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Effect of Abrupt Temperature Shift on the Growth of Mesophilic and Psychrophilic Yeasts

MAXWELL K. SHAW
Division of Food Preservation, C.S.I.R.O., Cannon Hill, Queensland, Australia

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Exponentially growing cultures of mesophilic and psychrophilic yeasts were subjected to abrupt changes in temperature. Temperature shifts made within the range in which the temperature characteristic, μ, is relatively constant (moderate temperatures) immediately induced growth at the normal exponential rate for the new temperature. Prior incubation at temperatures defined as moderate enabled some yeasts to grow for a few generations at temperatures higher than their normal maximal temperature for growth. Shifts made to or from temperatures above or below those in the moderate temperature range resulted in growth rates that were intermediate between the normal steady-state rates for the initial and final temperatures. A period of transient growth rate at the new temperature outside the moderate temperature range seems to be required before normal steady-state growth rates can be attained after such temperature shifts. The psychrophiles gave transient growth rates only below 10 C, whereas the mesophiles gave transient rates below 20 C. However, the psychrophiles cannot be distinguished from the mesophiles on the basis of the temperature characteristic, μ, which was found to be about 12,000 cal/mole for both types.

Ingraham and Bailey (3) pointed out that μ, the "temperature characteristic" of the growth rate of a given bacterial strain, is relatively constant near the center of the temperature range over which growth of that strain occurs; it decreases at higher temperatures, and at lower temperatures it increases dramatically. The temperature characteristic, μ, is defined by the equation: $\log k = \mu/2.303\ RT + C$, in which k is the specific growth rate, R is the gas constant, T is the absolute temperature, and C is a constant. The use of the temperature characteristic, μ, is adequately discussed in the texts of Porter (8) and Lamanna and Mallette (4).

Ng, Ingraham, and Marr (6) found with *Escherichia coli* ML30 that sudden temperature shifts, within the temperature range where μ is relatively constant for this species, resulted in an immediate change in its specific growth rate to the specific rate appropriate to the new temperature. However, when the temperature was changed rapidly to temperatures below the range where μ is constant, an immediate lag in growth occurred. This lag was later followed by a period of rapid exponential growth until the normal rate of growth for the particular organism at the lower temperature was attained. Converse temperature shifts, from a temperature below to temperatures within the range of relatively constant μ, caused growth of the organisms to be slow at first, but the rates finally attained were identical with the normal growth rates at the new temperature.

This paper reports the results of similar experiments in which the effects on the growth rates of mesophilic and psychrophilic yeasts of temperature shifts to and from both high and low temperatures were studied. The effect of temperature on the growth of these yeasts is discussed in terms of typical temperature characteristic values for mesophiles and psychrophiles, and the relationship between minimal and maximal growth temperatures is also considered.

MATERIALS AND METHODS

Organisms. Seven psychrophilic and two mesophilic yeasts were studied. The psychrophilic yeasts, isolated from beef stored at 0 C, were *Candida scottii* (Y1), *Trichosporon pullulans* (Y8 and Y9), *C. zeylanoides* (Y15 and Y71), *C. mycoderma* (H1), and *Rhodotorula mucilaginosa* (R1). The numbers relate to the culture collection in the C.S.I.R.O. Meat Research Laboratory at Cannon Hill, Queensland, Australia. The mesophilic strains, *C. tropicalis* and *C. krusei*, were obtained from the Department of Microbiology, University of Queensland.

Medium. The growth medium contained: yeast extract, 5 g; malt extract, 3 g; dextrose, 20 g; $(NH_4)_2\cdot$

HPO_4, 0.72 g; $NH_4H_2PO_4$, 0.26 g; and distilled water, 1 liter. The *p*H was adjusted to 5.5 with 10% (w/v) lactic acid, and the medium was autoclaved.

Culture apparatus. Cultures were grown in Pyrex T-tubes (6 inches by ⅝ inch; 15.2 by 1.6 cm) containing 10 ml of medium. The tubes, suitably mounted in a refrigerated water bath controlled at the appropriate temperature to within ±0.1 C, were rocked at 25 oscillations/min at an amplitude of about 6 inches. Baths maintained at temperatures less than 20 C were operated in cool rooms, to avoid condensation on the tubes and to prevent transient temperature rises during measurements of turbidity. In temperature-shift experiments, transfers were made when the cultures were in the mid-range of exponential growth. Usually a 1.0-ml sample of the growing culture was transferred to 9 ml of fresh medium at the new temperature, but direct transfers without dilution were also made.

