SUMMARY OF PROFESSIONAL ACCOMPLISHMENT

1. Name and surname.

Beata Furmanek-Blaszk

2. Diplomas, scientific/artistic levels attained (with titles), place and year of they were awarded, as well as title of doctorate.

Master of Science in Biology – University of Gdańsk, Faculty of Biology, Geography and Oceanology, November 1986

Doctorate Studies – University of Gdańsk, Faculty of Biology Geography and Oceanology Graduated with PhD in biology, April 1996. thesis: ”Characterisation of a bacteriocin from *Staphylococcus sp*.T”.

3. Scientific/artistic employment.

1986-1990 - Assistant, Department of Biochemistry, Faculty of Biology, Geography and Oceanology, University of Gdansk

1990-1996 - PhD student, Department of Microbiology, Faculty of Biology Geography and Oceanology, University of Gdansk

1996 (June to September*)* - Specialist, Department of Microbiology, Faculty of Biology Geography and Oceanology, University of Gdansk

1996 – 2010 Adjunct, Department of Microbiology, Faculty of Biology Geography and Oceanology, University of Gdansk

2010 – until now, Senior lecturer Department of Microbiology, Faculty of Biology Geography and Oceanology, University of Gdansk

4. Scientific achievement* according to the laws concerning scientific levels and titles (Art. 16 paragraph 2 of the Act of March 14, 2003) as well as levels and titles of art (Law Gazette No. 65, item. 595, as amended):

* if this achievement is the work/teamwork, you must provide statements of all its contributors, defining the individual contribution of each of them in its creation (appendix no 8)

a) title of scientific/artistic achievements

Genetic structure and properties of three type IIS restriction-modification systems recognizing asymmetric five-nucleotide specific sequences.

b) author/authors, title/titles of publications, year of publication, name of publishing house

1. **Furmanek B**., Gromek K., Sektas M., Kaczorowski T. (2001) Isolation and characterization of type IIS restriction endonuclease from *Neisseria cuniculi* ATCC 14688. FEMS Microbiology Letters, 196:171-176 (IF_{2001} = 1.806)

2. **Furmanek B**., Sektas M., Wons E., Kaczorowski T. (2007) Molecular characterization of the methyltransferase M1.NcuI from *Neisseria cuniculi* ATCC 14688. Research in Microbiology, 158:164-174 (IF_{2007} = 2.219)

3. **Furmanek-Blaszk B**., Boratynski R., Zolcinska N., Sektas M. (2009) M1.MboII and M2.MboII type II methyltransferases: different specificities, the same target. Microbiology, 155:1111-1121 (IF_{2009} = 3.025)

4**.** Katna A., Boratynski R., **Furmanek-Blaszk B**., Zolcinska N., Sektas M. (2010) Unbalanced restriction impairs SOS-induced DNA repair effects. Journal of Microbiology and Biotechnology, 20:30-38 (IF_{2010} = 1.224)

5. **Furmanek-Blaszk B**., Sektas M. (2015) The SfaNI restriction-modification system from *Enterococcus faecalis* NEB215 is located on a putative mobile genetic element. FEMS Microbiology Letters, 362:fnv028 (IF_{2015} = 1.858)

c) description/summary of scientific/artistic aims with regard to publications and achieved results, with a description/summary of their possible application(s)

Restriction-modification systems (R-M) are widely distributed among prokaryotic cells. R-M systems comprise of a restriction endonuclease and methyltransferases with the same DNA binding specificity. The restriction endonucleases recognize and cut specific DNA sequences, while cognate methyltransferases modify DNA by the addition of a methyl group to either adenines or cytosines in the recognition sequence protecting the DNA from a cognate restriction endonuclease (Wilson and Murray, 1991). Methylation of genomic DNA occurs immediately after replication. As a result of this, unmodified foreign DNA entering the cell is degraded by the host restriction endonuclease. Despite the fact that both enzymes comprising a specific R-M system recognize and bind to the same sequence, comparative analysis of their amino acid sequences show only marginal resemblance (Chandrasegaran and Smith, 1988). This suggests that the enzymes forming the R-M system bind to DNA in a different way so that it is likely they arose as a result of separate evolutionary processes. R-M systems play a central role in prokaryotic defense, while restriction endonuclease degrades DNA from viruses and other exogenous sources. In addition to these protective functions, their impact on the genetic diversity of bacteria can be postulated while another function that can be implied is the creation of molecular barriers to promote speciation (Vasu and Nagaraja, 2013).

