SHORT COMMUNICATION

Effect of Preincubation Temperature on the Heat Resistance of Escherichia coli Having Different Fatty Acid Compositions

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The heat resistance of Escherichia coli depends on the temperature of incubation prior to heat treatment at 50 °C. This dependence is affected by the fatty acid composition in bacterial membranes. The results are discussed in relation to the change in fluidity of membrane lipids.

INTRODUCTION

The effect of growth temperature is well-documented as one of the factors which can affect the heat resistance of bacteria (Elliker & Frazier, 1938; Hansen & Riemann, 1963; Beuchat, 1978). In general, a lower growth temperature produces bacteria more sensitive to heat. The lowering of the growth temperature also results in an increase in the proportion of unsaturated fatty acid in bacteria (Marr & Ingraham, 1962; Cullen et al., 1971); the corresponding alteration of heat resistance has therefore been associated with increased fluidity of the membrane phospholipids (Beuchat, 1978).

The temperature at which bacteria are held prior to heat treatment may also be a factor influencing heat resistance, but little attention has been paid to this point when testing the heat resistance of bacteria. Tsuchido et al. (1974) have indicated that the viability of Escherichia coli heated at 50 °C increased with increasing temperature of preincubation without nutrients.

This study was undertaken to examine the effect of the preincubation temperature on the heat resistance of E. coli in which the composition of fatty acids in membrane lipids was modified by changing the growth temperature and by using an unsaturated fatty acid auxotroph.

METHODS

Organisms. Escherichia coli strains W3110 and K1060 were used. The latter strain, which is a mutant deficient in the ability to synthesize or degrade unsaturated fatty acids and requires them for growth (Overath & Raufuss, 1967), was obtained from the E. coli Genetic Stock Center, Yale University, New Haven, Connecticut, U.S.A.

Culture conditions. Bacteria were grown for 16 h at 37 °C in a 100 ml flask containing 20 ml M9 minimal medium (consisting of 8.8 g Na2HPO4, 1.2 g KH2PO4, 5.0 g NaCl, 1.0 g NH4Cl and 0.25 g MgSO4.7H2O per litre; pH 7-0), supplemented with 2% (w/v) glucose and, for strain K1060, 0.01% (w/v) unsaturated fatty acid and 0.04% (w/v) Brij 58. A 3 ml portion of this culture was then inoculated into a 500 ml flask containing 100 ml fresh medium and incubated at 37 or 15 °C. All cultures were shaken on a rotary shaker. Bacteria were harvested by centrifugation at 3000 g for 5 min during the exponential growth phase (2 x 10^6 to 4 x 10^6 cells ml^-1), washed twice with 50 mM-Tris/HCl buffer, pH 8.0, containing 10 mM-MgSO4 (Tris/Mg buffer) (for strain W3110) or with M9 medium without fatty acid and Brij 58 (for strain K1060; to avoid loss of viability of this strain during harvesting), and then resuspended in the same amount of fresh buffer or medium as the culture volume. These operations were done at 37 °C and at room temperature, respectively, for bacteria grown at 37 and 15 °C. The
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flask containing the cell suspension was kept for 15 min at the same temperature as that for growth for temperature equilibration.

Preincubation and heat treatment. The cell suspension was divided into 10 ml portions and these were transferred to 100 ml flasks and incubated on a shaking incubator at various temperatures between 0 and 37 °C (±0.2 °C accuracy) for 30 min. After the preincubation, 2 ml samples were withdrawn and immediately added to 100 ml flasks containing 18 ml Tris/Mg buffer, pH 8.0 (for strain W3110) or M9 medium (for strain K1060) which had been preheated to 50 °C in an incubator (±0.1 °C accuracy). Incubation at 50 °C was continued with shaking at 100 strokes min⁻¹.

Viability counts. At intervals during the heating period 1 ml samples were taken, diluted serially in Tris/Mg buffer (for strain W3110) or in M9 medium (for strain K1060), and then plated on M9 containing 1 % (w/v) agar and, for strain K1060, 0.01 % (w/v) oleic acid and 0.04 % (w/v) Brij 58. These operations were done at room temperature, since the temperature after heat treatment did not affect the results. The plates were incubated at 37 °C for 2 d and the numbers of colonies were counted. Viability was expressed as a percentage of the initial viable counts.

Fatty acid analysis. Cells were harvested from 11 cultures by centrifugation at 4 °C and washed twice with deionized water. Phospholipids were extracted from the wet cells by the method of Bligh & Dyer (1959). Lipid extracts were dissolved in 10 ml 3 % (w/v) HCl in anhydrous methanol and the solution was esterified at about 100 °C under reflux for 3 h. Methyl esters of fatty acids were extracted twice with 10 ml hexane and the total combined fractions were evaporated. The fatty acid esters were dissolved in acetone and injected into a Hitachi gas chromatograph (model 063) equipped with a flame ionization detector. The stainless steel column (2 m × 0.2 cm) was packed with 15 % diethylene glycol succinate (DEGS) coated on to 60/80 mesh Neopak 1A (Nishio Industry Co.) and operated at 200 °C with helium as carrier gas. The temperatures of the injector and detector were 210 and 220 °C, respectively. Fatty acid methyl esters were identified by comparing their retention times with those of standard compounds. Areas under the curve were determined by triangulation.

