

Relationship of Critical Temperature to Macromolecular Synthesis and Growth Yield in *Psychrobacter cryopegella*

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Most microorganisms isolated from low-temperature environments (below 4°C) are eury-, not steno-, psychrophiles. While psychrophiles maximize or maintain growth yield at low temperatures to compensate for low growth rate, the mechanisms involved remain unknown, as does the strategy used by eurypsychrophiles to survive wide ranges of temperatures that include subzero temperatures. Our studies involve the eurypsychrophilic bacterium *Psychrobacter cryopegella*, which was isolated from a briny water lens within Siberian permafrost, where the temperature is –12°C. *P. cryopegella* is capable of reproducing from –10 to 28°C, with its maximum growth rate at 22°C. We examined the temperature dependence of growth rate, growth yield, and macromolecular (DNA, RNA, and protein) synthesis rates for *P. cryopegella*. Below 22°C, the growth of *P. cryopegella* was separated into two domains at the critical temperature ($T_{\text{critical}} = 4^\circ\text{C}$). RNA, protein, and DNA synthesis rates decreased exponentially with decreasing temperatures. Only the temperature dependence of the DNA synthesis rate changed at T_{critical} . When normalized to growth rate, RNA and protein synthesis reached a minimum at T_{critical} , while DNA synthesis remained constant over the entire temperature range. Growth yield peaked at about T_{critical} and declined rapidly as temperature decreased further. Similar to some stenopsychrophiles, *P. cryopegella* maximized growth yield at low temperatures and did so by streamlining growth processes at T_{critical} . Identifying the specific processes which result in T_{critical} will be vital to understanding both low-temperature growth and growth over a wide range of temperatures.

Evolutionary adaptation to low- or high-temperature conditions often leads to the commitment to exist only in those conditions (28). However, eurythermal organisms tolerate and grow at a wide range of temperatures while stenothermal organisms are restricted to a narrow range (3). Hence, a eurypsychrophilic bacterium would be capable of growing over a wide range of low temperatures, from –10 to 0°C at the lower limit to 20 to 30°C at the upper limit. One such eurypsychrophilic bacterium is *Psychrobacter cryopegella*, isolated from a low-temperature (–12°C), saltwater lens in Siberian permafrost (4). This bacterium displays its maximum growth rate (i.e., has an optimal growth temperature [T_{opt}]) at 22°C yet is able to reproduce at temperatures as low as –10°C and as high as 30°C.

Of major concern to organisms living over a wide range of temperatures is the fact that reaction rates decrease exponentially as temperature decreases. This also holds for bacterial growth rates (essentially a collection of chemical reactions). However, when bacterial growth rate (μ) is examined on an Arrhenius plot ($\log \mu$ versus $1/T$), two linear domains often appear below T_{opt} . The point at which the slope changes is designated the critical temperature (T_{critical}). The existence of T_{critical} has been reported for eurypsychrophiles, mesophiles, and thermophiles (5, 6, 9, 12, 13, 15, 22, 35). The lack of T_{critical} reports for stenopsychrophiles is probably because their growth rates have not been systematically examined at sufficiently low temperatures. It is hypothesized that T_{critical} exists

as a result of increased energy demands, the synthesis of stress proteins, and/or the use of alternate metabolic pathways at low temperatures (6, 10, 12).

Understanding the basis of T_{critical} is important because much of the earth's surface is permanently at or below 4°C and the microorganisms isolated from permanently cold ecosystems (e.g., sea ice, glacial ice, the deep sea, and permafrost) generally have T_{opt} values much greater than in situ temperatures ($T_{\text{in situ}}$) (2, 18, 23, 29, 31). Furthermore, eurypsychrophiles are isolated more frequently from low-temperature environments than are stenopsychrophiles (29, 37). Stenopsychrophiles are more adapted to low temperatures than eurypsychrophiles and subsequently have faster growth rates at 0°C (13, 29). In addition, stenopsychrophiles compensate for low growth rates at $T_{\text{in situ}}$ by having growth yields that peak around $T_{\text{in situ}}$ or remain constant from T_{opt} to $T_{\text{in situ}}$ (13, 15, 19). Unfortunately for eurypsychrophiles, not only is T_{opt} greater than $T_{\text{in situ}}$ but T_{critical} is also greater than $T_{\text{in situ}}$.

