Life in the cooler- starvation in the midst of plenty; and implications for microbial polar life

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ABSTRACT

The physiological reasons for imposition of a lower temperature limit for growth in microorganisms has been a matter of controversy. Recent work has demonstrated that there is a consistent decrease in the affinity of microorganisms for substrates as temperature decreases, thereby reducing their ability to sequester substrates from their environment at low temperature. This applies equally to psychrotrophs, mesotrophs or thermotrophs, over the range of temperature relevant to each physiological type. Therefore, at low environmental temperature microorganisms become increasingly nutrient-limited because of their reduced affinity for substrates. Addition of substrate may reverse the substrate limitation, or increase of temperature may remove the limitation by increasing affinity for substrate. The ecological implications of this are discussed with respect to uptake of both organic substrates by bacteria, and uptake of inorganic substrates by algae in polar regions.

Cardinal temperatures for growth

The upper temperature limit for growth is imposed by denaturation of key proteins, leading to cessation of growth. The reasons for the lower temperature limit for growth are less clear, although there seems to be consensus that at the low temperature limit there is loss of membrane function [e.g. 13]. Membrane structure and composition changes in respect to temperature, both within a species and between species adapted to different temperature régimes [see 25, 26 for reviews]. In microorganisms adapted to low temperature environments (psychrophiles and psychrotolerants) there tends to be an increased proportion of unsaturated membrane lipids, and a decreased proportion of branched chain lipids compared to species adapted to moderate (mesophiles) or high (thermophiles) ranges of temperature. Similar trends of change of membrane lipids can be observed within a single species when it is grown across its range of temperature [1, 11 28]. Furthermore, cold shock proteins may be produced when an organism is challenged by low temperature, some of which at least are enzymes such as *desaturase* enzymes associated with modification of the cell membrane in response to temperature [25,26].

Adaptation of membranes to temperature

Membranes are essentially colloidal solutions of phospholipids and proteins in a fluid phase, and it is only in this fluid phase that they are biologically functional. As temperature decreases membranes become increasingly viscous, with decreasing membrane fluidity [22,27], and at some temperature will undergo a phase change to a gel (semicrystalline)

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phase when biological function is lost [33]. The changes in membrane lipid composition in response to lowered environmental temperature, which are described above, seem to result in maintenance of the cell membrane in a biologically functional fluid phase to as low a temperature as possible (homeoviscous adaptation [27]. Unsaturated lipids tend to have lower melting points than the equivalent saturated lipids; while crosslinked lipids tend to have even higher melting points. Other things being equal, increased proportions of unsaturated lipids maintain membranes in a fluid condition to a lower temperature. High proportions of saturated and branched lipids in thermophile membranes increase the phase change temperature of the membrane, conferring stability at high temperature but making the membrane 'stiff' and biologically non-functional at a comparatively high minimum temperature for growth. Proteins embedded in the membrane, including key respiratory and transport proteins, function only when the membrane is in the fluid phase, and cease activity on the phase change to semicrystalline form.

Nutrient limitation in natural environments, and affinity for substrates

In virtually all natural environments vital resources, such as energy substrates, are present at very small, usually growth-rate-limiting, concentrations [e.g. 14]. Growth and survival depends, therefore, upon the ability of a species to sequester these sparse resources in extreme competition with other species competing for the same resources. The efficiency of active uptake by a microorganism from its external environment of any substrate at low concentration depends upon the affinity of the organism for that substrate. Affinity has been described most frequently by Michaelis-Menten saturation kinetics [e.g. see 9] described by two 'constants', μ_{max}- the maximum specific growth rate, and K_s the half saturation constant. Traditionally, the affinity of an organism for a substrate has been described by the value of K_s (or K_m for a particular substrate), the half saturation constant, but investigations to try to detect changes in the affinity of microorganisms for substrates by measuring solely changes in K_s (or K_m) values with temperature have generally failed to detect any consistent trends [e.g. 7,12]. However, half-saturation constants alone are poor indicators of affinity for a substrate, particularly at low substrate concentrations. Button and co-workers [see 2,3,9] have argued that the specific affinity (a_A, equivalent to μ_{max}/K_s) is a much more robust measure than K_s of the affinity of an organism for a substrate, and is independent of the actual mechanism of uptake. When we examine the few data sets which give values of μ_{max} and Ks at different temperatures, consistent trends start to be seen where they were not seen before [15]. Figure 1 shows trends of a_A with temperature for uptake of a variety of substrates by psychrophilic and mesophilic bacteria. The range of temperatures over which each bacterium grows, and the actual values of a_A, differ for each organism, reflecting their different physiological status with respect to temperature. However, in all cases there was a consistent trend of decrease in a_A as temperature decreased below the optimum temperature for growth, indicating a consistent decrease in the affinity of all the various bacteria for a variety of substrates as temperature declined. The trend applies not just to uptake of organic substrates, but also to affinity for nitrate by nitrate-respiring bacteria [16].

