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Influence of Changing Temperature on Growth Rate and Competition between Two Psychrotolerant Antarctic Bacteria: Competition and Survival in Non-Steady-State Temperature Environments

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Competition between two psychrotolerant bacteria was examined in glycerol-limited chemostat experiments subjected to non-steady-state conditions of temperature. One bacterium, a *Brevibacterium* sp. strain designated CR3/1/15, responded rapidly to temperature change, while a second, *Hydrogenophaga pseudoflava*, designated CR3/2/10, exhibited a lag in growth after a shift-down during a square-wave temperature cycle but not after a shift-up. The effects on competition and survival by these bacteria of both sine-wave and square-wave temperature changes between 2 and 16°C over a 24-h cycle time were examined, as well as square-wave cycles over 12 and 96 h. The changing proportion of each bacterium in the chemostat was determined by plate counting at regular intervals. Under a sine-wave temperature cycle *H. pseudoflava* outcompeted the *Brevibacterium* sp., but under square-wave temperature cycles the two bacteria coexisted because the lag by *H. pseudoflava* after the temperature shift-down favored the faster-responding *Brevibacterium* sp. The two bacteria thus exhibited different survival strategies, with *H. pseudoflava* adapted to effective competition under steady-state conditions and the *Brevibacterium* sp. adapted to rapid adaptation and survival in a changing environment. The degree of perturbation of the bacteria, expressed as a temperature challenge index (δtemp/δtime), was greater under a square-wave temperature cycle than under a sine-wave cycle of equivalent amplitude and frequency, and higher-temperature challenge favored the *Brevibacterium* sp. A computer model was developed to examine competition between the bacteria in transient environments. The frequency of the temperature cycle influenced competition, as with a longer cycle (96 h) the significance of the lag by *H. pseudoflava* decreased compared with that of a 24-h cycle, and *H. pseudoflava* predominated in a mixed culture with a 96-h cycle. The shift-down lag by *H. pseudoflava*, during which it adapted to low temperature, disadvantaged it in a changing temperature environment, but at a short cycle time (12 h) this disadvantage was countered by the incomplete loss of low-temperature adaptation between cycles and thus the carryover of some low-temperature adaptation. Also, it was demonstrated that, as well as consideration of the effect of temperature changes on inducing lags in growth, the loss of adaptation to low temperature between cycles had to be taken into account in the computer model if it was to reproduce the trends in the experimental data.

Environmental heterogeneity is vital to maintain community diversity, providing a multiplicity of niches within which species can coexist in close proximity while interspecies competition is reduced (42). Much research has been directed towards an understanding of spatial heterogeneity on the micro scale that is appropriate to microorganisms. Thus, microelectrode technology has demonstrated the micrometer scale spatial variability in many natural environments, which maintains community diversity (30). Laboratory model systems have been used to demonstrate the subtle mechanisms which lead to spatial ecological separation of microbial species, and hence their coexistence (37, 42). If they are not ecologically segregated, species may compete for environmental resources, and the most competitively efficient species will outgrow, and exclude, the less successful species.

In experimental analysis of microbial competition, chemostats have commonly been used so that growth-rate-limiting substrates can be maintained at low concentrations analogous to those in most natural environments. The basis of our current understanding of microbial competition for limiting substrates was established by Harder and Veldkamp and others (13, 15, 19, 22, 23), who showed that the outcome of competition in steady-state chemostats could be predicted from Monod-type hyperbolic curves which related growth rate, μ, to the concentration, S, of the growth-rate-limiting substrate. Species with higher affinities (low K_s values) for growth-rate-limiting substrates would be successful at low substrate concentrations, but at high substrate concentrations a high maximum specific growth rate, μ_{max}, was more important. However, most natural environments are not steady-state systems but are subjected to changes, or perturbations, of various time scales: diel, seasonal, interannual, and longer. Competition in non-steady-state environments may be different in that speed of response to rapid environmental perturbation may be as important for ensuring survival as adaptation to effective competition under steady-state conditions. Pickett et al. (25, 26) examined the response of *Escherichia coli* to square-wave perturbations in nutrient supply and found that there was a lag between stimulus and response which was independent of the amplitude of the cycle. The faster the perturbation is imposed, the more important to survival will be the speed of response of the species to that perturbation. Indeed, it has been shown theoretically (24, 35) that a more slowly growing species may outcompete a faster-growing species if the latter adapts more slowly to cycling in mixed cultures. Pavlou et al. (24) have pointed out that even when the specific growth rate of a species always exceeds that

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of a second species, extinction of either species or coexistence is still possible, depending on the response of the species to environmental perturbations.

Tilman (38) suggested that there is a range of perturbation in ecological systems, from level 1 (no perturbations [steady state]) through level 2 (an intermediate level in which perturbation of resources becomes a resource in itself) to level 3 (strong perturbations in which resources may be limited by environmental chaos). In steady-state systems such as chemostats (level 1), mixed populations of species competing for the same substrates cannot coexist unless the number of growth-rate-limiting substrates is equal to or greater than the number of competing species (36). However, in a non-steady-state system, the intermittent supply of nutrients (level 2) may maintain a more diverse community than the same nutrients supplied continuously. For example, in chemostats in which facultative or obligate thiobacilli were competing for acetate and thiosulfate, more than one species could coexist when the substrates were supplied alternately to the culture, but some of the same species were eliminated when the same substrates were supplied continuously and competition was under steady-state conditions (14, 18). Similarly, van Gemerden (41) showed continued coexistence of *Chromatium vinosum* and *Chromatium weissei* only under conditions of intermittent light-dark cycles. Periodic perturbations in plankton communities maintain community diversity, which declines if the perturbations are eliminated (16, 31). To date, most theoretical work in microbial ecology has concentrated on steady-state systems, level 1 of Tilman (38).

In previous research (23), we examined the growth rate kinetics of two psychrotolerant bacteria isolated from lake water and sediment (40) from Heywood Lake, Signy Island, Antarctica. One, designated *Hydrogenophaga pseudoflava* CR3/2/10 (referred to as 2/10), was isolated under conditions of constant low temperature, and a second bacterium, a *Brevibacterium* sp. strain designated CR3/1/15 (1/15), had initially been isolated in a chemostat under conditions of temperature cycling between 2 and 16°C over 24 h. The two organisms may be typical of different environments and temperature regimes, as Ellis-Evans and Wynn-Williams (10) have shown that gram-positive bacteria such as *Brevibacterium* spp. are more typically found washed in from soil in the catchment area surrounding the lake, whereas when isolations are done at low nutrient concentrations and constant low temperature gram-negative bacteria typical of the lake microflora (e.g., *Hydrogenophaga* spp.) are obtained. In the water immediately above the bottom sediment in Heywood Lake, there is a constant temperature of 2°C (0 to 6°C in surface water), but the surrounding soil environments can exhibit changes in temperature of as much as 40°C in 1 to 2 h, although 0 to 20°C is more typical (9). These temperature perturbations are damped with increased depth in the soil but at the soil surface resemble abrupt, square-wave changes.

