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Boundary Between Bacterial Mesophilism and Thermophilism

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ABSTRACT

BAUSUM, HOWARD T. (Fort Detrick, Frederick, Md.), AND THOMAS S. MATNEY. Boundary between bacterial mesophilism and thermophilism. J. Bacteriol. 90:50–53. 1965.—The temperature boundary between bacterial mesophilism and thermophilism has been identified as 44 to 52 C. Facultative thermophiles growing in the mesophilic range require a brief period of adaptation at intermediate temperatures before gaining the capacity to initiate growth at thermophilic temperatures. Obligate mesophiles cannot grow in the thermophilic temperature range, but obligate thermophiles may show limited growth at temperatures as low as 41 C.

Bacteria capable of growth at both 37 and 55 C may be defined operationally as facultative thermophiles (Allen, 1953), because their minimal and maximal growth temperatures extend into both the mesophilic and thermophilic ranges. Campbell (1955) demonstrated that such an organism grown at 55 C produces a remarkably heat-stable α-amylase, whereas the same organism produces a heat-labile enzyme at 37 C. The question remained as to whether this transition from mesophilism to thermophilism occurred gradually over the temperature range from 37 to 55 C, or rapidly during a brief span within this range.

The present study developed from the basic observation that facultative thermophiles growing at 37 C were rapidly inactivated when their growth temperature was suddenly elevated to 55 C, despite the fact that 55 C was ordinarily considered to be their optimal growth temperature. This finding suggested that bacteria growing in the mesophilic temperature range required adaptation at an intermediate temperature before growth could be initiated at thermophilic temperatures.

MATERIALS AND METHODS

Organisms. The bacterial strains employed, their sources, and their thermophilic properties are listed in Table 1.

Media. Brain Heart Infusion (BHI) broth (Difco) and nutrient broth (Difco) were used as the complete media in this investigation. Solid media for routine plating contained 1.5% agar (Difco); soft agar for use with membrane filters contained 0.75% agar. Potato-extract broth (Thorne, 1962) was used for sporulation. The minimal medium was prepared according to Spizizen (1958). A medium supplemented with manganese was suggested by Koffler (*personal communication*) for the growth of obligate thermophiles and contained 0.2% yeast extract, 1.0% casein hydrolysate, and 0.001% manganese sulfate.

Methods for rapidly changing the incubation temperature. Three techniques were found to accomplish the abrupt temperature change of cells in a growing culture. The first consisted of diluting the cells 1:100 into fresh broth previously equilibrated to the new temperature. Such cultures were aerated by sparging. The second method consisted of transferring a small volume of culture, less than 1 ml, into a test tube resting in a water bath set at the high temperature. In the third method, cells from a low-temperature culture were impinged onto the surface of membrane filters, and the latter were transferred to plates of soft agar pre-equilibrated to the desired high temperature. A sudden temperature challenge was not achieved by spreading 0.1 ml of cell suspension onto the surface of agar media pre-equilibrated to the high temperature.

Growth of bacilli and gradient temperatures of incubation. The aluminum sheet gradient plate adapted for liquid growth described by Landman, Bausum, and Matney (1962) was used in these investigations.

RESULTS

Effect of sudden temperature shifts in facultative thermophiles. The three facultative thermophilic

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TABLE 1. *Thermophilic bacilli*

Bacillus strain	Thermophilic type	Source
B. licheniformis Allen	Facultative	M. B. Allen
B. licheniformis 9945A	Facultative	ATCC*
B. subtilis P1	Facultative	C. B. Thorne
B. stearothermophilus 2184	Obligate	H. Koffler
B. stearothermophilus 7953	Obligate	ATCC*

* American Type Culture Collection.

strains cited in Table 1 were grown in BHI broth at 37 C. During the mid-log phase of growth (about 10^8 plating units per milliliter), the populations were challenged at 55 C by use of the membrane-filter technique. Samples incubated on soft BHI agar plates at 37 C indicated that about 100 to 200 viable plating units had been impinged per membrane filter. No colonies developed on the membrane filters incubated at 55 C. The *Bacillus licheniformis* Allen and *B. subtilis* P1 cultures growing at 37 C were challenged by use of the dilution method. After various intervals of incubation at 55 C, samples were withdrawn for routine viable plating at 37 C. From the data presented in Fig. 1, it is evident that the cells grown at 37 C were inactivated rapidly at 55 C, but the cells grown at 55 C were unaffected by similar treatment.

Spores of *B. licheniformis* Allen produced in potato-extract broth at 37 C were harvested, washed in distilled water, and pasteurized at 68 C for 15 min. The suspension was diluted and impinged on membrane filters, and the filters were placed upon prewarmed soft BHI agar at 55 and 37 C. The number of colonies arising on the two sets of membranes was essentially identical. Thus, spores produced at 37 C appear to be quantitatively capable of germination and growth at 55 C.

