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Adaptation of Psychrophilic and Psychrotrophic Sulfate-Reducing Bacteria to Permanently Cold Marine Environments

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The potential for sulfate reduction at low temperatures was examined in two different cold marine sediments, Mariager Fjord (Denmark), which is permanently cold (3 to 6°C) but surrounded by seasonally warmer environments, and the Weddell Sea (Antarctica), which is permanently below 0°C. The rates of sulfate reduction were measured by the $^{35}SO_4^{2-}$ tracer technique at different experimental temperatures in sediment slurries. In sediment slurries from Mariager Fjord, sulfate reduction showed a mesophilic temperature response which was comparable to that of other temperate environments. In sediment slurries from Antarctica, the metabolic activity of psychrotrophic bacteria was observed with a respiration optimum at 18 to 19°C during short-term incubations. However, over a 1-week incubation, the highest respiration rate was observed at 12.5°C. Growth of the bacterial population at the optimal growth temperature could be an explanation for the low temperature optimum of the measured sulfate reduction. The potential for sulfate reduction was highest at temperatures well above the in situ temperature in all experiments. The results from sediment incubations were compared with those obtained from pure cultures of sulfate-reducing bacteria by using the psychrotrophic strain ltk10 and the mesophilic strain ak30. The psychrotrophic strain reduced sulfate optimally at 28°C in short-term incubations, even though it could not grow at temperatures above 24°C. Furthermore, this strain showed its highest growth yield between 0 and 12°C. In contrast, the mesophilic strain ak30 respired and grew optimally and showed its highest growth yield at 30 to 35°C.

In cold marine environments, the optimum temperatures for respiration and growth are generally found to be well above the in situ temperature. Bacteria that are especially adapted to low-temperature habitats have been described as either psychrotrophic or psychrophilic. However, bacteria that apparently are not adapted to grow at low temperatures are often found in low-temperature environments. Hereafter, we use the following definitions for the classification of cold-adapted bacteria in this paper. The minimum temperature (T_{min}) is the lowest temperature for growth; the optimum temperature (T_{opt}) is the temperature with highest growth rate; the maximal temperature (T_{max}) is the highest temperature for growth. For psychrophilic bacteria, the T_{min} is <0°C, the T_{opt} is ≤15°C, and the T_{max} is ≤20°C. For psychrotrophic bacteria, the T_{min} is ≤0°C, the T_{opt} is ≤25°C, and the T_{max} is ≤35°C. For mesophilic bacteria, the T_{opt} is ~25 to 40°C and the T_{max} is ~35 to 45°C. It is important to note that whilst all of the definitions given above are concerned with growth, they are also used to categorize metabolic activity. As the results in this paper show, there can be a considerable difference between the cardinal temperatures for growth and metabolic activity, such as respiration.

Several studies have reported optimal metabolic activity at 30 to 40°C in bacterial populations from temperate marine environments despite an in situ temperature below 20°C (6, 20, 34, 50). Similarly in polar regions in which the temperature is constantly below 0°C, the optimal temperature for metabolic activity was found to be at least 10°C above the in situ temperature (29, 48). Many truly psychrophilic aerobic bacteria have been isolated from these areas (26, 39), but the majority of the isolated aerobes appear to be psychrotrophic rather than psychrophilic (9, 10, 46, 48).

In the deep sea, the temperature is generally below 5°C (18) and most of the organic matter is oxidized by aerobic bacteria, either in the water column or on the sea floor (7). In contrast, in some marine areas in Antarctica, anaerobic processes contribute substantially to the degradation of organic matter (29) probably because of high organic input (26). Although a considerable part of the degradation is anaerobic, only a few truly psychrophilic anaerobic bacteria have been isolated (11); among anaerobic bacteria, psychrotrophic bacteria appear to be predominant (18). The lowest temperature optimum for sulfate reduction, 21°C, was reported by Nedwell (29) for an Antarctic sediment. This was well above the in situ temperature of −1 to 1°C.

At higher temperatures, there is apparently a much closer adaptation of the bacterial metabolism to the ambient temperature. Around hydrothermal vents, the temperature optimum for sulfate reduction was found to be near the in situ temperature (23); bacteria isolated from deep-sea areas with hydrothermal vent activity and from coastal areas with volcanic activity had temperature optima near the in situ temperature (8, 40).

The aim of the present study was to examine how and to what degree anaerobic bacteria adapt their metabolism to low in situ temperatures. Two permanently cold sampling areas, Mariager Fjord in Denmark and the Weddell Sea of Antarctica, were chosen.

MATERIALS AND METHODS

Samples. Sediment samples from Antarctica were collected with a boxcorer at Station 124, Kapp Norvegia (71°08′6″S, 12°12′8″W), at a depth of 436 m and at Station 178 (69°56′9″S, 08°58′4″E) at a depth of 469 m in February and March 1991 during the ANT 1×/3 Expedition using the Polarstern in the Weddell Sea. The temperature at each of these stations is permanently below 0°C.

