marine ecosystems, because the latter are more influenced by nutrient input from terrestrial runoffs. Antarctic desert (dry valley) soils contain yeasts, which have simple growth requirements that enable them to colonize such a severely nutrient-limited habitat. Laboratory experiments reinforce these observations. Using mixed cultures of a psychrophile and a psychrotolerant in a chemostat, in which nutrient supply can be regulated independently of temperature, it was shown that the psychrophile became dominant at –2 °C, whereas the psychrotolerant did so at 16 °C; at intermediate temperatures, nutrient supply was the deciding factor as to which organism predominated.

2.3. Biotypes

A great variety of cold environments exists, including fresh and marine waters, sea-ice, polar and high alpine soils, snow, glacier ice, cold deserts, permafrost sediments, cold caves in sub-arctic or mountain areas, together with their associated plants, cold-blooded animals, and other organisms. These vary widely in their physico-chemical characteristics, including pH, water activity, light availability, salinity, and redox potential. Therefore, it is not surprising that a correspondingly broad diversity of cold-adapted microorganisms have evolved to colonize cold habitats, in which they contribute essentially to all the major nutrient cycles for carbon, nitrogen, phosphorus, and sulfur, as well as primary biomass production, nitrogen fixation, and photosynthesis. Cold-adapted microorganisms are also responsible for processes such as methanogenesis, acetogenesis, sulfate reduction and the biodegradation of natural or xenobiotic organic compounds (for instance, proteins, carbohydrates, lignin, hydrocarbons, and aromatics). Anaerobic respiration and *in situ* (de)nitrification are less frequently described. Microbial growth and metabolic activities have been recorded beneath 3–6 m of ice in permanently frozen lakes, in sub-glacial ice and sediments, in surface snow at the South Pole where highest summer temperatures remain well below zero (at least –10 °C), and at temperatures as low as –20 °C in permafrost samples. Some cold-tolerant bacteria, including *Xanthomonas campestris, Pseudomonas syringae,* and *Erwinia herbicola*, are often responsible for frost injuries in plants as they are able to induce ice nucleation on the leaves and fruits of crop plants under field conditions. Psychrotolerant bacteria, yeast, and fungi are also found in frozen or chilled foodstuffs where they are may cause spoilage; some bacteria (for example, *Clostridium botulinum*, *Bacillus cereus*, *Listeria monocytogenes*, and *Yersinia enterocolitica*) and fungi (for example, *Penicillium* and *Fusarium* spp.) produce toxins and so are pathogenic.

All of the major groups of microorganisms, namely bacteria, yeasts, fungi, and microalgae, have representative genera and species that are psychrophilic or psychrotolerant. Among bacteria, they are mainly Gram-negative, aerobic, non-spore-forming rods: many belong to the genera *Pseudomonas*, *Pseudoalteromonas*, *Alteromonas*, *Flavobacterium*, *Alcaligenes*, *Moraxella*, *Aeromonas*, *Psychrobacter*, *Polaribacter*, *Psychroflexus*, *Gelidibacter*, *Polaromonas*, *Shewanella*, *Colwellia*, *Xanthomonas*, *Vibrio*, *Serratia*, *Escherichia*, and *Proteus*. Some strains belong to the yellow-pigmented *Flexibacter-Bacteroides-Cytophaga* phylum. *Arthrobacter*, *Bacillus*, *Planococcus*, and *Micrococcus* dominate among Gram-positive cold-adapted bacteria. Bacterial isolates from particularly exposed cold habitats such as glaciers, as well as in sea-ice, are often pigmented, which would give them extra protection against UV radiation and free radicals. However, there does not seem to be a general correlation between degree of exposure to UV and the extent of pigmentation: some of the most exposed and irradiated habitats (for example, the Antarctic dry valleys) do not contain specially pigmented microbial populations, and some of the most psychrophilic bacteria are non-pigmented. Cold-adapted cyanobacteria, which form extensive mats on the bottom of lakes and melt ponds on ice, are commonly species of psychrotolerant *Phormidium* and *Nostoc*. The most common genus of cold-adapted yeast is *Candida* (including a number of species that have been reclassified as members of the psychrophilic genus *Leucosporidium*), followed by *Torulopsis*, *Cryptococcus*, *Rhodotorula*, and *Saccharomyces*. They are found in polar ice, snow and soils, as well as in chilled foods. The preponderant genera among cold-adapted higher fungi are *Penicillium* and *Cladosporium*, together with *Mortierella*, *Mucor*, *Chrysosporium*, and *Rhizopus*. Like bacteria, the fungi in Antarctic soils are more frequently psychrotolerant than psychrophilic. Snow algae are among the best cold-adapted microorganisms in respect of having very low optimum growth temperatures that are often below 10 °C and they also sometimes have a narrow growth temperature range. They are found mainly in the upper 1 cm layer of snow, giving it a distinctive red, green, yellow, or gray coloration.For more details about the biodiversity of low temperature environments, *Ice Ecosystems and Biodiversity* should be consulted. In order to survive and grow successfully in cold habitats, cold-adapted microorganisms have evolved a complex range of adaptations of all their cellular constituents, which will be outlined in the next section.

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Biographical Sketch

Nick Russell was a Prize Scholar and gained a First Class Honors degree in Biochemistry at the University of Cambridge in 1969, followed by a Ph.D. in 1973, at the same University, for his studies of the cold adaptation of amino acid transport in a psychrotolerant bacterium. After post-doctoral studies in the University of Cambridge, he was appointed successively as Lecturer, Senior Lecturer, and Reader in the Department of Biochemistry at the University of Cardiff, where his research was focused on the mechanisms of microbial adaptation to extreme environments, particularly low temperature or high salinity. Low-temperature research has included topics such as adaptive changes in cell membrane lipids, the cold-activity of enzymes, the biochemistry of cold-adapted food-poisoning bacteria, and climate-change effects on cold-adapted microbial ecosystems. Some of this research has been conducted in the field during several international expeditions to Antarctica working in a diverse range of cold habitats. In 1995, he was appointed as Professor of Food and Environmental Microbiology, and then Head of Biological Sciences at Wye College, University of London. Following the merger of Wye with Imperial College London, he was appointed Director of Research. Professor Russell has coordinated several EU research programs, including EUROCOLD and COLDZYME, which integrated European research on cold-adapted microorganisms with a particular focus on how their enzymes function and can be exploited in novel biotechnological applications. He is the author of more than 200 original publications, has served on the Editorial Boards of *Microbiology (UK)* and *Extremophiles* journals, and on the Council of the UK Society for Microbiology, and is a member of the Sub-Committee for Evolutionary Biology of the Scientific Committee for Antarctic Research.