Given that eurypsychrophiles predominate in low-temperature environments, where $T_{\text{in situ}}$ is lower than T_{critical} , the question arises as to how eurypsychrophiles function at temperatures below T_{critical} and T_{opt} . Thus, we have examined how the rate of growth of *P. cryopegella*, rates of DNA, RNA, and protein synthesis, and growth yield are affected by temperature. Here, we report the T_{critical} of *P. cryopegella* (4°C) and its relation to macromolecular synthesis and growth yield. While RNA, protein, and DNA synthesis rates decreased exponentially with decreasing temperatures, on a per-cell-division basis, RNA and protein synthesis reached a minimum at T_{critical} and DNA synthesis was constant over the entire temperature range. Growth yield peaked at about T_{critical} , with further decreases in temperature resulting in much reduced yields. The

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correlation of high growth yields with low RNA synthesis requirements per cell division was confirmed via examination of the mesophile *Shewanella oneidensis* MR-1.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The isolation and characterization of *P. cryopegella* (previously referred to as *Psychrobacter* sp. 5) from Siberian permafrost has been described (4). *S. oneidensis* MR-1 was provided by K. Nealon. *P. cryopegella* was grown in R2A broth (4) plus 3% NaCl (R3) while *S. oneidensis* was grown in Luria-Bertani Medium (LB) at various temperatures in a VWR model 2005 low-temperature incubator with shaking at 100 rpm (4). To determine growth rate, absorbance at 600 nm was monitored over time using a Spectronic 20D spectrophotometer (Thermo Spectronic, Rochester, N.Y.).

For macromolecular synthesis experiments conducted at temperatures at or above 0°C, *P. cryopegella* or *S. oneidensis* was first grown at the experimental temperature for 1 to 7 days, diluted (1:10) into 18 ml of fresh R3 in 30-ml polycarbonate straight-sided jars (Nalgene), and incubated for an additional 1.5 to 24 h prior to the addition of [³H]leucine or [³H]adenine. For experiments conducted at -4 and -7°C, *P. cryopegella* was grown overnight at 22°C, diluted (1:20) into fresh R3, and incubated for an additional 11 days at -4°C or 6 days at -7°C. Prior to the addition of [³H]leucine or [³H]adenine, 4-ml subsamples were removed to determine optical density at 600 nm and cell numbers by dilution plating. For killed controls, 0.16 g of trichloroacetic acid (TCA) was added.

Growth yield. Triplicate cultures of *P. cryopegella* were grown in 50 ml of LB3 (10 g of glucose, 30 g of NaCl, and 5 g of yeast extract [all per liter] [pH 7.0]) in 250-ml flasks at 20, 16, 10, 4, 0, -4, and -10°C with shaking at 100 rpm. The flasks were inoculated with a loopful of cells, and cultures were collected during exponential phase. Cultures grown at 0, -4, and -10°C were inoculated with 5 ml of an overnight culture grown at 16°C and incubated for 5, 6, and 20 days, respectively. Cell weights and glucose concentrations of inocula were also measured. Cells were collected by centrifugation for 10 min at 10,000 × g and 4°C. Cell pellets were washed with 5 ml of phosphate buffer, recollected by centrifugation, resuspended in 0.5 ml of phosphate buffer, transferred to preweighed Al trays, dried at 65°C for 3 days, and weighed. Supernatants were stored at -20°C and passed through 0.2-μm-pore-size filters prior to analysis using Sigma's glucose (GO) assay kit (GAGO-20).