R-M systems are mainly classified into four different types based on their subunit composition, sequence recognition, cleavage position, cofactor requirements, and substrate specificity (Roberts et al., 2003). Type II enzymes are the most widely studied and extensively utilized nucleases in genetic engineering. To date, more then 3800 type II restriction endonucleases have been identified, with about 300 different specificities having been discovered (Roberts et al., 2015). Type II R-M systems, with very few exceptions, consist of two separate proteins with independent enzymatic activities, a restriction endonuclease and a DNA methyltransferase. Restriction endonucleases recognize short, usually palindromic, sequences of 4 - 8 bp, and in the presence of Mg^{2+} cleave DNA within or in close proximity to the recognition sequence. Type II methyltransferases function by transferring a methyl group from S-adenosyl-L-methionine to adenine or cytosine generating N^6-methyladenine, N^4-methylcytosne or C^5-methylcytosine (Dryden, 1999). Type II enzymes, as the most heterogeneous, have been divided into several subsets. The type IIS enzymes are grouped as a subset on the basis of their recognition sites; asymmetric sequences that are cleaved by restriction endonuclease a fixed distance away from the site (Roberts et al., 2003). Type IIS endonucleases are accompanied by (i) large, individual proteins containing two functional domains within a single protein molecule, each of which is responsible for the modification of different strands or (ii) two separate methyltransferases, each of which modifies one strand of the recognition sequence. In addition, the latter are not the product of gene duplication but have been acquired or subjected to independent evolutionary processes. This is confirmed by the low level of similarity between proteins at the primary structure level (Madhusoodanan and Rao, 2010).

My research in the Department of Microbiology, focused among other things on type IIS R-M systems. Enzymes belonging to them have the ability to recognize - with extreme precision - short, asymmetric specific nucleotide sequences. As a model in my study I use two isospecific R-M systems: MboII from *Moraxella bovis* ATCC 10900 and NcuI from *Neisseria cuniculi* ATCC14688. These systems originate from different microorganisms but the enzymes from which they are composed, have the same DNA binding specificity and recognize the same nucleotide sequence 5'-GAAGA-3'/3'-CTTCT-5'. Comparative analysis of the enzymes forming the isospecific R-M systems is aimed at finding common structural and functional elements, and detecting differences at the genetic and biochemical level. Class IIS enzymes were selected due to their ability to accurately recognize very short specific sequences.

My first research concerned the comparison of restriction endonuclease NcuI **(publication 1)** with previously described isoschizomer MboII (Sektas et. al., 1992). In order to do this, R.NcuI was purified to electrophoretic homogeneity using standard chromatographic procedures. The calculated molecular weight of R.NcuI and R.MboII is approximately 48 kDa. The NcuI endonuclease recognizes the pentanucleotide sequence 5'-GAAGA-3'/3'-CTTCT-5' and cleaves the DNA 8 and 7 nucleotide downstream from the recognition site. Therefore, the R.NcuI is an isochizomer of the R.MboII enzyme. Due to the similar physicochemical properties and cleavage mechanism of both enzymes, an experiment aiming at antigenic cross-reactivity was conducted. The ability of antiserum prepared against R.MboII to cross-react with R.NcuI was examined. R.NcuI possesses a substantial antigenic similarity to R.MboII suggesting that both proteins have identical epitopes. Both enzymes may also have a similar architecture since antigenic similarity is generally correlated with three-dimensional similarity. The close relationship of R.NcuI and R.MboII is also apparent from the N-terminal fragment of the amino acid sequence of these proteins. The comparison of the amino acid sequence of N-terminal fragments R.NcuI and R.MboII show a high level of identity (85%).

