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Temperature-Dependent Anomalies in the Growth of Microorganisms[1]

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ABSTRACT

DAVEY, C. B. (North Carolina State University, Raleigh), RAYMOND J. MILLER, AND LARRY A. NELSON. Temperature-dependent anomalies in the growth of microorganisms. J. Bacteriol. 91:1827–1830. 1966.—Water in the liquid phase (0 to 100 C) has been shown, by others, to undergo subtle changes in its physical structure at approximately 15, 30, 45, and 60 C. It has been suggested that these temperature-dependent anomalies in the structure of water may have biological implications. After incubation in a polythermostat, direct cell counts were made to determine temperature-growth interactions for the four bacteria which were used to cover the temperature range from 5 to 70 C: *Pseudomonas fragi*, 5 to 25 C; *Streptococcus faecalis*, 20 to 40 C; *Bacillus coagulans*, 35 to 55 C; and *B. stearothermophilus* 1518 smooth, 50 to 70 C. In all cases, growth was suppressed at the predicted temperatures, suggesting a strong interaction between the structure of water and biological activity.

Evidence that liquid water and aqueous solutions undergo higher order phase transitions at specific temperatures has recently been reviewed by Drost-Hansen (2). Evidence was cited to show that these transitions take place not only in pure water but also in fairly concentrated aqueous solutions. That these anomalies in the structure of water may affect biological systems was suggested by Drost-Hansen (1). Subsequently, Oppenheimer and Drost-Hansen (5) showed a repression in the growth of a sulfur-reducing bacterium at 16, 31, and 43 C. Since this work has not been confirmed, it was decided to repeat and extend this type of experiment.

This work represents a systematic coverage of the temperature range from 5 to 70 C, with use of appropriate bacteria to cover various portions of the range, in an attempt to determine whether (i) temperature-dependent anomalies occur in the growth of the selected bacteria; (ii) if they occur, to define the temperatures; and (iii) if they occur, to determine which phase in the bacterial life cycle is most strongly influenced.

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MATERIALS AND METHODS

Four bacteria were employed to cover overlapping segments of the temperature range as follows: *Pseudomonas fragi*, 5 to 25 C; *Streptococcus faecalis*, 20 to 40 C; *Bacillus coagulans*, 35 to 55 C; *B. stearothermophilus* 1518 smooth 50 to 70 C. Media employed were APT broth (BBL), TDY broth [containing, in grams per liter: Trypticase (BBL), 20.0; dextrose, 5.0; yeast-extract, 5.0], TSoy broth (containing, in grams per liter: Trypticase, 17.0; phytone, 3.0; NaCl, 5.0; K_2HPO_4, 2.5; dextrose, 2.5), and nutrient agar (Difco). The bacteria were maintained on APT broth (*P. fragi*), TDY broth (*S. faecalis*), or nutrient agar (*B. coagulans* and *B. stearothermophilus*), and were cultured in APT broth (*P. fragi*), TDY broth (*S. faecalis*), and TSoy broth (*B. coagulans* and *B. stearothermophilus*) during experiments. Media employed were those well suited to the growth of the individual bacteria. Inoculum used in any experiment consisted of a 24-hr broth culture (*P. fragi* or *S. faecalis*) or a broth suspension of cells grown on an agar slant for 24 hr (*B. coagulans* or *B. stearothermophilus*). The two bacilli were very dependable in their growth during the first transfer from semisolid to liquid media, but were not dependable when carried through numerous liquid to liquid serial transfers. The reasons for this growth attenuation were not determined. However, it apparently was not related to carry-over of any nutrient or metabolite, since such carry-over would have been increased rather than decreased in liquid to liquid transfers. The incubation temperatures for the

various inocula were 18, 23, 47, and 55 C, respectively, for the appropriate bacteria.

The various experiments were conducted by culturing the bacteria in a polythermostat similar to that of Oppenheimer and Drost-Hansen (5) now commercially available from Labline, Inc., Chicago, Ill. Temperature in any one well was controlled to ± 0.2 C.