DNA and RNA synthesis rates. [³H]adenine incorporation rates into DNA and RNA were determined by standard procedures with minor modifications (16). Briefly, [2,8-³H]adenine (specific activity, 24.2 Ci/mmol; Perkin-Elmer) was added to a final concentration of 30 nM and samples were incubated for at least 10% of the expected doubling time at the experimental temperature. Subsamples were taken in duplicate at six or seven time points within this incubation period. At the time points, 1 ml of culture was filtered through a 25-mm-diameter Whatman GF/F filter in a glass filter tower, placed into a 1.5-ml Eppendorf tube, and stored at -80°C. Filters were removed from the freezer, and 1 ml of ice-cold 5% TCA, 1 mg of reagent RNA (baker's yeast; Sigma), and 1 mg of DNA (calf thymus; Sigma) were added. Samples were mixed thoroughly, incubated on ice for 1 h, and spun at 12,000 × g for 1 min, and the supernatant was discarded. Filters were washed three times with 1 ml of ice-cold 5% TCA and three times with 1 ml of ice-cold 95% ethanol. Residual ethanol was evaporated by gentle heating. Filters were mixed with 1 ml of 1 M NaOH, incubated at 37°C for 1 h, incubated on ice for 15 min, mixed with 0.25 ml of 9 M HCl-100% TCA-H₂O (2:1:1), incubated on ice for 15 min, and spun at 12,000 × g for 1 min. For [³H]RNA analysis, 0.625 ml of supernatant was transferred to a clean scintillation vial while the remaining supernatant was discarded. Subsequently, filters were washed two times with 1 ml of ice-cold 5% TCA and two times with ice-cold 95% ethanol. Residual ethanol was evaporated by gentle heating. Filters were mixed with 0.5 ml of 5% TCA, incubated at 95 to 100°C for 30 min, cooled on ice, mixed with 0.5 ml of 1 M NaOH, and spun at 12,000 × g for 1 min. For [³H]DNA analysis, 0.5 ml of supernatant was transferred to a clean scintillation vial containing 0.5 ml of 2 M HCl. Five milliliters of BetaMax Scintillation cocktail (ICN) was added to vials followed by analysis in a Beckman LS6000 scintillation counter for ³H.

Protein synthesis rates. [³H]leucine incorporation rates were determined by standard procedures (17). Briefly, [3,4,5-³H(N)]leucine (specific activity, 170 Ci/mmol; Perkin-Elmer) was added to a final concentration of 4.2 nM. Samples were incubated and subsampled as above. At time points, 1 ml of culture was filtered through a 25-mm-diameter Whatman GF/F filter in a glass filter tower, rinsed twice with 3 ml of ice-cold 5% TCA, and rinsed twice with 3 ml of ice-cold 80% ethanol. Filters were placed into clean scintillation vials and air dried. Five

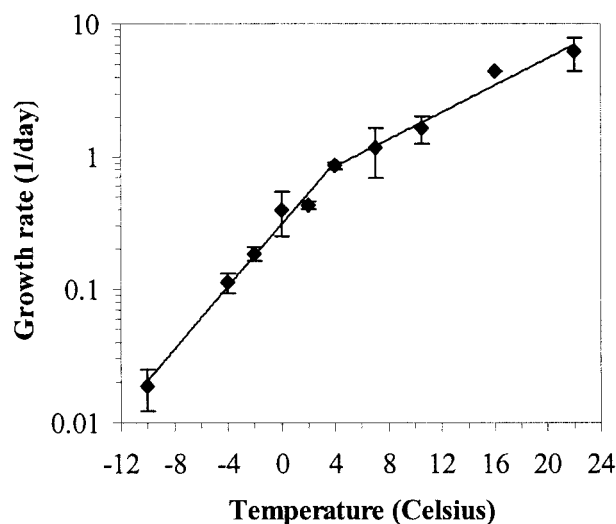


FIG. 1. Temperature dependence of *P. cryopegella* growth rate. Each point represents the average of two to four replicates. Standard deviations are shown. Note that the y axis is a log scale, and lines of best fit are shown.

milliliters of scintillation cocktail was added to the vials, incubated at room temperature for 2 days to maximize dispersion into the cocktail, and analyzed in a Beckman LS6000 scintillation counter as described above.

Calculations and statistical analyses. Specific growth rates (μ) were calculated by determining the slope of the straight-line portion of exponential growth (on a semilog plot). At least six time points over approximately three doublings were used to determine the slope of the line of best fit. The minimum acceptable r^2 value of the lines was 0.8 (average $r^2 = 0.959$). The average growth rate and standard deviation for each temperature were calculated by standard methods. For normalization purposes (see below), the growth rate at -7°C for *P. cryopegella* was determined from the line of best fit ($\mu = 0.308 e^{0.271 T}$, $r^2 = 0.987$) for data from 4 to -10°C.