Sediment samples from Mariager Fjord (Denmark) were collected in April 1990 with a grab sampler at a depth of 29 m. The bottom water of this threshold fjord is permanently anoxic below 18 to 20 m, and the in situ temperature ranges

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from 3 to 6°C throughout the year (3). Sediment samples from shallow but permanently water-covered sediments at Kysing Fjord (24) were collected at a depth of 1 m in Plexiglas tubes. The temperature at the sediment surface of this locality varies between 0 and 20°C throughout the year (24). All experiments were done with subsamples taken from the uppermost part of the black reduced zone of sediment.

Radiotracer experiments with sediment slurries. Sediment samples from Antarctica were stored for 6 weeks at 4°C in 1.0-liter glass jars, filled to the top with sediment and capped without headspace with tight-fitting screw-cap lids. Sediments from Mariager Fjord and Kysing Fjord were used within a few hours of sampling. From each sample, a slurry was made by a 1:1 dilution with oxygen-free seawater. Sediment samples from Antarctica were diluted with artificial seawater (35‰), whereas sediment samples from Mariager Fjord were diluted with seawater from the sampling site. A constant flow of oxygen-free N_2 prevented contact with atmospheric O_2. Sediment slurries were stored at 4°C overnight under an N_2 atmosphere before being used.

Sediment slurries were mixed under oxygen-free N_2, and 7- to 10-ml aliquots were dispensed into 10-ml test tubes flushed with oxygen-free N_2. Tubes were stoppered and preincubated for 1 h (until temperature equilibration was reached) at 1 to 3°C temperature intervals in a 185-cm-long, insulated aluminum temperature gradient block. The temperature was measured continuously and remained constant within ±0.1°C throughout incubation.

After preincubation, 1 to 10 μCi of $^{35}SO_4^{2-}$ (Isotope Laboratory, Risø, Denmark) in a 0.1-ml solution was injected into each tube. The isotope had been diluted in either water or a neutralized solution containing the organic compounds formate, acetate, propionate, butyrate, lactate, and ethanol to give final concentrations of 1 mM each compound in tubes. Tubes were thoroughly mixed with a vortex mixer and incubated for 4 or 24 h or 1 week. Incubations were stopped by the addition of 1 ml of 20% (wt/vol) Zn-acetate solution to each tube, after which these tubes were frozen immediately. Reduced ^{35}S was analyzed by the single-step chromium reduction method (13), and sulfate reduction rates were calculated by the method of Jørgensen (21).

Cultivation of bacteria. Anaerobic sulfate-reducing bacteria were cultivated in a bicarbonate-buffered, sulfide-reduced mineral medium containing the following (in grams per liter, unless stated otherwise): NaCl, 15.0; $MgCl_2 \cdot 6H_2O$, 2.0; KCl, 0.5; NH_4Cl, 0.25; KH_2PO_4, 0.2; $CaCl_2 \cdot 2H_2O$, 0.2; Na_2HCO_3, 1.75; $Na_2S \cdot 8H_2O$, 0.18; trace element solution (SL 10a) (52), 2 ml/liter; and vitamin solution (53), 2 ml/liter. All chemicals were of analytical grade. The pH was adjusted to 7.3. The medium was prepared under an O_2-free N_2 atmosphere by the method of Widdel and Bak (52). Substrates (electron donors and acceptors) were added before inoculation from sterile neutralized stock solutions to give the desired concentrations. Prior to inoculation, all cultures received sodium dithionite ($Na_2S_2O_4$) as an additional reducing agent to give a final concentration of about 5 μM. Sodium dithionite reduced the lag phase of bacterial growth in batch cultures but was not required for growth.

Pure cultures were obtained by repeated application of the deep-agar shake technique described by Widdel and Bak (52). Isolated strains were checked for purity under a microscope and by growth tests in complex medium. Stock cultures were kept at 6°C in the dark and transferred to fresh medium at monthly intervals. Bacterial strains were characterized by substrate tests.

Determination of growth rates. Bacteria were grown in batch cultures at either 10°C (strain ltk10) or 30°C (strain ak30). Experiments were carried out with bacteria collected in late exponential-growth phase. In all experiments, the substrates were sulfate (10 mM) and lactate (20 mM) for strain ltk10 and sulfate (10 mM) and acetate (10 mM) for strain ak30. Growth was monitored by measuring the light absorption at 500 nm with a spectrophotometer. The growth rate (r) of an exponentially growing culture was calculated from the following equation by linear regression in a plot of ln(OD) as a function of time (t): $OD = OD_0 \exp(rt)$, where OD is the optical density at t and OD_0 is the optical density at the start of incubation. There was a linear relationship between OD and the cell number in the OD range at which measurements for growth rate were taken.