The present work was undertaken to examine in a controlled manner the significance of environmental perturbation of temperature for competition between these two model psychrotolerant bacteria and the factors which influence the outcome of such competition. These will be of wider implication than just for competition between psychrotolerant bacteria and will throw light on the factors which influence competition between all types of bacteria in non-steady-state environments.

MATERIALS AND METHODS

Speed of response of the bacteria to temperature shifts. Cultures of both 1/15 and 2/10 were grown in FC2 medium (0.5 g of glycerol · liter^{-1}) (6) at 2 and 16°C to early exponential phase. Subsamples (10 ml) of each culture were then transferred to further optically matched tubes of FC2 medium in a temperature gradient block incubator (5, 39) over the range of 2 to 16°C. The tubes of medium quickly equilibrated to the temperature of their position along the gradient, and growth of the bacteria at each temperature was then monitored by periodically measuring the optical density of each culture tube in a nephelometer.

Competition between the two bacteria under conditions of changing temperature. Examination of the outcome of competition between the two bacteria was investigated in two ways. First, competition experiments were carried out in chemostat cultures which were subjected to controlled temperature changes.

Chemostats. Each chemostat vessel (0.5-liter volume) was water jacketted and the temperature of the water flowing through the jacket was controlled by a thermocirculator (model FH16; Grant Instruments, Cambridge, United Kingdom) and cooler (FC15; Grant Instruments). The temperature of the thermocirculator was in turn controlled by a microcomputer (BBC; Acorn Computers, Cambridge, United Kingdom) so that temperature changes could be programmed into the chemostat's operation. It was possible to impose either cyclical (sine-curve) temperature regimes, square-wave, stepped temperature changes, or constant temperature upon the chemostat vessels via control of the thermocirculator, and it was also possible to vary the period of the temperature cycle (ω). The temperature range used in the present work was 2 to 16°C over 12, 24, or 96 h. Response of the medium in the chemostat to a change of temperature was extremely rapid, equilibrating to a 14°C step change in water jacket temperature within 20 min. There was no measurable lag in the response of the temperature of the medium to a sine-wave change of 2 to 16°C in the water jacket temperature over 24 h.

In all experiments, the medium used was FC2 medium with 0.5 g of glycerol · liter^{-1}, and the dilution rate, D, was 0.02 h^{-1}. Starter cultures of the two bacteria were each grown at 16°C in FC2 broth to exponential phase. The turbidity of each culture was measured in a nephelometer, and the nephelometer reading was converted to biomass from a standard curve. The biomass of each organism inoculated into the chemostat was equalized by adjusting the volume of each culture in the inoculum. The chemostat vessel was filled and inoculated with the mixed inoculum of the two bacteria, and the medium pump (Multiperpex; LKB Ltd., Broma, Sweden) was switched on. The temperature regime for the experiment was initiated at inoculation. For the sine-wave experiments, the temperature was started at the midpoint of the temperature cycle; for the square-wave experiments, the temperature was started at the beginning of the low temperature part of the square wave.

Total biomass at any time was measured by aseptically withdrawing a sample (2 ml) directly from the chemostat vessel and measuring the optical density at 550 nm in a spectrophotometer. The proportion of each bacterium present in the sample was also determined by plate counts on agar medium.

Counts of bacteria. Preliminary experiments with paper discs of antibiotics on lawn plates of each bacterium had shown that penicillin (0.5 mg · l^{-1}) inhibited the growth of 1/15 without affecting 2/10, while trimethoprim (0.5 mg · l^{-1}) had the opposite effect. Samples of the culture were extracted aseptically from the chemostat vessel with a sterile long hypodermic needle and syringe, via a subaseal. Tenfold dilutions of this sample were made in sterile one-fourth-strength Ringer's solution, and aliquots (0.1 ml) of suitable dilutions were spread aseptically with a sterile glass rod over the surface

of sterile plates of FC2 agar. Triplicate agar plates at each dilution were prepared with each antibiotic addition so that the numbers of each bacterium could be estimated separately. Plates giving 30 to 300 well-separated colonies were selected for counts, and the number of each bacterium in the original sample was then calculated.

Chemostat experiments. A series of chemostat experiments was carried out to investigate the outcome of competition between the two bacteria in variable temperature environment. First, the chemostat was subjected to a cyclical, sine-wave temperature regime between 2 and 16°C over 24 h. This was followed by experiments in which square-wave, stepped temperature changes between 2 and 16°C were imposed over 12, 24, and 96 h.

In all cases, the progression of the chemostat culture with time was monitored by regular measurements of the culture density and of the numbers of the two bacteria.

Computer model predictions of competition. We have previously reported (23) measurements of the μ_{max}, K_s, and cell yield (Y) of these two bacteria over a range of temperatures from 2 to 16°C. The μ_{max} and K_s were found to be functions of temperature and could be described by Arrhenius functions. These relationships were used to construct computer models to describe growth of the bacteria and to predict the outcome of their competition under conditions of variable temperature.

Differential Monod-type equations were used as the basis of the model, written in Quickbasic. (A full listing of the model is available upon request.) The steady-state Monod parameters, μ_{max}, K_s, and Y, were substituted by mathematical functions, with temperature as a variable, describing their response to temperature change. The program carried out at 10-min intervals the iterations for estimating the biomass of each bacterium and the residual substrate in the chemostat. The datum output from a model run was saved and exported to graph-plotting software (Sigmaplot; Jandel Corp., Corte Madera, Calif.).