Ability of obligate thermophilic bacteria to withstand temperature challenge. The obligate thermophilic organisms listed in Table 1 were grown in manganese medium at 44 C, near their minimal growth temperature. During the logarithmic phase of growth, the bacteria were challenged by sudden transfer to 65 C, by use of the small-volume method without dilution. At various intervals after transfer, samples were withdrawn, and routine viable counts were performed upon manganese-agar plates, with subsequent incubation at 50 C. No inactivation and no evident delay in growth resulted from the sudden transfer from 44 to 65 C. Obligate thermophiles, therefore, do not exhibit an adaptive response to temperature shift comparable to facultative thermophiles.

Heat resistance as a function of growth temperature. The Allen strain of *B. licheniformis* was cultured on BHI broth at seven temperatures

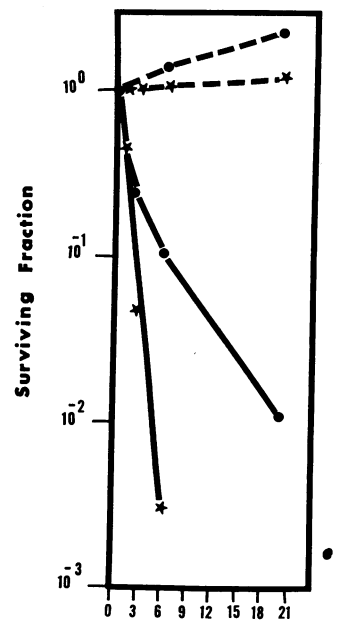

FIG. 1. *Effect of sudden temperature shifts to 55 C on viability. Solid lines = cultures pregrown at 37 C; broken lines = cultures pregrown at 55 C;* ● *= Bacillus subtilis P1;* ★ *= B. licheniformis Allen.*

ranging from 37 to 53.5 C. Cells from log-phase populations were diluted 1,000-fold in cold BHI. Small volumes (0.3 ml) were transferred to test tubes resting in water baths set at temperatures ranging from 48 to 58 C. After a 20-min incubation period at these higher incubation temperatures, routine plate counts were performed with nutrient-agar plates. The results recorded in Table 2 show a rise in heat stability at successively higher growth temperatures. For each growth temperature, the challenge temperature required to inactivate 90% of the population in 20 min was estimated. These LD_{90} temperatures are plotted in Fig. 2.

Thermal de-adaptation in a facultative thermophilic strain. The Allen strain of *B. licheniformis*

TABLE 2. *Response of Bacillus licheniformis, growing at various temperatures, to 20-min challenge at the indicated temperature of inactivation*

Growth temp	Temp of inactvation (C)								
	37	48	50	51	52	53	55	56.5	58
C									
37	100*	25	12	0	0	0	0	0	0
44.5	100	100	20	3	0	0	0	0	0
46.7	100	—	—	—	14	0	0	0	0
48.8	100	—	—	—	100	4	0	0	0
51.2	100	—	—	—	100	100	4	0	0
52	100	—	—	—	100	100	100	5	0
53.4	100	—	—	—	100	100	100	12	0

* Results expressed as per cent survivors.

was cultured at 49 C in Spizizen minimal broth. During the mid-log phase of growth, a portion of the undiluted culture was transferred to 37 C and allowed to continue incubation. At various intervals after this temperature switch, samples were withdrawn and diluted to 10^{-3} in cold BHI, and 0.3 ml was pipetted into tubes that were held at 55 C for 10 min. Viable counts of the surviving fractions appear as the control curve in Fig. 3. The cells appeared to enter an extremely heat-sensitive phase from which they recovered about 1 hr later. Several hours were required for final adjustment to the heat-stability level characteristic of 37 C cultures. After the initial transfer from 49 to 37 C, the optical density and viable count continued to rise with no noticeable lag.

To elucidate the mechanisms involved in thermal de-adaptation, the process was allowed to take place in the presence of several inhibitory substances: chloramphenicol (100 μg/ml), 5-methyltryptophan (500 μg/ml), 5-hydroxyuridine, 6-azauracil, 5-fluorodeoxyuridine (500 μg/ml), 2,4-dinitrophenol (500 μg/ml), and KCN (0.005 M). Conditions under which de-adaptation was tested were otherwise the same as described above. The effects of these substances on the de-adaptation process are presented in Fig. 3. Chloramphenicol and 5-methyltryptophan, both inhibitors of protein synthesis, were highly effective in preventing loss of thermal stability. 2,4-Dinitrophenol, an inhibitor of oxidative phosphorylation, was also effective. The uridine and deoxyuridine derivatives, believed to affect ribonucleic acid and deoxyribonucleic acid synthesis, respectively, were without marked effect.

