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Temperature Effects on Bacterial Movement

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Details are presented for the construction of a simple precision temperature-controlled chamber for investigating bacterial motile behavior. Independent of original incubation temperature, all species of motile bacteria observed showed a five- to sevenfold increase in average translational velocity (micrometers per second) as the environment temperature was incremented over the range from 10 to 50°C. Temperature jumps downward produced transient tumbling or reciprocal behavior responses, depending on the mode of flagellar distribution, in all species examined. Upward temperature jumps induced accelerated velocities without tumbling or reversal. A partial capacity adaptation to temperature was noted, in that the greatest average translational velocity at any given observation temperature occurred when the organisms were grown at temperatures less than the optimum.

The relative sparsity of information regarding temperature effects on bacterial behavior is due, in part, to a lack of adequate controls and precise instrumentation. Fast-response electronic transducers are particularly suited for innovations in developing simple and inexpensive precision temperature-controlled observation chambers characterized by rapid and accurate alteration capabilities. This paper describes experimental results of temperature effects on bacterial movements obtained with a device having the above-mentioned properties.

MATERIALS AND METHODS

Bacteria. The following bacteria were selected from the department collection: *Salmonella typhimurium, Pseudomonas aeruginosa, Spirillum serpens, Serratia marcescens, Bacillus polymyxa, Bacillus coagulans, Proteus mirabilis,* and *Pseudomonas fluorescens.* These organisms were grown on Trypticase soy agar (Baltimore Biological Laboratory, Cockeysville, Md.) slants in screw-capped glass tubes. Stock cultures were transferred monthly, incubated for 24 h at 30°C, and thereafter maintained at 20°C. For temperature range and motility experiments, bacteria were grown in screw-capped tubes containing 7 ml of Trypticase soy broth (Baltimore Biological Laboratory) for 12 h at 30°C on a reciprocal temperature-controlled shaker (Tem-Blok Incubator, model 2095; Lab-Line Instruments Inc., Melrose Park, Ill.). The bacteria were then inoculated into 5 ml of Trypticase soy broth in glass tubes placed into a 19-hole aluminum temperature gradient block (Lab-Line Instruments), which maintained temperatures between 51 and 15°C.

Motility measurement. The closed-circuit television recording method of Schneider and Doetsch (8) was used to determine swimming velocities (micrometers per second) and to observe behavioral responses to temperature variations. The velocities of the 10 fastest organisms were averaged.

Temperature-controlled chamber. A milling machine was used to cut an acrylic slide (1 by 3 inches [ca. 2.54 by 7.6 cm]) to create a chamber through which water may flow (Fig. 1). Small-diameter tubing (ID, 1.0 mm) was fitted to each end of a flat glass capillary tube (0.062 by 0.025 by 1.124 inches [ca. 0.157 by 0.063 by 2.855 cm]). The tube was sealed with epoxy (Coltana 2000) into the slide. Two glass cover slips (no. 2; 22 by 22 mm) were sealed with epoxy in place on the slide. The inlet and exit tubes were fitted in position in a drilled hole and sealed with epoxy at the side of the chamber. A thermistor (GB34P2; Fenwall Electronics, Framingham, Mass.) was placed in the water entry chamber, a cover slip cut to size was placed on top, and the entire slide was sealed around all joints with beads of epoxy. Two small brass tabs were attached to fit under the microscope stage arms to hold the device securely in place (Fig. 2).

Ice water (about 1°C) was gravity fed from a polyethylene container with a tubing nipple on the bottom. Polypropylene tubing (ID, 0.37 inch [ca. 0.95 cm]) led to a needle valve (no. 6072-10; Cole-Parmer, Chicago, Ill.). Heated (55°C) water was syphoned from a 1,000-W water bath (Lab-Line Instrument Co., Melrose Park, Ill.) via polypropylene tubing (ID, 0.37 inch) to a second needle valve. The two needle valves led via rubber tubing (ID, 0.12 inch [ca. 0.32 cm]) to a "Y" connector (no. 6295-10; Cole Parmer, Chicago, Ill.). A 3-inch length of rubber tubing (ID, 0.12 inch) was connected to the acrylic slide inlet nipple. The outlet tubing was routed to a discard bottle placed below microscope level. Care was taken to insure a constant positive flow through the chamber to prevent air bubbles from developing.