The goal of the study following on from the one outlined above, was to isolate and characterize the methyltransferase M1.NcuI and compare it with the properties of the isospecific M1.MboII enzyme **(publication 2)**. In order to do this, recombinant and wild-type methyltransferases M1.NcuI were purified to electrophoretic homogeneity using standard chromatographic procedures. Both proteins have similar biochemical properties. It was found that divalent cations, including Mg^{2+}, inhibit methylation activity. However, magnesium ions inhibit the recombinant methyltransferases M1.NcuI less efficiently than the native enzyme (IC_{50} = 0.35 mM and IC_{50} = 10 mM, respectively). This particular dissimilarity may be the result of different conditions affecting protein folding in the homologous and heterologous host, incorrect folding is linked to significant reduction in activity of recombinant proteins compared to their correctly folded counterparts. Previous studies show that to carry out the cleavage reaction, restriction endonuclease NcuI requires the presence of Mg^{2+} ions, while on the other hand, the same ions are a potent methyltransferase M1.NcuI inhibitor. The sensitivity of M1.NcuI to Mg^{2+} can shift the equilibrium of the R-M system towards the restriction of DNA which in turn provides the bacterial cell with an effective defense against exogenous DNA. Subsequent studies have shown that M1.NcuI and M1.MboII have similar biochemical properties, and also exhibit the same specificity. Methyltransferase M1.NcuI likewise M1.MboII (McClelland et al., 1985) catalyze the methylation reaction of the outer

adenine nucleotide in the recognition sequence 5'-GAAG^{m6}A-3'/3'-CTTCT-5'. The calculated molecular weight of M1.NcuI and M1.MboII is approximately 32 kDa. Due to the similar biochemical properties and methylation specificity of both enzymes, an experiment to study the immunological cross-reactivity of the anti-M1.MboII antibodies with M1.NcuI was conducted. For this purpose homogenous preparation of M1.MboII was used as an antigen for immunization of rabbits to obtain serum containing a polyvalent anti-M1.MboII antibodies It was found that the anti-M1.MboII serum is specific for both methyltransferases. These results indicate that the two enzymes may have a similar architecture since the antigenic is generally correlated with three-dimensional similarity. In addition to this, since the genera *Neisseria* and *Moraxella* are naturally transformable, it is likely that the R-M systems found in these strains may have been acquired from other species through horizontal gene transfer.

At the same time as the research described above, a study aimed at determining the NcuI R-M system's genetic structure was conducted. Thus initial primers for *ncuIR* gene amplification based on the partially known nucleotide sequence of the R-M MboII system (Bocklage et al., 1991) and similarity between enzymes comprising NcuI and MboII R-M systems were designed. As a template, the genomic DNA isolated from bacteria of *Neisseria cuniculi* ATCC 14688 was used. This approach allowed for the determination of the *ncuIR* gene fragment. To complete the nucleotide sequence of other genes forming NcuI R-M and flanking regions, the PCR technique was used. The NcuI and MboII R-M systems comprise a cluster of three linked genes oriented in the same direction that encode two methyltransferases and one restriction endonuclease: *ncuIM2→ncuIM1→ncuIR* and *mboIIM2→mboIIM1→mboIIR*. However, contrary to the close location of genes encoding M2.Ncu and M1.NcuI, in the case of the MboII R-M system *mboII2* and *mboIIM1*genes are separated by a 683 bp intergenic region (http://rebase.neb.com). Proteins forming the MboII and NcuI R-M systems share a high degree identity of 90% for M1.MboII/M1.NcuI, 88% for M2.MboII/M2.NcuI, and 87% for R.MboII/R.NcuI at the amino acid level. The presence and distribution of nine highly conserved amino acid sequence motifs and the target recognition domain in the enzyme structure suggested that M1.NcuI belonged to the N^6-adenine β-class of methyltransferases. The overall G+C content of genes encoding MboII and NcuI R-M systems were 32% and 31.6% respectively which is significantly lower than the average G+C content of genomic DNA of *Moraxella bovis* (45%) and *N .cuniculi* (44.5%). suggesting their acquisition by horizontal gene transfer.