In each experiment the culture tubes, each containing 10.0 ml of the appropriate medium, were prepared and placed in the polythermostat at least 8 hr prior to inoculation to allow time for temperature equilibration within the medium. Inoculum consisted of a loop of cells from the broth cultures of *P. fragi* or *S. faecalis* or two drops of the cell suspension of *B. coagulans* or *B. stearothermophilus*.

Bacterial multiplication was determined in every case by direct, individual, cell counts at the end of the incubation period. Incubation periods were 24 hr for *P. fragi*, 16 hr for *S. faecalis* and *B. coagulans*, and 8 hr for *B. stearothermophilus*. These times were the optimum for growth of the various organisms at the incubation temperature of the various inocula. Growth was halted at the end of the incubation period by placing the culture tubes of *P. fragi* and *S. faecalis* in a 50 C water bath and the culture tubes of both bacilli in an ice-water bath. Preliminary studies indicated that these treatments effectively halted growth without destroying cells.

In the experiment concerning the development with time of the temperature-related growth anomaly, *S. faecalis* was cultured as indicated, except that the individual cultures were serially sampled at 2-hr intervals for 24 hr.

RESULTS

The effect of temperature on the growth of the four organisms is shown in Fig. 1, 2, 3, and 4. Temperature-related anomalies were observed in the growth of all four organisms investigated. The anomalies were observed at or very near the four temperatures (15, 30, 45, and 60 C, each plus or minus 2 degrees) predicted by the physical data available for water and aqueous solutions.

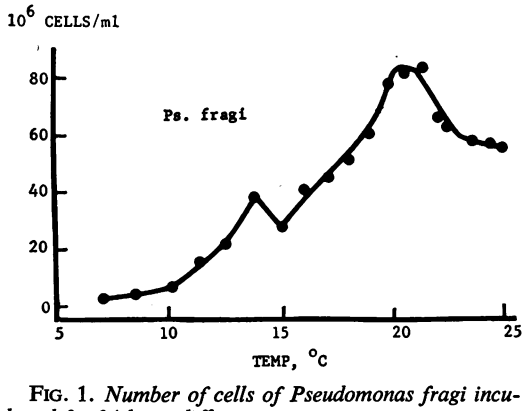

FIG. 1. *Number of cells of Pseudomonas fragi incubated for 24 hr at different temperatures.*

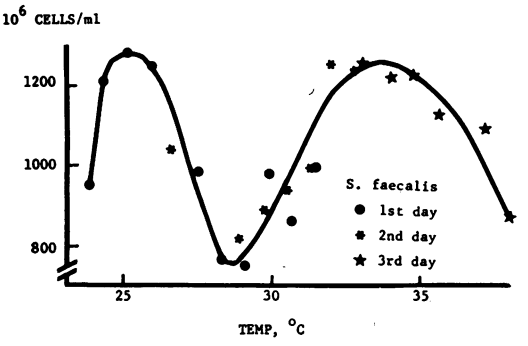

FIG. 2. *Number of cells of Streptococcus faecalis incubated for 16 hr at different temperatures.*

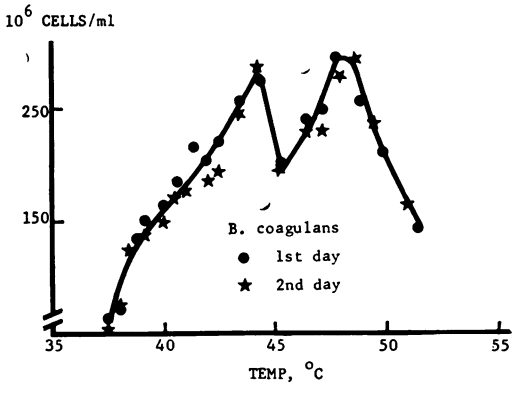

FIG. 3. *Number of cells of Bacillus coagulans incubated for 16 hr at different temperatures.*

Anomalies at temperatures other than the four predicted ones were not observed.

