Temperature Characteristics of Psychrotrophic and Psychrophilic Bacteria

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When the Arrhenius equation is applied to microbial growth rates, a temperature characteristic (μ_A) represents the activation energy. While these μ_A values describe the energetics favouring growth, there is still no convincing evidence from empirical data to support the idea of a widely applicable relationship between μ_A values and optimal growth temperatures, which may serve as a basis for the classification of psychrophiles, psychrotrophs, mesophiles and thermophiles. The μ_A values for the exponential growth rates of 16 psychrophilic and psychrotrophic bacterial strains, including 9 from the literature, varied from 3 to 33 (kcal mol⁻¹). A plot of μ_A versus growth temperature optimum deviated considerably from empirical equations reported recently (Mohr & Krawiec, 1980) for a different selection of strains. Moreover, Arrhenius profiles with two distinct slopes at suboptimal temperatures were not restricted to bacteria with growth temperature optima higher than 37 °C, as had been suggested in that report. A psychrotrophic strain of *Cytophaga johnsonae* with a growth temperature optimum of 23–25 °C was characterized by two different temperature characteristics. Furthermore, in the upper range of suboptimal temperatures (11–24 °C), temperature characteristics (μ_{A1}) were also affected by temperature acclimation of the inoculum.

INTRODUCTION

Growth–temperature relationships of bacteria are adequately described by temperature characteristics representing Arrhenius constants (μ_A) of the exponential growth rates (Arrhenius, 1908; Dean & Hinshelwood, 1966). However, some attempts to establish a relationship between μ_A and the temperature ranges used for the classification of psychrophiles, psychrotrophs, mesophiles and thermophiles (Ingraham, 1958) could not be confirmed (Shaw, 1967; Hanus & Morita, 1968; Tai & Jackson, 1969). Nevertheless, a relationship between μ_A and growth temperature optima has recently been described on the basis of two empirical equations (Mohr & Krawiec, 1980), suggesting a distinctive pattern for bacteria with growth temperature optima below 15 °C and 30 °C, i.e. psychrophiles and psychrotrophs, respectively (Morita, 1975). Our data, obtained from a set of psychrophilic and psychrotrophic freshwater isolates, do not confirm the general validity of those equations nor is there substantial published evidence for their assumption.

Mohr & Krawiec (1980) described the occurrence of complex Arrhenius profiles consisting of more than one negative slope. They suggested that only organisms with optimum growth temperatures above approximately 37 °C can have two distinct temperature characteristics at suboptimum temperatures. In contrast to this suggestion, we show here that disparate Arrhenius profiles can also occur in psychrotrophic bacteria with growth temperature optima far below 37 °C.

METHODS

Seven psychrophilic and psychrotrophic isolates from cold ($<5^{\circ}\text{C}$) freshwater environments were grown at 6, 10, 14, 18, 22, 26 and 30°C (shaking water bath, operated at 150 rev. min^{-1}) in 50 ml Erlenmeyer flasks containing 10 ml half-strength Difco nutrient broth. Growth rates were calculated from readings, taken at 12 h intervals, of apparent absorbance at 578 nm (0.2 ml microcuvette, 1 cm path-length).

A psychrotrophic strain of *Cytophaga johnsonae* (strain C21) had been isolated from lake water (Reichardt, 1974). Its exponential growth rates were determined in a shaking (40 strokes min^{-1}) Temperature Gradient Incubator (Scientific Industries Inc., Mineola, N.J., U.S.A.) constructed for duplicate incubation in side-arm tubes. These contained 10 ml of autoclaved basal mineral medium [containing (g l^{-1}): $(\text{NH}_4)_2\text{SO}_4$, 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; CaCl_2 , 0.003; FeCl_3 , 0.0003; together with 10 mM- $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer, pH 7.0] supplemented with 10 mM filter-sterilized *N*-acetylglucosamine as sole organic carbon and nitrogen source. Tubes were inoculated with 0.1 ml portions of late-exponential phase cultures grown at 10 or 23°C (apparent absorbance at 500 nm of 0.5; Bausch & Lomb Spectronic 20). Growth of cultures was monitored at 3–8 h intervals for up to 72 h using the same instrument. From semi-logarithmic plots of apparent absorbance, the exponential growth rates (k) were determined using the equation: $k = \ln(A_n/A_0)/\Delta t$, in which A_0 and A_n are the apparent absorbances at the beginning and end, respectively, of the time interval Δt .

