

Restriction endonuclease (REase)-methyltransferase (MTase) TaqII enzyme is isolated from *Thermus aquaticus* YT-1 (TaqII<sup>wt</sup>) and belongs to Type II restriction-modification (RM) system which functions as primitive immune system of microorganisms. In 2003 TaqII<sup>wt</sup> was classified to a new family *Thermus* sp. (Skowron i wsp., 2003) which REase members are from the border of 3 sub-Types: IIS/IIC and IIG. Moreover, enzymes from the family *Thermus* sp. also exhibit some features of Types I and III. Proteins from the family *Thermus* sp. represent a unusual group of enzymes, since despite the differences in recognition sequence they revealed a number of similarities to the amino acid sequence level (which is unique among REases recognizing different DNA sequences).

The results of the present study provide fundamental knowledge of the biochemical properties of the recombinant REase TaqII<sup>wt</sup>, verify of the enzyme specificity and propose conditions that allow a controlled change of the enzyme activity depending on the conditions used - the reaction buffer and cofactor S-adenosyl-L-methionine or its analogues: S-adenosyl-L-homocysteine or sinefungin.

In addition, the present study presents optimized overproduction procedure of recombinant protein TaqII which is toxic (and therefore problematic in cloning and expression) for mesophilic host bacteria *E. coli*. For this purpose was designed and chemically synthesized new, modified gene *taqIIRM<sup>syn</sup>* by: (i) modification of nucleotide sequence (changes in the codons without disclosing it in the amino acid sequence), (ii) reduction of GC base pars, (iii) decreasing the stability of duplexes of mRNA gene, and (iv) putting the sequence enabling partial relaxation of the secondary structure of mRNA. The Optimization of the gene sequence was carried out to:

- elimination of stable mRNA secondary structures that may hinder the translation TaqII<sup>wt</sup>,
- optimized the codons used by *E. coli*,
- increased *taqIIRM<sup>syn</sup>* gene expression in bacteria mesophilic, thereby increasing the level of overproduction of the recombinant protein.

Finally, the present study presents an optimal procedure for isolating TaqII<sup>syn</sup> protein prior to homogeneity with heating of the crude lysate at 65°C, the techniques of selective precipitation of nucleic acid, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation, ion exchange chromatography and gel filtration.