Conditioning of the membrane fatty acid profile of Escherichia coli during periodic temperature cycling

Tina Ivancic, Masa Vodovnik, Romana Marinsek-Logar and David Stopar

1Chair of Microbiology, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia
2Chair for Microbiology and Biotechnology, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domzale, Slovenia

The membrane fatty acid composition of Escherichia coli becomes conditioned during periodic temperature cycling between 37 and 8 °C. After several cycles of temperature change, the bacteria become locked into a low-temperature physiology. Even after a prolonged incubation at high temperature the membrane fatty acid composition of conditioned cells was similar to that of cold-stressed cells.

INTRODUCTION

Micro-organisms must constantly adapt to a variety of changing conditions and stresses in their local environment to survive and multiply. In the natural environment, periods of constant temperature or conditions where temperature is up- or downshifted only once, as in a typical cold- or heat-shock experiment, are rare. The driving question of this study is how bacteria adapt their membrane fatty acid composition during periodic temperature cycling. Although the effect of fluctuating temperatures on bacterial growth rate and survival has been demonstrated (Mitchell et al., 1994), there appears to have been no systematic study of how changing temperature during bacterial growth affects membrane composition. In this work we challenge the generally accepted homeoviscous model, which states that membranes consistently adapt to low temperature by increasing the ratio of the unsaturated fatty acids (UFAs) to saturated fatty acids (SFAs) (Sinensky, 1974).

METHODS

Bacterial growth. In all experiments Escherichia coli strain K-12 was grown in LB (Luria–Bertani) medium, consisting of 10 g tryptone l⁻¹, 5 g yeast extract l⁻¹ (both from Biolife Italina Srl) and 10 g NaCl l⁻¹ (Merck), in rubber-stoppered tubes on a rotary shaker at 200 r.p.m. in the dark. Bacteria in control cultures were incubated at a constant temperature of 37 °C. The data were corrected for different amounts of CO₂ dissolved in the liquid phase at different temperatures. Intracellular content of ATP during incubation using a 1 ml syringe with a hypodermic needle. The data were analysed using Student’s t-test for significant differences between the two sample means (n>3).

RESULTS AND DISCUSSION

The step-like growth of E. coli during temperature cycling is shown in Fig. 1(a). During temperature downshift from
37 to 8 °C bacterial growth stopped after approximately 30 min, when the temperature of the growth medium dropped to 15 °C or lower. Similarly during temperature upshift growth resumed approximately 30 min after the onset of temperature upshift, when the temperature in the growth medium reached 20 °C or higher. Production of CO₂ (Fig. 1b) correlated with the growth rate during temperature cycling, i.e. more CO₂ was produced when
cells were growing faster and less during slower growth. Unlike growth rate and CO₂ production, net ATP concentration (Fig. 1c) did not follow a 120 min period imposed by temperature oscillations. At the end of the experiment periodically fluctuated cells had fourfold higher net ATP concentration than cells constantly incubated at 37 °C. This may indicate that periodically cycled cells were under severe stress (Napolitano & Shain, 2005). The response of the fatty acid composition to periodic temperature fluctuations is shown in Fig. 2. During the first two temperature cycles the fraction of unsaturated cis-vaccenic acid (C18:1) periodically increased with decreasing temperature and decreased with increasing temperature. With progressing number of temperature cycles (n>3), however, the bacterial membrane lost its ability to change the fraction of C18:1 in response to temperature up- or downshift although cells were still able to grow. This is surprising since Garwin & Cronan (1980) demonstrated that unsaturation is a quick process, completed in less than 30 s, and does not require new protein synthesis. The lack of cell membrane response after three temperature cycles is not what homeoviscous adaptation predicts (Sinensky, 1974) and suggests that periodically oscillated cells maintain a homeostatic response to temperature fluctuations only when they are physiologically young. Not only UFAs but also SFAs changed during periodic temperature fluctuations. The fractions of both palmitic acid (C16:0) and C17:0 cyclopropane fatty acid in temperature-cycled cells were lower than in cells constantly incubated at 37 °C. An unexpected finding of this study is that the fatty acid profile of periodically cycled cells was more similar to that of cold-shocked bacteria than to that of cells constantly incubated at 37 °C. One would expect either a membrane composition that is intermediate between cold or warm cells or a membrane composition that consistently fluctuates between the two extremes. Neither outcome was observed. It is, however, possible that cells become conditioned not to respond to high temperatures during temperature fluctuations. To test this we extended the time of incubation at 37 °C after cells had completed either three or five temperature cycles. There was no change of fatty acid composition with prolonged incubation at 37 °C, when cells were already in the stationary phase (Fig. 2, dashed lines). This indicates that membrane fatty acid composition of stationary-phase cells is rather fixed and is not responsive to temperature fluctuations.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the Slovenian Research Agency (ARRS no. 1000-06-310164).

REFERENCES


Edited by: F. Sargent