Growth yield was calculated as follows: $Y_{\text{Glc}} = (m_{\text{cells}} \times \text{MW}_{\text{Glc}}) / [V \times ([\text{Glc}]_{\text{initial}} - [\text{Glc}]_{\text{final}})]$, where m_{cells} is the grams (dry weight) of cells produced, MW_{Glc} is the molecular weight of glucose in grams/mole, $[\text{Glc}]$ is the concentration of glucose in grams/liter, and V is the volume of the culture in liters. The average growth yield and standard deviation at each temperature were calculated by standard methods. Standard t tests were performed to verify the significance of differences in Y_{Glc} between temperatures.

Synthesis rates (k) were calculated from the linear portion of the ³H uptake versus time curve (duplicate samples minus killed controls). The slope and r^2 value for these lines were determined (r^2 ranged from 0.56 to 0.99, with an average of 0.86, for 21 slopes). The standard error of each slope (SE_b) was calculated as follows: $\text{SE}_b = \text{SE}_{Y|X} / \sqrt{\text{SS}_{XX}}$, where $\text{SE}_{Y|X}$ is the standard error of the regression and SS_{XX} is the sum of squares of X . The standard error of regression, $\text{SE}_{Y|X}$, is equal to $\sqrt{[(\text{SS}_{YY} - b \times \text{SS}_{XY}) / (n - 2)]}$, where b is the slope, SS_{YY} is the sum of squares of Y , and SS_{XY} is the sum of the cross-products of X and Y . The sum of squares of X is equal to $\sum (X_i - X_{\text{mean}})^2$ (analogous calculation for SS_{YY}). The sum of the cross-products of X and Y is equal to $\sum (X_i - X_{\text{mean}})(Y_i - Y_{\text{mean}})$.

Normalized rates, k^N , were calculated as follows: $k^N = k/\mu$, where k is the ³H uptake rate in zeptomoles/cell/day and μ is the specific growth rate in divisions/day. The standard deviation of the normalized rate was calculated as follows: $\sigma = k^N \times \sqrt{[(\text{SE}_b/k)^2 + (\sigma_{\mu}/\mu)^2]}$, where SE_b is the standard error of k and σ_{μ} is the standard deviation of the growth rate. Standard t tests were performed to verify the significance of differences in k^N between temperatures.

RESULTS

Growth rates and growth yields. When graphed on a semilog plot (Arrhenius relationship), the temperature dependence of growth rate was linear with two distinct slopes below T_{opt} (Fig. 1). The slope of the linear relationship changed at 4°C, which

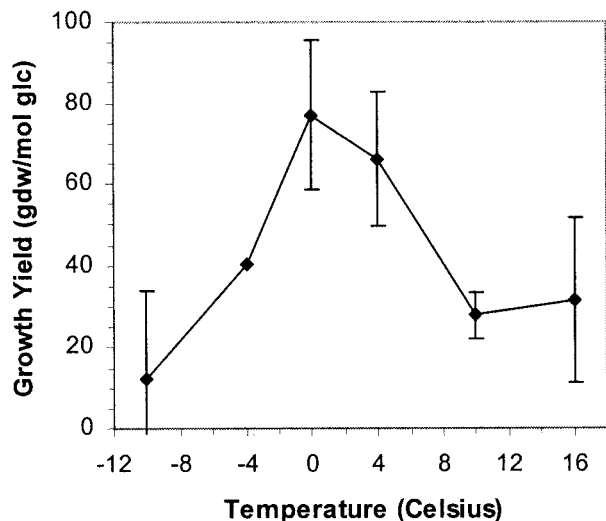


FIG. 2. Temperature dependence of *P. cryopegella* growth yield. Each point represents the average of triplicate samples (except for -10 and -4°C , which represent two and one sample, respectively). Standard deviations are shown.

is identified as the T_{critical} . These two slopes describe two domains of growth: low temperature (-10 to 4°C) and high temperature (4 to 22°C). The slopes of the lines of best fit for growth rate were 0.271 ($r^2 = 0.987$) for the low-temperature domain and 0.117 ($r^2 = 0.954$) for the high-temperature domain. In addition, the temperature dependence of growth yield (grams [dry weight] per mole of glucose) was determined (Fig. 2). Growth yield peaked at 0 to 4°C , or approximately T_{critical} , and declined rapidly as temperature decreased further. t tests confirmed that the reduced yields measured at 16 and -10°C were significantly different from the yields measured at 0 and 4°C ($P < 0.05$).

