

# Influence of Temperature on Growth Rate and Competition between Two Psychrotolerant Antarctic Bacteria: Low Temperature Diminishes Affinity for Substrate Uptake

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The growth kinetics of two psychrotolerant Antarctic bacteria, *Hydrogenophaga pseudoflava* CR3/2/10 (2/10) and *Brevibacterium* sp. strain CR3/1/15 (1/15), were examined over a range of temperatures in both batch culture and glycerol-limited chemostat cultures. The maximum specific growth rate ( $\mu_{\max}$ ) and  $K_s$  values for both bacteria were functions of temperature, although the cell yields were relatively constant with respect to temperature. The  $\mu_{\max}$  values of both strains increased up to an optimum temperature, 24°C for 2/10 and 20°C for 1/15. Strain 1/15 might therefore be considered to be more psychrophilic than strain 2/10. For both bacteria, the specific affinity ( $\mu_{\max}/K_s$ ) for glycerol uptake was lower at 2 than at 16°C, indicating a greater tendency to substrate limitation at low temperature. As the temperature increased from 2 to 16°C, the specific affinity of 1/15 for glycerol increased more rapidly than it did for 2/10. Thus 1/15, on the basis of this criterion, was less psychrophilic than was 2/10. The steady-state growth kinetics of the two strains at 2 and 16°C imply that 1/15 would be able to outgrow 2/10 only at relatively low substrate concentrations ( $<0.32$  g of glycerol · liter<sup>-1</sup>) and high temperatures ( $>12^\circ\text{C}$ ), which suggests that 1/15 has a less psychrotolerant survival strategy than does 2/10. Our data were compared with other data in the literature for bacteria growing at low temperatures. They also showed an increase of substrate-specific affinity with increasing temperature. Our results explain recent reports of decreased affinity for substrates by heterotrophic bacteria in polar seas, with consequently increased difficulty of substrate uptake at low temperature, and may also be significant in explaining the limitation of primary production in the Southern Ocean.

Psychrophiles, mesophiles, and thermophiles traditionally have been defined in relation to their cardinal temperatures for growth. For example, Morita (31, 32) differentiated between obligate psychrophiles, which are able to grow at 0°C, with an optimum temperature of  $<15^\circ\text{C}$  and a maximum temperature near 20°C, and facultative psychrophiles (psychrotrophs or, more properly, psychrotolerants), which grow at 0°C but which have an optimum temperature of  $>20^\circ\text{C}$ . Optimum temperature for growth has often been interpreted as the temperature at which the final stationary-phase culture density is maximal (35), but this is actually a measure of final cell yield rather than growth rate and is a composite function of the cell growth rate ( $\mu$ ), the affinity for the substrate ( $K_s$ ), and the cell yield ( $Y$ ) at the different temperatures. The classic work of Harder and Veldkamp (16) showed, for an obligate and a facultative psychrophile, that these growth rate parameters were not constants but were functions of temperature. The outcome of competition between the two physiological types varied at different temperatures, which reflected changes in the growth parameters with temperature. At  $-2^\circ\text{C}$  the obligate psychrophile always outgrew the facultative psychrophile, while the reverse was true at 16°C. At intermediate temperatures, the outcome of competition depended on the concentration of growth-rate-limiting substrate. Topiwala and Sinclair (45), in a careful study of the growth kinetics of *Klebsiella pneumoniae* (*Aerobacter aerogenes*) in chemostat cultures, showed that the kinetic growth parameters were strong functions of temperature.

Work on the interaction of environmental temperature with growth rates of bacteria is limited, yet it is of increasing importance as the ecological significance of low-temperature

polar regions becomes clear. The balance of evidence suggests that in most low-temperature polar environments psychrotolerant types (psychrotrophic, facultative psychrophiles) predominate over obligate psychrophiles (8, 11, 20, 47) despite some reports of high proportions of psychrophiles (22, 32, 49). Herbert and Bell (19) investigated the psychrophilic *Vibrio* sp. strain AF-1 and showed that the  $K_s$  values for different substrates changed at 15°C compared with those at 0°C. Whether there was an increase or decrease of  $K_s$  between these two temperatures varied with different substrates. The ecological implications of such interactions may be great. Recently, investigators (36, 50) have suggested that in Arctic seas at low temperature,  $<3.5^\circ\text{C}$ , bacteria require high concentrations of substrate to be active; one implication is that this is because of a decreased affinity for organic substrates at low temperatures. Similarly, a decreased substrate affinity at the low end of the temperature range has been described recently also for marine mesophilic bacteria (51). If these observations are correct generally, then the effect of substrate limitation on growth rates in energy-limited natural environments will be further exacerbated by low temperatures.

The present work was undertaken to investigate in detail the growth responses of two psychrotolerant bacteria to changes of their environmental temperature. Initially this study was carried out at steady-state temperatures in chemostats, but the intention was to extend this to investigate competition in non-steady-state environments (41). We report here the results of the steady-state temperature experiments.

## MATERIALS AND METHODS

**Bacterial isolates used.** The bacteria used in this study were two psychrotolerant bacteria isolated from lake sediment from Signy Island, South Orkney Islands, Antarctica (48). They were

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isolated from chemostat cultures on glycerol substrate, under conditions of either constant or cycling temperatures. Strain CR3/2/10 (hereafter referred to as strain 2/10) was isolated at a constant 8°C, while strain CR3/1/15 (strain 1/15) was isolated under conditions of sine-wave temperature cycling between 1 and 16°C over 24 h. Initially, both were identified as *Pseudomonas* spp., but on further examination strain 1/15 was found to be a gram-positive (although very variable), nonsporing, irregular rod which was able to grow aerobically and anaerobically. On the basis of total cell lipid analysis (43, 44), the fatty acid methyl ester profile of 1/15 resembled a *Brevibacterium* sp. (43a). Organism 2/10 was found to be a gram-negative, oxidase-negative rod that was motile but was a strict aerobe. By fatty acid methyl ester analysis, it was identified as *Hydrogenophaga pseudoflava* (previously *Pseudomonas pseudoflava* [43a]). These organisms are deposited with the National Collection of Industrial and Marine Bacteria (United Kingdom) (*Brevibacterium* sp. strain NCIMB 13216 and *H. pseudoflava* NCIMB 13215).