The thermistor was compared, via a resistance bridge circuit, to a linear taper potentiometer calibrated in 0.1°C increments. The imbalance in the

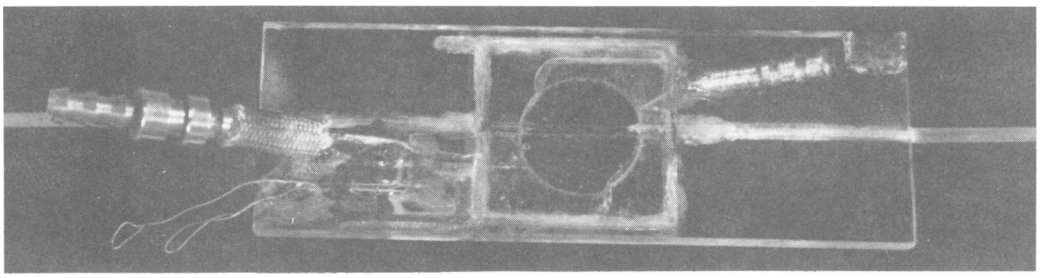

FIG. 1. *Overall appearance of temperature-controlled slide chamber.*

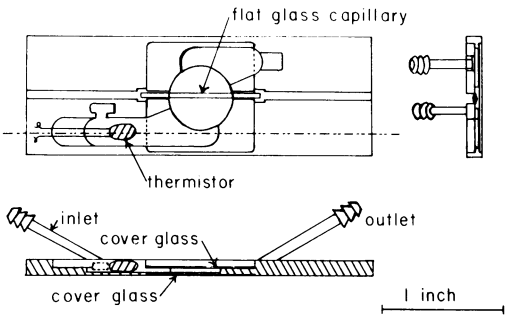

FIG. 2. *Construction details for temperature-controlled slide chamber.*

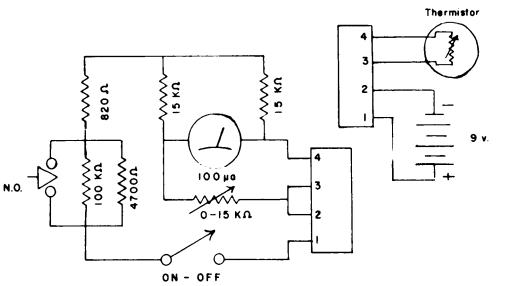

FIG. 3. *Wiring diagram for electronic thermometer.*

bridge was read by a 100-0-100 microammeter (Weston model 201). The unit was powered by a 9-V battery wired through the plug connector, which also connected the thermistor to the chassis. This insured that the unit did not remain on between experiments and facilitated changing the externally mounted battery. A momentary "on" push button switch increased the current flowing through the bridge, making it more sensitive for fine temperature adjustment (Fig. 3). When used, the calibrated dial was rotated until the meter needle read "0" in the center of the scale; the dial then indicated the thermistor temperature to ±0.1°C.

Temperature-controlled experiments. The culture to be observed was injected into the capillary tube with a 2-ml plastic syringe and 18-gauge blunt needle. Capillary tube ends were then plugged with a 0.5-cm length of 18-gauge wire. The temperature of the slide chamber was adjusted to that of the original incubation temperature of the culture to obviate exposure to sudden environmental changes upon injection. To provide an energy source and to avoid possible extraneous physical shocks, organisms were maintained undisturbed in the original culture medium.

The temperature of the hot water reservoir was held at 55°C, and higher temperatures were avoided to prevent the possible breakage of the capillary tube. Temperature jumps, over a range of ±30°C, could be achieved in less than 1 s by a flow adjustment of the heated and cooled water supplies fed around the culture-filled capillary tube.

RESULTS

In general, all cultures showed an increasing proportion of nonmotile organisms with age, and, except in certain instances, cultures of 18 h or less were used since they contained at least 50% motile organisms. Interestingly, there seemed to be no diminution of average translational velocity with culture age.

Motility and environmental temperature. Figures 4 to 7 relate the original incubation temperature of a culture to the average velocity observed at different environmental temperatures. The data for *P. mirabilis* (Fig. 4), *S. typhimurium* (Fig. 5), *S. serpens* (Fig. 6), and *P. fluorescens* (Fig. 7) are shown. It is clear that in every case, irrespective of the original incubation temperature, a striking increase in the average translational velocity occurred over the range from 10 to 50°C, and, indeed, all species observed moved about five to six times as fast at 50°C as at 10°C.

