

Stress proteins in the cytoplasmic membrane fraction of *Bacillus subtilis*

A primary habitat of the Gram-positive bacterium *Bacillus subtilis* is the upper layer of the soil. Within this ecosystem, *B. subtilis* experiences a wide variety of environmental challenges and nutrient limitations that induce several mechanisms to help the cells to survive. A process known as a general stress response belongs among them. Expression of about 150 genes is enhanced and their products called general stress proteins (GSP) minimize the cell damage. Although the size and structure, as well as the regulation, of many stress proteins have been fairly well elucidated the information about stress membrane proteins is very limited.

We characterized the membrane proteome of *Bacillus subtilis* 168 *trp₂* exposed to acidic pH and ethanol. Cells were 1) grown under optimum conditions in complex medium (pH 7.0) at 40°C with aeration; 2) grown in complex medium at pH 5.0 for 20 hours or 3) challenged with 3% v/v ethanol for 30 minutes during exponential growth. The cultures were harvested in the mid-exponential phase by rapid filtration. Isolated membrane fractions were analysed by an optimized two-dimensional gel electrophoresis. Two alternative methods of protein detection were compared: silver staining and radioactive labeling with L-[³⁵S]-methionine. In each experiment 4 parallel gels were prepared and about 400 protein spots were quantified using Molecular Imager scanner and PDQuest software. The stress induced proteins were identified by MALDI-TOF mass spectrometry.

The growth rate of *B. subtilis* grown in complex medium (pH 7.0) at 40°C is $\mu = 3.5 \text{ h}^{-1}$. Both stress factors (pH 5.0 and 3% v/v ethanol) reduced the growth rate to $\mu = 2.4 \text{ h}^{-1}$. Reproducible differences in expression as a function of ethanol shock or acid stress showed at least 25 membrane proteins. Nevertheless, only 5 proteins were significantly increased after ethanol shock or acid stress. Ethanol stress proteins were identified as YdaP, Ctc, YfhM, YjcH and YwaC. To acid stress proteins belong the following: AcoB, YkwC, SodA, YjcH and YwaC. Proteins YjcH and YwaC were increased after ethanol shock as well as acid pH treatment.

Some of stress proteins found in both proteomes were classified as general stress proteins responsible for stress signalling; the specific stress proteins are involved in lipid metabolism and in the detoxification capacity of the cell.