Modelling of μ_{max}. The temperature range used in these experiments lies within the range of linear response of an Arrhenius plot of μ_{max} versus the reciprocal of absolute temperature (1/T°K). The temperature characteristics describing the change of μ_{max} with reciprocal temperature were similar in batch and chemostat experiments (23). For the model, the equations describing the relationship of μ_{max} to temperature (T, in °C) used these temperature characteristics and were as follows for each organism:

$$\text{for 2/10, } \mu_{max} = e^{[-4{,}417/(T + 273)] + 13.28}$$

$$\text{for 1/15, } \mu_{max} = e^{[-7{,}925/(T + 273)] + 25.09}$$

Modelling of K_s. The calculated values for K_s for glycerol at 2 and 16°C gave Arrhenius plots which related K_s and 1/T°K over the range of 2 to 16°C. The K_s values for 2/10 increased with temperature, while those for 1/15 decreased (23). The change in value of K_s with temperature for each organism over the range of 2 to 16°C was therefore described as follows:

$$\text{for 2/10, } K_s = e^{[-2{,}702/(T + 273)] + 8.02}$$

$$\text{for 1/15, } K_s = e^{[8{,}601/(T + 273)] - 32.1}$$

Cell yield. As the yield coefficient is dependent on growth rate as well as temperature (27), the values for yield determined in batch cultures were not used is this model. Chemostat experiments (23) previously had given values for Y at 2 and 16°C for each bacterium, and it was assumed initially that yield changed linearly with temperature between these two extremes. The values for Y for any temperature was derived, therefore, as follows: for 2/10, $Y = (-0.007 \times \text{T°C}) + 0.4$, and for 1/15, $Y = (-0.007 \times \text{T°C}) + 0.26$.

When grown at 0.5 g of glycerol · liter^{-1} in batch culture, the cell yield of 1/15 was not linear between 2 and 16°C but exhibited an optimum at about 10°C (23). This would have no effect upon the square-wave temperature cycle, in which the temperature was at only 2 or 16°C, but it might influence the outcome of competition under the sine-wave temperature cycle, in which all temperatures intermediate to 2 and 16°C were encountered. Therefore, in a second version of the computer model the influence of this temperature effect on cell yield, and hence competition, was investigated for the sine-wave temperature cycle. The change in the cell yield of 1/15 in batch cultures over 2 to 20°C was described for this cycle by a second-order regression equation: $Y = -0.00376T^2 + 0.07479T + 0.1556$. The r^2 value for the fit of this equation to the data was 0.92. The computer model was run for the sine-wave temperature cycle described above, except that the cell yield of 1/15 at temperatures intermediate to 2 and 16°C was described by fitting the chemostat values of cell yield to the above equation. The effect of this modification of the cell yield of 1/15 on the predicted outcome of competition was examined.

Response time. Two versions of the model were used. In the first, it was assumed that when the temperature changed each organism immediately displayed the growth kinetics of the new temperature, i.e., the current temperature, with no adaptation period. In the second model, the response of organism 2/10 was assumed to lag for 6 h after a temperature shift-down from 16 to 2°C, as indicated by the experimental data for this organism (see below).

Effect of loss of low-temperature adaptation. Initially, in the model no consideration was given to the conservation of low-temperature adaptation by organism 2/10 during the high-temperature phase of the square-wave cycle. The results of the model of a square-wave cycle at a 12-h cycle time suggested that some degree of cold adaptation must be retained during a rapid alternation of temperature, i.e., adaptation to low temperature by 2/10 was not entirely lost during the 6-h elevated-temperature phase of a 12-h cycle (see Results).

Cold adaptation can be modelled in terms of a cold-adaptation index (α) which will be maximal ($\alpha = 1$) when fully induced, i.e., after a period of 6 h at 2°C, and 0 when cold adaptation is completely lost. When the temperature is shifted up to 16°C, α will decline because of the breakdown of cold-adaptation proteins (see Discussion), and its value at the end of the high-temperature phase will depend on the length of time that the organism was at that high temperature. The model simulation for the 24-h square-wave cycle conformed well to the experimental chemostat data when 2/10 was modelled with a 6-h lag. Thus it appeared from the experimental results that any adaptation to low temperature by 2/10 was entirely lost ($\alpha \rightarrow 0$) after 12 h at 16°C. For the 12-h square-wave cycle, the best fit of the model to experimental results was provided when the lag of 2/10 was reduced to 2.5 h upon the step-down in temperature (see Fig. 6). Thus the lag time was reduced by 58% ($\alpha = 0.58$), suggesting that cold adaptation was still 58% induced after a 6-h period at 16°C.

The loss of cold adaptation can be modelled by a negative logistic curve of the form $\alpha = K/[1 + e^{(a + bt)}]$ where K is the maximum for α and is equal to 1, a and b are constants which determine the slope and height of the logistic curve, respectively; and t is the elapsed time at 16°C and equal to $1/2\omega$.

If we substitute $\alpha = 0.58$ when $t = 6$ (from the 12-h cycle experiment) and assume a value of α close to 0 ($\alpha = 0.01$)

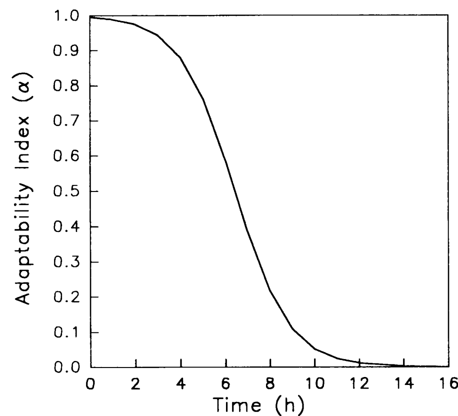

FIG. 1. Negative logistic curve modelling the loss of low-temperature acclimation by organism 2/10 after exposure to a higher temperature (16°C).

when $t = 12$ (from the 24-h cycle experiment), then we generate two equations which can be solved simultaneously to obtain the values of the constants a and b to give $\alpha = 1/(1 + e^{(-5.27 + 0.82t)})$. This provides a mathematical description for the turnover of cold-acclimation proteins at higher temperatures and the loss of cold acclimation by 2/10 (Fig. 1). The model run for the square-wave cycle over 12 h was repeated with a routine included to model the effect on growth and competition of loss of low-temperature adaptation by 2/10.

RESULTS

Response of bacterial growth to temperature shifts. Organism 1/15, grown at either 2 or 16°C, showed no lags in growth when transferred to fresh medium at different temperatures over the range of 2 to 16°C, in either shift-up or shift-down changes of temperature. By comparison, organism 2/10 grown at 2°C did not exhibit any lag in growth when shifted to higher temperatures, but a culture grown at 16°C, when tested for growth at lower temperatures, exhibited lags in growth of about 6 h at temperatures of <5°C. Thus, organism 1/15 was able to respond more readily to abrupt temperature changes than was organism 2/10.

