Vol. 66, No. 6

Effect of Temperature and Salinity Stress on Growth and Lipid Composition of *Shewanella gelidimarina*

DAVID S. NICHOLS,[1]* JUNE OLLEY,[1] HORACIO GARDA,[2] RODOLFO R. BRENNER,[2] AND TOM A. MCMEEKIN[1,3]

School of Agricultural Science[1] and Antarctic CRC,[3] University of Tasmania, Hobart, Tasmania 7001, Australia; and Instituto de Investigaciones Bioquimicas de la Plata, UNLP-CONICET, Facultad de Ciencias Médicas, La Plata, Argentina[2]

The maximum growth temperature, the optimal growth temperature, and the estimated normal physiological range for growth of *Shewanella gelidimarina* are functions of water activity (a_w), which can be manipulated by changing the concentration of sodium chloride. The growth temperatures at the boundaries of the normal physiological range for growth were characterized by increased variability in fatty acid composition. Under hyper- and hypoosmotic stress conditions at an a_w of 0.993 (1.0% [wt/vol] NaCl) and at an a_w of 0.977 (4.0% [wt/vol] NaCl) the proportion of certain fatty acids (monounsaturated and branched-chain fatty acids) was highly regulated and was inversely related to the growth rate over the entire temperature range. The physical states of lipids extracted from samples grown at stressful a_w values at the boundaries of the normal physiological range exhibited no abrupt gel-liquid phase transitions when the lipids were analyzed as liposomes. Lipid packing and adaptational fatty acid composition responses are clearly influenced by differences in the temperature-salinity regime, which are reflected in overall cell function characteristics, such as the growth rate and the normal physiological range for growth.

Antarctic sea ice is characterized by the presence of a unique bacterial community that is dominated by psychrophilic bacteria (2, 3, 12). Nichols et al. (25) have discussed the potential role of the temperature-salinity regime in selecting psychrophilic bacteria in sea ice. It has been suggested that understanding the physiological response of psychrophilic bacteria to combined temperature-salinity stress is very important for understanding the bacterial sea ice community. The importance of fluctuations in salinity that affect the composition of microbial populations in estuarine and brackish water ecosystems has been recognized (7, 30), and such fluctuations are very important when the survival and viability of psychrophilic marine bacteria are considered (12, 20, 21, 34). Changes in salinity similar to those observed in estuarine and brackish water environments also occur in Antarctic coastal waters and sea ice and are associated with the annual ice formation and melting cycle. However, researchers have attempted to relate changes in physicochemical parameters to a physiological mechanism for growth in only a few studies of psychrophilic bacteria (8, 11, 21, 26, 35).

Workers have proposed a number of models to describe the effect of temperature and/or salinity on bacterial growth. Most of these models are empirical and seek solely to summarize observations of bacterial growth under various conditions. Many are based on the Arrhenius equation and utilize Arrhenius kinetics to describe bacterial growth in response to temperature. The concept of a normal physiological range (NPR) for bacterial growth is derived from modelling of the bacterial growth rate by using Arrhenius models. Over a defined range of temperature, the growth rates of all bacteria obey Arrhenius kinetics, and this temperature range is designated the NPR. The boundaries of the NPR are defined at both high and low temperatures by deviations in the bacterial growth rate response from Arrhenius kinetics (22). Deviations in the bacterial growth rate outside this range have been explained by the postulated inactivation of one or more enzymatic processes essential for growth, although no single mechanism has been discovered yet (22).

In this study a temperature gradient incubator was used to investigate the effects of temperature-salinity regimes on the growth, lipid phase, and fatty acid composition of the psychrophilic sea ice bacterium *Shewanella gelidimarina*. Square root type models were used to establish limits for growth, while an Arrhenius model was used to estimate the NPR.

MATERIALS AND METHODS

Bacterial strains. *S. gelidimarina* ACAM 456[T] was first described by Nichols et al. (24) and was fully described by Bowman et al. (4). *S. gelidimarina* was maintained on slopes of Zobell's agar (42) and was cultured in Zobell's broth prior to experiments.

Temperature gradient incubator experiments. The effect of salinity on the rate of growth of *S. gelidimarina* at 20°C was determined by using a temperature gradient incubator (Toyo Kagaku Sangyo, Tokyo, Japan). The growth medium used was Zobell's broth; however, it contained distilled water rather than seawater. Sixteen incubation tubes were prepared with water activity (a_w) values of 1.00 to 0.892 (0 to 15% [wt/vol] NaCl) by adding NaCl to individual tubes. One incubation tube was also prepared by using nominal seawater salinity (3.5%, wt/vol), and another tube was prepared with natural seawater (a_w, 0.982). The tubes were inoculated with an actively growing culture, and growth was monitored by measuring percent transmittance at 540 nm until growth was complete. Cultures were shaken via oscillatory motion of the incubator through a 60° arc at a rate of 60 oscillations min^{-1}. Growth rates at each temperature were calculated by fitting a modified Gompertz function to the data (18). The growth temperature for each incubation tube was determined in triplicate by using a Fluke model 51 K/J thermometer and a Fluke model 80PK-1 thermocouple after growth ceased. The calculated growth rate data were then fitted to the modified four-parameter square root model of Miles et al. (19) for use with a_w data by using UltraFit software.

