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Effects of Growth Temperature on Ribosomes and Other Physiological Properties of *Escherichia coli*

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The amount of ribosomal protein relative to total protein remained constant when *Escherichia coli* B/r was grown at different temperatures. Some effects of growth temperature on bacterial physiology are discussed.

The rate of multiplication of heterotrophic bacteria can be varied widely by temperature or nutritional conditions. Different carbon sources are utilized with different efficiencies to produce cell mass, and supplemented building blocks (such as amino acids or nucleosides) relieve the bacterium from the necessity of producing them, hence allowing a higher proportion of the overall energy of the bacterium to be channelled into the protein-synthesizing machinery (8). Growth rates of bacterial cultures can thus be varied by modifying the composition of the external medium and are affected by corresponding changes in the macromolecular composition within the cells (3, 5, 8). In contrast, alteration of growth rate by cultivation at different temperatures (but identical medium) does not involve similar substantial rearrangements of cellular components (6, 10), or changes in cell size (C. L. Woldringh, personal communication), or changes in relative cycle parameters (9).

Total RNA in *Salmonella typhimurium* LT2, for example, was found (10) to comprise a similar fraction of cellular dry weight at 25 and at 37°C, suggesting a constant ribosomal fraction. These determinations, however, had been performed before RNA was classified into three main groups, and no further attempt to clarify this issue has been recorded in the literature. The results presented in Table 1 refine the original observation. The fraction of ribosomal protein in total protein (α_r) does not vary systematically with temperature in glucose-grown *Escherichia coli* B/r; thus, a major cellular constituent, the ribosome, is found invariant over a wide range of growth temperatures.

Ribosomes comprise about a quarter of the total dry weight of glucose-grown *E. coli* B/r and almost one-half of macromolecules involved in protein synthesis (8). These are coordinately regulated, and their relative amounts are therefore not likely to change with temperature. The relative amount of DNA is also not likely to change (9, 10). Adenylate charges (4) and relative amounts of many other proteins (6) remain essentially constant between 25 and 37°C. Plotting the log of growth rate (reciprocal of doubling time) of *E. coli* in a particular medium against the inverse of growth temperature yields a straight line, the so-called Arrhenius plot, over an even wider temperature range (6, 7). Thus, if other constituents do vary, they do so without affecting the efficiency of available energy utilization (1). This shows that a bacterium can be better described by the interactions between the various component enzymatic reactions to yield a system with a unified Arrhenius plot (as

TABLE 1. Fraction of ribosomal protein in total protein (α_r) as a function of temperature[a]

Temp (°C)	Doubling time (min)	α_r
30.0	80	0.114
33.5	58	0.116
37.0	55	0.115
39.5	52	0.111
42.0	50	0.114

[a] *E. coli* B/r (CP14)(12) was grown in glucose (0.4%) minimal medium A + B (12) for at least 10 generations in a reciprocating water bath shaker at the indicated temperature. Growth was monitored with an Eppendorf spectrophotometer at 436 nm, and α_r was determined (optical density value at 436 nm < 0.5) by a modification of Schleif's (11) technique (2, 12). Samples were pulsed with L-[^{3}H]leucine (10 μCi/0.025 μg per ml) for 1 min and chased with cold leucine (400 μg/ml). During the chase (100 min), RNA was labeled with [^{14}C]uracil (0.1 μCi/0.2 μg per ml) for 50 min and chased (400 μg/ml). Cells were pelleted, washed, and sonicated in the cold. Sonicate was layered on top of D_2O sucrose gradient (25 to 65%, with a shelf of 80%), which was centrifuged for 15 h at 38,000 rpm (SW41 rotor) and fractionated into scintillation vials. Each fraction was counted, and α_r was calculated as follows:

$$\alpha_r = \frac{(^{14}\text{C counts in rRNA}) \times (^3\text{H}/^{14}\text{C})_{70s}}{^3\text{H counts in total protein.}}$$

though cellular growth were a simple chemical process) rather than by the enzymes themselves, whose Arrhenius plots presumably vary considerably.

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