Macromolecular synthesis rates of *Psychrobacter*. DNA, RNA, and protein synthesis rates (k_{DNA} , k_{RNA} , and k_p , respectively) of *P. cryopegella* were measured from -7 to 16°C . These macromolecular synthesis rates generally followed the Arrhenius relationship (Fig. 3A). However, a deviation from the linear relationship occurred for k_{DNA} at T_{critical} , similar to growth rate. The slopes of the lines of best fit for k_{RNA} and k_p were 0.186 ($r^2 = 0.979$) and 0.194 ($r^2 = 0.991$), respectively. The slopes of the lines of best fit for k_{DNA} were 0.248 ($r^2 = 0.975$) for the low-temperature domain and 0.120 ($r^2 = 0.979$) for the high-temperature domain.

In order to reveal other temperature-dependent processes, synthesis rates were normalized (divided by growth rate), placing synthesis on a per-cell-division basis and removing time from the analysis. Normalized synthesis rates, k^N , in zeptomoles ($1 \text{ zmol} = 10^{-21} \text{ mol}$) of $[^3\text{H}]$ adenine or $[^3\text{H}]$ leucine incorporated per cell division are shown in Fig. 3B. There is no significant change in k_{DNA}^N with temperature (verified via t test, $P > 0.25$). The temperature dependencies of k_{RNA}^N and k_p^N were more complex (Fig. 3B). Both k_{RNA}^N and k_p^N reached a minimum at approximately 4°C and increased above and below 4°C . t tests indicated that increases in both k_{RNA}^N and k_p^N at the two temperature extremes were significant ($P < 0.05$) with respect to values at 4°C . However, values of both k_{RNA}^N and k_p^N at 0 and 7°C were not always significantly different from those at 4°C .

Macromolecular synthesis rates and yield of *S. oneidensis*

MR-1. For comparison, the DNA and RNA synthesis rates (k_{DNA} and k_{RNA} , respectively) and growth rates (μ) of the mesophilic *S. oneidensis* MR-1 were measured from 4 to 22°C (Fig. 4A). Both the DNA synthesis rate and growth rate generally followed the Arrhenius relationship ($k_{\text{DNA}} = 24.8 e^{0.083 T}$; $r^2 = 0.925$; and $\mu = 0.690 e^{0.087 T}$; $r^2 = 0.971$). Normalized respiration rates indicated that T_{critical} was about 12°C for *S. oneidensis* (data not shown); however, no obvious deviations from the linear relationship for k_{DNA} and μ occurred at T_{critical} . Below 18°C , RNA synthesis rates remained constant with temperature.

Normalized DNA and RNA synthesis rates are shown in Fig. 4B. As seen for *P. cryopegella*, there was no significant change in k_{DNA}^N with temperature (verified via t test, $P > 0.10$). The temperature dependence of k_{RNA}^N was more complex (Fig. 4B): k_{RNA}^N was approximately constant from 22 to 12°C and