Chemostat experiments. Figure 2 shows the results of the computer model prediction for competition between 2/10 and 1/15 under sine-wave temperature variation between 2 and 16°C over 24 h. It also shows the results of the actual chemostat experiment (Fig. 2a). It can be seen that the computer model predicted that organism 2/10 would outgrow 1/15, the latter

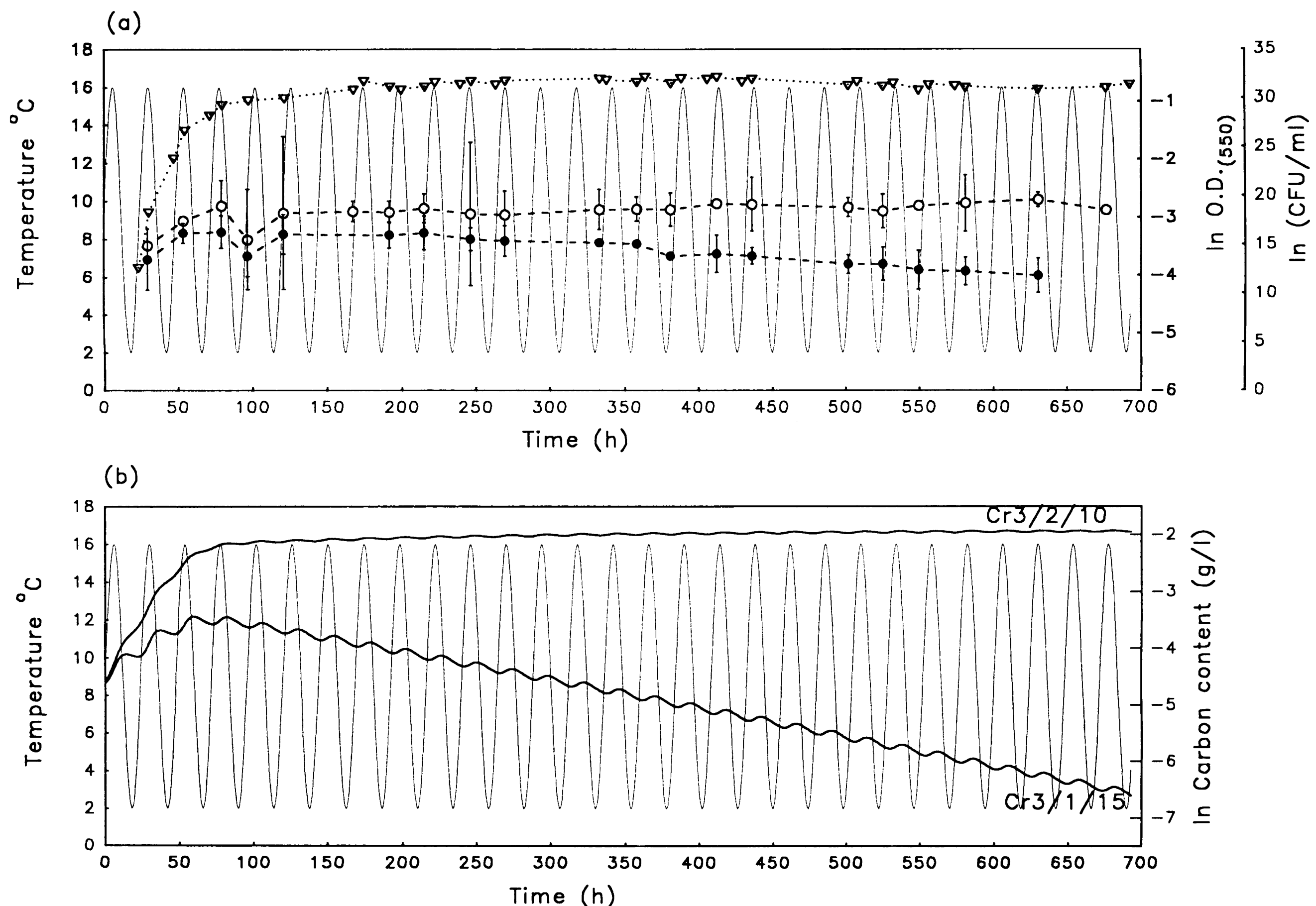

FIG. 2. Outcome of competition between organisms 2/10 and 1/15 in a glycerol-limited chemostat ($D = 0.02$ h^{-1}) under a sine-wave temperature cycle (2 to 16°C; cycle time = 24 h). (a) Chemostat temperature (solid line), optical density at 550 nm (OD_{550}) of the chemostat culture (▽), and plate counts of 2/10 (○) and 1/15 (●) during the experiment (bars indicate standard errors; $n = 3$). (b) Temperature (thin line) and biomasses (heavy lines) of 2/10 and 1/15 predicted by the computer model during the experiment.