The effect of temperature on the rate of growth of *S. gelidimarina* was determined by using a temperature gradient incubator. Preparations (20 ml) were incubated at temperatures ranging from −2 to 28°C at approximately 1°C intervals. Modified Zobell's broth was prepared with a_w values of 0.993 (1.0% [wt/vol] NaCl), 0.986 (2.5% [wt/vol] NaCl), and 0.977 (4.0% [wt/vol] NaCl). Tubes were inoculated with an actively growing culture, and growth was monitored as de-

* Corresponding author. Mailing address: School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tasmania 7001, Australia. Phone: 61 3 62 261831. Fax: 61 3 62 262642. E-mail: D .Nichols@utas.edu.au.

scribed above. The calculated square roots of growth rate data were then fitted to the model of Ratkowsky et al. (29) by using UltraFit software. Growth rate data were also fitted to the model of Rosso et al. (32) in order to estimate the optimal growth temperature (T_{opt}). The latter model contains T_{opt} as a parameter, and thus standard errors can be determined. The two models were found to give similar results for other parameters. Significance values were generated by performing a Student's t test.

Sampling and harvesting of cultures. The study to determine the effect of temperature on the rate of growth of *S. gelidimarina* at two stress-inducing salinities was repeated, and growth was monitored to ensure that the previous experimental conditions were replicated. When individual incubation tubes reached percent transmittance values of 27 to 30, corresponding to the mid-exponential phase, the cultures were immediately harvested by filtration onto glass fiber filters (Whatman) which previously had been heated at 400°C for 24 h. Sample filters were then frozen at −20°C until lipids were analyzed. The initial fatty acid composition data set was used as a guide for choosing lipid phase transition samples. Temperatures of 15.0 and 4.0°C (a_w, 0.993) and 13.5 and 5.0°C (a_w, 0.977) were selected based on abrupt changes in the ratio of branched-chain fatty acids to monounsaturated fatty acids. Due to the amount of lipid required, samples used for lipid phase transition measurements were grown in duplicate 100-ml portions of appropriate-salinity media at these temperatures. The cultures were harvested by centrifugation at 8,750 × g for 15 min, and each cell mass was washed in an isotonic saline solution. The cell mass that was collected was extracted directly as described below.

Lipid extraction and analysis. Filters were extracted by using a direct trans-esterification procedure (6) that yielded fatty acid profiles comparable to those obtained with the solvent extraction method of Bligh and Dyer (1, 41). Briefly, filters were placed in screw cap test tubes containing 3 ml of methanol-chloro-form-hydrochloric acid (10:1:1, vol/vol/vol). The tubes were heated at 80°C for 1 h and then cooled to room temperature. After Milli-Q water (1 ml) was added, the resultant fatty acid methyl esters were extracted three times with 1.5 ml of hexane-chloroform (4:1, vol/vol). Duplicate samples of biomass which was used for lipid phase analysis were extracted directly by using the method of Bligh and Dyer to obtain a total solvent extract. One sample was stored in chloroform prior to analysis as described below, and the other sample was transesterified as described above for filter samples in order to correlate the results with the gradient incubator results.

Fatty acid methyl esters were analyzed by using a Hewlett-Packard model 5890 II gas chromatograph and a model 5970A mass selective detector equipped with a cross-linked methyl silicone (film thickness, 0.33 μm) fused-silica capillary column (internal diameter, 0.22 mm; length, 50 m). The operating conditions were similar to those described by Nichols et al. (23). Fatty acid methyl esters in all of the samples were identified by comparing the component spectra to spectra of known standards. Double bond positions and the geometry of monounsatu-rated isomers of selected samples were determined by producing and analyzing dimethyl disulfide adducts (27). The data presented below are the sums of the values for individual fatty acid methyl esters identified in each category.

Lipid phase transition measurements. Solvent was evaporated from the total solvent extract material (0.2 mg), and 0.5 ml of buffer A (0.1 M Tris-HCl, 0.16 M NaCl; pH 8.0) was added. After 15 min of incubation at room temperature, samples were vortexed for 2 min, and suspensions were extruded through a 100-nm-pore-size filter by using a Liposofast extruder (Anestin Inc., Ottawa, Ontario, Canada). Extruded liposomes (0.5 ml) were mixed with 0.5 ml of a 2 μM fluorescent probe suspension (1,6-diphenyl-1,3,5-hexatriene [DPH] or 6-lauroyl-2,2-dimethylaminoaphthalene [laurdan]) in buffer A, and the preparation was vortexed before it was incubated at room temperature for 30 min. Samples were diluted 2.5-fold with buffer A prior to analysis. All data were obtained by using a model SLM 4800 spectrofluorometer and cuvettes (1 by 1 cm). The steady-state fluorescence anisotrophy (r_S), lifetime (τ), and differential polarized phase lifetime ($\Delta\tau$) of DPH were measured by using an excitation wavelength of 361 nm and observing the total emission at wavelengths of >389 nm through a sharp cutoff filter (type KV389). For τ and $\Delta\tau$ measurements the exciting light was modulated sinusoidally in amplitude at 18 or 30 MHz with a Debye-Sears modulator and was polarized with a Glan-Thompson polarizer. For τ measurements, emission was observed through a Glan-Thompson polarizer oriented 55° to the vertical. The phase shift and demodulation of the emitted light were measured relative to 1,4-bis(5-phenyloxazol-2-yl)benzene in ethanol and were used to compute the phase and modulation lifetimes. $\Delta\tau$ was determined from the phase shift between the parallel and perpendicular components of the emission observed between vertically and horizontally oriented emission polarizers. Data were interpreted by using the hindered wobbling rotation model (16, 40). The rotational correlation time (τ_R) and the limiting anisotropy (r_∞) were calculated as described previously (9).