Determination of growth yield. Here the growth yield is defined as the amount of biomass produced for a given amount of substrate assimilated. The substrate turnover was determined from the sulfate turnover, i.e., the sulfate reduction rate (SRR), and SRR per cell were calculated. It was assumed that no CO_2 was fixed to balance the oxidation levels of organic carbon in substrates, as it is zero in lactate and acetate as well as in biomass. The growth rate of culture was used to obtain the amount of biomass produced per unit of time. The biomass C (in moles per cell) was determined with a CHN analyzer. By dividing the biomass C increase per cell by the substrate turnover per cell, the biomass C produced (in moles per mole of substrate assimilated) was calculated. Two methods were used to estimate the total biomass from the biomass C measurement. (i) By assuming that the overall composition of biomass is $C_4H_7O_3$ (16, 53), 1 mole of carbon equals 25.8 g of biomass (dry weight); (ii) by using the factors that the molar weight of carbon is 12.0 g/mole, the molar carbon/wet weight ratio is 0.092 (32), and the dry weight/wet weight ratio is 25% (33), 1 mol of carbon is equivalent to 32.6 g of biomass (dry weight). The first method does not take into consideration that biomass consists of more than carbon, hydrogen, and oxygen, whereas the second estimate is less accurate. Therefore, we used the average factor of 29.2 g of biomass (dry weight) per mol of carbon assimilated.

To ensure that the biomass per cell was constant for cultures grown at different temperatures, the biomass per cell was measured at three different growth temperatures, near the T_{min}, between the T_{min} and T_{opt}, and near the T_{opt}. For strain ltk10, these temperatures were 5, 10, and 15°C; for strain ak30, they were 10, 20, and 30°C, respectively. The carbon content per cell was 1.3×10^{-7} μmol for strain ak30 and 1.1×10^{-7} μmol for strain ltk10. No temperature effect was found.

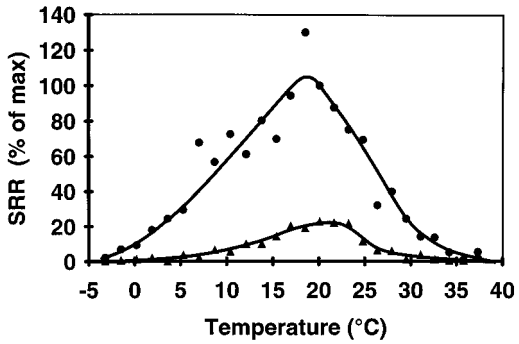

FIG. 1. Sulfate reduction by psychrotrophic bacteria in sediment samples from Station 178, Antarctica. Incubation was for 24 h with (●) or without (▲) a mixture of electron donors added. The highest activity was 6 nmol of SO_4^{2-} g of slurry^{-1} day^{-1} at 18 to 19°C.

Radiotracer experiments with pure cultures. Aliquot volumes (8 to 10 ml) of each of the two bacterial cultures in the exponential phase of growth were dispensed into 10-ml test tubes with glass pipettes. Tubes were flushed with N_2-CO_2 (88:12) and stoppered. Preincubation, incubation, and the determination of sulfate reduction rates were carried out by the procedures described above.

Enumeration of bacteria. Bacteria were counted in a Burker-Türk counting chamber.

Chemical determination of sulfate. The concentrations of sulfate in sediment slurries and pure cultures were determined by nonsuppressed ion chromatography (Waters) or by the turbidimetric $BaSO_4$ precipitation method (41).

Arrhenius plot and Q_{10}. The effect of temperature on bacterial sulfate reduction rates or growth has previously been modelled by using the integrated form of the Arrhenius equation (2, 50). The activation energy (E_a; in joules per mole) was estimated from the slope in an Arrhenius plot of ln(k) as a function of T^{-1} (4) as follows:

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{R} \cdot \frac{1}{T}\right) \quad (1)$$

where k is the rate of reaction, A is the Arrhenius constant, R is the gas constant (8.31 J K^{-1} mol^{-1}), and T is the absolute temperature (K). The E_a value is not activation energy in the chemical sense but a measure of the temperature response of the microbial community in total. It has therefore also been called the temperature characteristic μ (25).

Q_{10} is the factor by which the rate of reaction increases with a temperature increase of 10°C. Q_{10} was calculated by the following equation:

$$Q_{10} = \exp\left[\frac{E_a \cdot 10}{RT(T+10)}\right] \quad (2)$$

RESULTS

Temperature profiles of sediment sulfate reduction rates. The temperature profile of SRRs in sediment samples collected from Station 178, Antarctica, had an optimum at 18 to 19°C when organic substrates were added. Without added substrates, the SRR optimum was at 20 to 22°C (Fig. 1). Sediment samples from Station 124, Kapp Norvegia, Antarctica, incubated for 1 week showed an optimum SRR at 12°C with electron donors added, whereas in the absence of added electron donors, activities were low at all temperatures (Fig. 2).

