## Significance of the Temperature Characteristic of Growth<sup>1</sup>

## F. J. HANUS AND RICHARD Y. MORITA

Departments of Microbiology and Oceanography, Oregon State University, Corvallis, Oregon 97331

Received for publication 9 October 1967

The van't Hoff-Arrhenius plot is commonly used in chemical kinetics to determine the activation energy of chemical reactions. If log<sub>e</sub> of the reaction rate is plotted as a function of the reciprocal of the absolute temperature, the slope of the straight line obtained is proportional to the energy of activation of the reaction. Microbiologists have substituted bacterial growth rate for reaction rate in the van't Hoff-Arrhenius equation to obtain the temperature characteristic of growth  $(\mu)$ ;  $\mu$  is then analogous to activation energy. Unfortunately, attempts have been made to relate the classification of a species as a mesophile or psychrophile to the  $\mu$  value calculated for the species.

Our purpose was to determine whether this relationship existed with several closely related species of vibrios having different temperature requirements (Table 1). The experiments were carried out in a multiple-temperature water bath with carefully controlled temperatures. Growth vessels consisted of 1-liter round-bottom flasks with provisions for continuous sparging and aseptic sampling. This procedure allowed us to determine 10 growth rates simultaneously. Thus we eliminated three possible sources of error, which may have been important in some earlier work: (i) the medium could be made up in one batch and, therefore, would be identical at each growth temperature; (ii) the same culture could be used as an inoculum for the various growth temperatures; and (iii) sufficient experimental points (usually 10) were used to accurately define the slope of the  $\mu$  value curve.

SDB medium (R. D. Haight and R. Y. Morita, J. Bacteriol. 92:1388, 1966) was employed throughout these experiments. Stock cultures were maintained on agar slopes. A  $1\%$  inoculum of log-phase cells was added to each of the 1-liter round-bottom flasks which contained 500 ml of medium. All flasks were vigorously aerated. The amount of growth was determined by measuring the optical density of the growth medium at

<sup>1</sup> Published technical paper no. 2393, Oregon Agricultural Experiment Station.

420  $m\mu$  in a colorimeter (Bausch and Lomb Spectronic 20). The  $\mu$  values were calculated from the formula:

$$
\log_{\rm e} \text{ growth rate} = -\mu/\text{RT}
$$

(For details, see J. L. Ingraham, J. Bacteriol. 76: 75, 1958.)

Figure 1 shows the generation rate of Vibrio marinus MP-1,  $V$ . marinus PS-207, and  $V$ . metschnekovii as a function of the temperature of growth. Table <sup>1</sup> lists maximal and optimal







FIG. 1. Generation rate as a function of temperature (C) for Vibrio marinus MP-I  $(\Box)$ , V. marinus **PS-207** ( $\bigcirc$ ), and *V*. metschnekovii ( $\bigtriangleup$ ).



FIG. 2. van't Hoff-Arrhenius plot for Vibrio marinus. MP-L. The logio of the growth rate is plotted as a function of the reciprocal of the absolute temperature.



FIG. 3. van't Hoff-Arrhenius plot for Vibrio marinus, **PS-207.** The  $log_{10}$  of the growth rate is plotted as a unction of the reciprocal of the absolute temperature.

growth temperatures. Figures 2, 3, and 4 give the van't Hoff-Arrhenius plots of the data in Fig. 1. The slopes of these curves are proportional to the  $\mu$  values which are listed in Table 1 for all three organisms. If optimal growth temperature and  $\mu$  are related, as has been previously suggested by Ingraham (J. Bacteriol. 76:75, 1958), then MP-1 should possess the lowest  $\mu$ value.

Ingraham (J. Bacteriol. 76:75, 1966) reported that a psychrophilic pseudomonad and Escherichia coli had  $\mu$  values of 9,050 and 14,200, respectively. Our recalculation of Ingraham's data and his recalculation (personal communication) indicate that the values given were in error by a factor of two. The corrected values would be 18,100 for the psychrophile and 28,400 for the mesophilic E. coli. Both of the corrected values are higher than any value we calculated for psychrophilic or mesophilic vibrios. They are considerably higher than the values Shaw

published for mesophilic and psychrophilic yeast (approximately 12,000; J. Bacteriol. 93: 1332, 1967). The  $\mu$  value of 28,400 for E. coli is not in agreement with the earlier calculation of 14,000 made by Johnson and Lewin (J. Cellular Comp. Physiol. 28:47, 1949) or the later calculation of 16,000 made by Ng, Ingraham, and Marr (J. Bacteriol. 84:331, 1962). However, no valid comparison is possible, because each investigator employed a different growth medium for E. coli. These discrepancies make it difficult to substantiate the earlier supposition that psychrophiles have a lower  $\mu$  value than do mesophiles.

Shaw (J. Bacteriol 93:1332, 1967) could find no difference in  $\mu$  values for some mesophilic and psychrophilic yeasts. Brownlie (as cited by M. K. Shaw, J. Bacteriol. 93:1332, 1967) could find no consistent difference in the  $\mu$  values for mesophilic and psychrophilic gram-positive bacteria; however, no data were presented. These investigations together with our results, indicate that there is no consistent difference in the  $\mu$ values of mesophiles and psychrophiles. Whereas  $\mu$  values may be a property of the particular species studied or of the medium in which the organism is grown, there appears to be little reason to try to correlate the  $\mu$  of an organism with its temperature range of growth.

This investigation was supported by Public Health Service Training grant <sup>5</sup> Ti GM <sup>704</sup> MIC from the National Institute of General Medical Science and by National Science Foundation Grant GB 6548. F. J. H. is a National Institute of Health predoctoral trainee.



FIG. 4. van't Hoff-Arrhenius plot for Vibrio metschnekovii. The  $log_{10}$  of the growth rate is plotted as a function of the reciprocal of the absolute temperature.