**Determination of effect of temperature on growth rate parameters.** The effect of environmental temperature on growth rate constants ( $K_s$ , maximum growth rate [ $\mu_{\max}$ ], and cell yield [ $Y$ ]) were investigated in both batch cultures and chemostat culture.

**Batch culture experiments.** In order to measure the  $\mu_{\max}$  and  $Y$  of each organism at different temperatures, batch culture experiments were carried out in a temperature gradient aluminium block incubator (2, 47) over the temperature range of 2 to 25°C. Optically standardized test tubes containing 10 ml of FC2 medium (3, 48) with glycerol (0.5 and 1 g · liter<sup>-1</sup>) as the substrate were placed in the vertical holes in the temperature gradient block, to give approximately 2°C temperature steps over a range of 2 to 25°C. Each tube was aerated continuously with sterile air via a long hypodermic needle penetrating into the medium. The air stream into each culture tube was humidified and equilibrated to the correct temperature by first being passed through a tube of distilled water at the same position in the aluminum block. The tubes were allowed to equilibrate to temperature for 20 min and then were inoculated.

Inocula were prepared from exponential-phase cultures of each bacterium grown in FC2 glycerol medium at 16°C. The cells were then centrifuged, washed once by resuspension in sterile one-fourth-strength Ringer's solution, centrifuged again, and resuspended in Ringer's solution to give a faintly turbid suspension. This suspension (0.2 ml per tube) was used as the inoculum.

**Determinations of  $\mu_{\max}$ .** After inoculation, growth at each temperature was monitored by periodic measurements in a nephelometer of the turbidity of the culture in each tube. The  $\mu_{\max}$  at each temperature was established by linear regression analysis to determine the slope of the linear part of the semilogarithmic plot of optical density (OD) against time for each incubation temperature.

**Cell yield.** When the culture entered stationary phase, the cell yield was determined by withdrawing a sample of the culture. The sample was centrifuged, and the residual glycerol concentration in a subsample of the clear supernatant was measured by an enzymatic assay (Boehringer GmbH, Mannheim, Germany). From the residual glycerol, the assimilated carbon could be calculated by the difference from the inflow concentration. The carbon content of the cells was determined from a calibration curve which related nephelometer readings of each culture to cellular carbon content. The calibration curves were established for each bacterium by injecting known amounts of bacterial cells, suspended in distilled deionized

water, into a carbon analyzer (model 915C; Beckman Instruments, Ltd.). Potassium phthalate standards were used to calibrate the carbon analyzer. Cell yields were expressed, therefore, in carbon values: moles of carbon assimilated per mole of substrate (glycerol) carbon utilized. This is a more direct measure of assimilation efficiency than cell dry weight.

**Substrate uptake efficiency.** The residual concentration of the growth-rate-limiting nutrient (glycerol) at stationary phase in batch cultures reflects the affinity of the uptake system for the substrate (35), which is inversely related to the  $K_s$ . Although it is not possible to determine directly the actual  $K_s$  from measurements of residual substrate in stationary-phase batch cultures, changes of residual substrate concentration at different temperatures do give an at least qualitative idea of the change of affinity for the substrate with temperature.

**Chemostat experiments.** Chemostat cultures were set up in order to measure, independently of batch cultures, values for  $\mu_{\max}$  and  $Y$  and to provide values for  $K_s$  at different temperatures. Two growth temperatures, 2 and 16°C, were used for chemostat cultures of each organism growing on FC2 medium with three different concentrations of glycerol, 0.25, 0.5, and 1.0 g · liter<sup>-1</sup>. Dilution rates ( $D$ ) were set at 0.02 h<sup>-1</sup> for *H. pseudoflava* and 0.01 h<sup>-1</sup> for the *Brevibacterium* sp. After inoculation of the chemostat vessel, the progress of each chemostat to steady state was monitored by withdrawing a sample (1 ml) of the culture aseptically from the chemostat vessel and measuring the OD at 550 nm (OD<sub>550</sub>) in a spectrophotometer. The chemostat was deemed to be at steady state when the variation in standard error of at least six OD readings measured over at least one turnover time was <2% of the mean OD value.

**$K_s$  values.** When steady state was attained, determinations of  $K_s$  were carried out on three consecutive days by aseptically withdrawing with a hypodermic syringe a small volume of culture directly from the chemostat vessel via a long hypodermic needle through a septum. The cells were immediately filtered off from the medium as they were withdrawn through a membrane filter (0.45- $\mu$ m pore size) in the hypodermic syringe. The  $K_s$  was determined (35) from a measurement of the residual concentration of glycerol at each steady state from the formula  $K_s = s (\mu_{\max} - D)/D$ , where  $s$  is steady-state glycerol.

**$\mu_{\max}$  values.** The dilution rate of the chemostat was then increased to higher than the critical dilution rate,  $D_{\text{crit}}$ , to induce washout, and the  $\mu_{\max}$  was determined from the slope of a plot of  $\ln \text{OD}_{550}$  against time. This permitted checks against the  $\mu_{\max}$  values measured in the batch culture experiments.