The temperature profile of SRRs in Mariager Fjord sediment samples (Fig. 3) showed a mesophilic temperature response, with activities from below 0 to 45°C and an optimum at 30 to 35°C. A similar result was obtained with sediment samples from Aarhus Bay (20). The in situ temperature in Aarhus Bay varies from 3°C in winter to 15°C in summer, while the in situ temperature in the central region of Mariager Fjord never

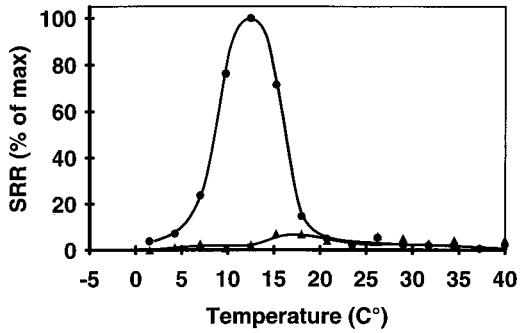

FIG. 2. Sulfate reduction by psychrophilic bacteria in sediment samples from Station 124, Kapp Norvegia, Antarctica. Samples were incubated for 1 week with (●) or without (▲) a mixture of electron donors added. The highest activity was 8 nmol of SO_4^{2-} g of slurry^{-1} day^{-1} at 12.5°C.

exceeds 6°C (3). Despite this difference, the temperature responses of these two populations of sulfate-reducing bacteria for sulfate were similar.

Isolated strains. Two strains of sulfate-reducing bacteria were isolated from Kysing Fjord sediment samples. Strain ltk10 was isolated at 10°C with lactate as the electron donor and thiosulfate as the electron acceptor, whereas strain ak30 was isolated at 30°C with acetate and sulfate as substrates.

The cells of strain ltk10 were rod shaped, 4.0 to 5.5 μm in length and 1.8 to 2.1 μm in width, and were unusual in containing gas vacuoles. In the stationary growth phase, these bacteria did not divide and long cells were observed. This strain was able to grow over the temperature range from 0 to 24°C, with a T_{opt} of 18 to 19°C (Fig. 4A). The doubling time at 20°C was 22.5 h. On the basis of physiological and phylogenetic characteristics, strain ltk10 belongs to the new genus *Desulforhopalus*, which is closely related to the genus *Desulfobulbus* (20a).

The cells of strain ak30 were small and oval to rod shaped, 2.5 to 3.2 μm in length and 1.6 to 1.8 μm in width. This strain was able to grow from 6 to 38°C, while the T_{opt} was 33 to 35°C (Fig. 5A). The doubling time at 32°C was 18 h. On the basis of physiological and morphological data, strain ak30 was characterized as a subspecies of *Desulfobacter curvatus*, originally described by Widdel (51).

Temperature profiles of the SRRs of these bacterial strains. Both strains were able to respire with sulfate at temperatures below the T_{min} and above the T_{max}.

Strain ltk10 reduced sulfate over the temperature range from −2.5 to 38°C, with an optimum of 1.12 mM SO_4^{2-} day^{-1}

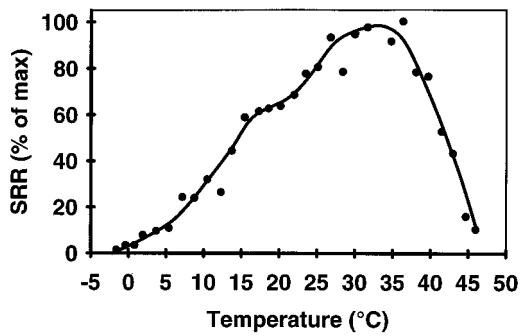

FIG. 3. Sulfate reduction by mesophilic bacteria in sediment samples from Mariager Fjord, Denmark, without substrate added. The highest activity was 300 nmol of SO_4^{2-} g of slurry^{-1} day^{-1} at 30 to 35°C.

