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Summary of PhD study

„Characteristics and physical and chemical properties of atypical restriction endonucleases of IIS class”

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At the Department of Molecular Biotechnology under the guidance of prof. Piotr Skowron the new restriction specificity derived from thermophilic organisms is researched. As a result of the analysis of samples taken from geothermal environment in Egypt, the *Bacillus* sp. strain was isolated that carries the newly discovered restriction-modification system (R-M), and which was designated BilI in accordance with the accepted nomenclature. Phenotypic properties of the strain were analyzed and the conditions for its cultivation determined. The strain species was analyzed using the mass spectrometry MALDI-TOF. The analysis revealed that the isolated strain belongs to the *Bacillus ilicheniformis* species. The purification procedure using the selective precipitation method and chromatography was developed for REase BilI.

The REase's size was determined using the gel filtration techniques. Assuming that the protein investigated is globular and symmetrical, it was determined to be 59 kDa (kilodalton). This result was confirmed by measuring the migration in a polyacrylamide gel under denaturing conditions in the presence of reference proteins. The optimal conditions for digestion of the DNA were determined for the enzyme. The optimal conditions are: temperature 47 °C, pH = 9.0, minimum concentration of Mg^{2+} ions required for a complete digestion of the substrate - 0.1 mM. Also, the optimal concentration of NaCl in the reaction buffer was determined - 10 mM. Designated restriction pattern of DNA of bacteriophage lambda (λ) and T7 turned out to be identical to the BsaI enzyme. The same method of cutting the DNA was confirmed by sequencing. Thus, the discovered enzyme is an isoschizomere of BsaI. The enzyme is characterized by the activity at lower temperature optimum than the growth optimum of the strain of origin. This suggests that *Bacillus ilicheniformis* could get the R-M system being researched by horizontal transfer of genes from another species / strains.

In the second part of the study a functionally atypical REase-MTase TspDTI was analyzed, which is the R-M system derived from the thermophilic bacteria *Thermus* sp. DT

(Skowron et al., 2003). The role of TRD2 and *coiled coil* regions in recognizing the DNA-cut sequences by the TspDTI enzyme was investigated.

Also the influence of the NPPW motive on the methyltransferase activity (MTase) was analyzed. The characteristic features of this enzyme are: thermal stability, two enzymatic activities - restricting and methylating in one polypeptide, and incomplete digestion of the substrate DNA. A series of targeted mutations was carried out. Activity in the obtained mutational variants of bacterial lysates were investigated. Cultures were grown in the *Escherichia coli* (*E. coli*) BL21(DE3) expression strain. Expression of the proteins was carried out under the control of the bacteriophage λ P_R promoter and induced by heat. The enzyme variants that showed restriction activity were purified. Physical and chemical properties of one of the mutational variants were compared with the native TspDTI enzyme.

It was demonstrated that removal of the TRD region causes the total absence of recognition of the cut DNA sequence by the enzyme. Also removal of the *coiled coil* region produces the same effect. Changing only the residual amino acid (aa) in the NPPW motive caused an overall decrease in methylating enzyme's activity as well as decrease in REase's activity by 14%. Also, the physical and chemical properties of TspDTI were compared with that of the altered variant. The results show that the mutation did not cause significant changes in the reaction optimum of the temperature and pH as compared to the unaltered enzyme. In conclusion, we managed to separate two coupled enzymatic activities within the TspDTI protein and received the variant, which is functionally the equivalent of conventional Type II REase, which have separate enzymes encoding REase and MTase.