RESULTS

Escherichia coli W3110 grown at 37 °C was preincubated for 30 min at 0 and 37 °C in Tris/Mg buffer, pH 8.0, and then transferred, with 10-fold dilution, to fresh buffer preheated to 50 °C. The viability of cells preincubated at 37 °C was higher than that of cells preincubated at 0 °C. After preincubation at 37 and 0 °C the times taken to kill 90% of the cells were 13 and 7 min, respectively. A similar phenomenon was observed when M9 medium was used instead of Tris/Mg buffer for washing, incubating and heating the cells, although the heat resistance was increased slightly. When cells were incubated at 0 °C for 30 min, rewarmed to and kept at 37 °C for 30 min and then heated at 50 °C, the resultant survival curve was similar to that for cells preincubated at 37 °C without prior chilling. These observations indicate that the alteration of the heat resistance was reversible.

Since the growth temperature affects the fatty acid composition as well as the heat resistance of bacteria, we compared the heat resistance of E. coli W3110 preincubated at various temperatures, using cells grown at 15 °C (15 °C-grown cells) and cells grown at 37 °C (37 °C-grown cells), in relation to the difference in their fatty acid compositions. As shown in Table 1, growth of E. coli W3110 at 15 °C resulted in an increase in the relative proportions of palmitoleic (C₁₆:₁) and cis-vaccenic (C₁₈:₁) acids and a decrease in that of palmatic acid (C₁₆:₀). Thus, the ratio of the content of total saturated fatty acids to that of the sum of total unsaturated fatty acids and cyclopropane fatty acids was 2:1 for 37 °C-grown cells but was 0.8:1 for 15 °C-grown cells. Since fatty acids are mainly located in membrane phospholipids and unsaturated fatty acids largely contribute to the membrane fluidity (Cronan & Gelmann, 1975), the above results suggest that the membrane fluidity was different for these cell populations.

There was a marked difference in the effect of preincubation temperature on the heat resistance of 15 °C- and 37 °C-grown cells (Fig. 1). For 37 °C-grown cells, the viability after heating at 50 °C for 15 min decreased gradually with decreasing temperature of preincubation below about 20 °C, while it was approximately constant above this temperature. On the other hand, the viability of 15 °C-grown cells after exposure to heat remained constant in the range of preincubation temperature tested.
Table 1. Fatty acid composition of Escherichia coli W3110 grown at 15 and 37 °C

Values shown are the mean of results for two separate samples.

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>Growth temp. ...</th>
<th>15 °C</th>
<th>37 °C</th>
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<tr>
<td>14:0</td>
<td></td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>42.4</td>
<td>52.5</td>
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<td></td>
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<td>17:0cyc</td>
<td></td>
<td>1.4</td>
<td>3.0</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>18:1</td>
<td></td>
<td>22.2</td>
<td>11.5</td>
</tr>
<tr>
<td>19:0cyc</td>
<td></td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td>44.3</td>
<td>56.0</td>
</tr>
<tr>
<td>UFA</td>
<td></td>
<td>54.9</td>
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</tr>
<tr>
<td>SFA/UFA</td>
<td></td>
<td>0.81</td>
<td>1.32</td>
</tr>
</tbody>
</table>

* In fatty acid designations, the first number indicates the number of carbon atoms and the second the number of double bonds; cyc indicates cyclopropane fatty acid; SFA and UFA indicate total saturated and total unsaturated plus cyclopropane fatty acids, respectively. The 18:1 acid is cis-vaccenic acid.

Fig. 1. Effect of preincubation temperature on the heat resistance of Escherichia coli W3110 grown at 15 °C (●) and 37 °C (○). Cells were preincubated at the indicated temperatures for 30 min and then heated at 50 °C for 15 min in Tris/Mg buffer, pH 8.0. Values are means of results from two independent experiments.

To establish the importance of the fatty acid composition of the cells in the effect of preincubation temperature on heat resistance, we investigated this effect further using an unsaturated fatty acid auxotroph, E. coli K1060. In agreement with the results of other investigators (Haest et al., 1972; Yatvin, 1977), this strain incorporated added oleate or linolenate (unsaturated fatty acids) into the membrane during growth. The amounts incorporated were 50.5 and 26.3%, respectively, of the total fatty acids in the cell and accounted for almost all of the total unsaturated fatty acids. Myristic and palmitic acids (saturated fatty acids) accounted for 7.1 and 38.2%, respectively, of total fatty acids for cells grown in oleate-supplemented medium (oleate-grown cells) and 7.1 and 61.1%, respectively, for cells grown in the presence of linolenate (linolenate-grown cells).