**Yield.** Cell yield was determined for each culture from the analyses of residual glycerol concentration and measurements of the carbon content of the cells at steady state.

## RESULTS

**Batch cultures. (i)  $\mu_{\max}$ .** The  $\mu_{\max}$  values measured for each organism in the temperature gradient block are shown in Fig. 1 as Arrhenius plots, which were linear between 2 and 20°C. The optimum temperature for strain 2/10 was about 24°C ( $\mu_{\max}$ , 0.27 h<sup>-1</sup>), and for strain 1/15 it was 20°C ( $\mu_{\max}$ , 0.11 h<sup>-1</sup>). The temperature characteristics, describing for each organism the relationship between  $\mu_{\max}$  and temperature, were determined from fitting a linear regression to this part of the Arrhenius plot. The temperature characteristics for 2/10 and 1/15 were 50.1 (standard error, 6.45) and 56.9 (standard error, 7.79) kJ · K<sup>-1</sup> · mol<sup>-1</sup>, respectively, indicating that 2/10

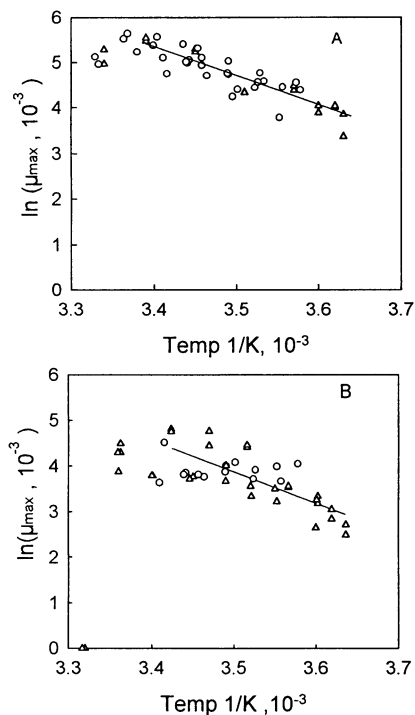


FIG. 1. Arrhenius plot showing the relationship between  $\mu_{\max}$  and temperature for *H. pseudoflava* 2/10 (A) and *Brevibacterium* sp. strain 1/15 (B) grown in batch culture in temperature gradient block incubators at 0.5 g of glycerol  $\cdot$  liter $^{-1}$  ( $\Delta$ ) and 1 g of glycerol  $\cdot$  liter $^{-1}$  ( $\circ$ ). The straight line shows the linear regression for the datum points over the range of temperatures indicated by the line ( $P < 0.05$ ).

showed a smaller response of  $\mu_{\max}$  to increased temperature than did strain 1/15.

(ii)  $K_s$ . The residual concentrations of glycerol at stationary phase in the batch cultures at each temperature are shown in Fig. 2 and 3. It can be seen that for organism 2/10 (Fig. 2) the residual glycerol concentration remained low ( $< 0.02$  g  $\cdot$  liter $^{-1}$ ) over the range of temperature from 2 to 20°C but increased at temperatures of  $> 20^\circ\text{C}$ , indicating that the affinity of the uptake system for glycerol had declined. When the residual concentration of glycerol was plotted as an Arrhenius plot it was approximately linear, indicating an exponential response of the uptake system to temperature change, with the affinity of the uptake system for glycerol increasing as temperature decreased.

In contrast, organism 1/15 exhibited a completely different response to temperature change (Fig. 3). The residual glycerol concentration remained low over the temperature range of 7 to 25°C. When the temperature dropped to  $< 7^\circ\text{C}$ , the residual glycerol concentration greatly increased, showing that there was a dramatic decrease in affinity for glycerol by this organism at low temperatures. Again, when plotted as an Arrhenius plot there was a linear relationship, but with a slope opposite to that for strain 2/10, showing that there was a decrease in the affinity of the glycerol uptake system for the substrate as the temperature decreased.

(iii) Cell yield. The response of the cell yield coefficient to temperature at two different starting concentrations of substrate is shown in Fig. 4. For 2/10 the cell yield seemed to be independent of temperature over the range tested. It averaged 0.43 (standard deviation,  $\pm 0.01$ ) with 0.5 g of glycerol  $\cdot$  liter $^{-1}$

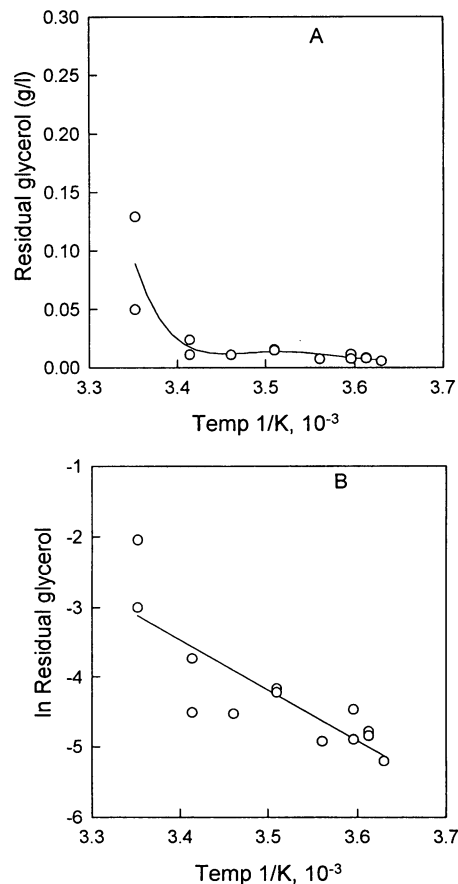


FIG. 2. (A) Residual glycerol concentrations for organism 2/10 at stationary phase in batch cultures grown in a temperature gradient block incubator. (B) Arrhenius plot of the data in panel A. The straight line shows the linear regression ( $P < 0.05$ ).