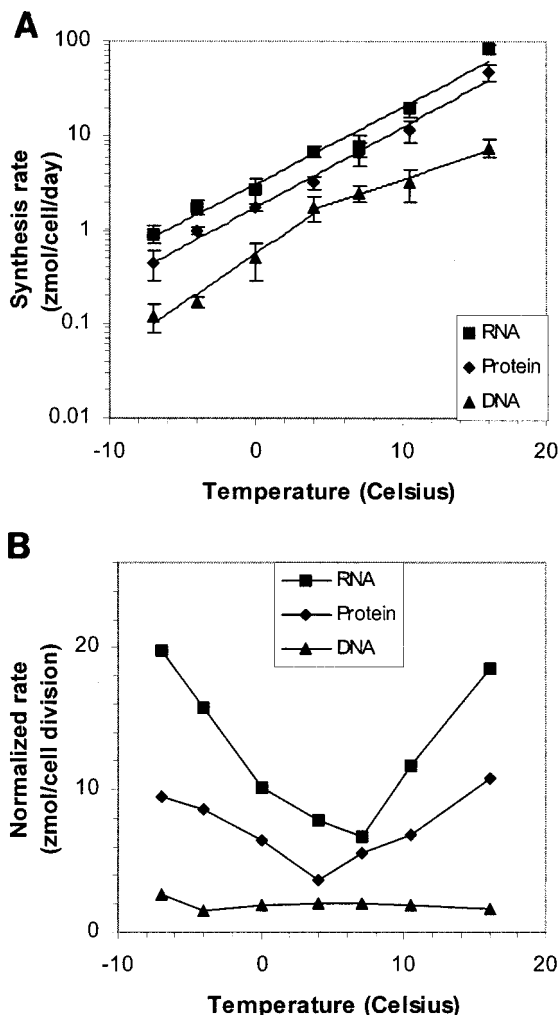


FIG. 3. (A) Temperature dependence of *P. cryopegella* macromolecular synthesis rates. Each point represents the slope of the linear portion of the tritium uptake versus time curve for two samples minus killed controls. Standard errors of the slopes are shown. Note that the y axis is a log scale, and lines of best fit are shown. (B) Temperature dependence of *P. cryopegella* normalized macromolecular synthesis rates. ($1 \text{ zmol} = 10^{-21} \text{ mol}$.)