In connection with the above, the discovery of a second methyltransferases in the MboII and NcuI R-M systems raised the question of what the biological role might be for the

tandem modification enzymes in the proper regulation and functioning of the whole systems **(publication 3).** To obtain answers to this question it was decided to compare the substrate specificity of the two methyltransferases and their ability to protect host DNA against R.MboII action. As it is extremely popular for recombinant protein expression, the T7 promoter system present in the pET24a vector was used for protein purification. The calculated molecular weight of M2.MboII and M2.NcuI is approximately 32 kDa. The biochemical characteristics determined for M2.MboII and M2.NcuI were very similar. Both methyltransferases methylate the internal cytosine residues of the strands carrying 3'-CTT^{m4}CT-5'. Interestingly, M2.MboII and M2.NcuI contrary to M1.MboII and M1.NcuI can also, with comparable efficiency, modify the specific targets in single-stranded DNA. The primary biological role of this phenomenon may be to facilitate the natural transfer plasmid DNA between strains by the transformation pathway, in which incoming single-stranded DNA may shape the bacterial genomes. Sequence analysis of the M2.MboII and M2.NcuI revealed the presence of highly conserved motifs common to N^4-cytosine β-class methyltransferases. Despite the fact that the two methyltransferases of MboII and NcuI R-M systems recognize and bind to the same DNA sequence, comparative analysis of the amino acid sequences showed a marginal resemblance (14% identity) at the amino acid level. The difference between the two methyltransferases of the same system was confirmed by a Western-blot hybridization assay. These results indicate that both enzymes are not the result of a single gene duplication but rather were acquired or were subjected to independent evolution.

The enzymes of the MboII R-M system are functionally identical with those of the NcuI R-M system. However, while the two MboII methytransferase genes are separated by 684 nucleotides the genes encoding the NcuI R-M system are closely linked. Earlier data available in the database REBASE indicated that this region is one nucleotide shorter. In this DNA fragment two ORFs, 654 and 375 bp long were identified, which are in the same reading frame with no sequence similarity to any known expressed genes. A PCR procedure with chromosomal DNA coming from the geographically distant *M. bovis* strains showed the presence of a 684 bp DNA fragment identical to that of *M. bovis* ATCC 10900. Considering the location of this DNA fragment between the *mboIIM2* and *mboIIM1* genes and its presence in other *M. bovis* strains, *orf654* might encode the protein involved in the regulation of the MboII R-M system. The location of *mboIIM2* in the genetic structure of the MboII R-M system suggests independent evolutionary routes at least for this element among MboII-like systems.

Although a great number of R-M systems have been identified, relatively little has been determined about regulation of their expression. Most publications exhaustively review this topic from a biochemical and genetic viewpoint, providing a timely overview of current research whereas tight regulation of the expression of modification and restriction that prevents premature activity of the restriction endonuclease is essential for bacterial cells. Therefore, in order to assess the impact of each methyltransferase on cell viability an incomplete MboII R-M system was constructed lacking a *mboIIM1* or *mboIIM2* gene. Firstly, it was demonstrated that the natural $P_{mboIIM2}$ promoter is too weak to protect restriction sites against R.MboII digestion in the *E. coli* host, as the gene can only give a sufficient level of protein to protect all R.MboII sites after its fusion with an additional constitutive promoter. In contrast, the expression of the *mboIIM1* gene from a natural promoter is fully sufficient to give protection to all cellular MboII sites. Furthermore, it has been shown that overproduction of R.MboII in *E. coli* cells is only possible in the presence of both MboII methyltransferases. Comparison of the viability of the host cells and level of R.MboII production dependent on the presence of MboII methyltransferases, separately or together showed that the most stable and fast-growing were cells with both methyltransferase activities. A complete MboII R-M system consisting of two methyltransferases and the *mboIIR* gene is the most stable functionally and genetically.