and was slightly lower, at  $0.32 \pm 0.01$  with 1 g  $\cdot$  liter $^{-1}$ . In contrast, organism 1/15 showed some effect of temperature on cell yield. With 0.5 g of glycerol  $\cdot$  liter $^{-1}$ , there was an optimum Y value of 0.6 at around 10°C, declining to 0.3 or less at higher and lower temperatures. At the higher glycerol concentration (1 g  $\cdot$  liter $^{-1}$ ), the yields were lower (0.2 to 0.3) and declined with increased temperature. By comparison, Ng (33) reported for *Escherichia coli* that Y, in terms of carbon assimilated, was 0.37 at 10°C and 0.53 at 30°C. Reduction in cell yields at higher substrate concentrations also has been reported before (1, 30).

**Chemostat cultures.** Table 1 shows the results of the series of chemostat experiments with the two bacteria at two temperatures and three substrate concentrations. The reproducibility of the chemostat results was checked with a repeat experiment with organism 1/15 on 0.5 g of glycerol  $\cdot$  liter $^{-1}$  at 2°C, and the values obtained in the two runs were virtually identical (Table 1).

The OD values of the cultures at steady state indicated that the chemostats were not glycerol limited at 1 g  $\cdot$  liter $^{-1}$ , as in general the steady-state OD did not increase proportionately with the doubling in substrate concentration from 0.5 to 1 g  $\cdot$  liter $^{-1}$ . In contrast, the chemostats seemed to be glycerol limited ( $\leq 0.5$  g of glycerol liter $^{-1}$ ) as the steady-state OD increased proportionately with the doubling of glycerol from 0.25 to 0.5 g  $\cdot$  liter $^{-1}$ . It should be noted that organism 1/15 was

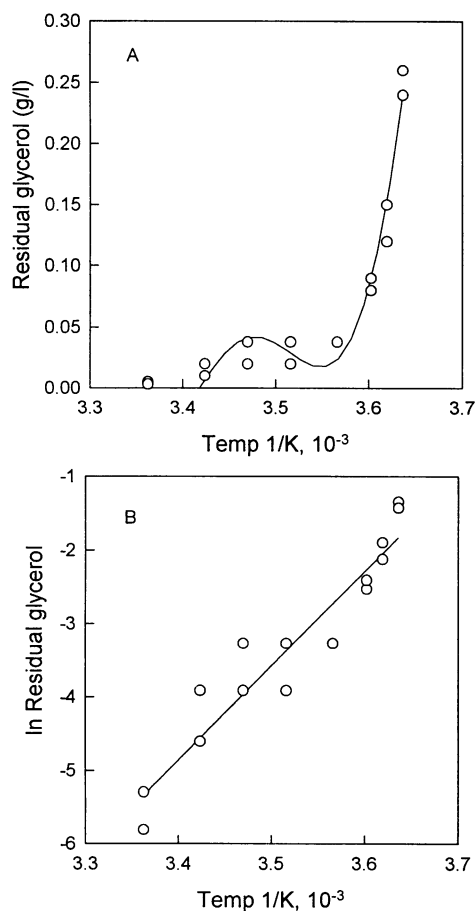


FIG. 3. Residual glycerol concentrations for organism 1/15. For explanation, see the legend to Fig. 2.

completely unable to grow at 2°C at 0.25 g of glycerol · liter<sup>-1</sup>, although it was able to grow at this temperature on higher concentrations of substrate.

The values of  $\mu_{\max}$  measured in the chemostat at 2 and 16°C corresponded well with those measured in batch cultures at the same temperatures. For example, with 0.5 g of glycerol · liter<sup>-1</sup>, for organism 2/10 at 16°C the  $\mu_{\max}$  in batch culture was 0.14 h<sup>-1</sup> and that in the chemostat was 0.139 h<sup>-1</sup>, while at 2°C the  $\mu_{\max}$  was 0.048 h<sup>-1</sup> in batch culture and 0.055 h<sup>-1</sup> in the chemostat; for 1/15 at 16°C the  $\mu_{\max}$  was 0.062 h<sup>-1</sup> in batch culture and 0.095 h<sup>-1</sup> in the chemostat, while at 2°C it was 0.019 h<sup>-1</sup> in batch culture and 0.024 h<sup>-1</sup> in the chemostat.

The  $K_s$  values measured reflected those inferred from residual substrate concentrations in the temperature gradient block incubator. For 2/10, the  $K_s$  for glycerol at 16°C was 0.26 g · liter<sup>-1</sup>, and this decreased at 2°C to 0.14 g · liter<sup>-1</sup> (with 0.25 g of glycerol · liter<sup>-1</sup>); that is, the affinity for glycerol apparently increased at the lower temperature. In contrast, for 1/15 the  $K_s$  at 16°C was 0.1 g · liter<sup>-1</sup> but at 2°C increased dramatically to 0.4 g · liter<sup>-1</sup> at 0.5 g of glycerol · liter<sup>-1</sup>, and the organism was completely unable to grow at the lower glycerol concentration of 0.25 g · liter<sup>-1</sup>. Thus the affinity of 1/15 for the substrate decreased markedly at low temperature to the extent that the organism was unable to grow at the lowest glycerol concentration that we used.

