The influence of the expression level of the EcoRI restriction-modification system on the restriction effectivity of the invasive DNA Karolina Wilkowska

Restriction-modification systems are one of the most common bacterial defense mechanisms against invasive DNA. The balance between restriction and modification is the reason for stability of R-M system in the bacterial cell, and also the effectivity of the restriction in the viral DNA. So far, there are not any known data about the optimal production's level of protein – restriction endonuclease and DNA methyltransferase - which give the bacteria the maximal protection. In this thesis the dependence was examined on the model R-M EcoRI system, which was isolated from Escherichia coli. The studies were carried on two plasmids, and each of them had different expression level of R-M genes, which allowed to show that the low level of EcoRI gene expression (plasmid pACYCeco) give the high effectivity of the restriction of the invasive DNA bacteriophage λ , in opposite to the high level of the expression (plasmid pIM-RM), where restriction was lower for about 3 rows of difference, probably caused by the protective modification of the viral DNA. The analysis of the level of the gene expression showed 10-times higher level transcription level of the ecoRIM gene in the plasmid pIM-RM, and the 14-times higher of the ecoRIR, which next, is observed as a 6-times higher production's level of the both proteins in the plasmid pIM-RM. In this thesis the importance of the genetic elements in the EcoRI operon was examined, it has influence on the regulation of its expression, which has influence on the biological functionality of the R-M system, like main promoter sequence, the ribosome-binding site, or transcription signals out of the operon for example the presence of the additional strong promoter up to the EcoRI R-M sequence or the presence of the gene resistance (chloramphenicol in both of the plasmids). Using the modification of the promoter region of the operon and the number copy of R-M EcoRI determinant, it has been shown that the possible change is reversesable for the effectivity of the system. The decreasing of the number of the copy of the plasmid, examined in the strain E. coli MM294 with mutation pcnB80 increased 1000 times in the effectivity of the restriction of the invasive DNA with plasmid pIM-RM, in comparison to the wild type strain. Moreover, it was observed the toxic influence of the continuous, high production's level of the R-M proteins on the viability bacterial cells affects the induction of the cell SOS response, which is observed as formation of the filaments of the bacterial cell caused by the autorestriction of its own chromosome and the inductions of the SOS response. That effect was decreased through the additional source of the protection methyltransferase.