Each point in Fig. 1, 2, 3, and 4 is the average of duplicate determinations. There were at least three replicate counts in each determination with a maximal variation of 15% within counts. The duplicates were always within 10% of each other. The curves indicated in Fig. 1, 3, and 4 were each reproduced three times, and the curve in Fig. 2 was reproduced eight times. That these growth curves can be replicated is shown in Fig. 3 where the values obtained on two different days are plotted simultaneously. To see whether various segments of the curve could be obtained at different times, Fig. 2 was obtained on three successive days with successive days overlapping.

As expected from classical concepts of microbiology, the lag phase in the growth of *S. faecalis* was affected in inverse relation to temperature (Fig. 5). However, the log phase (Fig. 6) and the maximal cell density (Fig. 5) were both found to be adversely affected near 30 C. The log-phase plot (Fig. 6), although based on somewhat fewer

data than might be desired, nevertheless clearly indicates that the slope of the 30 C line falls out of place in the family of lines. The cell density per unit volume of medium reaches a maximal value considerably below that reached by other cultures at temperatures both above and below 30 C (Fig. 5). The development of the growth anomaly with time indicates that 30 C is the critical temperature, especially at 6 and 8 hr (Fig. 7).

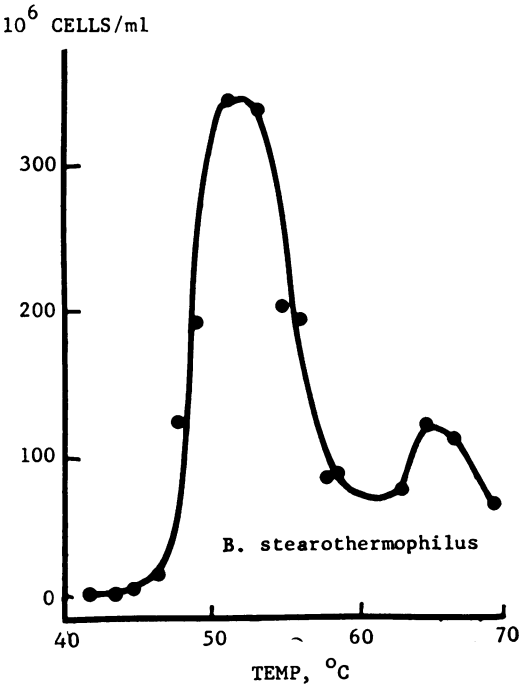

FIG. 4. *Number of cells of Bacillus stearothermophilus 1518 smooth incubated for 8 hr at different temperatures.*

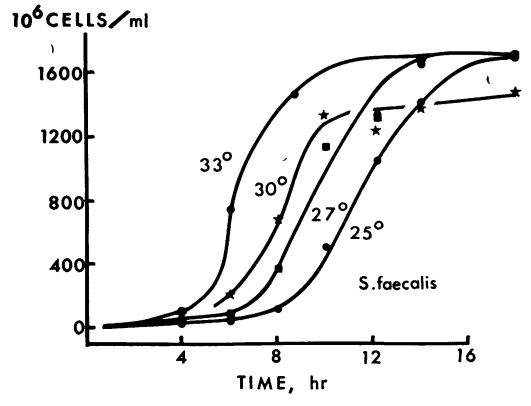

FIG. 5. *Time-temperature interaction in the growth of Streptococcus faecalis.*

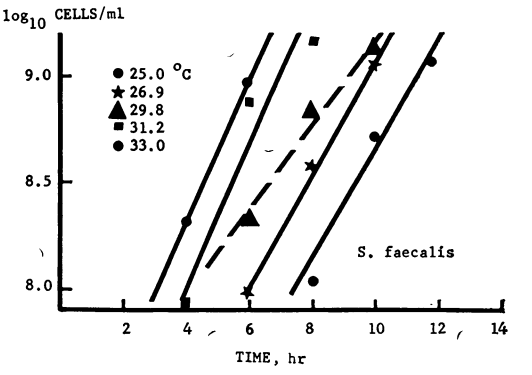

FIG. 6. *Effect of temperature on the log phase of growth of Streptococcus faecalis.*