The actual values of  $K_s$  measured were high compared with

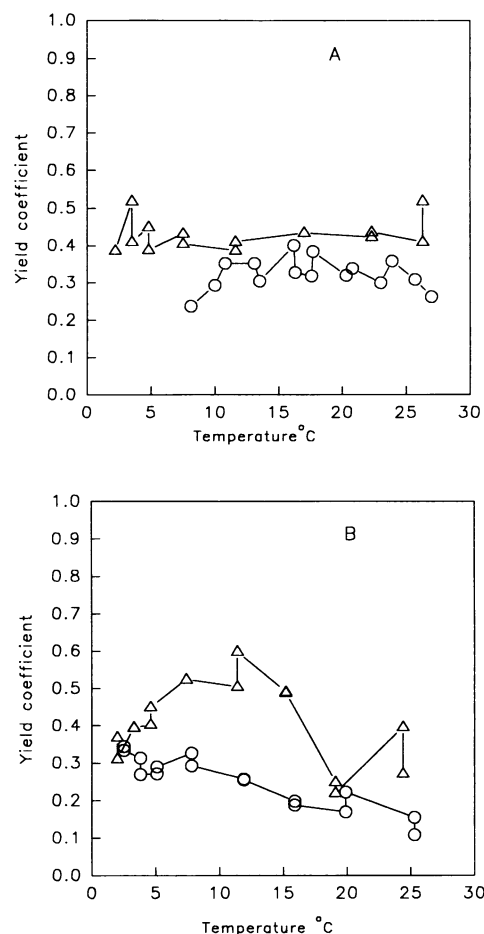


FIG. 4. Cell yields (ratio of grams of carbon assimilated to grams of carbon utilized) for 2/10 (A) and 1/15 (B) in batch culture experiments in a temperature gradient block incubator. Data for two initial concentrations of glycerol in the growth medium are shown (symbols are as in Fig. 1).

previously published values for carbohydrates (for an example, see reference 19). However, Law and Button (26) showed that the presence of other substrates could influence the limiting concentration of a substrate in a chemostat. For example, the addition of arginine reduced the limiting steady-state glucose concentration 600-fold, while addition of 20 amino acids led to an even greater reduction. Thus, the apparently high  $K_s$  for glycerol in the present study may be the result of the relatively poor nutrient status of the growth medium used.

Yield coefficients for strains 2/10 and 1/15 at 2°C in the chemostat were similar to those measured in batch cultures at the same temperature. However, at 16°C chemostat yields were lower than those in gradient block batch cultures at the same temperature: for 2/10,  $Y = 0.29$  at 0.5 g · liter<sup>-1</sup>, compared with  $Y = 0.41$  in batch culture, and for 1/15,  $Y = 0.37$  compared with  $Y = 0.5$  in batch culture. These differences may be due to the effect of growth rate, which affects cell yields (25). In batch cultures, such as in the temperature gradient block experiments, growth rate would be at maximum for the exponential part of the growth cycle ( $\mu_{\max} = 0.14$  h<sup>-1</sup> for 2/10 and 0.06 h<sup>-1</sup> for 1/15 at 16°C), whereas in the chemostats the growth rates were set at 0.02 h<sup>-1</sup> and 0.01 h<sup>-1</sup> for 2/10 and 1/15, respectively. The effects of differing growth rates would

TABLE 1. Kinetic growth rate parameters of the two psychrotolerant bacteria measured in chemostat experiments with glycerol as the substrate

Organism	Temp (°C)	Glycerol concn (g · liter <sup>-1</sup> )	Steady-state mean OD <sub>550</sub>	$\mu_{\max}$ (h <sup>-1</sup> )	Residual glycerol (g · liter <sup>-1</sup> )	Yield coefficient	$K_s$ (g · liter <sup>-1</sup> )	Specific affinity (liters · g <sup>-1</sup> · h <sup>-1</sup> )
<i>H. pseudoflava</i> 2/10	2	0.25	0.348	0.069	0.055	0.43	0.135	0.511
		0.5	0.559	0.055	0.110	0.39	0.193	0.285
		1.0	0.592		0.391	0.27		
	16	0.25	0.272	0.130	0.047	0.29	0.259	0.502
		0.5	0.442	0.139	0.045	0.29	0.268	0.519
		1.0	0.508		0.36	0.21		
<i>Brevibacterium</i> sp. strain 1/15	2	0.25	NG <sup>a</sup>					
		0.5	0.257	0.024	0.31	0.27	0.434	0.055
		0.5	0.234	0.024	0.32	0.27	0.448	0.054
	16	1.0	0.160		0.78	0.13		
		0.25	0.223	0.098	0.015	0.19	0.090	1.089
		0.5	0.671	0.095	0.015	0.37	0.104	0.913
		1.0	0.539		0.39	0.22		

<sup>a</sup> NG, no growth.

therefore be expected to be large at 16°C. In contrast, the growth rates in the chemostats at 2°C were closer to  $\mu_{\max}$  values, and the smaller difference between yields measured in the chemostats and the batch cultures at this lower temperature might be expected.

By comparing yield coefficients in batch cultures ( $\mu = \mu_{\max}$ ) and chemostats ( $\mu < \mu_{\max}$ ), it appears that for both organisms the yields were greater at higher growth rates. For 2/10, the yield increased at low temperature (Table 1) and with lower substrate concentrations. In contrast, with 1/15 at 0.5 g · liter<sup>-1</sup>, there was a decrease in the cell yield at the low temperature. These differences in changes of yield with temperature in chemostats must be purely related to temperature effects, as growth rates were constant. In the batch experiments, changes in cell yields could have resulted from different growth rates as well as different temperatures.