The heat resistance of oleate- and linolenate-grown cells of strain K1060 was determined after preincubation at different temperatures. The viability of oleate-grown cells heated at 50 °C for 45 min was constant at about 1-1% after preincubation at temperatures ranging from about 15 to 37 °C but decreased to 0.15% after preincubation at 0 °C; this pattern is similar to that described above for 37 °C-grown cells of strain W3110. In the case of
linolenate-grown cells. The heat resistance was the same (about 0.10% viability) for all preincubation temperatures tested between 0 and 37 °C.

In all of the above experiments, no growth or death of cells occurred during preincubation.

DISCUSSION

In agreement with our preliminary data (Tsuchido et al., 1974), the heat resistance of E. coli depended on the preincubation temperature. The results obtained indicate that the difference in the heat sensitivity as affected by the preincubation temperature did not arise simply from the difference between the preincubation and heating temperatures, but from the difference in the physicochemical state of cells at various holding temperatures before heating.

A decrease in the growth temperature causes an increase in the proportion of unsaturated fatty acids in membrane phospholipids of E. coli (Marr & Ingraham, 1962), as we also observed (Table 1). It has been suggested that this change is due to the ability of cells to maintain constant membrane fluidity irrespective of the temperature of growth (Sinenskey, 1974). The unsaturated fatty acid auxotroph of E. coli, strain K1060, also seemed to maintain constant membrane fluidity by changing the proportion of unsaturated fatty acid incorporated into membrane phospholipids according to the degree of unsaturation, as suggested by Esfahani et al. (1969).

In general, lowering the growth temperature is known to result in cells more sensitive to heat (Hansen & Riemann, 1963) and a correlation has therefore been proposed between the growth temperature, the ratio of saturated to unsaturated fatty acid, and the heat resistance (Beuchat, 1978). Yatvin (1977) has also reported that E. coli K1060 grown in linolenate-supplemented medium has a lower resistance to heat than when grown in oleate-supplemented medium. He suggested that the heat resistance of this mutant was correlated with the degree of unsaturation of fatty acid incorporated into membrane phospholipids. The higher resistance of cells grown at a higher temperature and of oleate-grown cells agrees with our results, except for cells incubated at a low temperature before heating. We observed that at a preincubation temperature of 0 °C the order of the heat resistance of strain W3110 was inverted between 15 °C- and 37 °C-grown cells (Fig. 1) and the difference between oleate- and linolenate-grown cells of strain K1060 diminished markedly.

In E. coli, membrane functions such as substrate transport, the activities of membrane-associated enzymes and the maintenance of cell integrity depend on the membrane fluidity (Cronan & Gelmann, 1975). It seems likely that membrane fluidity influences the thermal stability of a certain structure(s) or function(s) essential for survival under heat stress. When the incubation temperature of cells is above a critical level, a gel–liquid crystalline phase transition of their membrane phospholipids should occur. Variation in the growth conditions of cells would influence the temperature of this phase transition as a result of the change in the fatty acid composition. Sinenskey (1974), using a spin-labelling technique, showed that the temperature of the phase transition of membrane lipids in E. coli was usually 14 to 16 °C lower than the growth temperature of the cells, i.e. 21 to 23 °C for 37 °C-grown cells and −1 °C for 15 °C-grown cells. Moreover, Overath et al. (1970) reported from their observations on the efflux of methyl thiogalactoside and force/area isotherms of lipid films that the temperatures of the phase transition for the membrane lipids of the unsaturated fatty acid auxotroph of E. coli were 15 °C and 4 to 6 °C for oleate- and linolenate-grown cells, respectively. These temperatures are close to those at which the preincubation started to reduce the heat resistance of cells, as observed in our study. The results suggest that the heat resistance of cells may depend on the physicochemical state of the membrane before heating, or on the phase transition of membrane phospholipids. The involvement of membrane fluidity has also been indicated for bacterial susceptibility to cold shock (Farrell & Rose, 1968; Paton et al., 1978) and γ-irradiation (Yatvin, 1976).
Although several possible targets of heat action, such as DNA, RNA, protein and membrane, have been proposed (for reviews, see Tomlins & Ordal, 1976, and Beuchat, 1978), our results suggest that the damage to a certain membrane-associated structure or function which depends on the membrane fluidity may be linked with the heat resistance of bacteria. Alternatively, the repair process influenced by the membrane fluidity in heat-injured cells might contribute to an apparent heat resistance.

In addition, our findings show that the temperature for holding cells prior to heat treatment should be taken into consideration when testing their heat resistance.

REFERENCES