Measurement of growth. Growth was followed by periodically measuring turbidity with a photoelectric nephelometer, the response of which was directly proportional to yeast concentration.

The specific growth rate, k, in hr^{-1}, was calculated from the formula:

$$k = \frac{dx}{xdt} = \frac{2.303\ (\log_{10} x_2 - \log_{10} x_1)}{t_2 - t_1}$$

in which x_1 and x_2 are the turbidities at times t_1 and t_2, respectively. The specific growth rate, k, should not be confused with the more commonly used growth rate, R, which is expressed in base 2 logarithms.

$$R = \frac{\log_2 x_2 - \log_2 x_1}{t_2 - t_1} = k/0.693$$

RESULTS

Normal temperature ranges for growth. Specific growth rates of the yeasts were determined at 10 temperatures, and are represented in the form of Arrhenius plots in Fig. 1. On the basis of these data, the psychrophilic and mesophilic yeasts are not readily grouped into distinct classes; rather, there is a gradation of optimal and maximal temperatures in passing from one class to another. It is apparent, however, that the yeasts growing best at low temperatures also have the lowest maximal temperatures for growth. *T. pullulans* (Y9) had a specific growth rate of 0.024 hr^{-1} at 0 C, and failed to grow above 25 C; the specific growth rate for *C. zeylanoides* (Y15) was 0.016 hr^{-1} at 0 C, and no growth occurred above 30 C; the specific growth rate of *Rhodotorula mucilaginosa* (R1) was 0.010 hr^{-1} at 0 C, and this yeast failed to grow above 35 C. Specific growth rates at moderate temperatures were closely similar for all strains examined, and at 20 C the k values ranged only from 0.19 to 0.25.

Temperature characteristic (μ) values. The temperature characteristic (μ) values can be calculated from the slope of the straight line of best fit passing through the points in the middle region of the temperature range where the slope is relatively constant (Fig. 1). By applying this method, it appears that μ is constant for both mesophiles and psychrophiles. The average value was 12,300 cal/mole, and the values ranged from 11,900 [*C. zeylanoides* (Y15)] to 13,400 [*R. mucilaginosa* (R1)]. There was no consistent difference in μ values between mesophilic and psychrophilic species.

Step-up experiments. The growth rate of *T. pullulans* (Y9) was measured after suddenly exposing this psychrophile to increases in temperature. These "step-up" experiments can be divided into three types: (a) those involving temperature transfers within the range where μ is relatively constant (termed "moderate" temperatures), (b) those where changes were from lower temperatures to moderate temperatures, and (c) those involving changes from moderate temperatures to higher temperatures. Typical results for each type of transfer are shown in Fig. 2. Cultures subjected to type (a) transfers immediately changed their specific growth rates to those characteristic of the same organisms growing at the higher temperature. In a type (b) transfer, however, the initial growth rate was less than the normal rate at the new, higher temperature for up to two generations. When cultures were subjected to type (c) transfers (from 15 to 25 C), the initial specific growth rate persisted for 3.5 generations and was higher than the steady-state (0.13 hr^{-1}) later attained at the new, higher temperature.

A fourth type of "step-up" in temperature involved transferring a culture of *T. pullulans* (Y9) from a moderate temperature (15 C) to a temperature above the maximal temperature for growth (30 C) of this psychrophile. After such a temperature shift, a transient specific rate of 0.24 hr^{-1} was maintained for 0.72 generations (2 hr) before growth ceased.

Figure 3 shows, in the form of Arrhenius plots, the transient rates occurring on "step-up" and the normal specific growth rates of *T. pullulans* (Y9). The respective transient rates after shifts from 5 C to 10, 15, and 20 C fit on a line parallel to, and beneath, the line drawn through the steady-state growth rates for 10, 15, and 20 C. Similarly, the fast transient rate at 25 C (transfer from 15 C) fits on the extension of the same steady-state line. When these experiments were repeated with the mesophile *C. tropicalis*, the same patterns held as for the psychrophile, but there was a general upward shift of all temperature ranges. Transfers of type (a) to 20, 25, and 30 C were made from 10 C, and in all these