## DISCUSSION

By using the  $\mu_{\max}$  and  $K_s$  values obtained for the two bacteria in chemostats, it was possible to construct  $\mu$ -substrate concentration ( $S$ ) curves for each temperature (Fig. 5). At 2°C, it can be seen that for any concentration of glycerol, organism 2/10 would have the higher specific growth rate and hence would survive in the chemostat, while 1/15 would be washed out. Initially, this was surprising as 1/15 was regarded as more psychrophilic, with a lower optimum temperature than 2/10 (47). At 16°C, 1/15 would outgrow 2/10 up to an  $S$  of 0.32 g of glycerol · liter<sup>-1</sup>, at which  $\mu$  was 0.074 h<sup>-1</sup>. At higher values of  $S$  the growth rate for 1/15 would be exceeded by the growth rate of 2/10, and 1/15 would be washed out. Thus, the apparently more psychrophilic bacterium, 1/15, was successful only at the higher temperature.

The values of  $\mu_{\max}$  and  $K_s$  for each bacterium at temperatures between 2 and 16°C were calculated by using the linear Arrhenius relationships with temperature for each of the variables described above. These values were used to calculate the  $\mu$ - $S$  curves for each bacterium at temperatures intermediate to 2 and 16°C, and hence the crossover points of growth rates at each temperature. These are illustrated in a phase-plane diagram (Fig. 6) which shows the domain of temperature and glycerol concentration within which either 1/15 or 2/10

dominates by having the greater value for  $\mu$ . It can be seen that 1/15 outgrows 2/10 only when there is a combination of high temperature (>12°C) and low glycerol concentration.

Organisms usually have been classified in relation to temperature on the basis of their cardinal growth temperatures (31, 32). Neither of the organisms used in this study could be regarded as an obligate psychrophile as defined by Morita (32), but rather were psychrotrophic, or more properly, psychrotolerant. The balance of evidence suggests that psychrotolerant, not psychrophilic, microorganisms predominate in most low-temperature environments (8, 40, 47). Organism 1/15 has been reported previously (48) to be more psychrophilic than 2/10, as its optimum temperature for production of the highest final culture density was at about 10°C, compared with 21°C for 2/10. This might be associated with the fact that 1/15 appears to have a maximum cell yield at about 10°C when grown in batch culture at 0.5 g of glycerol · liter<sup>-1</sup>. In the present work, 1/15 had a marginally lower optimum temperature for  $\mu_{\max}$  (20°C) than organism 2/10 (24°C), and again it might be considered to be the more psychrophilic of the two organisms. However, the results of our study have shown that the conventional classification of the organism's physiology in relation to temperature is not necessarily an indicator of its competitive, and thus survival, ability at a particular temperature. In fact, because of the greater affinity of 2/10 for glycerol at low temperature, it was always able to outcompete 1/15 at temperatures of <12°C. Organism 1/15 was successful only at low glycerol concentrations combined with relatively high temperatures (>12°C). This also emphasizes that the determination of a temperature optimum for growth based on measuring final culture densities at different temperatures may be misleading. Such a measure represents an aggregate response of  $\mu$ ,  $K_s$ , and  $Y$  to temperature change, and the effect of temperature change upon each variable individually may be different.

These results can be explained in terms of the observed changes in growth rate parameters with temperature. In particular, the  $K_s$  values seemed to be crucial in determining the outcome of competition between these two psychrotolerant organisms. Organism 2/10 at 2°C, which was more characteristic of temperature in its native environment, had a much lower  $K_s$ , which allowed it to outcompete 1/15 for the growth-rate-limiting substrate, glycerol. Most natural environments are energy or substrate limited, and the  $K_s$  value is, in reality,

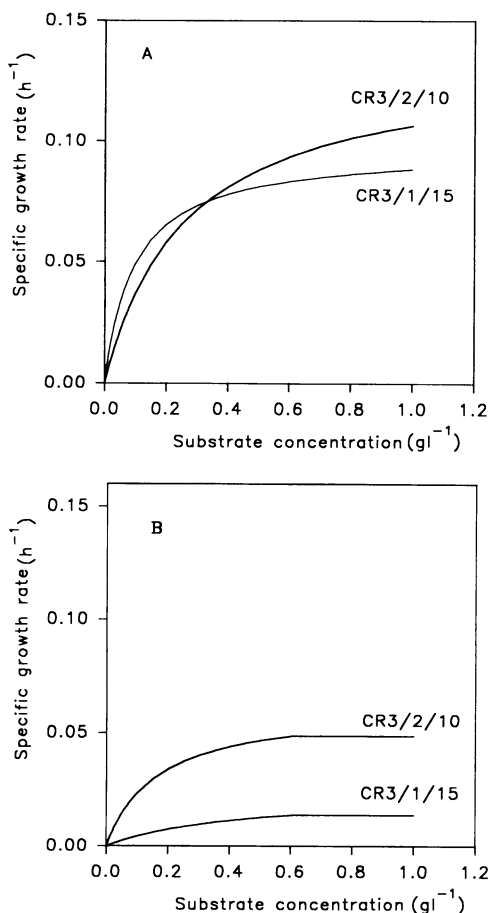


FIG. 5. Theoretical  $\mu$ - $S$  curves drawn for 2/10 and 1/15 by using the values of  $\mu_{\max}$  and  $K_s$  determined in the chemostat experiments at 16°C (A) and 2°C (B).

the factor which most influences the outcome of competition in situ. In nature, most microorganisms never achieve their  $\mu_{\max}$ , or anything approaching it, and its ecological value remains of theoretical interest only. (This is, of course, why many ecological reaction rates can be modelled by first-order kinetics.)