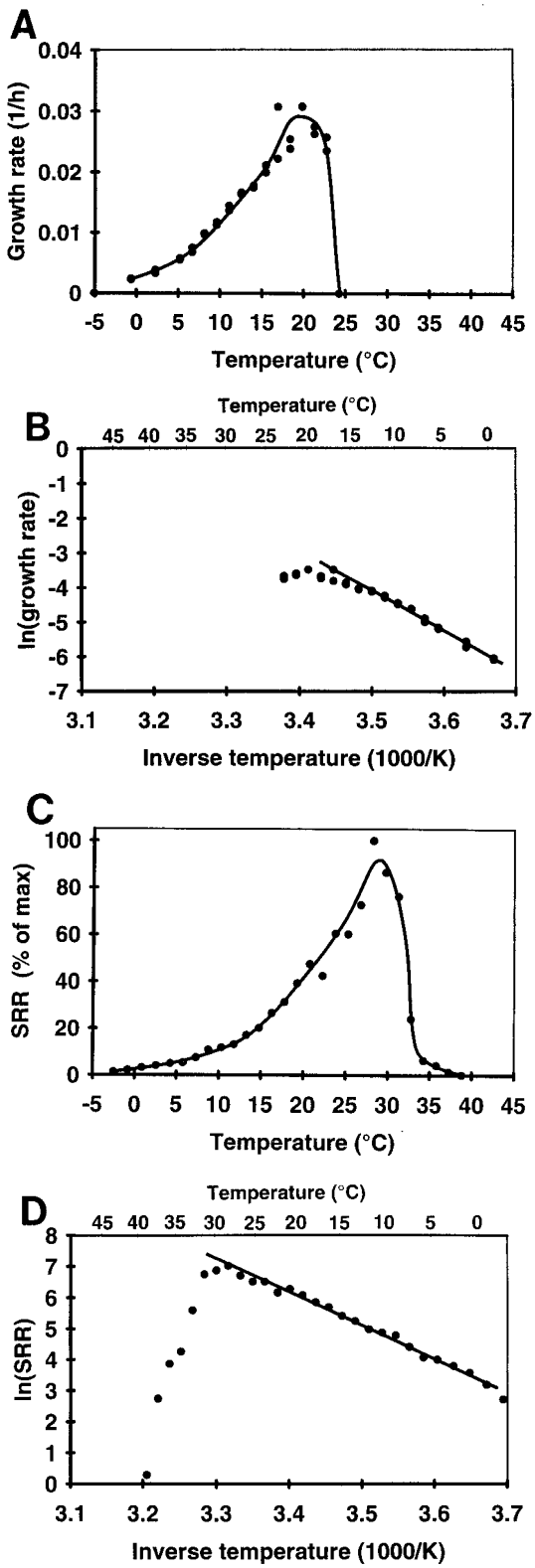

FIG. 4. (A) Growth rates of ltk10, a psychrotrophic sulfate-reducing bacterium isolated from Kysing Fjord, Denmark, at different temperatures. The optimal growth rate was at 18 to 19°C. (B) Arrhenius plot of the data in panel A. (C) Relative SRRs in cultures of ltk10 at different temperatures. Cultures were pregrown at 15°C and incubated for 24 h. The highest activity was at 28°C. (D) Arrhenius plot of the data in panel C.

