Growth Rate of Enterobacteriaceae at Elevated Temperatures: Limitation by Methionine

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The effect of elevated temperatures on growth rate was studied in five strains of Enterobacteriaceae. In all the strains tested a shift to the elevated temperature resulted in an immediate decrease in growth rate which was due to limitation in the availability of endogenous methionine. The first biosynthetic enzyme of the methionine pathway—homoserine transsuccinylase—was studied in extracts of Aerobacter aerogenes, Salmonella typhimurium, and Escherichia coli and was shown to be temperature sensitive in all of them.

The decrease in growth rate of mesophilic bacteria at temperatures above the optimum was generally regarded as the result of competition between killing and the continuous growth of those cells that remained viable (5). However, it was found that the addition of various specific nutrients to the medium shifts the temperature optimum upward in various organisms, suggesting that the temperature sensitivity of one or another specific enzyme may limit the growth of a wild-type organism at elevated temperatures, as it is known to do in temperature-sensitive mutants (3). This suggestion was supported by studies on the effect of elevated temperatures on Escherichia coli (7, 8). The results indicated that when cultures of E. coli growing in minimal medium were shifted from 37 C to higher temperatures, up to 45 C, the growth rate immediately assumed a new, lower value characteristic of that temperature. This effect was shown to be due to a decreased synthesis of methionine, since in the presence of added methionine growth was equally rapid at 44 and 37 C, whereas without methionine (even with 19 other amino acids) growth was only 20% as rapid at the higher temperature (7). The cause of the decrease in growth rate was shown to result from an immediate, irreversible impairment of the activity of the first enzyme of the methionine biosynthetic pathway, homoserine transsuccinylase (HTS) (8), which thus appears to be the most heat-sensitive biosynthetic enzyme in the cell.

The pathway to methionine appears to be the most temperature-sensitive reaction in five wild-type strains of E. coli (7). This uniformity might reflect evolutionary stability of HTS or, alternatively, suggest the possibility of a selective advantage. To learn more about this mechanism of control of growth rate at elevated temperatures, we examined other representatives of the Enterobacteriaceae. The data presented in this paper show that in all strains tested (representing three different tribes) the growth rate at elevated temperature is restricted by the supply of endogenous methionine and that HTS is temperature sensitive in Aerobacter aerogenes and Salmonella typhimurium as well as in E. coli.

MATERIALS AND METHODS

Bacteria and growth conditions. Bacterial strains used were E. coli B, A. aerogenes, Klebsiella pneumonia, Serratia marcescens (kindly provided by R. Zak), and S. typhimurium LT-2 (kindly provided by D. Gutnick).

Growth conditions were as described previously (7). All experiments were carried out in minimal medium A (2) containing 0.5% glucose. Temperature was controlled with a precision of ±0.2 C. Culture volumes were small to assure quick equilibration upon shifts to new temperature. Equilibration times were, in fact, shorter than 1 min. Cultures were aerated by shaking, and growth was followed in the Klett-Sum- merson photoelectric colorimeter using filter no. 54. A turbidity of 30 Klett units corresponds to about 10⁴ cells per ml.

HTS activity. The formation of O-succinyl homoserine was measured by the conversion of [¹⁴C]homoserine to [¹⁴C]succinyl homoserine (9) which was separated from [¹⁴C]homoserine by the method of Nagai and Flavin (6). The reaction mixture contained (in a final volume of 0.2 ml): 20 μmol of potassium phosphate buffer, pH 7.6; 0.2 μmol of succinyl coenzyme A (CoA); 15 nmol of [¹⁴C]homose- rine (4 mCi/mmol); and 700 μg of protein (determined by the method of Lowry et al. [4]). Reaction was initiated by the addition of succinyl CoA to pre-
warmed reaction tubes, and 0.1 ml of 1 N KOH was added to stop the reaction. The reaction was linear for 6 min at 33 C. For assay of succinyl homoserine the tubes were put in boiling water to achieve conversion of O-succinyl homoserine to N-succinyl homoserine (6), cooled, and vigorously mixed with Dowex 50 (H +). The unadsorbed succinyl homoserine was counted in Bray solution (1) in a Packard Tri-Carb liquid scintillation spectrometer.

Extraction and partial purification of HTS. The following steps were carried out in the cold. For preparation of extracts, cells were harvested at about 6 x 10^9 cells per ml, washed once with cold potassium buffer (pH 7.6, 0.05 M), and resuspended in a minimal volume of the same buffer. Cells were broken up by sonic disruption using the Branson Sonifier model B-12 (10 s at intensity 3). Cell debris was removed by 15 min of centrifugation at 15,000 rpm. The bulk of the nucleic acids was removed from the extracts by the dropwise addition of 5% streptomycin sulfate, up to 0.2 volumes, followed by centrifugation after 10 min. The fraction precipitating between 35% and 55%-saturated (NH4)2SO4 was placed on a column of Sephadex G-25 (6 by 1 cm), and HTS was eluted with 0.05 M potassium phosphate buffer (pH 7.6). At this stage the enzyme could be stored at 4 C for several days.

