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Motility of *Colwellia psychrerythraea* Strain 34H at Subzero Temperatures

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We examined the Arctic bacterium *Colwellia psychrerythraea* strain 34H for motility at temperatures from −1 to −15°C by using transmitted-light microscopy in a temperature-controlled laboratory. The results, showing motility to −10°C, indicate much lower temperatures to be permissive of motility than previously reported (5°C), with implications for microbial activity in frozen environments.

The earth is primarily a cold biosphere, with most of the world's ocean waters at temperatures below 5°C. Liquid water and microbial life also persist at much colder temperatures within frozen environments such as lake (18) and sea ice (10). Yet, only very recently has any attention been paid to how the basic bacterial trait of motility may be expressed in the cold (this question was addressed theoretically by Price [17] and empirically by Allen and Deming, who showed motility to −1°C, along with chemically directed movement or chemotaxis [D. M. Allen and J. W. Deming, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. I-52, 2002]). The lowest temperature considered in prior studies of either motility (21) or chemotaxis (22) has been 5°C.

To better understand constraints on a variety of bacterial activities at extremely low temperatures, we have been investigating wintertime Arctic sea ice samples by using newly developed, nondestructive (nonwarming and nonmelting) methods for direct visualization of bacteria within the three-dimensional network of brine inclusions in the ice (10). Such in situ observations have suggested that life at subzero temperatures in sea ice depends closely upon the morphology and distribution of the brine inclusions themselves and that association with surfaces is important for sustained activity at temperatures as low as −20°C (K. Junge, H. Eicken, and J. W. Deming, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr. N-201, p. 524, 2001). Bacterial motility as a means to reach and associate with a surface has been studied mainly in the context of biofilm formation and nutrient acquisition (14–16) and invariably under moderate conditions (typically, at room temperature in low-viscosity or seawater strength salt solutions). How the more extreme conditions of sea ice brines (subzero temperatures, high salt, and high viscosity) may affect bacterial attachment or motility is currently unknown.

The idea of bacterial movement in ice has inspired the development of theories about its possibility (17) and would be consistent with bacterial survival strategies in temperate marine environments (7), but no direct measurements have been reported. To examine potential motility of bacteria at temperatures relevant to wintertime sea ice, we adapted our subzero microscopy methods to the investigation of bacterial motility at temperatures from −1 to −15°C. The Arctic marine bacterium *Colwellia psychrerythraea* strain 34H (GenBank accession number AF396670) (5, 6, 9), already the subject of chemotaxis studies at growth-permissive temperatures from 13 to −1°C (Allen and Deming, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol.), served as our model psychrophilic strain. The observed minimum temperature for growth of this strain is −5°C (8). The species is commonly found in sea ice (1, 11), the coldest marine habitat on Earth (with liquid brine temperatures as low as −35°C) (13).

Motility was studied in a temperature-controlled laboratory housing a microscope equipped for both transmitted-light and epifluorescence microscopy in conjunction with a computerized imaging system for use at subzero temperatures (10). First, a motile culture of *C. psychrerythraea* strain 34H, grown to late logarithmic phase in marine broth at −1°C, was examined for continued motility at −1°C. In an attempt to achieve a "live" staining approach that would facilitate high-contrast, automated image processing, the culture was stained in a solution containing 2, 4, or 20 μg of the DNA-specific fluorescent stain 4′,6′-diamidino-2-phenylindole (DAPI)–2HCl/ml after suspension in 5 or 20% glycerol in marine broth (salt concentration, 34 ppt) or in an artificial brine with a salt concentration of 200 or 300 ppt (10). The latter facilitated tests at subzero temperatures by preventing ice formation. Then, aliquots of the culture supplemented with 0, 5, 10, or 20% glycerol were equilibrated to −5°C for several days and examined for motility at high magnification (final digital magnification, ×3,230) by using transmitted-light microscopy (not epifluorescence, since live staining worked only at −1°C) and time-lapse photography (10). Temperatures were then lowered stepwise to further examine the strain for motility at −8, −10, −12, and −15°C. Once each temperature was reached, essentially continuous observations were made for approximately 1 h. Local temperatures were measured frequently on the bench top where the microscopic slides were prepared and on the microscope stage itself to ensure that observations were made at the desired temperature. The examination unit held the temperature within 1°C during sample processing and analysis, as ver-