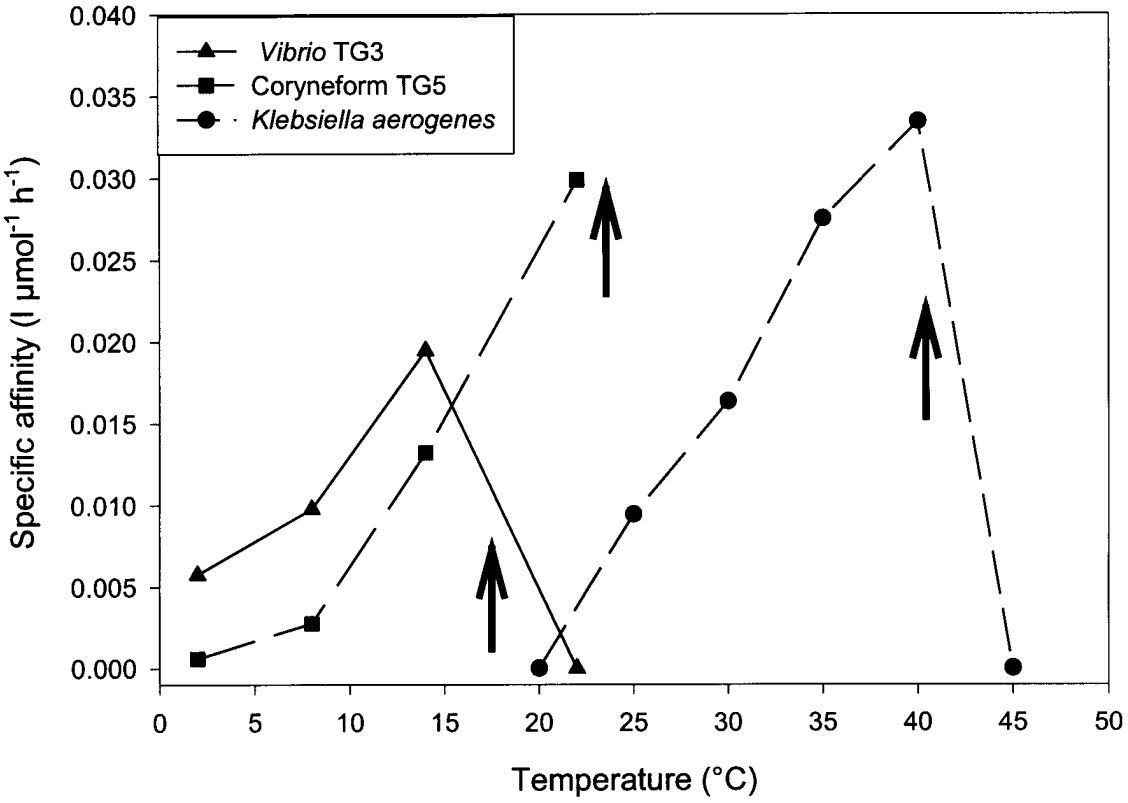

Figure 1. Specific affinity for glucose versus growth temperature for psychrophilic *Vibrio* TG3, a psychrotolerant coryneform (TG5), both from a freshwater Antarctic lake; and a mesophilic *Klebsiella aerogenes*. Data for *K.aerogenes* from [29]; for the other two from [7], with additional data courtesy of the authors). Arrows indicate the optimal temperature for growth of each organism.

Effect of membrane fluidity on affinity for substrates

The trend of decreased affinity with temperature below the optimum might be expected if decreasing temperature reduced the fluidity of the cell membrane, making it 'stiffer' and reducing the efficiency of transport proteins embedded in the membrane [18,30]. The trend of change of a_A with temperature provides the first consistent evidence that the lower limit of temperature for growth is, indeed, determined by the lowest temperature at which a species can maintain membrane fluidity and active transport across the membrane. Furthermore, there seems to be progressively decreased specific affinity for substrate with lowering temperature below the optimum, rather than constant affinity across the growth temperature range with a sudden decrease near the minimum temperature for growth.

Evidence of effect of low temperature on affinity for substrates

What evidence is there from the natural environment that uptake affinity for substrates is indeed inhibited by decreased temperature ? In a key paper, Pomeroy et al [19] showed that in Arctic seawater off Newfoundland the respiratory rate of the bacterial community was inhibited at low temperature (-1°C) but was stimulated either by higher temperature or by higher concentrations of substrates (glucose, proteose peptone). Wiebe et al [31] hypothesised that there was an interaction between temperature and substrate requirement

such that higher substrate concentrations were necessary at temperatures near the lower temperature limit of a species. Even mesophilic marine bacteria, isolated from the southeastern subtropical shelf waters of the USA, were inhibited by lack of substrate at temperatures near their minimum for growth, despite the minimum temperature being high, near 10°C [32]. Growth rates were increased either by a rise in temperature or by addition of additional substrates. These workers concluded that there was an enhanced requirement for substrate near their lower temperature limit for growth in both mesophilic and psychrotolerant bacteria.

