STUDY OF GENES RESPONSIBLE FOR OSMOADAPTATION IN *BACILLUS SUBTILIS*

Abstract

Bacteria are probably the most adaptable organisms. This feature is partly attributed to their development of strategies controlled by stress. In our laboratory we use *Bacillus subtilis* as a model organism; a grampositive colonizer of upper layers of soil. For this habitat are typical frequent and sudden changes in wetness. To hypertonic environment *B. subtilis* responds by accumulation of potassium ions followed by transport or synthesis of compatible solutes. In my thesis I focused on obtaining information about genes responsible for osmosensoric and osmoadaptation mechanisms in *Bacillus subtilis*.

I have prepared osmosensitive mutants by unspecific insertional mutagenesis using minitransposon Tn*10*. Eight of these mutants were analysed genetically. For estimation of the numer of insertions in individual mutants restriction analysis and hybridisation were used. In only two mutants I identified only one insertion of mini-Tn*10* in chromosome responsible for osmosensitive phenotype. Transcriptional profiles of these mutants were analysed and compared with the profiles of wild strain of *B. subtilis*. Transcriptional profiles were obtained by cultivation at both normal and osmotic conditions. My results show that the insertion in mutants is presumably located in regulation protein which participates in termination of stress response. Using inverse PCR and conscruction of chromosome library I sought to find the exact localization of insertion of mini-Tn*10* in genome of these mutants. I was not fully successful, but according to results I have got I expect that the insertion is localized near the transposase gene. In the genome of *Bacilus subtilis* I found two regions containing transposase genes. Near one of them are located genes of sigma B regulon and in proximity of the second one is probable transcriptional regulator of which transcription is activated during osmotic stress.

Keywords: *Bacillus subtilis*, osmotic shock, potassium transport, osmosensoric proteins, unspecific insertional mutagenesis, bacterial mRNA isolation

Klíčová slova: *Bacillus subtilis*, osmotiký šok, transport draslíku, osmosenzorické proteiny, nespecifická inzerční metageneze, izolace bakteriální mRNA