Laurdan fluorescence intensity and generalized polarization (GP) spectra were determined as described by Garda et al. (10). All spectra were corrected for any background signal by subtracting the signal obtained with unlabelled samples. An excitation wavelength of 360 nm (emission intensity spectra) and an emission wavelength of 430 nm (excitation intensity spectra) were used. GP spectra were determined by determining the excitation intensity spectra at 440 nm (I_{440}) and 490 nm (I_{490}) for emission and the emission intensity spectra at excitation wavelengths of 340 nm (I_{340}) and 410 nm (I_{410}). GP data for the
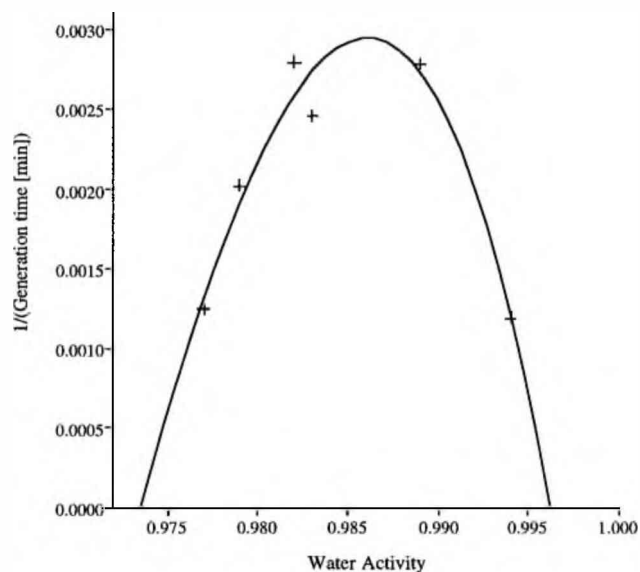

FIG. 1. Plot of growth rate (1/Generation time [min]) versus a_w for *S. gelidimarina* grown in Zobell's broth containing NaCl at different concentrations. The growth temperature was 20°C.

excitation and emission bands were obtained by using the (I_{440} − I_{490})/(I_{440} + I_{490}) and (I_{410} − I_{340})/(I_{410} + I_{340}) ratios, respectively.

RESULTS

Growth rate. *S. gelidimarina* grew at a narrow range of salinities, which is characteristic of marine bacteria (Fig. 1). The minimum a_w for growth was 0.974 ± 0.004 (4.6% [wt/vol] NaCl). This bacterium required NaCl for growth, and the maximum a_w was 0.996 ± 0.002 (0.7% [wt/vol] NaCl). No growth was detected in 10 of the 16 incubation tubes in which the a_w values were outside this range during the experiment. The optimum a_w for growth under experimental conditions was 0.986 (2.5% [wt/vol] NaCl).

The response of the growth rate of *S. gelidimarina* to temperature depended on the salinity (Fig. 2 and Table 1). The maximum growth temperatures (T_{max}) of *S. gelidimarina* at the three a_w values tested were significantly different ($P < 0.001$) from each other; higher T_{max} values were observed at lower a_w values. The T_{opt} values were also significantly different ($P < 0.001$) from each other, and the highest T_{opt} was observed at an a_w of 0.986. The theoretical minimum growth temperatures (T_{min}) under different conditions were not significantly different ($P > 0.05$) (Table 1). However, a significant increase ($P < 0.05$) in the theoretical growth range was observed when the data for an a_w of 0.986 and an a_w of 0.977 were compared due to an increase in the T_{max} under low-a_w conditions.

Fatty acid composition. The fatty acid compositions of *S. gelidimarina* cultures grown at a_w values of 0.993 and 0.977 at all of the temperatures at which growth occurred are shown in Fig. 3. The total values are the sum of values for the following major components: saturated fatty acids (12:0, 13:0, 14:0, 15:0, 16:0, 17:0, and 18:0), monounsaturated fatty acids (14:1ω5c, 15:1ω6c, 15:1ω8c, 16:1ω7c, 17:1ω8c, 18:1ω7c, and 18:1ω9c), branched-chain fatty acids (i13:0, i14:0, and i15:0), and polyunsaturated fatty acids (PUFA) (18:4ω3, 20:4ω3, and 20:5ω3). There were generally higher proportions of saturated and monounsaturated fatty acids in samples grown at an a_w of 0.977, while higher proportions of branched-chain fatty acids and PUFA were present in samples grown at an a_w of 0.993.
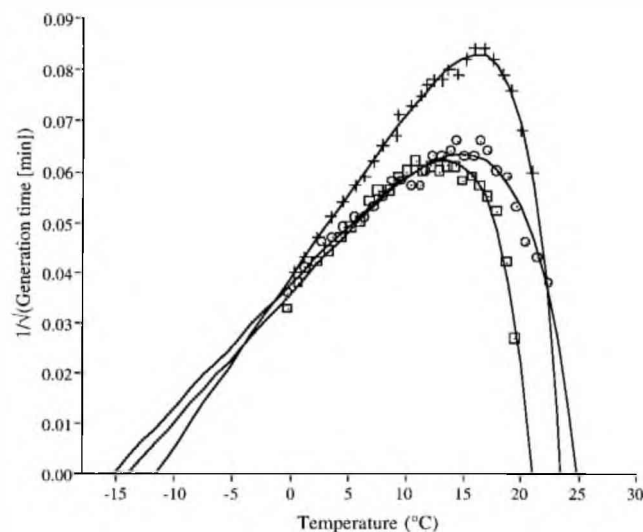
FIG. 2. Plots of growth rate (1/$\sqrt{\text{Generation time [min]}}$) versus temperature for *S. gelidimarina* grown in Zobell's broth at three a_w values. Symbols: $\square$, a_w of 0.993 (1.0% [wt/vol] NaCl); +, a_w of 0.986 (2.5% [wt/vol] NaCl); $\odot$, a_w of 0.977 (4.0% [wt/vol] NaCl).