Chemicals. [14C]L-homoserine was purchased from CEA-France; CoA, L-homoserine, L-methionine, and Dowex 50W were purchased from Sigma Chemical Co. Succinyl CoA was synthesized as described by Schlessinger (9). Sephadex G-25 was from Pharmacia.

RESULTS

Effect of elevated temperature and methionine on growth. S. typhimurium strain LT-2 proved to be similar to E. coli in response to elevated temperature. At 44 C growth rate was reduced to about 15% relative to that at 37 C; addition of methionine restored the 37 C growth rate (Fig. 1). Growth of A. aerogenes was affected by temperature to an even greater extent; an 80% reduction in growth rate was obtained at 42 C, whereas above 43 C no growth was detected for over 4 h (Fig. 2). Nevertheless, the addition of methionine restored the maximal 37 C growth rate at 42 C and had a considerable effect even at 44 C, restoring the growth rate to 40% of the 37 C value. Similar results were obtained with K. pneumoniae.

Another member of the Enterobacteriaceae, S. marcescens, was still more sensitive to elevated temperature than the other strains described. The effects of temperature and of methionine were similar to those in the Aerobacter-Klebsiella group but occurred at temperatures which were 1 C lower. Thus, growth was completely arrested at 42 C and was restored to 50% of the 37 C value by methionine.

Specificity of the effect of methionine. In all the strains tested the stimulatory effect of methionine occurred only at temperatures above 37 C (Fig. 1 and 2). At the elevated temperature
a concentration of 10 μg of methionine per ml was as effective as the concentration of 50 μg/ml usually employed. In addition, methionine was the only amino acid that could overcome the inhibitory effect of elevated temperatures. The results of Fig. 3 indicate that in A. aerogenes methionine could not be replaced even by a mixture of all the other amino acids.

**Effect of temperature on activity of HTS.** In *E. coli* the decreased growth rate at elevated temperature was found to result from the temperature sensitivity of the first biosynthetic enzyme in the methionine pathway, HTS. Since the effect of temperature on other strains of *Enterobacteriaceae* was similar to that found in *E. coli* we looked at the direct effect of temperature on the activity of HTS in two strains, *S. typhimurium* and *A. aerogenes*. The results, summarized in Table 1, indicate that HTS of both strains are similar to that of *E. coli* in being extremely temperature sensitive.

### DISCUSSION

In the experiments described here, the response of growth rate to elevated temperatures was studied in five wild-type strains from three tribes of the *Enterobacteriaceae*. In all cases, the growth was limited by the temperature sensitivity of one of the methionine biosynthetic enzymes, presumably as in *E. coli*, the first in the pathway (Table 1). This temperature limitation is, apparently, not specifically involved in host-parasite relationships since it occurs in soil organisms, such as *A. aerogenes*, as well as in pathogenic organisms, such as *K. pneumoniae*.

All the bacterial strains studied here responded to a shift from 37 C to higher temperatures by an immediate change in the growth rate. The new growth rate had a lower value which was characteristic of the temperature and of the bacterial strain. Although the strains varied in the response to temperature, the inhibition of growth could be shown in all cases to be due to limitation in the endogenous supply of methionine. This uniformity of limiting growth rate in response to elevated temperature by limiting methionine synthesis could simply reflect an unusual genetic stability of the temperature-sensitive enzyme HTS.

An alternative explanation for the temperature sensitivity of the methionine pathway in *Enterobacteriaceae* would assume that the ability to control the growth rate at elevated temperature can be advantageous to mesophilic organisms.

![Fig. 3. Effect of methionine and other amino acids on growth of *A. aerogenes* at 41 C. A culture of *A. aerogenes* growing exponentially was divided into three batches containing methionine (50 μg/ml) (●), amino acid mixture without methionine (19 amino acids, 10^-4 M of each) (x), and no addition (○). All three batches were incubated at 41 C, and turbidity was measured as described in the legend to Fig. 1.](image)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Succinyl homoserine formed pmol/min per mg of protein</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33 C</td>
<td>42 C</td>
</tr>
<tr>
<td><em>A. aerogenes</em></td>
<td>106</td>
<td>50</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>271</td>
<td>56</td>
</tr>
<tr>
<td><em>E. coli</em> B</td>
<td>210</td>
<td>138</td>
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*HTS was partially purified and its activity was determined as described. The reaction was for 4 min at the given temperatures.
bacteria and has been selected for independently in many bacterial strains. An efficient way of controlling growth rate would be by controlling the availability of methionine which is required for initiation and continuation of protein synthesis, biosynthesis of purines, various methylation reactions, and synthesis of polyamines. Such a control system has been shown in *E. coli* (7, 8), where the availability of methionine provides a mechanism by which an especially sensitive response to elevated temperatures leads to control of growth rate. This sensitive response is achieved by rapid and reversible decrease of the activity of HTS upon shifting to elevated temperatures. The reversibility of the changes in enzyme activity allows for fine and immediate adjustment of growth rate in response to temperature.

The hypothesis that the temperature sensitivity of methionine biosynthesis has been selected for is further supported by the finding that HTS is also the most temperature-sensitive enzyme in *Bacillus polymyxa* (10, 11). This independent evolution, in two unrelated groups of bacteria, of the same temperature-sensitive biosynthetic step is probably not coincidental but reflects a selective advantage.

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**LITERATURE CITED**