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ified with a Hobo temperature logger (Onset Computers Corporation, Pocasset, Mass.) (10). We also monitored temperature by immersing a thermometer (accuracy within 0.1°C) in a tube containing the glycerol-amended culture. Time-lapse image sequences were recorded with an MTI DC330E 3CCD color camera and a color frame grabber (Scion CG-7) at one frame per 0.2 s. At the magnification used, 1 μm on the sample slide corresponded to 10 linear pixel dimensions. From the resulting movies, the *x-y* positions of individual moving bacteria (10 to 29) were determined manually in successive frames (3 to 60) with the image analysis software NIH Image version 1.62a (19). The results were used to estimate the average bacterial swimming speeds (at −5, −8, and −10°C) and, when motility ceased (at −15°C), Brownian motion.

At −1°C, cells of *C. psychrerythraea* strain 34H maintained motility in 5 and 20% glycerol and at DAPI concentrations of 2 and 4 but not 20 μg/ml. Live staining was not readily achieved at lower temperatures (obliging the use of transmitted light at temperatures at and below −5°C), nor was motility observed at the high salinities tested (200 and 300 ppt) at −1°C. The successful live staining of motile bacterial cells with low DAPI concentrations at −1°C, however, will enable future use of automated, high-contrast image analysis techniques for the study of larger numbers of organisms (both attached and motile) than was possible with the manual image analysis approach used here.

Tests for motility at temperatures below −1°C clearly revealed that cells of *C. psychrerythraea* strain 34H, when suspended in glycerol-amended marine broth medium, were motile at subzero temperatures from −5 to −10°C (Fig. 1). Average swimming speeds (28 to 43 μm s^{-1}) (Table 1) were comparable to those of cultured enteric and marine bacteria at mesophilic temperatures (15 and 45 μm s^{-1}) (15). No significant differences in average swimming speeds were observed as a function of glycerol concentration. The maximum individual burst velocity (maximum speed observed from one frame to another) (14) of 107 μm s^{-1} was observed at −8°C.

At −12°C, the vast majority of cells were clearly nonmotile, precluding a reliable estimation of swimming speeds of the remaining cells. At −15°C, motility had ceased completely, such that only Brownian motion was observed. Ice formation in the culture tubes or on the microscopic slides prohibited further testing for motility in glycerol-amended marine broth at temperatures below −15°C. At all of the test temperatures, however, we observed occasional random or inadvertent ice formation due to sudden ice nucleation events (e.g., introduction of a pipette tip), a common problem when working at subzero temperatures. Whenever ice formed, cells ceased being motile within a few minutes. This observation is consistent with bacterial attachment to ice walls within the spatially constricted liquid brine network of natural sea ice, as inferred from our earlier work (Junge et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.). It could also be explained by detrimental effects on motility ensuing from the sudden increases in salinity that occur when brine channels are newly formed (as indicated by the cessation of motility in tests of highly saline brines at −1°C). Induction and cessation of motility have been proposed to be closely coupled to changes in ambient nutrient concentration (14); based on these first motility results at subzero temperatures, the way in which differences in salinity, temperature, viscosity, and amount of space available also link to nutrient availability within a frozen ice matrix merits further study.