Temperature jump effects. Table 1 shows the mode of directional change of organisms characterized by various flagellar arrangements. Tumbling organisms ceased translational movement and appeared to rotate end-over-end or spin before resuming a normal run. Reversing organisms changed direction of movement by 180° and reciprocated or oscillated over a distance of a few cell lengths. In both cases these typical responses lasted from 2 to 10 s before normal motility behavior resumed. Responses to upward or downward temperature jumps were almost instantaneous.

In Table 2 responses of seven different organisms to upward and downward temperature

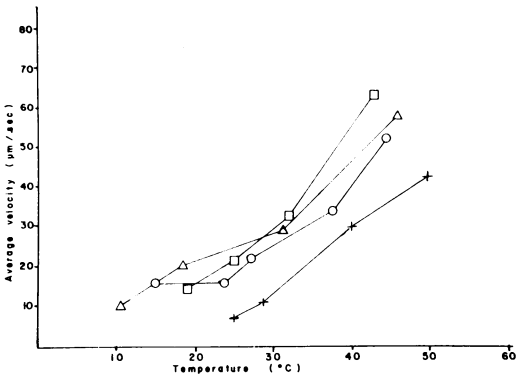

FIG. 4. *Effect of temperature on the velocity of P. mirabilis. The cultures were originally incubated at 41.0°C (+), 35.2°C (○), 27.3°C (△), and 21.3°C (□).*

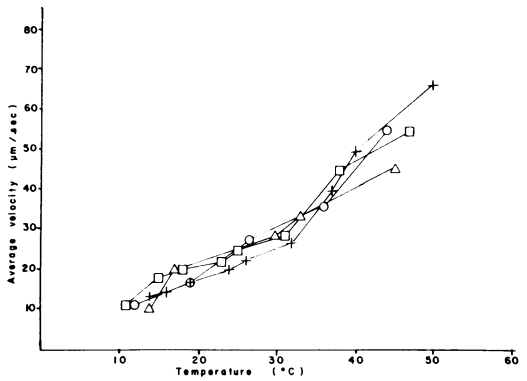

FIG. 5. *Effect of temperature on the velocity of S. typhimurium. The cultures were originally incubated at 43.0°C (+), 35.2°C (○), 27.3°C (△), and 23.4°C (□).*

jumps are summarized. A temperature drop induced tumbling or reciprocating direction changes. A step up produced a transitory acceleration and suppression of normal tumbling or reciprocation.

S. marcescens did not exhibit any of the responses to temperature jumps. *S. serpens* exhibited atypical behavior. A temperature jump to over 43°C induced a rapid reciprocation having a very short mean free path length. This effect is similar to that produced by *P. fluorescens* when exposed to low concentrations of aliphatic alcohols (1).

DISCUSSION

Thermistor lag time was approximately 0.6 s, similar to that estimated to be characteristic of the capillary tube and culture liquid, and, hence, behavioral responses of the bacteria being observed could be precisely assayed.

A water-cooled chamber has been described (5), but in this case the water regulated the temperature of a copper plate on which a standard glass microscope slide was placed. Since there is a considerable amount of material through which the heat must be conducted, a longer lag time occurs before arriving at the desired temperature, and this cannot be changed more rapidly than 0.15°C/s, whereas the capillary tube slide chamber has relatively little conductive mass and the temperature can be changed throughout its entire range in less than 1 s.

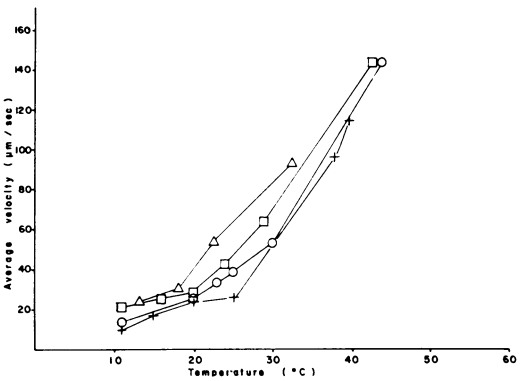

FIG. 6. *Effect of temperature on the velocity of S. serpens. The cultures were originally incubated at 41.0°C (+), 35.2°C (○), 30.0°C (△), and 17.4°C (□).*

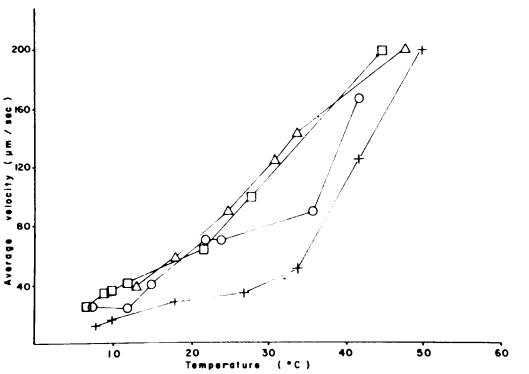

FIG. 7. *Effect of temperature on the velocity of P. fluorescens. The cultures were originally incubated at 37.0°C (+), 21.3°C (○), 17.4°C (△), and 4.0°C (□).*