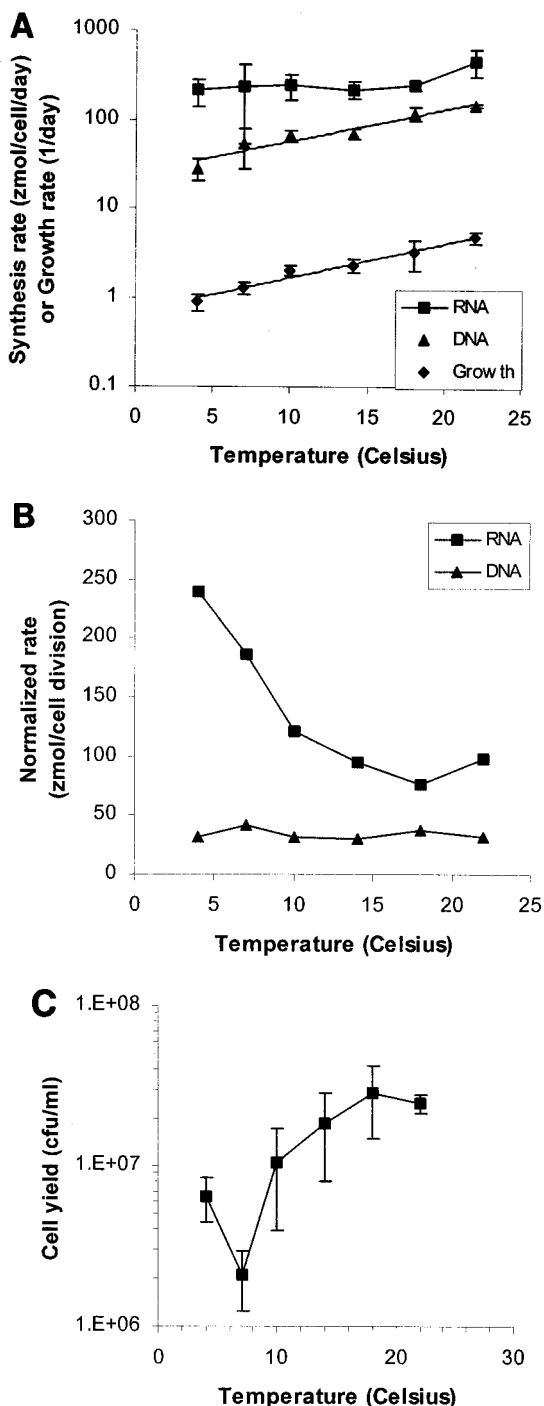


FIG. 4. Analogous data for the mesophile *S. oneidensis* MR-1. (A) Temperature dependence of macromolecular synthesis and growth rates. Each point represents the slope of the linear portion of the tritium uptake versus time curve for two samples minus killed controls (for growth rate, each point represents the average of two to four samples). Lines of best fit are shown for DNA synthesis and growth rates. The standard error (synthesis rates) or standard deviation (growth rate) is shown. Note that the y axis is a log scale. (1 zmol = 10^{-21} mol.) (B) Temperature dependence of normalized macromolecular synthesis rates. (C) Yield versus temperature. Cell yield was measured as the number of cells present at the beginning of stationary phase at each temperature. Each point represents the average of at least three samples; standard deviations are shown.

increased dramatically below 12°C, $T_{critical}$. t tests indicated that the increase in k_{RNA}^N below $T_{critical}$ was significant ($P < 0.05$) with respect to values at temperatures above $T_{critical}$.

The yield of *S. oneidensis* MR-1 was estimated as the number of cells present at early stationary phase during growth in LB at various temperatures (Fig. 4C). Yield was highest and approximately constant from 14 to 22°C; t tests showed no significant difference between cell yields at these temperatures ($P > 0.15$). Below 14°C, yield decreased significantly ($P < 0.05$) as temperature decreased.

DISCUSSION

Organisms that live over a wide range of temperatures must compensate for slow reaction rates at low temperatures to remain competitive. Some psychrophiles maximize or maintain their growth yields at low temperatures (below T_{opt}) to compensate for low growth rates (13, 15, 19). Our data indicate that, similar to the psychrophilic sulfate-reducing bacterium (SRB) LSv514, growth yield of *P. cryopegella* steadily increased as temperature decreased from 16 to 0°C (19). A variety of responses of growth yield to temperature were seen in psychrophilic SRB isolated from Arctic marine sediments. For example, growth yield first increased and then remained constant as T decreased from T_{opt} to 11°C to 0°C, growth yield remained constant from T_{opt} to -1.8°C , and growth yield peaked at T_{opt} and decreased with decreasing T (15, 19). Currently, there is insufficient data to determine whether or not the decrease in growth yield of *P. cryopegella* seen below 0°C and the subsequent maximum growth yield at about $T_{critical}$ is typical of psychrophiles (especially given the variation in trends seen for psychrophilic SRB alone). To date, six of seven psychrophiles examined maximized or maintained growth yield at low temperatures. Whether or not psychrophiles as a class exhibit this behavior is unknown, because the temperature dependence of growth yield in psychrophiles has not been extensively studied nor has the effect of other physical or chemical variables on growth yield. In comparison, the growth yield of mesophiles generally peaks close to the T_{opt} and can remain constant over parts of the growth temperature range where growth rate is constant (1, 15, 20).

We examined the temperature dependence of growth rate and DNA, RNA, and protein synthesis rates to elucidate the basis of $T_{critical}$. In *P. cryopegella*, the temperature dependence of both DNA synthesis rate and growth rate changed at $T_{critical}$. The slopes for both linear domains of k_{DNA} and growth rate were similar, suggesting that these two processes are tightly coupled. It is not likely that a temperature-dependent change in the DNA replication machinery caused $T_{critical}$ because no such change has ever been reported, nor has the control of growth rate by DNA synthesis. Cell reproduction probably slowed for other reasons, causing the growth rate and DNA synthesis rate to be regulated accordingly. The temperature dependence of protein and RNA synthesis rates in *P. cryopegella* did not change at $T_{critical}$, indicating that its ribosomes and RNA polymerases remained functional at temperatures below $T_{critical}$, as has been demonstrated for other psychrophiles (32–34). (Note: protein and RNA elongation rates are temperature dependent, while the numbers of ribosomes and RNA polymerases remain constant with temperature [9, 12, 30]). In

contrast, when grown at the same temperature but at different growth rates, bacteria alter their protein synthetic capacity by changing the number of ribosomes (21, 24). Genotypic adaptation of ribosomes to low temperatures has been demonstrated in cold-tolerant strains of *Bacillus cereus* that were shown to substitute A and T for G and C, when compared to mesophilic strains (26). Conversely, in mesophiles the temperature dependence of protein synthesis rates changes at T_{critical} , reflecting the inability of mesophilic ribosomes to function at low temperatures (7, 9, 32–34).

