Abstract

Bacillus subtilis, a model Gram-positive soil bacterium, employs two distinct mechanisms in its membrane adaptation to low temperature: 1) Long-term adaptation to suboptimal temperature is accomplished by increasing the ratio of anteiso- to iso-branched fatty acids in the membrane lipids. 2) After a sudden temperature decrease, the oxygen-dependent fatty acid desaturase (Des) is induced which desaturates fatty-acyl chains incorporated in membrane lipids. The transcription of the gene encoding desaturase, *des*, is activated by the decrease of the membrane order, via two-component system DesK-DesR.

In this work, I studied the influence of cultivation conditions on the mechanisms of *B. subtilis* membrane adjustments for a low temperature employing fatty acid analysis, fluorescence spectroscopy, differential scanning calorimetry and methods of molecular biology.

In the first part of this work, I examined the impact of the cultivation medium on the composition and biophysical features of the *B. subtilis* cytoplasmic membrane during growth under the optimal (40 °C) and suboptimal (20 °C) cultivation temperature. I compared the nutrient-rich complex medium containing glucose and the mineral medium supplemented with either glucose or glycerol. The results obtained showed the crucial importance of medium composition for the membrane adaptation. The differences in membrane fatty acid profiles recorded in the particular media were probably induced by the different levels of branched fatty acid precursors, which resulted from the different metabolic pathways employed by the cells. At the optimal temperature (40 °C), the cells grown on nutrient-poor medium with glycerol were the ones that exhibited the highest membrane fluidity and the lowest transition temperature (T_t) of the membrane lipids. On the contrary, at the lower temperature (20 °C), the cells cultivated in complex medium had the most fluid membranes. The extent of adaptation, expressed as the difference of T_t obtained for the cells cultivated at the optimal and suboptimal temperature, was the greatest where the rich medium was used. However, the choice of cultivation medium did not have any substantial effect on the induction of fatty acid desaturase after the cold shock.

In the second part of my work, I focused on the adaptation of *B. subtilis* membrane to a low temperature under anaerobic conditions that were predicted to inhibit Des activity. I found that in anaerobiosis, as opposed to aerobic growth, the induction of *des* expression after the temperature downshift (from 37 °C to 25 °C) was not down-regulated. However, the transfer from anaerobic to aerobic conditions rapidly restored the down-regulation. Under both aerobic and anaerobic conditions, the induction of *des* expression was substantially reduced by the addition of external fluidizing oleic acid and was fully dependent on the DesK-DesR two-component regulatory system. Fatty acid analysis proved that there was no desaturation after *des* induction under anaerobic conditions despite the presence of high levels of the Des protein product, which was shown by immunoblot analysis. The cold adaptation of *B. subtilis* in anaerobiosis is, therefore, mediated exclusively by the increased anteiso/iso- ratio of branched-chain fatty acids and not by the temporarily increased level of unsaturated fatty acids that is typical under aerobic conditions. The degree of membrane fluidization, as measured by 1,6-diphenyl-1,3,5-hexatriene fluorescence anisotropy, was found to be similar under both aerobic and anaerobic conditions.

Keywords: *Bacillus subtilis*, cytoplasmic membrane, cold adaptation, anaerobiosis, membrane fluidity