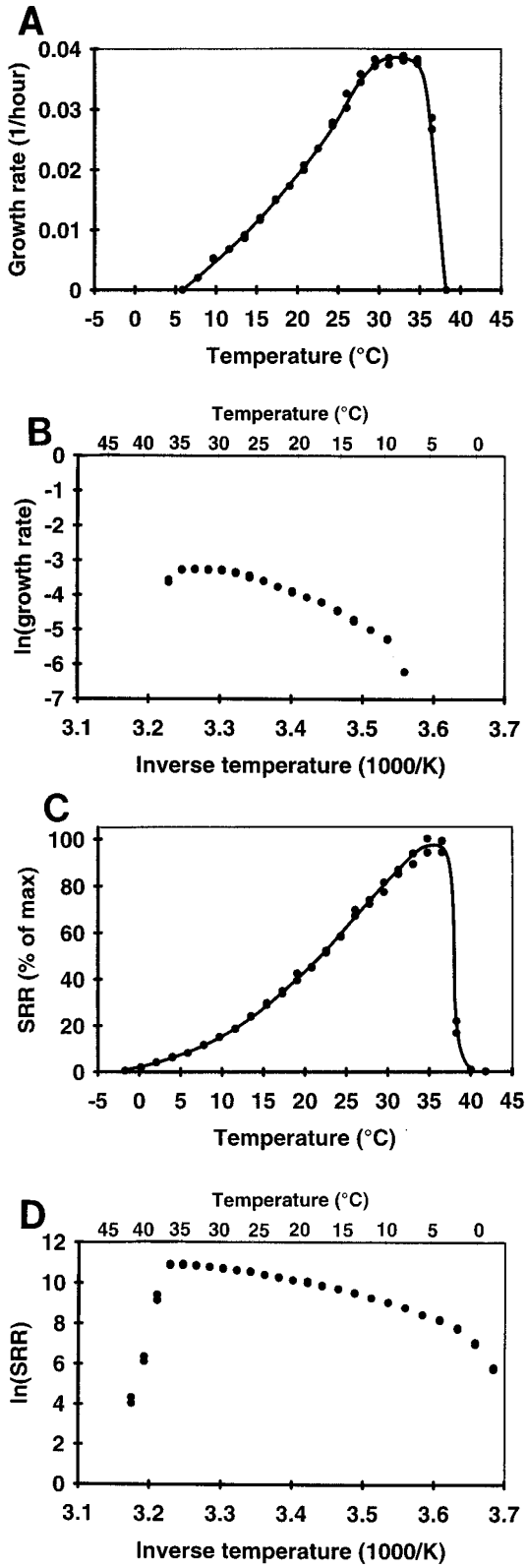

FIG. 5. (A) Growth rates of ak30, a mesophilic sulfate-reducing bacterium isolated from Kysing Fjord, Denmark, at different temperatures. The optimal growth rate was at 33 to 35°C. (B) Arrhenius plot of the data in panel A. (C) Relative SRRs in cultures of ak30 at different temperatures. Cultures were pregrown at 30°C and incubated for 4 h. The highest activity was at 35°C. (D) Arrhenius plot of the data in panel C.

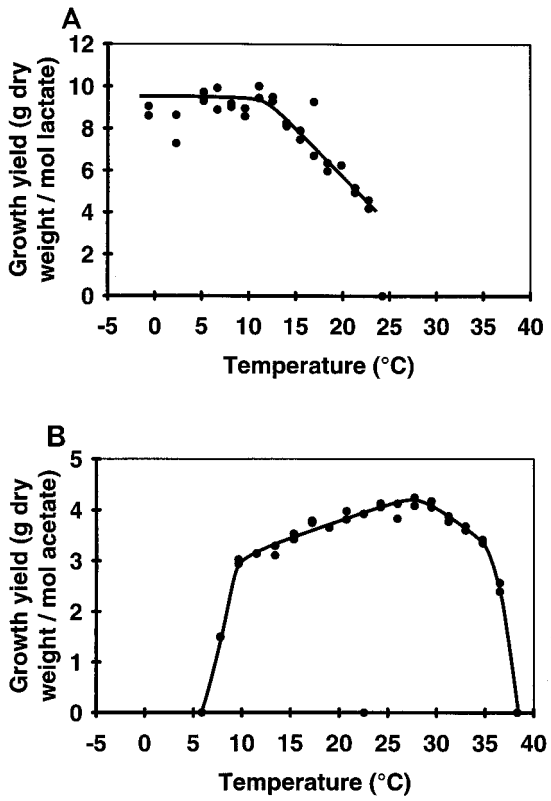

FIG. 6. (A) Growth yield of the psychrotrophic sulfate reducer ltk10 at different temperatures. The maximal growth yield was in the temperature range from 0 to 12°C. (B) Growth yield of the mesophilic sulfate reducer ak30 at different temperatures. The maximal growth yield was at 28°C.

at 28°C (Fig. 4C) or 4.5×10^{-4} nmol cell^{-1} day^{-1}. The Arrhenius plot of the same data showed a linear relationship from −1 to 28°C (Fig. 4D). In this temperature range, the E_a was 86.1 kJ mol^{-1}, while the Q_{10} was 3.5 in the range from 10 to 20°C.

Strain ak30 reduced sulfate at temperatures between 0 and 40°C, with an optimum of 56.2 mM SO_4^{2-} day^{-1} at 35°C (Fig. 5C) or 1.05×10^{-3} nmol cell^{-1} day^{-1}. The Arrhenius plot of these data gave a straight line only over the temperature range from 17 to 36°C (Fig. 5D). Within this temperature range, the E_a was 48.3 kJ mol^{-1}, while the Q_{10} was 1.9 in the range from 20 to 30°C.

Growth yield at different temperatures. The highest growth yield of 9 to 10 g (dry weight)/mol of lactate assimilated for strain ltk10 was achieved over the temperature range from 0 to 10 or 12°C, with a maximum of 10.0 g (dry weight)/mol of lactate at 11°C (Fig. 6A), whereas the growth yield for strain ak30 peaked at 28°C (Fig. 6B), with 4.2 g (dry weight)/mol of acetate assimilated. The differences in the optimum growth yields of strains ltk10 and ak30 were primarily due to the difference in free energy derived from the oxidation of lactate and acetate, as can be seen in the following equations: $acetate^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ ($\Delta G^{\circ\prime} = -47.6$ kJ per reaction) and $lactate^- + 1/2SO_4^{2-} \rightarrow acetate^- + HCO_3^- + 1/2HS^- + H^+$ ($\Delta G^{\circ\prime} = -80.3$ kJ per reaction). The $\Delta G^{\circ\prime}$ values (pH 7.0) were calculated from the data of Thauer et al. (43).