The percentage of saturated fatty acids increased linearly as the temperature increased at both a_w values, while the proportions of monounsaturated components revealed the opposite trend (Fig. 3). For both a_w values the proportion of branched-chain fatty acids responded to temperature in a parabolic manner centered around a temperature range (ca. 9 to 11°C for an a_w of 0.993; 10 to 12°C for an a_w of 0.977) slightly below the T_{opt} (Fig. 3 and Table 1). A constant percentage of PUFA was present at both a_w values until the temperature was around T_{opt}.

Of note were the distinct areas of compositional variability in the fatty acid temperature profiles at both a_w values. At an a_w of 0.977 large increases in the standard deviations were apparent for the values for monounsaturated fatty acids, branched-chain fatty acids, and PUFA at temperatures from 4 to 6°C and at 13°C (Fig. 3); this was clearly shown by the variance in the proportions of branched-chain and monounsaturated fatty acids (Fig. 4). At an a_w of 0.993 the two data sets for the pre-T_{opt} range (9 to 10°C) (Fig. 3) implied that there was a similar level of increased variability.

NPR. Estimates of the NPR were obtained from the linear portions of the Arrhenius plots, as shown in Fig. 5 (for an a_w of 0.993, ca. 1 to 12°C; for an a_w of 0.977 ca. 2 to 15°C). The percentage of monounsaturated fatty acids plus branched-chain fatty acids was inversely related to the Arrhenius curve at both salinities. The minimum proportion of monounsaturated fatty acids plus branched-chain fatty acids observed occurred at the temperature at which the growth rate was maximal, T_{opt}.

The temperatures at which fatty acid compositional variability occurred at both an a_w of 0.993 and an a_w of 0.977 (Fig. 3 and 4) corresponded to sample temperatures that were immediately inside the boundaries of the NPR (e.g., for an a_w of 0.977, variable region at 4 to 6°C and boundary at 2°C and variable region at 13°C and boundary at 15°C) (Fig. 4 and 5). When the proportions of branched-chain and monounsaturated fatty acids were added (Fig. 4), the variability in the data for the temperature ranges shown in Fig. 3 decreased markedly for the data obtained at an a_w of 0.977.

Lipid phase transition. Large (100-ml) cultures of *S. gelidimarina* were grown under the conditions described above that produced perturbations in the total fatty acid composition for the lipid phase analyses (at an a_w of 0.993, 4.0 and 15.0°C; at an a_w of 0.977, 5.0 and 13.5°C). While the increased culture volume resulted in minor changes in the fatty acid composition, the overall trends apparent from the gradient incubator sample data were supported (data not shown). The temperature dependence of the DPH r_S for the lipids in samples was determined at 1 to 24°C (Fig. 6). No abrupt change in slope was observed, indicating that there was no abrupt phase transition in this temperature range. Liposomes from cultures grown at low temperatures also exhibited lower r_S values, and the slopes were not altered by the a_w. However, the growth temperature did have a significant effect on liposome lipid properties. The r_∞ values indicated that the lipids from *S. gelidimarina* cells grown at higher temperatures were more ordered than the lipids from cells grown at a low temperature (Table 2). An increase τ_R was observed for the lipids from samples grown at 15°C compared with the lipids from samples grown at 4°C at an a_w of 0.993. However, the τ_R of lipids from samples grown at 13.5°C was less than the τ_R of lipids from samples grown at 5°C at an a_w of 0.977 (data not shown).

The emission spectra of laurdan in the liposomes obtained from *S. gelidimarina* total lipid extracts predominantly exhibited a blue band at ~435 nm (Fig. 6), which is characteristic of lipid bilayers in the gel state or in the liquid ordered state normally found in membranes containing cholesterol (28). However, a relaxed emission shoulder at ~490 nm, which is characteristic of the liquid state, was also present. The shoulder was less pronounced when we examined the lipids from samples grown at higher temperatures (13.5 and 15°C), indicating that there was a higher degree of lipid packing in liposomes in these samples than in samples grown at the lower temperatures (4.5 and 5°C). The degree of relaxed emission from the lipids from samples grown at low temperatures also increased as the analysis temperature increased. These spectral changes could be quantified by using the GP parameter, which related the relative fluorescence intensities at the two wavelengths. Lower GP values indicated that the degree of probe mobility was higher or the degree of lipid packing was lower (Table 3). The wavelength dependence of the GP parameter on the excitation and emission bands at 4.5 and 14.3°C is shown in Fig. 6. The GP values for all of the samples decreased as the

TABLE 1. Cardinal growth temperatures for *S. gelidimarina* grown at different a_w values

a_w	Salinity (%, wt/vol)	T_{min} (°C)	T_{opt} (°C)[a]	T_{max} (°C)	Theoretical growth temp range (°C)[b]	Generation time at T_{opt} (min)
0.993	1.0	-14.0 ± 1.4[c]	13.4 ± 0.2[c]	21.0 ± 0.2[c]	35.6 ± 1.6[c]	259
0.986	2.5	-11.6 ± 0.5	16.1 ± 0.1	23.5 ± 0.2	35.1 ± 0.7	146
0.977	4.0	-15.3 ± 1.9	14.8 ± 0.3	24.9 ± 0.3	39.3 ± 1.8	251