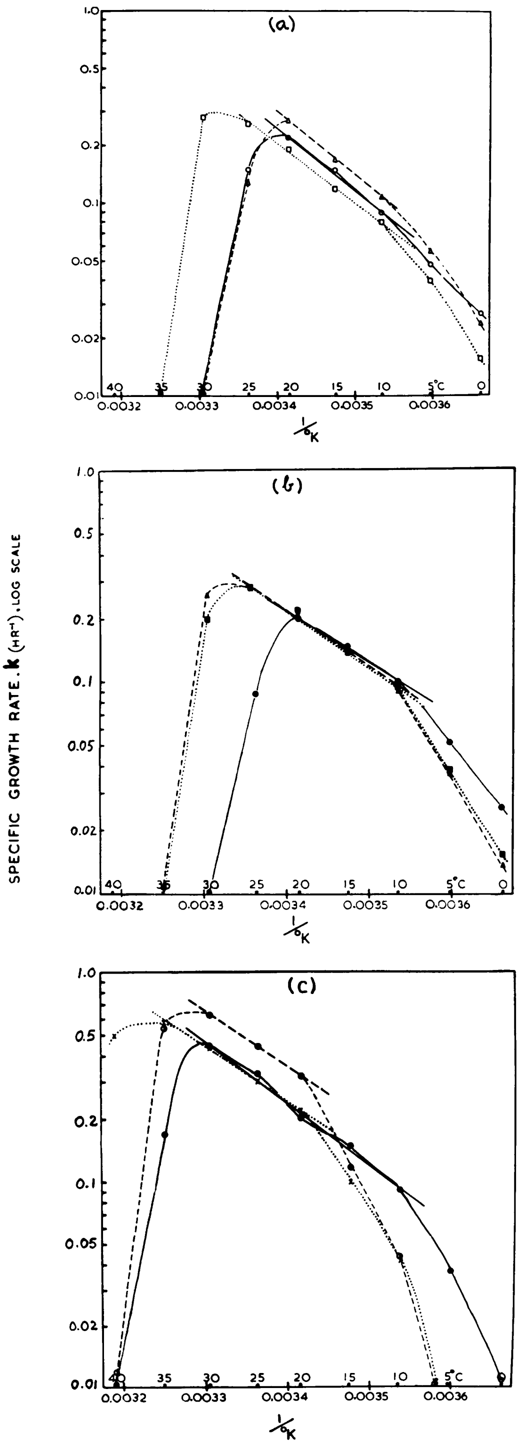

FIG. 1. *Arrhenius plot of normal specific growth rates, as a function of absolute temperature, for mesophilic and psychrophilic yeasts. Symbols:* ○, *Candida scottii (Y1);* □, *C. zeylanoides (Y15);* ▲, *C. zeylanoides (Y71);* ◓, *Rhodotorula mucilaginosa (R1);* × *Candida krusei;* △, *Trichosporon pullulans (Y9);* ●, *T. pullulans (Y8);* ■, *C. mycoderma (H1); and* ◑, *C. tropicalis.*

FIG. 2. *Effect of increases in temperature on the growth of Trichosporon pullulans (Y9): (a) 10 to 20 C; (b) 5 to 15 C; and (c) 15 to 25 C.*

instances transient growth rates were encountered. Transfers of type (b) gave the higher steady-state rate immediately. A transfer from 25 to 35 C [type (c)] gave a transient rate of 0.87 hr^{-1} for about 5 hr before the steady rate was attained (k, 0.54 hr^{-1}). A transfer from 25 to 40 C (above the maximum for growth) resulted in a transient growth rate of 0.58 hr^{-1} for 3.75 generations before growth ceased.

Figure 4 shows normal growth rates of *C. tropicalis* and the transient rates occurring on "step-up," both plotted in the form of an Arrhenius plot. Once again, the transient rates resulting after shifts from 10 C to 15, 20, 25, and 30 C, respectively, fit on a line parallel to and beneath a straight line drawn through the steady-state growth rates for 20, 25, and 30 C. Similarly, the fast transient rate at 35 C (after shifting from 25 C) fits on the extension of the same steady-state line.

Five other yeasts, *C. scottii* (Y1), *C. zeylanoides* (Y15), *C. mycoderma* (H1), *R. mucilaginosa* (R1), and *C. krusei* gave the same general results.