The maximal growth yield of 4.2 g/mol of acetate for strain ak30 is comparable to the growth yield of 4.4 g (dry weight)/mol of acetate assimilated (recalculated from 4.8 g [dry weight]/mol of acetate dissimilated) found for *Desulfobacter*

postgatei during respiration with sulfate (53). The maximum growth yield of 10.0 g/mol of lactate for strain ltk10 is higher than the yields recorded for *Desulfovibrio vulgaris* (6.0 to 7.8 g [dry weight]/mol of lactate assimilated) (27, 44) and more than double the growth yields found for *Desulfovibrio gigas*, *Desulfovibrio desulfuricans*, and *Desulfovibrio africanus* (1.7 to 4.1 g [dry weight]/mol of lactate assimilated) (27, 45). The low yields found for *Desulfovibrio* species may be due to suboptimal growth conditions.

DISCUSSION

Sulfate-reducing bacteria isolated from a Danish estuary. Strain ltk10 had an unusual morphology in that these cells contained gas vacuoles. Jørgensen and Bak (22) found sulfate-reducing bacteria with gas vacuoles to be the most abundant morphological type in the oxic and oxidized layers of marine sediments. Species with gas vacuoles, such as strain ltk10, may thus be among the dominant sulfate-reducing bacteria in this zone of sediment; consequently, the temperature characteristics of cold-adapted strain ltk10 may be typical for a large proportion of the sulfate-reducing bacteria in marine surface sediments of temperate regions.

The psychrotrophic strain ltk10 exhibited its highest SRR at 28°C (Fig. 4C), whereas the T_{opt} was 10°C lower, at 18 to 19°C (Fig. 4A). The cardinal temperatures for respiration show a mesophilic response, whereas the cardinal temperatures for growth show that this strain is psychrotrophic. This strain was able to survive at temperatures above the T_{max}, i.e., at temperatures up to ca. 30°C (data not shown). After about 24 h, cells slowly became deformed and started to lyse. In the range from T_{max} to optimal respiration temperature (ca. 23 to 30°C), this bacterium generated sufficient energy from respiration to repair or regenerate temperature-denatured enzymes, ribosomes, etc. The ability to respire at temperatures above the T_{max} may be the explanation for survival at high temperatures and may be normal among bacteria adapted to low temperatures. Thus, an aerobic psychrophilic *Pseudomonas* sp. has been shown to respire at up to 10°C above the T_{max} (17). Bacteria adapted to higher temperatures are apparently not able to respire at temperatures much above the T_{max}. The mesophilic strain ak30 showed little temperature difference between the T_{opt} and the respiration optimum, as the T_{opt} was 30 to 35°C (Fig. 5A) and the respiration optimum was 35°C (Fig. 5C). Similarly, the thermophilic sulfate reducer *Desulfotomaculum kuznetsovii* p60 has been shown to have the same temperature optimum (62°C) for growth and respiration (20).

A comparison of the growth yields of the psychrotrophic strain ltk10 and the mesophilic strain ak30 shows that strain ltk10 had its highest growth yield over the temperature range from 0 to 10 or 12°C, whereas strain ak30 had its highest growth yield at 28°C, which is close to its T_{opt}. It is obvious that strain ltk10 is much better adapted to low temperatures than is indicated by the optimum temperature for respiration alone, whereas there is a closer correspondence between the respiration and growth yield optima for the mesophilic strain ak30. In contrast, Nedwell and Rutter (31) did not observe any significant temperature effect on the growth yields of two psychrotrophic aerobic bacteria. Apart from this study, there are only a few experiments on the effect of temperature on growth yield, so we do not know if cold-adapted bacteria normally have their highest growth yields at low temperatures. Herbert and Bell (19), however, reported that the T_{opt} for a psychrophilic *Vibrio* sp. was more than 10°C higher than the temperature with the highest growth yield.

The use of cardinal temperatures for growth in the temperature characterization of bacteria (9, 28, 37, 47) (this paper) has several disadvantages. Instead of focusing on the temperature at which the highest growth rate is found, it may be more informative ecologically to examine the relative growth rates at low temperatures. In 1889 (4), Arrhenius showed that the rates of chemical reactions may increase exponentially with changes in temperature. If the reaction rate is plotted logarithmically in an Arrhenius plot as a function of the inverse absolute temperature (equation 1), the reaction would show a linear decline with decreasing temperatures. This relation also holds for enzymatic reactions, as long as the enzymes stay intact and functional (5). Harder and Veldkamp (17) showed that the Arrhenius plot of the growth rate of a psychrophilic *Pseudomonas* sp. decreased linearly with temperatures down to 0°C and below, whereas for a psychrotrophic *Pseudomonas* sp., the growth rate deviated from linearity at temperatures below 3°C. Apparently, only the rate of the enzyme activity of this psychrophile was affected by low temperatures, as judged from the linear relationship, whereas membrane transport and/or other functions of this psychrotrophic strain were adversely affected at temperatures below 3°C. This psychrophilic strain was thus able to extend its normal functions to lower temperatures, i.e., it adapted to low temperatures.