[a] Determined by using the model of Rosso et al. (32).
[b] T_{min} to T_{max}.
[c] Fitted value $\pm$ standard error of the fitted value. The values were determined by using the fitted models shown in Fig. 2.
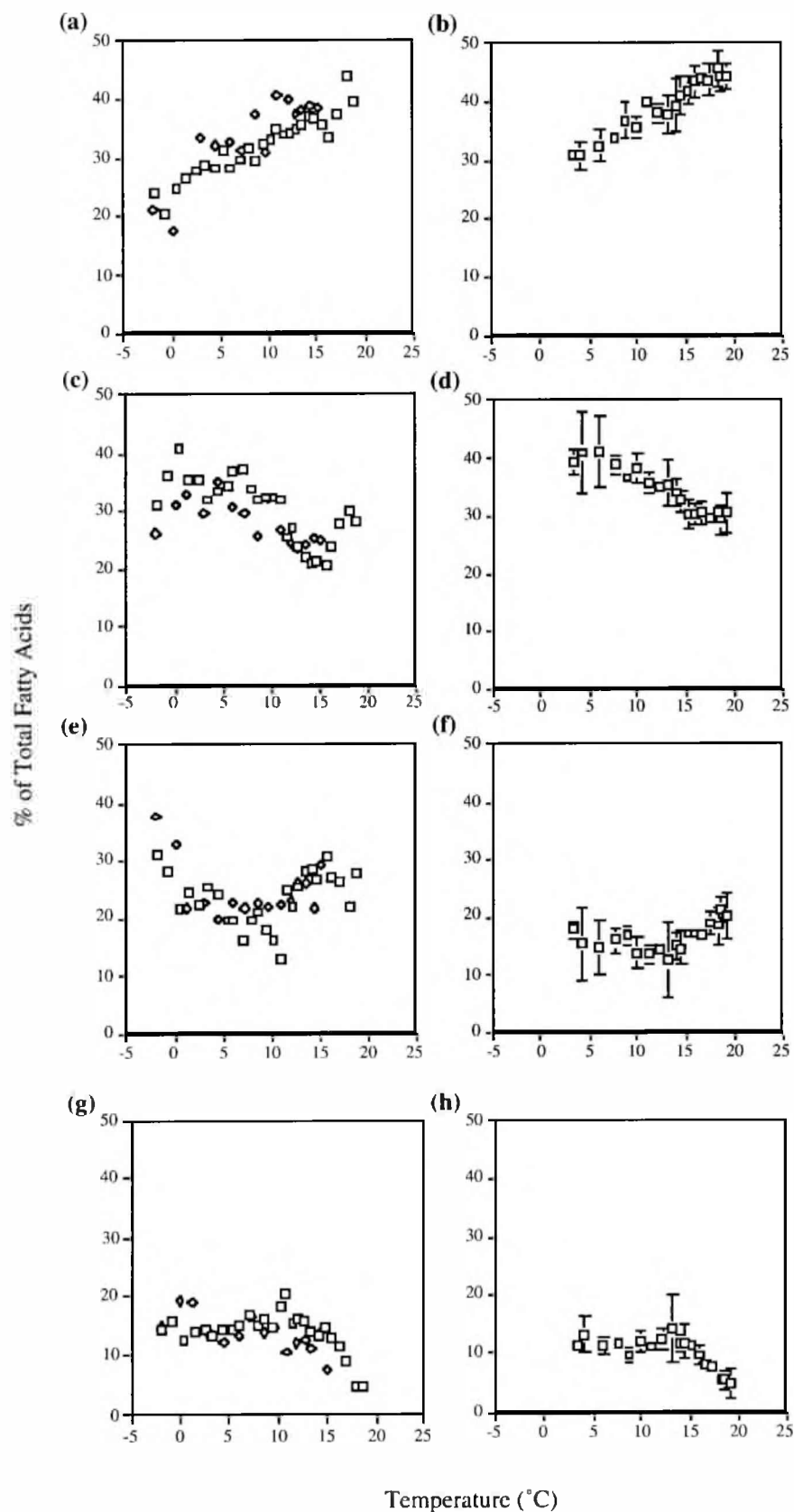
% of Total Fatty Acids

Temperature (°C)

FIG. 3. Plots of *S. gelidimarina* fatty acid compositions for the entire biokinetic temperature range at an a_w of 0.993 (1.0% [wt/vol] NaCl) for two data sets (□ and ◇) and at an a_w of 0.977 (4.0% [wt/vol] NaCl). The data points for a_w 0.977 represent means based on values in three data sets, and standard deviations are indicated by error bars. (a and b) Total saturated fatty acids at an a_w of 0.993 (a) or 0.977 (b). (c and d) Total monounsaturated fatty acids at an a_w of 0.993 (c) or 0.977 (d). (e and f) Total branched-chain fatty acids at an a_w of 0.993 (e) or 0.977 (f) (g and h). Total polyunsaturated fatty acids at an a_w of 0.993 (g) or 0.977 (h).
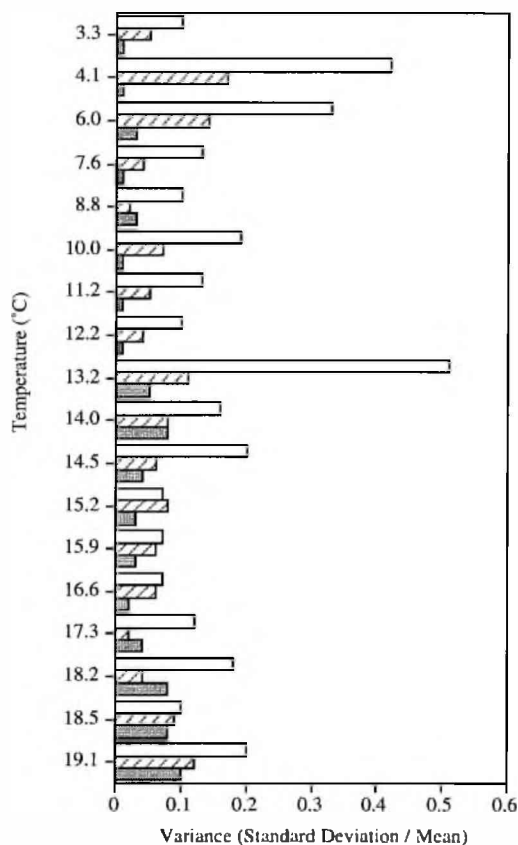
FIG. 4. Variances in the percentages of branched-chain and monounsaturated fatty acids for the entire biokinetic temperature range for *S. gelidimarina* grown at an a_w of 0.977, based on data from three data sets. Open bars, branched-chain components; cross-hatched bars, monounsaturated components; shaded bars, branched-chain and monounsaturated components.