Although the above results are based on light scattering, a similar result was obtained by counting viable cells; e.g., *C. scottii* (Y1), transferred from 5 to 15 C, gave by light scattering a transient rate of 0.112 hr^{-1} for 0.84 generations and by viable-cell counts a rate of 0.115 hr^{-1} for 0.8 generations.

Step-down experiments. Three types of "step-down" transfers were performed: (a) within the moderate range of temperatures (20 to 10 C), (b) from moderate to low temperatures (15 to 5 C), and (c) from high to moderate temperatures (25 to 15 C).

Figure 5 shows representative experiments with the psychrophile *T. pullulans* (Y9).

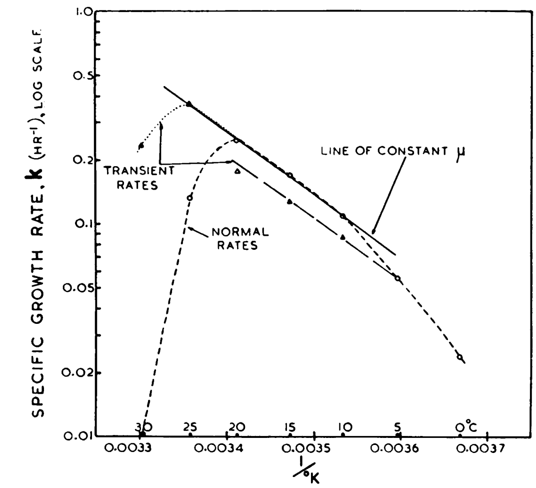

FIG. 3. *Arrhenius plots of both normal and transient specific growth rates of Trichosporon pullulans (Y9), based on data obtained in step-up experiments. Symbols:* ○, *normal specific growth rates;* △ *with solid line, transient specific growth rates from group (b) transfers; and* △ *with dotted line, transient specific growth rates from group (c) transfers.*

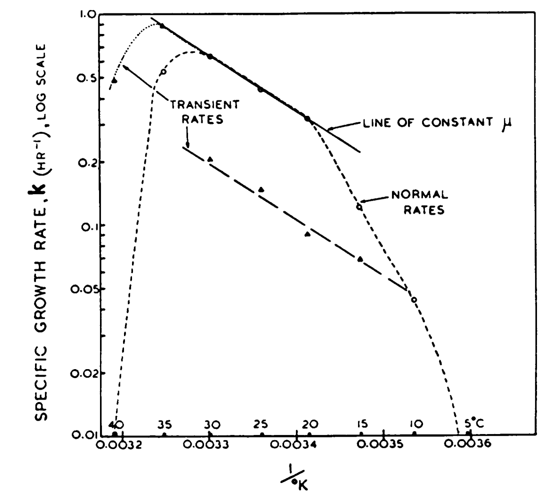

FIG. 4. *Arrhenius plots of both normal and transient specific growth rates of Candida tropicalis based on data obtained in step-up experiments. Symbols:* ○, *normal specific growth rates;* △ *with solid line, transient specific growth rates from group (b) transfers; and* △ *with dotted line, transient specific growth rates from group (c) transfers.*

Transfers of type (a) again showed no transient growth rates, unlike transfers of types (b) and (c) which gave complex results. After a type (b) transfer, there was an initial period of no turbidity increase; this lag was followed by growth at a faster rate than the normal steady-state rate for the lower temperature, but finally the growth rate fell to this steady-state rate (see Fig. 5b). Viable-cell counts established that no increase in numbers occurred during the period of constant turbidity. The magnitude of the temperature shift increased the lag period, but, whether the initial temperature was 20, 15, or 10 C, the fast transient growth rate after the initial lag was much the same, being about 0.11 hr^{-1}. The relationship between the duration of the lag period at 5 C and the temperature from which the shift to 5 C was made is shown in Fig. 6 to be substantially linear.