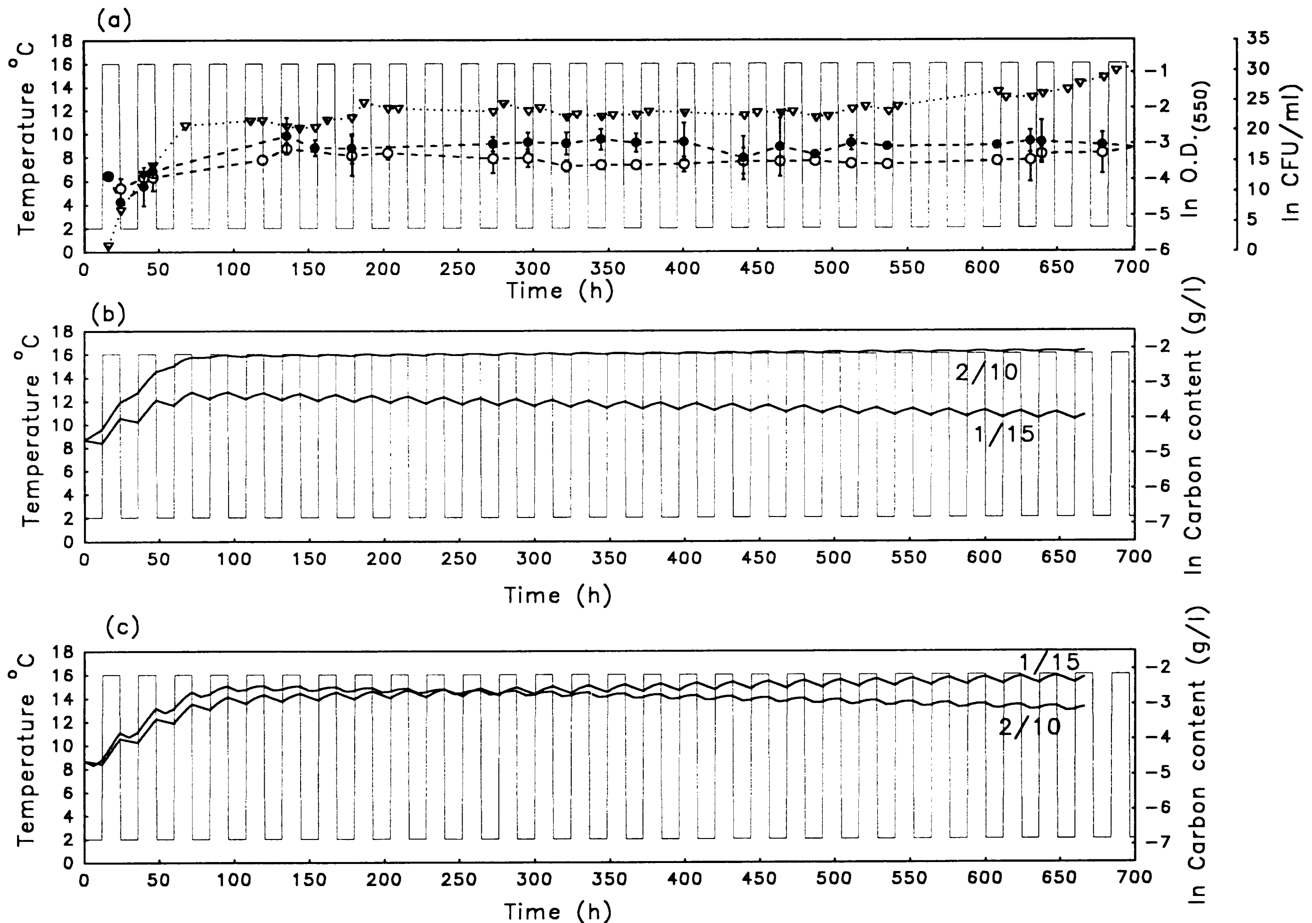

FIG. 3. Outcome of competition between organisms 2/10 and 1/15 in a glycerol-limited chemostat (D = 0.02 h^{-1}) under a square-wave temperature cycle (2 to 16°C; cycle time, 24 h). (a) Chemostat temperature, optical density, and plate counts as described for Fig. 2a. (b) Predicted biomasses when the model does not include a lag for 2/10. (c) Predicted biomasses when the model includes a 6-h lag for 2/10 after the step-down temperature shift.

being outcompeted after about 80 h and its numbers decreasing with time. The measurements of optical density at 550 nm in the chemostat run showed that the bacterial community increased over the first 100 h after inoculation and apparently achieved a steady state. The results of the plate counts indicated that both organisms increased in numbers over the first 100 h but then 2/10 maintained a slowly increasing population density while 1/15 appeared to be gradually excluded. Its numbers steadily decreased during the remainder of the experiment, although it had not been excluded entirely when the experiment terminated after 700 h of operation. The numbers of 1/15 organisms remaining after 630 h were 5% of those at the peak after 80 h, while residual biomass predicted from the model (Fig. 2b) was 8% of that at 80 h. Similarly, linear regression analyses between these two times (P <0.01 in both cases) showed that the washout rate was −0.005 h^{-1} for the computer model and −0.007 h^{-1} for the experimental counts. The rate of decrease of 1/15 with time predicted by the computer model was therefore very similar to that actually detected in the chemostat. (Note that the slopes appear different in Fig. 2 because of the difference in natural logarithm scales for the two independent variables.)

Figure 3 shows the experimental results (Fig. 3a) and the predictions of the computer models for competition under stepped, 24-h-period temperature changes. Two forms of the computer model output are illustrated (Fig. 3b and c), first with instantaneous responses by both bacteria to temperature change and second with a 6-h lag built in for 2/10 on the step-down part of the temperature cycle. It can be seen that without a lag, the model predicts that 2/10 will outcompete 1/15, which will be slowly excluded over 700 h (Fig. 3b). This is similar to the previous case with the sine-wave temperature. When a lag for 2/10 is included in the model (Fig. 3c), the prediction changes. It seems that while 2/10 initially grows faster than 1/15, after about 200 h the latter starts to outcompete 2/10, which slowly declines. Thus, the inclusion of a lag period in response to lowered temperature apparently favors the bacterium which is able to respond more rapidly to the temperature shift. The results of the measurements during the chemostat experiment show that a steady-state optical density at 550 nm was achieved after about 200 h in the chemostat. The plate counts showed that 1/15 and 2/10 apparently coexisted, with greater numbers of 1/15 than of 2/10, conforming to the data generated by the computer model which included the shift-down lag for 2/10 (Fig. 3c).

The results of a similar stepped-temperature experiment over a 96-h cycle are shown in Fig. 4. The model simulations (Fig. 4b and c) show that, with or without a lag for 2/10 in the