It has previously been proposed (4, 5, 14, 15, 18, 26) that the ability of an organism to sequester substrate is best defined not by  $K_s$  value alone, but by specific affinity. Specific affinity ( $a_A$ ) emphasizes the importance of taking into account, when comparing substrate-sequestering abilities of microorganisms, not only the half-saturation constant ( $K_s$ ) but also the maximum growth rate ( $\mu_{\max}$ ) and is approximated by  $\mu_{\max}/K_s$ . At 2°C the  $a_A$  of 2/10 was 0.285 liter  $\cdot$  g<sup>-1</sup>  $\cdot$  h<sup>-1</sup>, while that for 1/15 was 0.055 liter  $\cdot$  g<sup>-1</sup>  $\cdot$  h<sup>-1</sup> (Table 1). At 16°C the  $a_A$  values were 0.519 liter  $\cdot$  g<sup>-1</sup>  $\cdot$  h<sup>-1</sup> for 2/10 and 0.913 liter  $\cdot$  g<sup>-1</sup>  $\cdot$  h<sup>-1</sup> for 1/15. Therefore, in both cases the  $a_A$  for substrate was lower at 2°C than at 16°C, making substrate limitation more likely. The  $a_A$  of 2/10 for substrate was greater than that of 1/15 at low temperature. For both organisms,  $a_A$  increased with increased environmental temperature to 16°C but more markedly for strain 1/15 so that at 16°C it outcompeted 2/10 for glycerol at low glycerol concentrations. The responses of these same two bacteria on glucose apparently are different from those on glycerol (12), as it has been reported that at 5°C strain 1/15 took up glucose faster than did 2/10, while the relative rates were reversed at 20°C. It has been suggested (12) that the

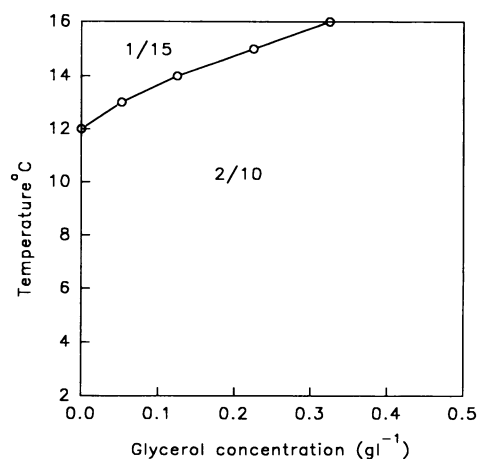


FIG. 6. Phase plane diagram to show the domains of dominance (faster growth) by either 2/10 or 1/15 at different combinations of temperature and substrate concentration.

glucose uptake system of the apparently more psychrophilic 1/15 was better adapted to function at low temperature than that of the more psychrotolerant 2/10, but the affinities of the two bacteria on glucose were not reported, so direct comparison with our data is not possible. The difference in the responses of the two bacteria to low temperature could not be explained by changes in membrane lipid composition (12). Changes in membrane lipids did occur but could not be correlated with temperature; a lack of correlation seems to be the norm (39, 40).

Herbert and Bell (19), in a study of the psychrophile *Vibrio* sp. strain AF-1, also reported that  $K_s$  was temperature dependent and that whether the values were higher or lower at 15°C than at 0°C was dependent on the organic substrate considered. Sugars such as glucose which supported a high growth rate had the lowest  $K_s$  values at 0°C, whereas those such as lactose which gave a low growth rate showed the lowest  $K_s$  values at 15°C. When we recalculated these data (Table 2) for *Vibrio* sp. strain AF-1 (19), the  $a_A$  for the variety of substrates tested increased with environmental temperature, similar to our data. The  $a_A$  for eight of nine substrates tested increased from 0 to 8°C, and for six of the nine substrates tested the  $a_A$  increased over the range of 0 to 15°C. Thus it appears that even with an obligately psychrophilic bacterium such as *Vibrio* sp. strain AF-1 the ability to take up substrates at low temperature declines when the  $a_A$ , and not solely the  $K_s$ , is considered. Similarly (Table 2), for a *Vibrio* sp. from an Antarctic lake the  $K_s$  for glucose was greatest at 0°C and decreased as the temperature rose; however, the reverse was the case for a *Corynebacterium* sp. from soil from the surrounding catchment area, which underwent regular temperature fluctuations (10). When the  $a_A$  values of these two bacteria for glucose were calculated, both showed increasing affinity with increased temperature from 2 to 22°C, and the  $a_A$  of the *Vibrio* sp. at any temperature was always greater than that of the coryneform. Furthermore, in the data available in the literature, the trend of decreased  $a_A$  values at lower temperatures seems not to be confined to psychrotolerant types. A mesophilic *K. pneumoniae* (*A. aerogenes*) strain showed an identical trend (45) over the range of 20 to 40°C (Table 2). We have not found data for thermophilic bacteria. In most cases, the  $a_A$  seems to increase up to the optimum temperature reported for the bacterium, although the actual values of  $a_A$  for different substrates at

TABLE 2. Comparison of specific affinity values available in the literature for a range of different physiological types of bacteria