In contrast to transfers of type (b), a transfer from 25 to 15 C (type c) resulted in an initial lag (1.7 hr) followed by growth at transient rate (*k*, 0.09 hr^{-1}) that was less than the steady-state rate

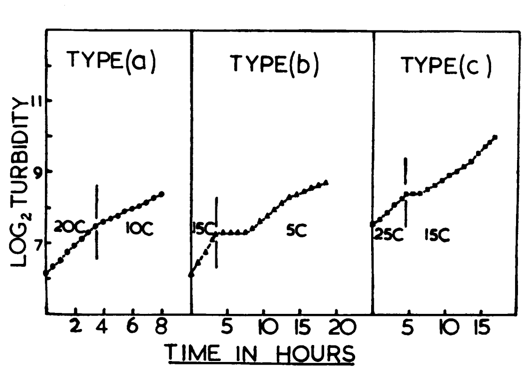

FIG. 5. *Effect of decreases in temperature on the growth of Trichosporon pullulans (Y9): (a) 20 to 10 C; (b) 15 to 5 C; and (c) 25 to 15 C.*

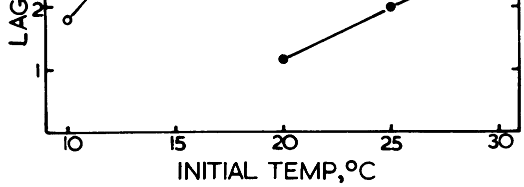

FIG. 6. *Effect of the magnitude of the temperature shift on the duration of the lag period in step-down experiments. Symbols:* ○, *lag periods obtained with Trichosporon pullulans (Y9) on transferring to 5 C;* ●, *lag periods obtained with Candida tropicalis on transferring to 10 C.*

for 15 C (k, 0.17 hr^{-1}) finally attained (see Fig. 5c).

Similar experiments with the mesophile *C. tropicalis* gave, in general, substantially the same results as were obtained with the psychrophile, except that once again the whole pattern of results was shifted to a high temperature range. A linear relationship between the magnitude of the temperature shift down to 10 C was found, as in Fig. 6.

To establish the generality of these results for both mesophilic and psychrophilic yeasts, the five other strains mentioned earlier (see Materials and Methods) were subjected to selected temperature changes. The same patterns held, in that the mesophiles and psychrophiles responded similarly, although they reacted over different ranges of temperature.

Discussion

The results presented here agree with the results of Ng et al. (6) on *E. coli*. The results with *E. coli* (6) showed that transient rates occurred on transfer to temperatures below 20 C. The experiments with yeast cultures included temperature shifts to and from high temperatures, and showed that transient growth rates can occur under these conditions also.

The data show a consistent difference between mesophiles and psychrophiles in the range of temperatures over which μ is relatively constant. One consequence of this difference is that, when the temperature shift is downwards, transient rates occur at a much higher temperature for mesophiles (20 C) than for psychrophiles (10 C). Conversely, when the transfer is to higher temperatures, transient growth rates become evident at a much lower temperature for psychrophiles (20 C) than for mesophiles (30 C).

The data presented here do not suggest a fundamental difference in behavior between mesophiles and psychrophiles. Nevertheless, they do show that, although the optimal temperatures may be similar for both types of organism, the minimal temperature at which shifts can be made without lag or transient rates are much higher for mesophiles than for psychrophiles.

It was possible to obtain growth for some time above the normal maximal temperature by previously incubating cultures at a moderate temperature. Hagen and Rose (1) have reported similar results with a psychrophilic *Cryptococcus* sp.

Although no clear distinction is apparent between mesophiles and psychrophiles with regard to their maximal temperatures for growth, there seems to be some correlation between rates of growth at 0 C and maximal growth temperatures. Thus, psychrophilic species giving the fastest rate of growth at 0 C also have the lowest maximal temperature for growth.

The parallel relationship between normal and transient growth rates suggests an immediate response to the temperature shifts in obedience to the Arrhenius function. The increase (or decrease) of the transient rate to the normal specific growth rate then involves correction (or establishment) of whatever alteration (or damage) has occurred at the high (or low) temperature.

In *E. coli*, growth at low temperatures can cause the derangement of regulatory processes (5–7). Perhaps, therefore, certain enzyme systems are over-repressed or inhibited at low temperatures, thus resulting in a lower enzyme level or activity at low temperatures. This could be the alteration occurring at low temperatures.

It has been reported that μ values for mesophilic bacteria are substantially higher than those for psychrophiles (2), but Brownlie (*personal communication*) found no consistent difference in μ values for mesophiles and psychrophiles among gram-positive bacteria. As already indicated, all strains of mesophilic and psychrophilic yeasts examined in the present work were found to have μ values of about 12,000 cal/mole.

Acknowledgments

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