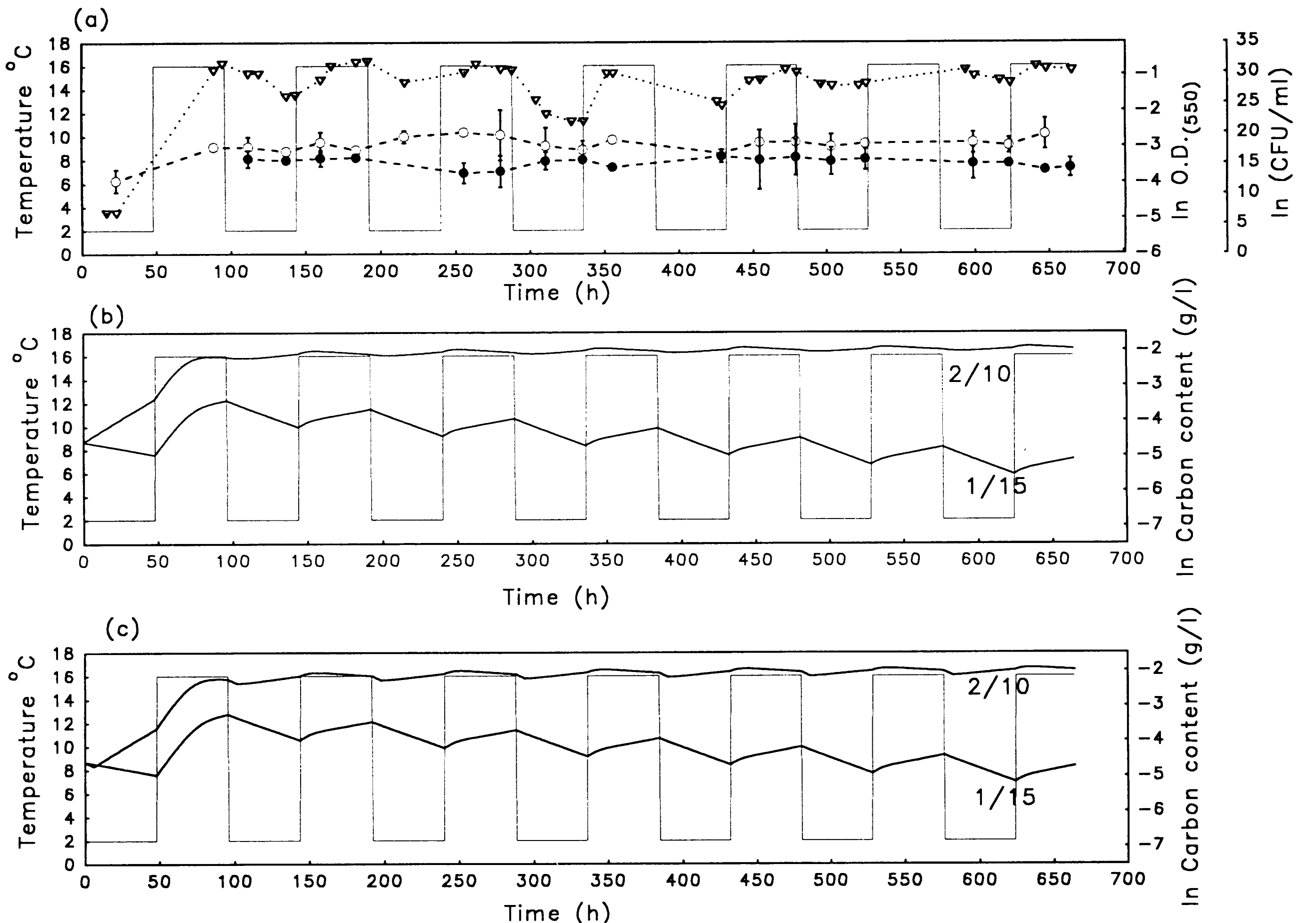

FIG. 4. Outcome of competition between 2/10 and 1/15 in a glycerol-limited chemostat (D = 0.02 h^{-1}) under a square-wave temperature cycle (2 to 16°C; cycle time, 96 h). Data are as described for Fig. 3.

downshift step of the model, organism 2/10 is predicted to outcompete 1/15, which gradually declines in the culture although it is not completely excluded during the 700 h of the model simulation. Presumably, over this longer cycle (96 h) the 6-h lag by 2/10 has proportionately less significance to its overall competitivity with 1/15, at least compared with the 24-h cycle. Both computer models for the 96-h cycle predict that, during the low-temperature part of the cycle, the rate of growth by 1/15 is slowed and washout exceeds growth rate ($D > \mu$); for 2/10, after the initial lag, the biomass increased slightly during the remainder of the low-temperature phase. In contrast, during the high-temperature period, for 1/15 μ was $>D$ and the biomass increased again, whereas for 2/10 there was a slight decrease during the high-temperature step as it was outcompeted by 1/15. Thus, periodic changes in the culture biomass were predicted by the model. The data from the chemostat experiment (Fig. 4a) corroborated this prediction to some extent, and clear oscillations in the optical density of the culture were seen, decreasing during the low-temperature phase and increasing during the elevated-temperature phase. However, the plate counting of each bacterium was too infrequent to detect the predicted sequence of variation of numbers within each temperature cycle. The plate counts showed that the cell numbers conformed to the model predictions to the extent that 2/10 was the numerically dominant organism during this experiment, although no clear trend of decrease of 1/15 with time, as predicted by the computer model, could be detected. However, the model predicted only low rates of change of the two bacteria with time, and the frequency of plate counts, at random times in the temperature cycle, was insufficient to detect the overall trends of counts with time against a background of variation of numbers within the longer temperature cycle.

In the last experiment, we investigated a 12-h stepped-temperature cycle (Fig. 5), in which it might be predicted that the downshift lag might be even more significant to the competitivity of 2/10 than when ω is 24 h. When the chemostat experiment was run, the two bacteria coexisted in an apparently steady state after about 100 h, with 2/10 numerically dominant (Fig. 5a). As this result seemed to contradict fundamentally the computer model prediction, the chemostat experiment was repeated, but with similar results. The computer model again predicted that when there was an instantaneous response to temperature change, 2/10 slowly would exclude 1/15 (data not shown). However, when a 6-h downshift lag was included (Fig. 5b), the model predicted that the outcome would be reversed and 1/15 would exclude 2/10 relatively rapidly. This, then, seemed to confirm that the significance of the lag to competitive success increased as the cycle time decreased. The organism able to respond quickly to temperature change, 1/15, apparently benefitted competitively in an increasingly variable environment. However, the predictions of