TABLE 1. *Mode of direction change by bacteria subjected to temperature jump*

Organism	Method of direction change	Flagella arrangement
P. aeruginosa	Reversal	Polar
S. typhimurium	Tumbling	Peritrichous
B. coagulans	Tumbling	Peritrichous
B. polymyxa	Tumbling	Peritrichous
S. serpens	Reversal	Bipolar
P. fluorescens	Reversal	Polar
S. marcescens	Tumbling (rare)	Peritrichous

TABLE 2. *Responses of bacteria to temperature jumps*

Organism	Temp jump (°C)	Response
B. polymyxa	40 to 30	Tumbled 3 s before resuming straight paths
	35 to 27	Tumbled 2 s
	27 to 22	Slowed velocity, no tumbling
	33 to 48	Immediate acceleration, no tumbling
	35 to 55	Acceleration, very long paths with no tumbling
S. serpens	40 to 12	Slows, straight paths, motility lost and not regained on raising temperature
	43 to 30	Slows, straight paths, slowly resumes curved paths, as small as 24 to 48 μm in diameter
	30 to 20	Slows, straight paths, slowly resumes curved paths, as small as 24 to 48 μm in diameter
	12 to 21	Immediate transitory acceleration in straight paths, then resumes curved paths within seconds
	21 to 31	Immediate transitory acceleration in straight paths, then resumes curved paths within seconds
	25 to 50	Immediate acceleration, MFP[a] reduced to 4.5 μm, reciprocates rapidly (seven reversals in 2.7 s)
		Immediate acceleration, MFP[a] reduced to 4.5 μm, reciprocates rapidly (seven reversals in 2.7 s)
	25 to 43	Immediate acceleration, reciprocates, MFP = 48 μm
P. fluorescens	50 to 27	Reverses direction often, erratic paths
	34 to 18	Reverses direction often, erratic paths
	25 to 15	Slows, straight paths, no reversals
	24 to 18	Slows, straight paths, no reversals
	22 to 16	Slows, straight paths, no reversals
	20 to 7.5	Slows, straight paths, no reversals
	15 to 25	Accelerates straight 2 s, then resumes erratic paths
	25 to 40	Accelerates straight 2 s, then resumes erratic paths
	40 to 50	Accelerates straight 4 s, then resumes erratic paths
S. marcescens	30 to 20	Slowed, no change in straight swimming pattern (tumbling rarely observed)
	20 to 13	Slowed, no change in straight swimming pattern (tumbling rarely observed)
	20 to 25	Velocity increased, continued straight pattern
	20 to 50	Slowed, motility ceased after 2 s, straight swimming pattern
P. aeruginosa	42 to 38	Immediate reciprocation, MFP = 50 μm, in many individuals
	43 to 33	Immediate reciprocation, MFP = 50 μm, in many individuals
	34 to 25	Immediate reciprocation, MFP = 23 μm, in many individuals
	15 to 19	Immediate acceleration, straight paths, wandering and occasional reversals after 3 s
	19 to 23	Immediate acceleration, straight paths, wandering and occasional reversals after 3 s
	23 to 33	Immediate acceleration, straight paths, wandering and occasional reversals after 3 s
	33 to 43	Immediate acceleration, straight paths, wandering and occasional reversals after 3 s
	43 to 50	Immediate acceleration, straight paths, wandering and occasional reversals after 3 s

TABLE 2.—*Continued*

Organism	Temp jump (°C)	Response
S. typhimurium	37 to 26	Tumbled 10 s before resuming straight paths
	23 to 20	Tumbled 10 s before resuming straight paths
	32 to 20	Tumbled 5 s before resuming straight paths
	45 to 14	Tumbled 5 s before resuming straight paths
	20 to 30	Immediate acceleration in straight paths
	20 to 26	Immediate acceleration in straight paths
	30 to 40	Immediate acceleration in straight paths
	26 to 44	Immediate acceleration in straight paths
B. coagulans	48 to 33	Tumbled 2 s before resuming straight paths with an occasional tumble
	48 to 42	Tumbled 3 s before resuming straight paths with an occasional tumble
	42 to 35	Tumbled 5 s before resuming straight paths with an occasional tumble
	35 to 42	Immediate transitory acceleration in straight paths
	42 to 48	Immediate transitory acceleration in straight paths
	30 to 50	Immediate transitory acceleration in straight paths

[a] MFP, Mean free path.