Bacterium and substrate	$a_A$ (temp) <sup>a</sup>	Reference
<i>Vibrio</i> sp. (psychrotolerant, optimum 17°C), glucose	<b>0.00576</b> (2°C), <b>0.00978</b> (8°C), <b>0.01946</b> (14°C), NG (22°C)	10
Coryneform (psychrotolerant, optimum 21°C), glucose	<b>0.00059</b> (2°C), <b>0.00275</b> (8°C), <b>0.01322</b> (14°C), <b>0.02987</b> (22°C)	10
<i>Vibrio</i> sp. strain AF-1 (obligate psychrophile, optimum 16°C)		19
Lactose	<b>0.00023</b> (0°C), <b>0.0025</b> (8°C), <b>0.0033</b> (15°C), 0.000035 (23°C)	
Galactose	<b>0.00019</b> (0°C), <b>0.00156</b> (8°C), <b>0.01128</b> (15°C), NG (23°C)	
Mannose	<b>0.00005</b> (0°C), <b>0.00024</b> (8°C), <b>0.00208</b> (15°C), 0.00023 (23°C)	
Ribose	<b>0.00022</b> (0°C), <b>0.00233</b> (8°C), <b>0.0091</b> (15°C), 0.00015 (23°C)	
Xylose	<b>0.00025</b> (0°C), <b>0.00216</b> (8°C), <b>0.00559</b> (15°C), 0.00021 (23°C)	
Sucrose	<b>0.0025</b> (0°C), <b>0.0036</b> (8°C), <b>0.0044</b> (15°C), NG (23°C)	
Glucose	<b>0.004</b> (0°C), <b>0.0055</b> (8°C), 0.0016 (15°C), NG (23°C)	
Maltose	<b>0.0048</b> (0°C), <b>0.0072</b> (8°C), 0.0056 (15°C), NG (23°C)	
Fructose	0.0040 (0°C), 0.0030 (8°C), 0.0014 (15°C), NG (23°C)	
<i>K. pneumoniae</i> ( <i>A. aerogenes</i> ) (mesophile, optimum 39.5°C), glucose	NG (20°C), <b>0.0094</b> (25°C), <b>0.0163</b> (30°C), <b>0.0275</b> (35°C), <b>0.0334</b> (40°C), NG (45°C)	45

<sup>a</sup> Values are given in liters per micromole per hour, in contrast to liters per gram per hour in Table 1. NG, no growth. Boldface type indicates an increase in specific affinity with temperature.

various temperatures vary widely, presumably reflecting differences in their uptake systems.

The general decreased  $a_A$  for substrates at low temperatures suggested by our study and other data in the literature, even for psychrophilic bacteria, may be extremely important in providing an explanation for reports of small bacterial cells in Arctic seawaters (36) where at low temperature (<3.5°C) respiration by the microbial community was stimulated only by high organic substrate concentrations. Wiebe et al. (50) confirmed that with psychrotolerant bacteria from Arctic seawater at temperatures of <10°C there was a marked effect of substrate concentration on generation time, i.e., there was an interaction between temperature and substrate concentration such that at lower temperatures a higher substrate concentration was required to maintain the same growth rate. That this decreased affinity for substrates at low temperatures may be a general phenomenon is supported by the report (51) of a similarly enhanced substrate concentration requirement at low temperatures by marine mesophilic bacteria from waters of the Georgia Shelf (United States) when they were growing near their minimum growth temperatures. That observation suggests that the preponderance of bacteria isolated from the Arctic samples were psychrotolerant types, exhibiting the diminished  $a_A$  for substrate at low temperatures exemplified by both of our bacteria but in extreme form by 1/15.

It might be argued that 2/10 was more successful as a psychrophile than 1/15, despite the apparently lower optimum temperature of the latter. If, however, the change of affinity with temperature by the two organisms varied with different substrates, as indicated for 1/15 and 2/10 on glycerol or glucose (this work and references 12 and 19), this would increase the metabolic niches available to bacteria within the microbial community and could help to maintain community diversity under the dual stresses of low concentrations of substrates and low environmental temperatures. The reason for such a differential response to changing temperature in the various bacteria remains to be explained, however, but must reflect differences in the substrate uptake systems. Moreover, the results reported here reflect growth and competitive success in steady-state environments, in which the temperature regime is constant. The outcome of competition may be very different in non-steady-state environments, in which other factors, such as

speed of response to environmental perturbation, may be significant (see reference 41).

Decreased affinity for substrates at low temperatures may be even more important ecologically if it extends to the inhibition of uptake by active transport of inorganic nutrients by phytoplankton. High residual nitrate concentrations in Antarctic seawater have been attributed previously (27) to inhibition of primary production by trace elements such as iron, but the evidence is equivocal (7, 46), with some work supporting the hypothesis (28, 29) but other work contradicting it (21). Other workers have suggested that preferential uptake of recycled nitrogen as ammonium, accounting for up to 95% of the N demand of phytoplankton, may spare nitrate (13, 23, 24, 34, 38, 42). In the Southern Ocean, measurements indicate relative preference indices for inorganic N components in the order ammonium > urea > nitrate (37), but the relative rates of uptake of nitrate and ammonium may change with temperature (see reference 9). Decreased affinity for substrates at low temperature also may contribute to high residual nitrate concentrations at low temperature. Olson (34) reported that in the Scotia Sea an increase of temperature from 0 to 15°C stimulated uptake of both nitrate and ammonium, suggesting that the phytoplankton were not optimally adapted to low temperature. Hayes et al. (17) reported that addition of nitrate stimulated primary production in water of the Southern Ocean, despite the presence of relatively high nitrate concentrations, but that addition of phosphorus, trace metals, or vitamins did not. Jacques (21) has demonstrated previously that the kinetics for silicate uptake by diatoms in Antarctic seawater changed at low temperature so that affinity for silicate decreased. Rather than primary production being limited always by a single nutrient or other environmental factor, where nutrient concentrations are low they may interact to limit primary production (6, 9). Thus, in different areas of polar oceans primary production may be limited by different nutrients or combinations of nutrients (including iron, ammonium, and nitrate), further complicated by the decrease in affinities for substrates at low temperature. Further work needs to be carried out to examine the extent to which low temperature diminishes substrate affinity and hence inhibits the uptake of nutrients by living organisms.

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