

Abstract

Bacillus subtilis is gram-positive soil bacteria. In its natural environment it is constantly exposed to changes of chemical and physical conditions, including changes of osmolality. It responds to high osmolality by transporting of potassium ions and after that transporting and/or synthesising of compatible solutes. In last years the mutant strain *Bacillus subtilis* L-42 was isolated with non-specific insertional mutagenesis (mini *Tn10*) in our laboratory. This strain displays limited growth and inability to cope with hyperosmotic shock in a defined medium with potassium concentration of < 1 mmol/l. Insertion of transposon was located in *yxkO* gene which encodes a protein of unknown biological function. Some other data also indicate a possible role of disruption of *yxkO* gene in regulation of expression of *hag* gene, which encodes flagelin – a pivotal protein of bacterial flagellum.

The goal of this thesis was to clarify if the disruption of *yxkO* gene influences motility and whether is affected the transcription of *hag* gene.

With integrative vector pMUTIN4 a mutant strain with specific mutation of *yxkO* gene was prepared. Vector was pasted into chromosome of *Bacillus subtilis* strain 1A839 – genotype of this strain allows to extrude the known transcriptional regulation of *hag* gene. Cell's motility was observed by light and electron microscopy. Gained data prove the effect of disruption of *yxkO* gene on motility and also shows that the loss of motility after hyperosmotic shock is caused by loss of flagellum – probably by gradual degradation – and not by inhibition of its locomotive. We also wanted to observe the effect of mutation on the transcription of *yxkO* and *hag* genes by Northern and Southern blot methods, but no data was gained. At least we detected the presence/absence of transcripts with PCR method. Despite of the data which should documented the changes of transcription is not complete it seems that YxkO does not control the transcription of *hag* gene. It is possible that it controls the expression of flagelin on posttranslational level. Additionally it was documented that disruption of *yxkO* gene has the pleiotropic effect - it causes not only changes of motility, but also inability to cope with high osmolality in medium with low concentration of potassium ions (< 1 mmol/l) and disruption of cell cycle, respectively inability to sporulate.