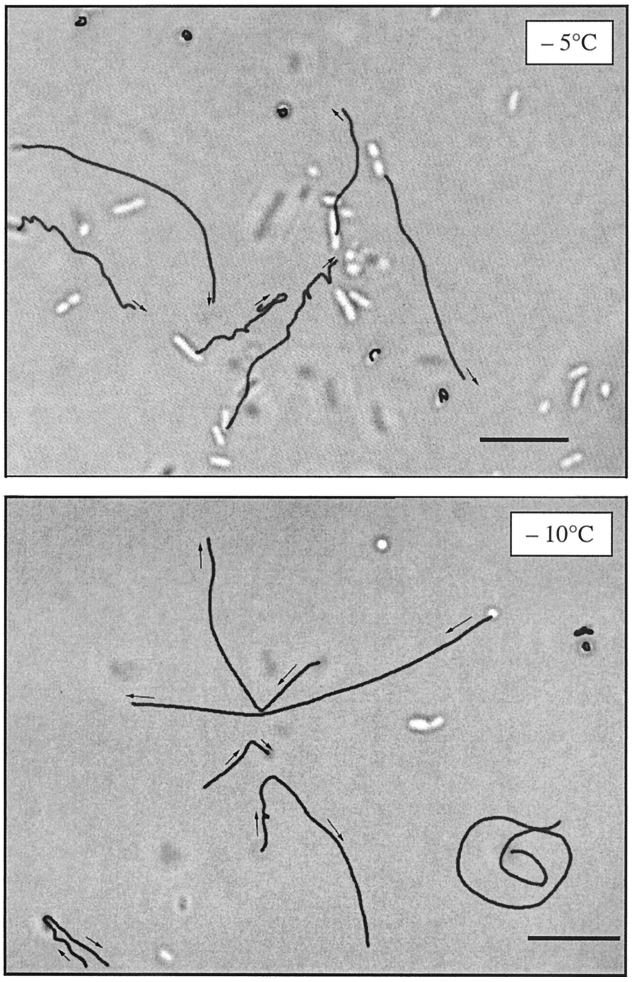

FIG. 1. Microscopic images of cells of *C. psychrerythraea* strain 34H (motile and nonmotile) taken at −5°C (upper panel) and −10°C (lower panel), with graphic representations of trajectories with time obtained by time-lapse photography (scale bar, 10 μm).

Since our observations at test temperatures below −5°C were limited in time, the issue of transient motility arises. Swimming speeds held reasonably constant (Table 1) over the duration of the experiment (6 h) from −5 to −12°C, indicating an ability to maintain swimming rates with prolonged exposure to the cold (no statistically significant difference between rates at −5 and −10°C was observed). When swimming speeds of

TABLE 1. Average swimming speeds of cells of *C. psychrerythraea* strain 34H at subzero temperatures

Temperature (°C)	Swimming speed (μm/s) ± SD (range)	*n*
−5	43 ± 24 (3.9–73)	29
−8	29 ± 20 (4.4–75)	21
−10	28 ± 16 (3.7–55)	22
−15	1.1 ± 0.18[a] (0.8–1.4)	10

[a] Represents Brownian motion, not swimming speed.

individual bacteria were plotted over time, we found no indication of a reduction in speed (though our sample size was too small for a statistical evaluation; data not shown). In the sea ice microenvironment characterized by marked fluctuations of temperature (and salt concentration), a bacterium that experiences more extreme temperatures can benefit even from transient movement to seek a better location (e.g., a nutrient-rich or cryoprotective exopolymer-rich spot) (12).

While more tests are needed to resolve the lower temperature limit for motility and its link to other variables, as well as the general question of motility as a survival strategy in subzero sea ice brines, the results presented here clearly indicate that bacteria can perform basic functions at temperatures far below 0°C and that the temperatures permissive of motility are much lower (by 15°C) (21) than previously considered. The availability of the whole genome sequence of *C. psychrerythraea* strain 34H (B. Methe, M. Lewis, B. Weaver, J. Weidman, W. Nelson, A. Huston, J. Deming, and C. Fraser, Ninth Genome Sequencing Contractor and Grantee Workshop, Washington, D.C., January 2002) may soon allow for additional insight into adaptations allowing motility at such low temperatures. Present observations of motility at −10°C and above, coupled with the results of our previous research determining surface association as a survival strategy in subzero brines, especially at −10°C and below (Junge et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.), lead us to the testable hypothesis that a temperature threshold (coinciding with a threshold in salinity and the availability of space) exists below which attached bacteria will be favored over motile ones.

In the meantime, the direct observation of bacteria swimming at −10°C adds to the growing body of evidence, obtained by using either tracer technology in permafrost (−20°C) (20), Antarctic snow (−17°C) (2), and glacial ice (−15°C) (3) or respiratory stain and molecular probes in Arctic sea ice (−20°C) (Junge et al., Abstr. 101st Gen. Meet. Am. Soc. Microbiol.), for microbial activity at extremely low temperatures in frozen habitats. The concept that liquid inclusions in the frozen environments of Earth provide adequate habitats for active microbial populations extends the discussion of the possibility of life elsewhere in the solar system, such as the icy and likely briny surface layers of Europa's ocean (4).

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