Subsequently, we normalized synthesis rates in order to reveal temperature-dependent processes that were masked by the primary effect of decreasing temperature (exponential decrease in reaction rates). Normalized rates allowed examination of synthesis requirements on a per-cell-division basis rather than time. There was no significant change in k_{DNA}^N with temperature in *P. cryopegella*, because the same DNA synthesis requirement exists for each cell division. Specifically, one genome must be synthesized per cell division. Both k_{RNA}^N and k_P^N were at a minimum at T_{critical} , indicating that cell reproduction required the least amount of RNA and protein synthesis at this temperature. Conversely, cell reproduction at temperatures above and below T_{critical} required the synthesis of additional RNA and proteins (or higher levels of the same RNA and proteins). Indeed, temperatures of 10°C and below induced the expression of both cold acclimation proteins (CAPs) and cold shock proteins (CSPs) in psychrophilic bacteria (7, 8, 14, 27, 36). Furthermore, as temperature decreased, more CAPs and CSPs are produced and expressed at higher levels (14, 27, 36). To date, cold response studies have examined 5°C temperature increments and found that expression of CAPs and CSPs changed at all increments (14, 27, 36). This study examined 3 to 6°C increments, and statistically significant changes in RNA and protein synthesis rates were seen between most of the increments. However, how these data relate to production of CAPs and CSPs is unclear. Above T_{critical} , the k_{RNA}^N and k_P^N may increase due to the production of additional proteins and/or increased protein turnover caused by higher rates of denaturation and degradation (9, 11, 25, 38). It is striking that cell reproduction required the least amount of RNA and protein synthesis at T_{critical} and that growth yield peaked at T_{critical} .

While this study focused on T_{critical} in psychrophiles, the synthesis rates, normalized rates, and growth yield were examined in the mesophile *S. oneidensis* MR-1 for comparison. The growth yield of *S. oneidensis* MR-1 displayed thermal characteristics typical of mesophiles (yield peaked at high temperatures). Likewise, DNA synthesis rates and growth rates decreased with T according to the Arrhenius relationship. Unlike most mesophiles however, *S. oneidensis* MR-1 maintained RNA synthesis rates from 18 to 4°C. Regardless of this ability, the normalized RNA synthesis rate of *S. oneidensis* MR-1 also changed at T_{critical} , as seen in *P. cryopegella*. In addition, minimal normalized RNA synthesis rates corresponded to temperatures with the highest yields. Therefore, *S. oneidensis* MR-1 grew most efficiently (that is, it minimized RNA synthesis requirements for cell division) at temperatures above T_{critical} , while *P. cryopegella* did so only at T_{critical} .

In summary, the most beneficial survival strategy for psychrophiles may be to maximize growth yield, not growth rates, at

low temperatures. *P. cryopegella* accomplished this by streamlining growth processes (cell reproduction required the synthesis of the least amount of protein and RNA) at a fairly low temperature (4°C, T_{critical}). In addition, we demonstrated that at T_{critical} growth of *P. cryopegella* was separated into two domains and, previously, that below T_{critical} the respiration requirement for cell reproduction increased dramatically (4). Identifying the cell processes that lead to T_{critical} is vital to understanding low-temperature growth. Prior to this study, no basic mechanisms involved in maximizing or maintaining growth yields at low temperatures had been identified. This type of growth strategy may be an essential part of being able to live over a wide range of temperatures. While this study presented one approach to define the basis of T_{critical} , it remains to be determined whether or not T_{critical} is a pivotal temperature in psychrophiles with respect to global stress response.

ACKNOWLEDGMENTS

This work was supported by a NASA Astrobiology Institute (NAI) grant to K.H.N.

Thanks to M. Thomashow and J. Tiedje for facilitating the completion of experiments at Michigan State University that were begun at the University of Southern California.

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