To calculate temperature characteristics, a specific version of the Arrhenius equation applicable to microbial growth rates (Dean & Hinshelwood, 1966) was employed, i.e. $k = Ae^{-\mu_A/RT}$, in which μ_A , the temperature characteristic, is here substituted for the activation energy, R is the gas constant ($1.987\text{ cal K}^{-1}\text{ mol}^{-1}$), T is the absolute temperature (K), and A is a constant. In Arrhenius plots showing $\ln k$ (h^{-1}) as a function of $1/T$ (K^{-1}), the negative slopes of the linear portions of the curves obtained at suboptimal growth temperatures equalled $-\mu_A/R$, with r^2 values describing their goodness of fit.

RESULTS AND DISCUSSION

Temperature characteristics have generally been given the units kcal mol^{-1} . However, SI guidelines require the use of the joule ($1\text{ cal} = 4.184\text{ J}$), and the meaning of the molar dimension in connection with bacterial growth is obscure. Hence, we suggest calculating Arrhenius constants in kcal mol^{-1} but without expressing the units, until a meaningful alternative is found. This is in agreement with the original description of μ_A (Arrhenius, 1908) and a few later publications (Hanus & Morita, 1968; Baker, 1974).

General validity of the Mohr–Krawiec equations

In order to check the general applicability of the empirical relationship between temperature characteristics (μ_A) and growth temperature optima of Mohr & Krawiec (1980), μ_A values of seven of our isolates and nine from the literature were plotted against growth temperature optima up to 30°C (Fig. 1). The μ_A values of these psychrophilic and psychrotrophic bacteria varied over a relatively wide range, from 3 to 33. The empirical curve obtained by Mohr & Krawiec (1980) for a corresponding range of growth temperature optima, i.e. $\leq 30^{\circ}\text{C}$, was described by the equation: $\mu_1 = (2.73 \times 10^5) t^{-0.738}$, with μ_1 corresponding to our μ_{A1} . This equation was based on μ_1 values ranging from 12.7 to 51.9, and suggested a very steep increase of the temperature characteristic in the lower range of the growth temperature optima, i.e. below 25°C . The steepness was caused exclusively by two psychrophiles with extremely high μ_1 values (51.9 and 39.0). However, when considering the μ_A versus temperature optimum plot obtained for a larger number of psychrophilic and psychrotrophic bacteria (Fig. 1), it seems unlikely that the μ_A values on which the empirical equation of Mohr & Krawiec (1980) was based, were characteristic of the majority of bacteria with low temperature optima. The temperature characteristics of most of our psychrophilic and psychrotrophic isolates and those based on literature values for other bacteria of these groups were considerably lower. Moreover, the Arrhenius constants of the two *Vibrio marinus* strains used in the empirical curve of Mohr & Krawiec (1980) (39.0 and 23.2) were much higher than those reported in an earlier paper (16.2 and 16.4, respectively; Hanus & Morita, 1968). Thus, the empirical equations presented by Mohr & Krawiec (1980) are not applicable to other psychrophilic and psychrotrophic bacteria nor are they

Fig. 1. The μ_A values of psychrophilic and psychrotrophic bacteria and ranges of optimal growth temperatures. ○, Gram-negative rods with respiratory metabolism, isolated from Rhône Glacier, Switzerland; ●, *Bacillus* sp., from a subalpine stream, S.W. Germany; □, pseudomonads, from profundal lake sediment (1000 m, Lake Baikal, Siberia); ■, *Cytophaga johnsonae* C21, from a eutrophic lake, West Germany ($\mu_{A1} = 4.7$ and $\mu_{A2} = 33.3$); △, *Vibrio marinus* MP1 (Hanus & Morita, 1968); ▲, *V. marinus* PS-207 (Hanus & Morita, 1968); ▼, *Pseudomonas fluorescens* (estimated value of $\mu_{A1} = 3.2$) (from Lynch *et al.*, 1975); +, psychrotrophic bacteria, from Antarctic peat (Baker, 1974). Data points from the present study are marked by horizontal bars indicating the length of the temperature intervals employed during measurement of the optimal growth temperatures.