wavelength along the excitation band increased and increased as the wavelength along the emission band increased. This pattern is characteristic of lipids in the liquid crystalline or liquid ordered state and not the gel state (28). It is therefore probable that a large proportion of the lipid domains in the liposomes from all of the samples had characteristics of the liquid ordered state and that a small proportion of liquid crystalline lipid domains was also present.

DISCUSSION

S. gelidimarina exhibited a narrow salinity range for growth, which is typical of marine bacteria. T_{max} was also a function of salinity. This phenomenon has been observed for other marine bacteria, including *Moritella marinus* (21) and *Vibrio anguillarum* (11). However, the major effect of high salinity on the growth temperature range was evident only when the temperature was greater than T_{opt}, and salinity had little effect on the growth rate at low temperatures (<10°C) or on T_{min}. It should be stressed that the square root models used to fit cardinal temperatures in this study yield estimates of these parameters that are more accurate than the estimates obtained from Arrhenius plots, which tend to be curvilinear at suboptimal growth temperatures in the region up to 18°C greater than T_{min} (18). However, as shown in Fig. 5, when growth of the bacterium does not extend significantly into this suboptimal temperature region, the natural log of growth rate (ln k) remains directly proportional to the reciprocal of absolute temperature

(1/temperature (K) within the NPR. Using an Arrhenius plot to estimate the NPR for growth of psychrophilic bacteria is therefore appropriate.

An increase in salinity was accompanied by an increase in the proportion of saturated fatty acids in *S. gelidimarina*, which correlated with a higher degree of lipid order at temperatures greater than 10°C in liposomes, compared to liposomes from cells grown at a low salinity. The liposomes from cells grown at a high salinity and a high temperature exhibited greater retention of lipid packing with increased temperature than the corresponding low-salinity liposomes (Fig. 6). Concurrently, the
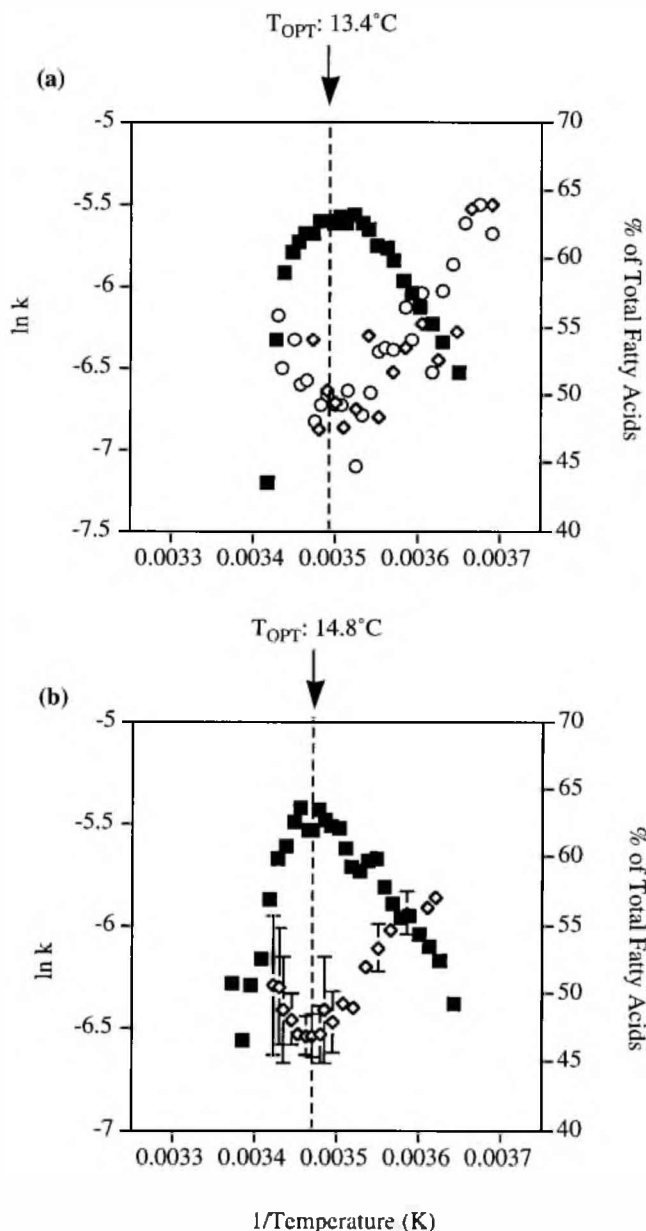

FIG. 5. Arrhenius plots of growth rate (■) versus temperature for *S. gelidimarina* at two salinities. Also shown are data for the percentage of total monounsaturated and branched-chain fatty acids versus temperature. (a) Growth at an a_w of 0.993 (1.0% [wt/vol] NaCl). The data from two fatty acid data sets are indicated by different symbols (○ and ◇). (b) Growth at an a_w of 0.977 (4.0% [wt/vol] NaCl). ◇, means based on three fatty acid data sets. The error bars indicate standard deviations.

