

frozen soil is not entirely analogous to that in the dry soil.

Interestingly, from a thermodynamic point of view, the potential of water is changed in an analogous manner by the presence of dissolved salts. The freezing point falls below 0°C, increasingly so as the concentration of salts increases. The recent discovery of bacteria living in salt deposits and also apparently of enormous age (Vreeland et al. 2000) raises scientific questions similar to those discussed in this paper. How do the halophytic bacteria resist the osmotic forces (similar in magnitude to the forces induced by freezing) which would be expected to extract almost all the water from the cells?

When soils are at -2 or -3°C, the remaining water is in layers so thin that a bacterium could not be fitted in. The microorganism may be partly frozen in the ice, or perhaps somehow makes room for itself. Added to this problem is that the small quantities of water themselves become an increasingly strong osmotic solution. The concentration of dissolved salts in most soils is small but, since solutes are expelled from ice on freezing, the small fraction of liquid water remaining can assume a strong concentration (Williams, et al. 1998). Thus some permafrost bacteria may have even more in common with the salt deposit bacteria. Most bacterial species, of course, would be killed by the high salt concentrations in the water layers in the freezing soils.

The ability of microorganisms to live under freezing conditions depends on the presence of liquid water but it must also be available water. If the cell becomes dehydrated, there is a lack of water for chemical reactions, the effect of the lack of water on protein structure, and shrinking of the plasma membrane occurs with collapse of the transport system.

Yeasts and moulds are capable of growing at temperatures much lower than those required for bacterial growth. Adaptation to growth, or at least survival, at low temperatures has been extensively studied in the Antarctic sea-ice, where a range of flagellates have been observed, which grow at around -2°C. Water inside cells in *Mytilus edulis* and *Littorina rudis* does not freeze at -20°C (Kanwisher, 1955). There is an active bacterial life in frozen Antarctic saline lakes at temperature as low as -48°C according to Meyer et al. (1962).

So-called organic cryoprotectors are complex mechanisms protecting cells from ice crystallization. Some organisms are able to live for years in a supercooled state (DeVries, 1982). Supercooling is a thermodynamically unstable (and

temporary) state, in which the temperature has fallen below the freezing point without freezing occurring; it is quite distinct from the unfrozen water with the reduced potential described above. When freezing of supercooled water does occur, rather large and disruptive ice crystals form suddenly. It seems unlikely that the water present in bacteria could remain in the supercooled state over such long periods of time as in the permafrost and any such abrupt freezing would probably be fatal.

Denaturation of protein can occur under freezing conditions. Psychrophilic bacteria are adapted to their cold environment by having unsaturated fatty acids in their plasma membranes. Some psychrophiles have been found to contain polyunsaturated fatty acids, which generally do not occur in prokaryotes (the group of organisms, including bacteria, which do not have a membrane around the cell nucleus). Some microorganisms when frozen, may go into an anabiotic state becoming inactive. Human sperm are routinely frozen yet retain their viability, although it is not known for how long they can do so.

Thus today, there is much and varied evidence in favour of the survival of microorganisms in ice and frozen ground. But are the individuals that have been found themselves of enormous age, or are they descendants of innumerable generations?

Relationship with unfrozen water: can the microorganisms divide?

The adaptive responses to low temperature are poorly characterized for microorganisms. Metabolic activity and especially the ability of microorganisms to divide is greatly limited in the conditions of the environment within the permafrost. For example, the bacteria *Pseudomonas* grow about ten thousand times slower at -2°C than at 26°C, but they do still grow (Harder, Veldkamp, 1971). It was shown that many microorganisms cease growing near 0°C (Foter and Rahn, 1936).

There are many conditions that can affect cell division (Green et al., 1989). These conditions include the availability of water, nutrients, salt concentration, pH, and temperature, and they are associated with enzyme function, protein stability, and plasma membrane integrity.

In spite of evidence of their activity, it remains unclear whether the microorganisms can divide in the frozen soil. The single bacterial cell is trapped and not even free to move or expand within the unfrozen water layer. Where could its offspring possibly go? At any rate they could not be produced at even a minute fraction of the rate associated with bacteria at above-freezing temperatures.