Fig. 2. Arrhenius plots for a psychrotrophic strain of *Cytophaga johnsonae* (C21) after growth of the inoculum at 10 or 23 °C. Data points contributing to the negative slopes in each experiment were grouped by linear regression analysis to produce two straight lines with r^2 values maximized over the entire range of suboptimal growth temperatures tested. Temperature characteristics calculated for the upper (μ_{A1}) and lower (μ_{A2}) ranges of suboptimal temperatures, together with r^2 values (in parentheses) were: inoculum grown at 10 °C (●), $\mu_{A1} = 4.7$ (0.64), $\mu_{A2} = 33.3$ (0.98); inoculum grown at 23 °C (○), $\mu_{A1} = 12.3$ (0.93), $\mu_{A2} = 32.0$ (0.85).

independent of the different techniques used to measure exponential growth rates (as shown for *Vibrio marinus*).

One of our psychrotrophic isolates (*Cytophaga johnsonae* C21) showed two distinct temperature characteristics (see below), although its temperature optimum was only 23–25 °C. According to Mohr & Krawiec (1980), such disparate Arrhenius profiles can only be expected for bacteria with growth temperature optima higher than 37 °C. Furthermore, calculations of μ_A values based on published data of another psychrotrophic bacterium (*Pseudomonas fluorescens* strain E20) indicated that this was not a singular finding (Fig. 1; Lynch *et al.*, 1975).

Effect of temperature acclimation of the inoculum on temperature characteristics

Deviations within the negative slope of Arrhenius plots have been interpreted as due to uncoupling of energy production from energy utilization (Senez, 1962; Ng, 1969). Such

deviations may also occur as a result of insufficient temperature acclimation which should be considered as an intrinsic factor influencing growth rate measurements in a temperature gradient (Shaw, 1967; Zachariah & Liston, 1973). Therefore, temperature-dependent exponential growth rates of *Cytophaga johnsonae* C21 were determined, not only after the lapse of approximately one generation time, but also by using inocula from cultures adapted to different temperatures (Fig. 2). The first negative slope for the temperature range from 24–12 °C corresponded to a temperature characteristic (μ_{A1}) of 4.7 when the cells in the inoculum were acclimated to 10 °C, whereas the temperature characteristic was 12.3 when the cells in the inoculum were acclimated to 23 °C. On the other hand, the temperature characteristics calculated from the second negative slope of the Arrhenius plots (μ_{A2}) were almost identical (33.3 after 10 °C acclimation, and 32.0 after 23 °C acclimation). These results suggested an adaptation in the upper temperature range, rather than in the lower as described for psychrotrophic strains of *Pseudomonas* (Zachariah & Liston, 1973).

CONCLUSIONS

Our data on psychrophilic and psychrotrophic bacteria deviate considerably from the observations and conclusions of Mohr & Krawiec (1980) who referred to only three isolates with comparably low growth temperature ranges. As a measure of the activation energy for growth at suboptimal temperatures, temperature characteristics (μ_A) describe primarily the energetic favouring of substrate-specific growth reactions, and are not necessarily linked to certain growth temperature ranges or temperature optima (Dean & Hinshelwood, 1966; Kovacs *et al.*, 1968; Hanus & Morita, 1968; Reichardt, 1971; Baker, 1974; Morita, 1975). Our results show that neither the empirical relationship between optimal growth temperatures and temperature characteristics (μ_A) nor the reported restriction of disparate Arrhenius profiles to mesophiles and thermophiles, as suggested by Mohr & Krawiec (1980), are generally applicable phenomena.

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