(a)

(b)

(c)

high-salinity, low-temperature liposomes exhibited greater retention of lipid packing when they were analyzed at a high temperature, while the high-salinity, high-temperature liposomes also exhibited a greater increase in packing when they were analyzed at a low temperature (Table 3). These findings imply that growth at a high salinity resulted in a lipid composition that could increase membrane packing in liposomes and a tendency to retain a higher degree of packing at high temperatures. This effect was correlated with the ability of cells to withstand higher growth temperatures without a detrimental effect on low-temperature growth. The implication is that a loss of lipid packing in the native membrane may have been the limiting factor for the growth of *S. gelidimarina* at high temperatures, while a more tightly packed membrane did not inhibit growth at low temperatures.

The bulk salinity of Antarctic sea ice has been reported to range from 0.4 to 0.9% in November and December (33), the spring-summer period when maximal heterotrophic activity occurs (14). At this time brine salinities are likely to be low due to the flushing effect of ice melt and saline depletion resulting from the preceding period of high-salinity brine drainage over winter (12). Thus, *S. gelidimarina* probably experiences low-salinity conditions during the period when maximal metabolic activity occurs. While the maximal growth rate apparently is not adapted to this environmental characteristic, the estimated NPR is shifted to lower temperatures at an a_w of 0.993. The generally higher proportion of branched-chain fatty acids and PUFA in samples at an a_w of 0.993 is also correlated with greater stability of physical lipid characteristics at low salinity values. These results suggest that there may be preferential membrane adaptation to low-salinity conditions. However, the similarity of the fatty acid profiles suggests that similar adaptive strategies with respect to temperature occur at high and low salinity values.

Suutari and Laakso (36) and Suutari et al. (37) studied changes in the fatty acid compositions of two bacilli and a yeast, respectively, over their characteristic temperature ranges for growth. A close temperature sampling regime, such as the one used in this study, revealed similar trends in the percentages of certain fatty acid components, which were species specific, within narrow temperature ranges near the limits for growth. The results of these studies foreshadowed the results of the present study. However, in neither study did the researchers replicate the data sets to assess compositional variability, nor did they consider the correlation of fatty acid composition with the NPR of the organisms studied.

When *S. gelidimarina* was studied, there were two regions where there was increased variation in fatty acid composition over the growth temperature range at an a_w of 0.977, as shown by the increased standard deviation of data points at 4 to 6°C and at 13°C (Fig. 3 and 4). Similar variation was implied by the scatter of data points in the two data sets obtained at an a_w of 0.993. The temperature at which the variations occurred was 2°C inside the estimated boundaries of the NPR at both high and low temperatures. Furthermore, the percentage of total monounsaturated and branched-chain fatty acids was closely correlated with the response of the growth rate to temperature, and there was little variation in the three data sets, in contrast
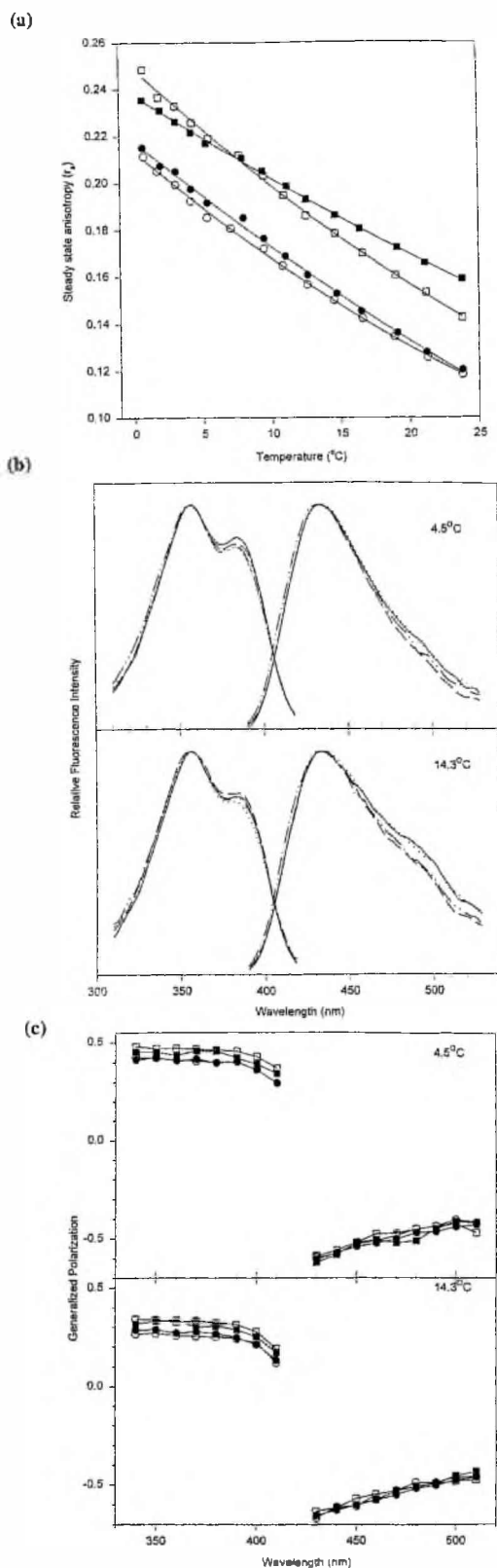
FIG. 6. (a) Temperature dependence of the r_S of DPH in liposomes made with total lipid extracts from *S. gelidimarina* grown under the following conditions: a_w of 0.993 (1.0% [wt/vol] NaCl) and 4.0°C (○), a_w of 0.993 (1.0% [wt/vol] NaCl) and 15.0°C (□), a_w of 0.977 (4.0% [wt/vol] NaCl) and 5.0°C (●), and a_w of 0.977 (4.0% [wt/vol] NaCl) and 13.5°C (■). (b) Fluorescence excitation and emission spectra of laurdan in liposomes grown under the following conditions: a_w of 0.993 (1.0% [wt/vol] NaCl) and 4.0°C (——), a_w of 0.993 (1.0% [wt/vol] NaCl) and 15.0°C (- - -), a_w of 0.977 (4.0% [wt/vol] NaCl) and 5.0°C (······), and a_w of 0.977 (4.0% [wt/vol] NaCl) and 13.5°C (-·-·-). (c) GP spectra for laurdan in liposomes grown under the following conditions: (1.0% [wt/vol] NaCl) and 4.0°C (○), a_w of 0.993 and 15.0°C (□), a_w of 0.977 (4.0% [wt/vol] NaCl) and 5.0°C (●), and a_w of 0.977 (4.0% [wt/vol] NaCl) and 13.5°C (■).