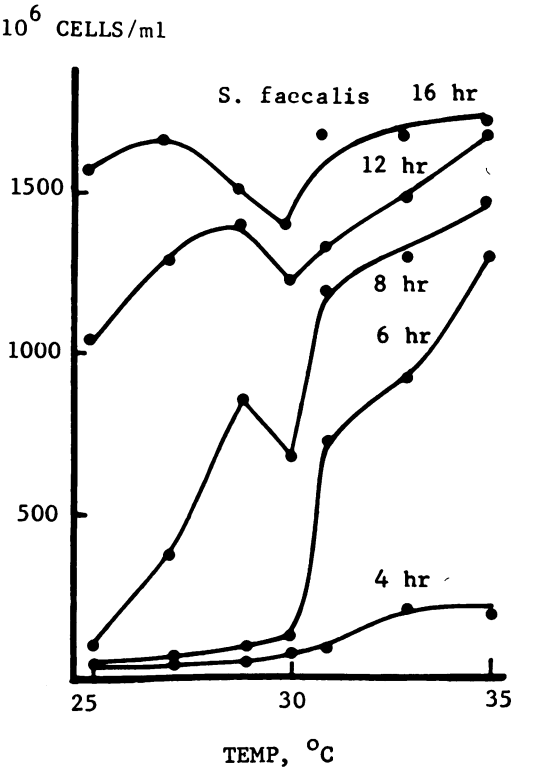

FIG. 7. *Number of cells of Streptococcus faecalis at different temperatures and times.*

DISCUSSION

The effect of temperature on the growth of the four bacteria was found to be that predicted by Oppenheimer and Drost-Hansen (5) and to occur at the temperatures where there is an anomalous change in the structure of water and aqueous

solutions. In all four cases (Fig. 1, 2, 3, and 4), a repression in growth occurred within ±2.0 degrees of 15, 30, 45, or 60 C.

Most of the evidence for anomalous structure changes in water or aqueous solutions has not been analyzed statistically. Lavergne and Drost-Hansen (3) have supported some of the physical evidence with statistical analysis. Since the results in Fig. 2 show the largest repression of growth and the largest separation of peaks, these data were analyzed statistically. A standard regression analysis with deviations from a quadratic, cubic, or quartic fitted regression line was performed. The fit of the quartic line was significant at the 10% level. Neither the quadratic nor cubic components were significant. Statistical analysis, thus, also predicts three flex points for these data.

Drost-Hansen (2) concludes that structural elements of different, discrete types coexist in water and aqueous solutions at any one temperature. The molecules within these structural units exhibit cooperativeness, which gives rise to the thermal anomalies. If this is the general character of water and aqueous solutions, there are several possible explanations for the anomalies in biological systems. The effect could be external to the biological body and reduce diffusion of nutrients and waste products to and from the system or cell. The effect could also be internal, that is, within the body or cell itself. The movement of fluids across membranes could be affected by the change in water structure. Or, it could be that water will allow efficient formation of enzymes or coupling of enzymes and substrates when one phase of water predominates between the anomalous temperatures, but at or near the specific temperatures, the formation or coupling is inefficient, and the biological activity is reduced. Finally, oxygen solubility may be reduced at the critical temperatures. However, a search of the literature on oxygen solubilities did not yield data supporting this possibility.

These results, although offering no additional proof of temperature-related structural anomalies of water, do provide some fairly strong circumstantial evidence in support of the concept. The observed temperature-related anomalies in the growth of the four bacteria seem to be greater in magnitude than most, if not all, of the published physical data, indicating the highly sensitive nature of biological systems to subtle alterations in their microenvironment (4).

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