Influence of Temperature on Steady-State Growth of Colonies of *Pseudomonas fluorescens*

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Diameters of surface colonies of *Pseudomonas fluorescens* were observed to increase linearly with time at temperatures from 30 to 0 C.

The influence of temperature on bacterial growth in liquid culture is well known (2). There have been, however, few studies of its influence on the growth of bacterial colonies. Pirt (4) studied the influence of temperature on the radial increase of surface colonies of *Escherichia coli*, *Klebsiella aerogenes*, and *Streptococcus faecalis*, but only over the limited range of 37 to 25 C. Haines (1), using the increase of the length of hyphae as a measure of growth, studied the influence of temperature on the surface growth of several actinomycetes. He observed linear increases of hyphal length with time. In another report, we (Palumbo, Rieck, and Witter, Bacteriol. Proc., p. 33, 1964) proposed that a linear increase of colony diameter with time represents the steady-state growth of bacterial colonies. Here we present data which further support that proposal by demonstrating that changes in temperature produce only changes in the rate of diameter increase.

We employed a psychrophilic strain of *Pseudomonas fluorescens* originally obtained from Sinclair and Stokes (5) and grew the colonies on glucose-salts-agar [glucose-salts broth (3) + 1.5% agar (Difco)]. This organism is known to grow from 0 C to a maximum temperature of 33 C in glucose-salts broth (S. A. Palumbo, Ph.D. thesis, Univ. of Illinois, 1967). Special flat-bottom petri dishes containing 30 ml of the agar medium were inoculated by the surface plating technique to give three to five colonies per plate. The plates were then incubated at temperatures from 30 to 0 C at 5-degree intervals. Diameters of 10 colonies at each temperature were measured periodically with a calibrated micrometer eyepiece; the diameters were averaged, and the average diameter was plotted versus time. A family of straight lines is observed (Fig. 1). Because of the slow rate of diameter increase and the long period (260 hr) before macroscopic colonies (0.45 mm) were visible, the 0 C plot is omitted. However, it was also linear. For each temperature, the rate of diameter increase with time in millimeters per hour was measured, and this data is presented in the form of an Arrhenius plot (Fig. 2). The best straight line was calculated by the least sum of squares method. From the slope, the temperature characteristic or µ was then calculated by the equation: µ = (slope) (2.303) (R), where R is the universal gas constant. A µ of 12,480 cal is obtained.

The µ of 12,480 cal for the temperature influence on surface growth is less than the 20,950 cal obtained for the same organism grown in glucose-salts broth (S. A. Palumbo, Ph.D. thesis, Univ. of Illinois, 1967). Thus, temperature influences the growth of surface colonies much less than the growth of liquid cultures. For all temperatures tested, the diameters of surface colonies of *P. fluorescens* increased linearly with time.

The observed changes in the growth rate of surface colonies of *P. fluorescens* at different temperatures cannot be explained solely on the

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![Fig. 1. Influence of temperature on the increase of colony diameter with time for *P. fluorescens* on glucose-salts-agar.](http://jb.asm.org/)

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basis of temperature-limited diffusion of nutrients to the colonies. By using diffusion constants from the *International Critical Tables* (6), the $\mu$ values for diffusion of hexose and ammonium sulfate were calculated and found to be 4760 and 5350 cal, respectively. Thus diffusion is much less sensitive than growth to changes in temperature. Another possible influence could be accumulated toxic metabolic products. However, Sinclair and Stokes (5) demonstrated that this strain did not produce autoinhibitory products during recycling studies in the glucose-salts broth.

We thus can discount the roles of nutrient diffusion and toxic products as principal causes of the observed changes in growth rates of surface colonies and conclude that temperature did cause these changes in the rate of colony diameter increase at the different temperatures. The influence of temperature follows the Arrhenius relationship as does growth in liquid culture.

**LITERATURE CITED**