Adaptation to high temperature in a facultative thermophilic strain. The Allen strain of *B. licheniformis* was cultured at 37 C in Spizizen minimal broth. During the log phase of growth, portions were removed to a 49 C water bath where incubation was continued. After various intervals, samples were withdrawn and challenged at 55 C for

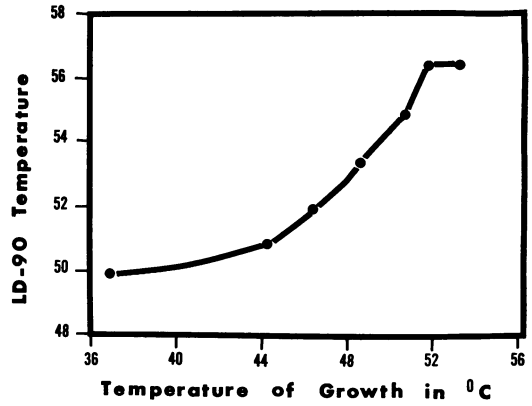

FIG. 2. *Effect of growth temperature on heat resistance. The temperature required to inactivate 90% of the population in 20 min is plotted against growth temperature.*

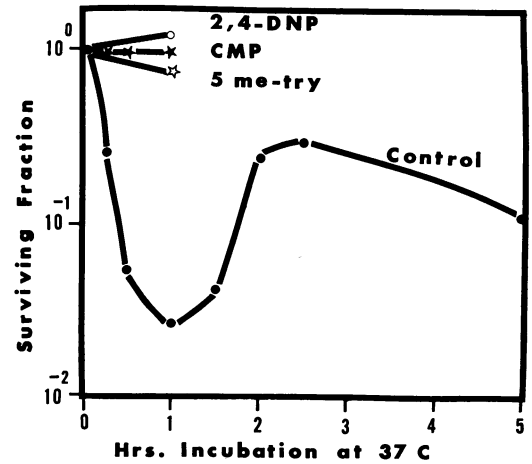

FIG. 3. *Thermal de-adaptation of Bacillus licheniformis Allen. Inhibition by 2,4-dinitrophenol (2,4-DNP), chloramphenicol (CMP), and 5-methyltryptophan (5 me-try).*

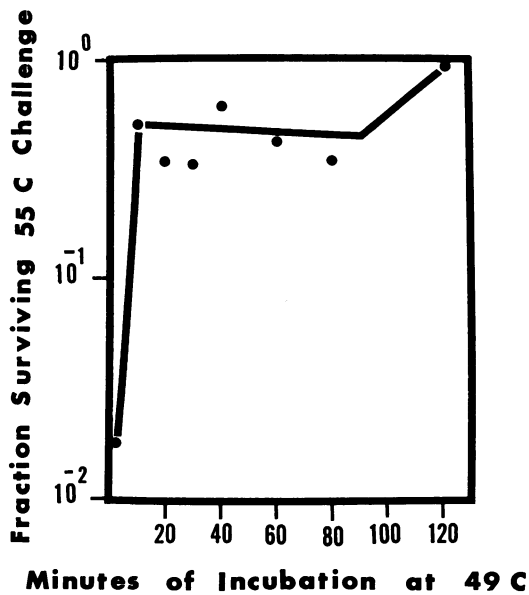

FIG. 4. *Thermal adaptation of Bacillus licheniformis Allen. Cells grown at 37 C were transferred to 49 C and incubated for varying periods of time prior to challenge at 55 C for 10 min.*

10 min. Thermal stability was acquired within 10 min after transfer to 49 C (Fig. 4).

DISCUSSION

Several generalizations may be drawn from the above findings. Facultative thermophiles appear to be unique in their ability to display both the mesophilic and the thermophilic types of metabolism. They appear to shift from mesophilism to thermophilism between growth temperatures of 44 and 52 C (as judged by the thermal challenge data of Fig. 2). Campbell (*personal communication*) obtained comparable results by determining the heat stability of an α-amylase produced by a facultative thermophile at graded temperatures of incubation between 37 and 55 C. Only the heat-labile, or mesophilic, form was produced at temperatures below 46 C; the heat-stable, or thermophilic, α-amylase was formed at temperatures above 50 C.

The conversion from mesophilism to thermophilism by actively growing bacteria requires a brief exposure to an intermediate temperature. The nature of this adaptation was approached by examining the reverse process, namely, the change from thermophilism to mesophilism. This de-adaptation was measured by timing the loss of ability to withstand a sudden return to high growth temperatures. This loss also occurred rapidly at 37 C, and preliminary-inhibition studies suggested that protein synthesis was required in the metabolic conversion.

Spores produced by facultative thermophiles at 37 C are capable of germination and growth at 55 C. Perhaps this finding may be useful in the selection of thermophilic mutants from mesophilic strains.

Mesophilic bacteria are incapable of growing in the thermophilic temperature range. The genetic capacity to grow at 55 C has been transformed to mesophilic cultures of *B. subtilis* (McDonald and Matney, 1963). Recent results suggest the involvement of more than one genetic locus (McDonald, *personal communication*).

Obligate thermophiles appear to have only the thermophilic type of metabolism. They thrive on a sudden shift in incubation temperature from 44 to 65 C, and do not grow well at temperatures below the metabolic shift range (44 to 52 C).

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