The velocity of all species examined was found to increase as the environmental temperature increased (Fig. 4 to 7). In the Arrhenius-van't Hoff relationship, $\log k = [C - E]/[2.303\ R\ (1/T)]$, where k is the reaction rate, C is a constant, E is the energy of activation, R is the gas constant, and T is the absolute temperature. This predicts that the velocity should, in fact, increase logarithmically with temperature. Furthermore, the temperature coefficient $Q_t = k_{t2}/k_{t1}$, usually taken at 10°C increments and designated Q_{10}, would also be constant over the entire temperature range if the velocity increased as a logarithmic function of temperature. A few of our experimental results show that this is the case. *S. typhimurium* incubated at 43°C exhibited Q_{10} values of $Q_{10-20} = 1.7$, $Q_{20-30} = 1.5$, and $Q_{30-40} = 1.4$ (Fig. 5). Q_{10} values for *S. serpens* incubated at 41°C were $Q_{10-20} = 2.5$, $Q_{20-30} = 2.2$, and $Q_{30-40} = 2.09$ (Fig. 6). All curves with relatively constant Q_{10} values are for organisms grown at the highest incubation temperature used. The remaining curves for other species tested are linear, and their velocities were less than predicted by the above equations by an amount that increases as the temperature is raised. This could be due to either a loss in efficiency of conversion of flagellar movement for a given energy input as the temperature is raised or an inverse log relationship between energy input to the flagella and velocity due to mechanics of flagellar action.

Most eucaryotic organisms exhibit rapid declines in motility above 30 to 35°C (3, 9). We saw in this work, however, that there is an increase in velocity with temperature throughout the experimental range. All of the bacteria investigated showed some degree of motility adaptation to incubation temperature. In most cases, velocity-temperature curves are displaced, but parallel, depending upon the temperature at which the bacteria were incubated. The greatest motile activity was found in bacteria grown at the lowest temperature. Motility adaptation to temperature is only a partial adaptation, since an organism grown at 20°C, for example, does not have the same velocity at 20°C as the same organism grown at 40°C exhibits at 40°C.

Recent observations on chemotactic behavior (2, 4) indicate that bacteria suppress their tumbling when encountering an increasing concentration gradient of attractant, whereas tumbling is associated with negative chemotactic responses (10). *B. polymyxa* and *S. typhimurium* were observed to tumble from 2 to 10 s upon a downward temperature jump, after which time they resumed a normal run typical for that temperature. However, when an upward temperature jump was applied to these bacteria, they simply accelerated without showing any other response.

P. fluorescens and *P. aeruginosa* are polarly flagellated, and they change direction (180°) by simple reversal, rather than by tumbling. In a similar manner, when a downward temperature jump was applied, they oscillated for 3 to 4 s before resuming a normal run, but when an upward temperature jump was applied, they accelerated without showing any other response. Thus, a downward jump produces responses similar to those induced by an increasing concentration gradient of repellent, whereas an up-

ward jump appears to induce behavior similar to an increasing concentration gradient of attractant. Maeda et al. (5) observed similar responses with *Escherichia coli*. Their observation slide could not change temperature faster than 0.15°C/s, but they did observe decreases in tumbling with a duration of 1 min in the course of a 2-min temperature increase. The tumbling frequency increased with temperature decreases.

One could speculate that, as a bacterial organism is suddenly cooled, its outer surface will cool before the interior. The cell wall layers will contract more rapidly than the cytoplasmic membrane, which, in turn, may be physically compressed. This temperature differential-induced compression could act to depolarize the membrane, possibly promoting an increase in the tumble frequency. Naitoh and Eckert (6, 7) observed that membrane depolarization can be induced by mechanical pressure on *Paramecium* and *Euplotes*.

Since the uncoordination of *S. serpens* at a high temperature is unique among the bacteria studied, this organism differs in some way as regards the control of its flagella. Bacterial species vary in their lipid content, and it is possible that *S. serpens* has a particular lipid with a melting point of about 40°C in a critical area of the membrane, so as to "short out" or depolarize the membrane and inactivate the direction-changing apparatus. This may correspond to the similar effects produced by lipid- and membrane-active substances.

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