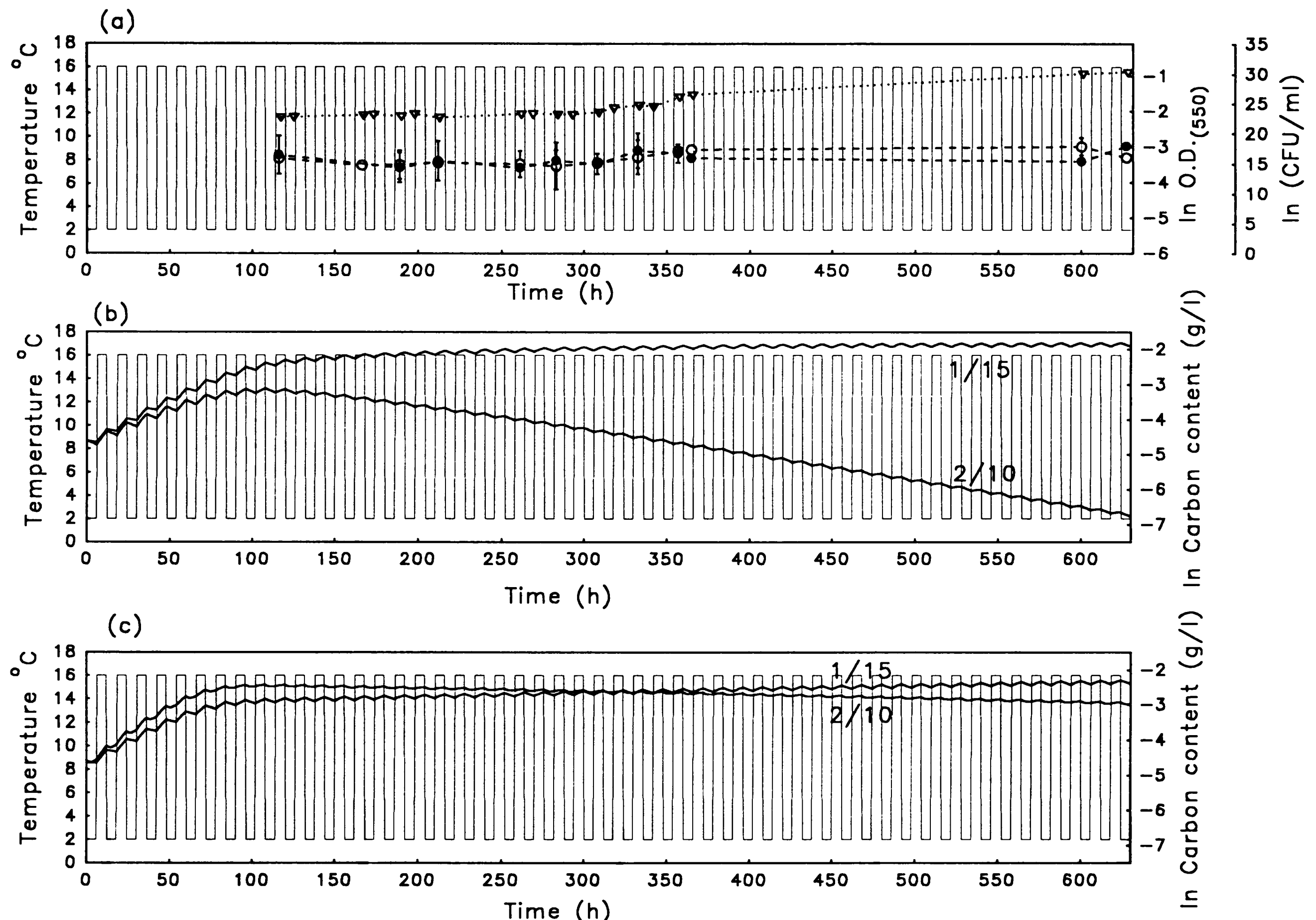

FIG. 5. Outcome of competition between 2/10 and 1/15 in a glycerol-limited chemostat (D = 0.02 h^{-1}) under a square-wave temperature cycle (2 to 16°C; cycle time, 12 h). (a) Results of the chemostat experiment, as described for Fig. 3a. (b) Predicted biomasses with a 6-h lag for 2/10 after the step-down temperature shift. (c) Predicted biomasses with a lag for 2/10 which allows for retention of at least partial low-temperature adaptation between temperature cycles.

the computer model, even with a 6-h downshift lag, did not reproduce the results measured in the experimental chemostat, and the computer model was further modified to account for retention of cold adaptation during short cycle times (see Discussion).

Modelling loss of adaptation to low temperature. When the model included consideration of the conservation of low-temperature acclimation by 2/10 during the 12-h cycle, the predicted outcome of competition was as shown in Fig. 5c. It is clear that when carryover of residual low-temperature acclimation was considered, the model output much more closely mimicked the results of the chemostat experiment.

DISCUSSION

The investigation of the speed of response to changed temperature by the two psychrotolerant bacteria suggested that 1/15 was able to respond without a lag to either an increase or a decrease of environmental temperature, at least over the range of temperatures tested. In contrast, while 2/10 was able to adapt without delay to increases of environmental temperature, a lag occurred when temperatures dropped to <5°C. Lag periods in response to cold shocks have been reported before a variety of microorganisms, e.g., *E. coli* (8, 18). Araki (2, 3) demonstrated a lag in growth rate below 7°C induced by cold shock when the psychrotolerant bacterium *Vibrio* sp. strain ANT-300 was subjected to a step-down temperature shift. Cold shock has been associated with the induction and transient production of cold shock proteins (2–4, 12, 18) and also with the production of cold-acclimation proteins which are produced continuously rather than transiently (32). Some cold shock proteins may be desaturase enzymes, associated with changes in the degree of unsaturation of the acyl chains of membrane lipids (33, 34). An increase in the degree of unsaturation of the membrane lipids commonly occurs in response to low temperature and is thought to help maintain the membrane in a fluid, biologically active state under the new, lower-temperature regime. Fukunga and Russell (11) have previously reported for the two bacteria used in the present study that the membrane lipid compositions responded to changes in both temperature and nutrient status of the growth medium.

Previous work (23) has shown that in constant-temperature environments organism 2/10 generally is better adapted to compete for glycerol because of a higher affinity for the substrate, while organism 1/15 is successful only at relatively high (>12°C) temperatures combined with a relatively low (<0.32 g of glycerol · liter^{-1}) substrate concentration. At any given constant temperature, the organism better adapted to

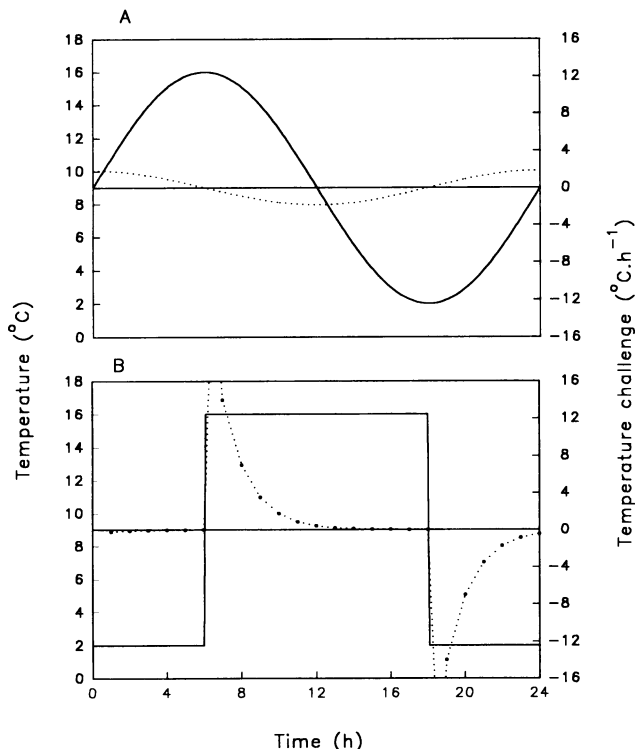

FIG. 6. Change of temperature challenge (τ) with time in a sine-wave (A) and square-wave (B) temperature regime (2 to 16°C; cycle time, 24 h). Solid lines show temperature and broken lines indicate temperature challenge.

