Abstract

Soil environment harbours a very high variety of diverse microorganisms. Thus soil microorganisms are the richest source of secondary metabolites such as antimicrobial compounds, volatiles and enzymes. The aim of my study are restriction endonucleases (REases) which paved the road to the genetic engineering era, as they provided biologists with a tool to study and manipulate DNA by enabling the generation of consistently sized DNA fragments. They are currently used for a wide range of applications, including gene cloning, molecular diagnostics, forensic analyses, studies on the phylogenetic and taxonomic diversity of microorganisms, improvement of techniques of DNA analysis and sequencing and assembling genomes and metagenomes.

Thermostable Type IIS restriction endonucleases (REases) are an excellent tool for genetic manipulations because of their ability to cleave DNA outside of the recognition sequence and their high thermal stability. Extreme natural environments are a rich source of new REases. Therefore in this study soil samples and biofilms obtained from Icelandic hot springs were investigated. During my studies I isolated thermophilic *Geobacillus* sp. bacteria from Icelandic geothermal areas. Biochemical analysis of R.GeoICI led to the discovery of the REase recognition sequence and cleavage site, which place it within the Type IIS. Interestingly, in the genome of the thermophilic bacterium *Geobacillus* sp. we found a gene coding for a methylotransferase (MTase) similar to the RM.TspGWI MTase. This could be a result of horizontal gene transfer.

Prof. Piotr Skowron's team discovered and defined a new REase-MTase *Thermus* family of enzymes in 2002-2003. These REases have combined properties of three Types of REases: IIS, IIC and IIG, and have a high amino acid (aa) sequence homology, despite having different substrate DNA recognition sequences. They show similarities to Type I and Type III REases. They are all bifunctional, with REase and MTase domains located within a single polypeptide. Their enzymatic activities can be regulated by a cofactor/allosteric effector S-adenosyl-L-methionine (SAM) or its analogues sinefungin (SIN) and S- adenosyl-L-homocysteine (SAH).

RM.TsoI which belongs to the REase-MTase Thermus family was characterized and cloned by collaborating research teams led by prof. Piotr Skowron and prof. Lubys Arvidas. RM.TsoI shows no significant changes in enzymatic activity specificity in the presence of SAM or its analogues. In the present study, we decided to design and synthesize a new structural analogue of SAM - S-adenozyl-L-cysteine (SAC). Interestingly, this compound induces a change in the activity of RM.TsoI towards more frequent cutting of substrate DNA, classifying the complex RM.TsoI/SAC to a small group of ultra-frequently cleaving REases which are essential in creating genomic libraries.

The RM.TaqII enzyme is the most thoroughly characterized biochemically representant of the REase-MTase Thermus family. It was isolated from the thermophilic bacterium *Thermus aquaticus.*

Because a structural characterization of the syn-RM.TaqII has not been done, in this study I decided to determine its physico-chemical properties in order to better understand the mechanism and preliminary structure of these enzymes. This will help understand chemically-induced, affinity star restriction specificity mechanisms.