The growth and respiration rates of the psychrotrophic strain ltk10 were linear to below 0°C in an Arrhenius plot (Fig. 4B and D). Consequently, on the basis of the relative activities at low temperatures, strain ltk10 is psychrophilic by the definition of Harder and Veldkamp (17). In contrast, the mesophilic strain ak30 showed an Arrhenius plot of respiration and growth that deviated from linearity at temperatures below 10°C (Fig. 5B).

Temperature response of sulfate reduction in sediments from temperate environments. Sulfate is the predominant electron acceptor for organic matter oxidation in reduced and sulfidic marine sediments, such as those studied here. The respiration rates of sulfate-reducing bacteria in such marine sediments are generally limited by the availability of suitable electron donors. The temperature profiles of sulfate reduction in sediments with substrate addition and short-term incubation thus show the temperature characteristics of in situ populations of sulfate-reducing bacteria in the absence of substrate limitation. When substrate is not added, the SRR and its temperature profile are likely to reflect the temperature characteristics of substrate production through enzymatic hydrolysis of organic polymeric material and through a variety of bacterial fermentation pathways. Accordingly, the temperature optimum for substrate production by enzymatic hydrolysis and bacterial fermentation in the Antarctic sediment samples (i.e., sulfate reduction without substrate addition) was 4 to 5°C higher than that for sulfate-reducing bacteria alone (i.e., sulfate reduction with substrate addition).

It is important to note that when the activities of natural populations of bacteria are measured in terms of oxygen consumption (15, 34, 35), sulfate respiration (12, 14, 30, 50), or electron transport system activity (36, 42, 48, 49), only the temperature response of respiration is observed. Especially in a population of cold-adapted bacteria, respiration measurements could indicate the presence of a mesophilic population, even though the population is indeed psychrotrophic.

The present results from pure cultures raise the question of whether it is possible to discriminate among mesophilic, psychrotrophic, and psychrophilic bacterial populations solely on the basis of respiration temperature profiles. The relative activities at low temperatures give more information about the adaptations of bacterial populations than does the optimum temperature for respiration. The relative temperature depen-

dence of metabolic activity can be described by the Q_{10} factor. A low Q_{10} shows that the activity is relatively high at low temperatures.

In sediment samples from Mariager Fjord, we observed the highest activity at 30 to 40°C (Fig. 3), despite the fact that the in situ temperature at the sediment surface never exceeded 6°C. The Q_{10} for sulfate reduction was 3.9 (2 to 12°C), which is similar to the Q_{10} obtained from temperate environments with temperatures of up to 20°C (38, 50). This suggests that the population of sulfate-reducing bacteria in Mariager Fjord sediment samples consisted of the same bacterial temperature groups as the populations in sediment samples from temperate environments with temperatures of up to 20°C.

Sulfate reduction in sediments from Antarctica. The highest SRRs over 24 h were observed at 18°C in the experiment with the addition of electron donors and at 20°C without an electron donor added (Fig. 1). These optima are in the same temperature range as the optimum in sulfate reduction found by Nedwell (29) in Antarctic sediments. Even though the temperature optimum was 10°C lower than those found in sediment samples from temperate environments (1, 20, 50), it was well above the in situ temperature of −1 to 0°C. However, the activities at 0 and 5°C in Antarctic sediment samples (10 and 29%, respectively, of maximal activity [Fig. 1]) were relatively high compared with the SRRs in Mariager Fjord sediment samples (4 and 10%, respectively, of maximal activity [Fig. 3]). The high activity at a low temperature was also expressed by a Q_{10} of 1.5 (2 to 12°C) for the Antarctic sediment samples (Fig. 1), which is low compared with the Q_{10} values of 3.0 to 3.9 for temperate sediment samples (38) (Fig. 3).

The highest SRR over 1 week was observed at a temperature as low as 12.5°C (Fig. 2). To our knowledge, this is the lowest published temperature optimum for an anaerobic bacterial process in nature; nevertheless, this temperature optimum is 13°C higher than the in situ temperature.

An important difference between the two experiments is the length of incubation. Experiments with relatively short-term incubations reveal the respiratory activity of the bacterial population present, whereas long-term incubations offer bacteria time for growth. As was seen with strain ltk10 and a psychrophilic *Pseudomonas* sp. (17), the T_{opt} was about 10°C lower than the temperature optimum for sulfate reduction. The SRRs in experiments with long-term incubations could therefore increase at temperatures at which these bacteria grow fastest, whereas the rates would stay constant or even decrease at temperatures at which these bacteria do not grow. The sulfate reduction optimum at 12.5°C for Antarctic sediment samples after 1 week of incubation is thus likely to reflect also the growth rate optimum of a predominantly psychrophilic population.

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