TABLE 2. Effect of NaCl concentration on the r_∞ of DPH in lipids from *S. gelidimarina* grown under different conditions

Growth conditions	r_∞ under the following measurement conditions[a]:			
	1.0% NaCl and 4.5°C	1.0% NaCl and 14.4°C	4.0% NaCl and 4.5°C	4.0% NaCl and 14.4°C
1.0% NaCl, 4.0°C	0.112 ± 0.004	0.074 ± 0.006	0.112 ± 0.007	0.073 ± 0.005
1.0% NaCl, 15.0°C	0.154 ± 0.004	0.101 ± 0.004	0.157 ± 0.005	0.103 ± 0.006
4.0% NaCl, 5.0°C	0.118 ± 0.004	0.076 ± 0.004	0.118 ± 0.005	0.077 ± 0.006
4.0% NaCl, 13.5°C	0.131 ± 0.008	0.093 ± 0.009	0.133 ± 0.010	0.093 ± 0.010

[a] The data are means ± standard deviations based on three replicate analyses.

to the data obtained for the individual branched-chain and monounsaturated components (Fig. 3 and 4). This implies that the proportion of total monounsaturated and branched-chain fatty acids is strongly regulated in response to temperature and/or cell function. These observations also imply that the physiological roles of branched-chain and monounsaturated fatty acids in *S. gelidimarina* are interchangeable; that is, at transitional temperatures around the boundaries of the NPR, the proportions of individual branched-chain and monounsaturated fatty acids are not crucial to cell function as long as the total proportion is adequate.

These observations indicate that there is a physiological basis for the NPR for bacterial growth. The fact that the deviation for the relationship between relative cell yield and temperature is similar to the deviation for the growth rate of *Escherichia coli* has led to the suggestion that the transition of a catabolic enzyme(s) from an active state to an inactive state may define the NPR (31). The number of isozymes involved in the initiation of fatty acid biosynthesis (predominantly β-keto-acyl-acyl carrier protein synthase I [KAS-I] through KAS-III), which play an important role in branched-chain fatty acid synthesis versus monounsaturated fatty acid biosynthesis (13, 17), provides a tempting hypothesis for the observed changes and variability in fatty acid composition near the estimated boundaries of the NPR. It has been suggested that the fatty acid desaturation activity and the availability or utilization of potential fatty acid chain primers explain the variations in the fatty acid compositions of *Bacillus subtilis* and *Bacillus megaterium* near their temperature extremes for growth (37). While the exact in vivo roles of KAS isozymes in the selection of fatty acid chain primer molecules in the genus *Shewanella* remain unclear, it is probable that a number of KAS isozymes exhibit overlapping in vitro activities for the initiation of branched-chain fatty acid synthesis versus monounsaturated fatty acid biosynthesis (17, 38, 39). It is also known that the KAS enzymes of *E. coli* (particularly KAS-II) are thermally regulated (17), while KAS-III has recently been identified as the central determinant of branched-chain fatty acid production (5). In addition, other fatty acid biosynthetic enzymes are sensitive to NaCl (15). One possible explanation for the observed regula-

tion of branched-chain and monounsaturated fatty acid composition by temperature and salinity is the function of different KAS isozymes.

Studies on the in situ effects of temperature and salinity variations on psychrophilic and psychrotrophic populations have not been performed. However, previous evidence and the results of this study indicate that these physicochemical parameters may be selective. Lipid packing and adaptational fatty acid composition responses are clearly influenced by the temperature-salinity regimen, which may be reflected in overall cell function characteristics, such as the growth rate and the NPR. In future studies researchers must also address the potential role of the frequency, duration, and rate of salinity variations in bacterial cell function.

ACKNOWLEDGMENTS

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TABLE 3. GP of laurdan fluorescence in liposomes of
S. gelidimarina lipids measured at 4.5 and 14.3°C

Growth conditions	GP at[a]:	
	4.5°C	14.3°C
1.0% NaCl, 4.0°C	0.399 ± 0.009	0.248 ± 0.007
1.0% NaCl, 15.0°C	0.462 ± 0.013	0.321 ± 0.006
4.0% NaCl, 5.0°C	0.400 ± 0.015	0.264 ± 0.003
4.0% NaCl, 13.5°C	0.457 ± 0.011	0.305 ± 0.010

[a] GP was calculated from the emission intensities at 440 and 490 nm with excitation at 380 nm. The data are means ± standard deviations based on three replicate analyses.

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