that set of steady-state conditions will outgrow the other. However, the data presented here show that in a variable environment the speed of response to environmental change may be as important as, if not more important than, the steady-state competitive ability. The importance of the speed of response to an organism's competitive ability will be related to the speed of change in the environment. The perturbation imposed by the environmental temperature change may be described by a temperature challenge index, τ, with units of °C · time^{-1}, analogous to δtemp/δtime. In a sine-wave temperature cycle in which temperature is described by temperature = $\sin(2\pi/\omega t) \cdot A$, where A is amplitude of temperature cycle, t is the elapsed time (in hours), and ω is the cycle time (in hours), the degree of perturbation (dT/dt) will vary with time according to the formula $dT/dt = \cos(2\pi/\omega t) \cdot (2\pi/\omega)A$. That is, the environmental perturbation (dT/dt) will be 0 at the maximum and minimum temperatures of the cycle and maximum at the midpoint temperature. For a sine-curve temperature cycle for which A = 7°C and ω = 24 h, as we used, the maximum temperature challenge in the sine-curve temperature regime is ±1.8°C · h^{-1} (Fig. 6A). Competing bacteria will be subjected to a regular cycle of challenge during which they must adapt to this changing temperature. In contrast, in a stepped-temperature regime the perturbation will be greatest immediately after a temperature shift but will then decrease with time until the next shift. Figure 6 shows the magnitude and change of temperature challenge with time under each temperature regime. Immediately after the stepped temperature shift τ = ∞ (Fig. 6B), but it decreases exponentially with time. For example, after 1 h τ was 14°C · h^{-1}, while after 6 h it had dropped to <2°C · h^{-1}. During the initial period of great perturbation, the speed of physiological response by a species to the perturbation will be important in terms of its competitive ability for survival. After some time dT/dt will approach 0, the system will again approach steady state, and competition between species again will be on the basis of steady-state growth kinetics. Clearly, the greater the proportion of the time that a perturbation operates, the greater the significance of speed of adaptive response, as opposed to steady-state adaptation.

When a sine-wave temperature cycle was imposed (Fig. 2), organism 2/10 maintained its competitive advantage over 1/15, implying that the cyclically varying temperature was not sufficient to confer an advantage upon 1/15 through its faster response. The maximum temperature challenge during the sine-wave temperature cycle was only ±1.8°C · h^{-1}, and the requirement for speedy adaptation to the perturbation was not great. In contrast, when the same amplitude of temperature change over the same cycle time (ω = 24 h) was imposed as a square wave, the perturbation was large and the outcome of competition was very different (Fig. 3). Organism 1/15 was not excluded from the environment during the square-wave cycle but coexisted in higher numbers than 2/10. Organism 2/10 was inhibited for 6 h after the temperature shift-down, by which time τ was <1.8°C · h^{-1}. This emphasizes that 2/10 would not have been inhibited during the sine-wave cycle, when τ was always <1.8°C · h^{-1}.

When the cycle time was extended (96 h), the significance to competition of the adaptive lag phase by 2/10 after temperature shift-down was proportionately reduced, and, although the two bacteria coexisted, 2/10 predominated (Fig. 4). Thus is appears that each bacterium adopts a different strategy for survival. The *H. pseudoflava* strain (2/10) appears to be best adapted to effective competition under steady-state, or only mildly perturbed, conditions, similar to those in the lake environment. The *Brevibacterium* sp. (1/15) is less well adapted for steady-state competition but responds quickly to large perturbations of its environmental temperature. This counterbalances to some extent its poorer steady-state competitivity, at least over the range of temperature and glycerol concentrations in which it could effectively grow (23). Thus, 1/15 is better adapted than 2/10 to effective competition under non-steady-state temperature environments, typical of the catchment soil environment in which gram-positive bacteria predominate, than under steady-state conditions. The difference may be compared to a k survival strategy for 2/10 but an r survival strategy for 1/15 (21).

It could have been predicted that under an even shorter square-wave cycle time (12 h) the competitive significance of rapid adaptation would be even more clear and that 1/15 would outcompete 2/10. Experimentally this did not prove to be the case (Fig. 5a). However, this discrepancy might reasonably be attributed to other processes which had not been considered initially in the computer model. All cellular components undergo continual turnover, i.e., constant synthesis and degradation. The induction of adaptive proteins, which may be enzymes, in response to challenges by environmental conditions may be followed by their gradual loss from the cells if the selective response is not maintained or if they are only transiently produced. Pickett et al. (26) have pointed out that an inductive response is probably swifter than deactivation, because once adaptive proteins have been induced there is no subsequent adaptive advantage to making deactivation rapid. Repeated, frequent perturbation of a bacterium might therefore be expected to increase the level of activity of the perturbed cells, as induction of increased activity would exceed deactivation between perturbations. Exponentially growing

mesophiles usually have low rates of protein turnover, suggesting that most proteins made during active growth are relatively stable (7, 20). Microorganisms living in extreme environments may have very much faster rates of protein turnover (1, 17, 28, 29). In our chemostat experiments, loss of adaptive proteins from a population was a function both of washout of induced cells from the chemostat and of intracellular turnover and breakdown of the induced components in the remaining cells. In view of the low dilution rate used, most of the loss of low temperature adaptation from the culture in the chemostat was due to intracellular turnover and not to washout.

In the case of the short 12-h temperature cycle, it is probable that the cells of 2/10 did not completely lose their cold-adaptive proteins during the high-temperature phase, before the low-temperature phase returned. When conservation of low-temperature adaptation by 2/10 was included in the computer model (Fig. 5c), the results better coincided with the experimental data from the chemostat. This suggests that the 2/10 cells remained at least partially adapted to low temperature during the short cycle time. The adaptive lag, after the temperature downshift, diminished in duration under the shorter cycle time and therefore became less significant to reducing the competitive ability of the organism. This observation emphasizes that in a chemostat the balance of the speed of adaptive induction and the loss of adaptive induction to survival depend very much on the relative rates of environmental perturbation and dilution (a similar conclusion has been derived theoretically [24, 35]). In a natural environment, loss of adaptation will be a function only of intracellular turnover of adaptive proteins and not of disappearance by washout.

In conclusion, the experiments described here have shown that temperature perturbation provides temporal heterogeneity in the environment, which can increase the diversity of the microbial communities. Temporal heterogeneity permits the coexistence of species which would otherwise competitively exclude each other from the environment. The coexistence of 2/10 and 1/15 is the consequence of their different survival strategies in relation to temperature and substrate availability. Organism 2/10 is well adapted to competition under steady-state or mildly perturbed conditions of temperature, when it outcompetes 1/15; however, 1/15 is better adapted to competition under rapidly changing, non-steady-state, transient temperatures by virtue of its rapid response to temperature change. In turn, these differing survival strategies may be adaptive for the environments from which these two psychrotolerant bacteria originated: a stable temperature regime in aquatic sediments for 2/10 but a very variable temperature regime in soil for 1/15.

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