Ecological implications of the interaction between temperature and affinity for substrates

Our observations of the relationship between temperature and specific affinity for organic and inorganic substrates provide a mechanism to explain the effects of substrate addition at low temperature on natural communities of marine bacteria [19,31]. Any substrate which is taken up by some form of active transport is likely to become increasingly less available as temperature decreases because the ability of bacteria to sequester the substrate declines at low temperature. The minimum concentration to which an organism can remove substrate from its environment is a function of its affinity for the substrate [20]. In general therefore, progressively larger pools of unavailable residual substrate will remain in an environment as temperature decreases, because the decreasing affinity of the organism(s) for a substrate at low temperature prevents any further uptake. As a_A is directly related to and influenced by temperature, a corollary is that at low temperature the presence of even a relatively high concentration of substrate in the environment does not imply that the organisms present are not substrate limited. We cannot compare directly the substrate concentrations which are limiting at low temperature with those which are limiting at high temperature, because of possible changes in specific affinity for the substrate.

This emphasises the synergy between temperature and substrate concentration in controlling the availability of a substrate to an organism at low temperature. In effect, for any organotrophic microorganism, which in the vast majority of natural environments are anyway existing under conditions of low substrate availability and severe energy limitation, low temperature exacerbates starvation because of increased inaccessibility of substrates even when what may be considerable concentrations of substrates remain present in the environment.

Effect of low temperature on uptake of inorganic substrates

The above arguments on how low temperature may influence uptake of organic substrates also apply to inorganic substrates taken up by active transport processes. Again, it has been argued that low temperature influences the ability of algae to take up nitrate [21], but investigation of that ability has usually been restricted to measurements of K_s values for nitrate at different temperatures. As with uptake of organic substrates, there has been no consistent pattern of variation of K_s with temperature (e.g.12). Our recent work with both bacteria and algae [23,24] has shown that when affinity for nitrate is measured by specific affinity, rather than just by K_s, there is a consistent trend of decreased $a_{A(nitrate)}$ with lowered temperature. In contrast, specific affinity for ammonia ($a_{A(amm)}$) shows very little response to temperature. It has been demonstrated also for higher plant roots that nitrate uptake is much

more affected by temperature than ammonium uptake [e.g. 4,5,10]. It appears that the different effects of temperature on uptake of both nitrate and ammonium is consistent across bacteria, algae and higher plants. This consistency is possibly not surprising as the biochemical requirements to assimilate nitrate and ammonium are identical to all organisms assimilating them, and evolutionarily these mechanisms are therefore likely to be strongly conserved. The lesser effect of temperature on ammonium uptake may be consistent with at least some passive transport of NH_3, across the membrane contributing to total ammonium uptake. Passive transport is less affected by decreased fluidity of the membrane at low temperature. This difference in the effect of temperature on uptake of ammonium or nitrate will have profound effects on which source of nitrogen is able to be used at low temperature. In the Southern Ocean during most of the summer growth season primary production is commonly supported by assimilation of ammonium rather than nitrate, despite the usually much higher concentrations of the latter [6,8,17]. The *f ratios* for nitrogen uptake are commonly <0.5, indicating predominant use of ammonium rather than nitrate. These observations are consistent with an affinity for nitrate significantly decreased by low temperature, to the extent that even relatively high concentrations of nitrate remain unavailable in the environment at low temperature. Such decreased affinity at low temperature is less significant to ammonium uptake, and ammonium can therefore be sequestered to a much lower concentration even at low temperature.

Conclusions

Reduction in fluidity of biological membranes as temperature lowers below the optimum temperature for a species has profound effects upon the uptake of substrates from that species' environment: where usually such substrates are anyway in greatly limited availability. The effect of low temperature is to reduce the affinity of that species for any substrates which are taken up by active transport processes dependent upon transporter molecules embedded in the membrane, presumably because the 'stiffening' of the membrane with lowered temperature reduces the transporter proteins' efficiency. This reduced efficiency is exhibited as a consistent loss of affinity for the substrate as temperature decreases below the species' optimum temperature for growth. In effect, the minimum concentration to which the substrate can be sequestered from the environment rises as temperature decreases, leaving an increasing concentration of unavailable substrate, so exacerbating the tendency to 'starvation' at low temperature.

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