The MboII R-M system consists of three genes coding for the restriction endonuclease and two separate methyltransferases. M1.MboII modifies the last adenine on the top strand recognition sequence, and M2.MboII transfers the methyl group to the internal cytosine in the bottom strand. When one of methyltransferases is absent, as a result of DNA replication, only one newly synthesized DNA molecule is specifically methylated (hemimethylation) while the second molecule is devoid of specific DNA methylation. The structure of the MboII R-M system makes it possible to study the impact of incomplete methylation of a specific sequence on the level of intracellular autorestriction after removing one of the methyltransferase. These experiments were designed to investigate the influence of a functionally unbalanced MboII R-M system on the ability of cells to repair mitomycin C-induced DNA damage **(publication 4)**. As a source for an incomplete MboII R-M system, plasmids carrying the *mboIIR* and *mboIIM2* genes under the control of P_{lac} promoter were used, so the expression was induced by adding IPTG to the culture. In our study the *E. coli* ER1992 strain deficient in methylation-dependent restriction systems, containing a *dinD1::lacZ*$^+$ reporter gene, was used. The dinD1 locus is a DNA damage inducible gene that is expressed in *E. coli* when the SOS response is triggered. Application of the incomplete MboII R-M system under the control of the inducible

P_{lac} promoter allowed for the examination of its influence on the process of post-replicative DNA repair due to damage caused by the action of mitomycin C. The sudden appearance of a large number of non-methylated DNA regions resulted in greater mortality of the bacterial cells containing the MboII R-M system by autorestriction of chromosomal DNA. Over time, these sites were methylated. Fluorescent staining using DAPI and analysis of the degree of chromosomal DNA degradation confirmed that the restriction endonuclease MboII is responsible for the death of the bacterial cells. To investigate whether this phenomenon is unique or common in bacteria containing any type of R-M system, a similar experiment was performed using bacterial cells possessing different variants of the EcoRI R-M system, cultivated under sublethal concentrations of mitomycin C. It was found that the SOS induced DNA repair process is more effective in strains lacking the type II R-M system or comprising the type I R-M system, and less effective in the case of bacteria with chromosomally located EcoRI so the concentration of both protein components is at very low level. Our research showed that the presence of the functioning type II R-M system can cause a decrease in the efficiency of DNA repair induced by the genotoxic agent (mitomycin C) and, consequently, reduce the competitiveness of such cells against bacteria lacking the type II R-M system in the presence of compounds which damage DNA structure.

Continuing research into the characteristics of the type IIS R-M system, I consequently turned my attention to the enzymes forming the SfaNI R-M system present in the *Enterococcus faecalis* NEB215 strain **(publication 5)**. Both proteins recognize the asymmetric specific sequence 5'-GCATC-3 '/ 3'-CGTAG-5'. The enzyme R.SfaNI has been used in molecular biology for many years, yet its properties are poorly understood. An additional prerequisite to undertake research on the restriction endonuclease SfaNI was the development of a DNA sequencing method by indexer walking, by the employees of the Microbiology Department (Gromek and Kaczorowski, 2005). In this method type IIS endonucleases are used to produce DNA fragments with nonidentical 4-nucleotide 5′ overhangs. Using standard chromatographic procedures, restriction endonuclease R.SfaNI was purified to electrophoretic homogeneity, and then used for N-terminal amino acid sequencing and the cloning of the SfaNI R-M system. The calculated molecular weight of R.SfaNI is approximately 72 kDa and the biochemical characteristics determined for the enzyme resembles conventional type II restriction enzymes. The complete nucleotide sequence of the SfaNI R-M system was obtained using a strategy based on the selection of self-modifying recombinant plasmids that have become resistant to the R.SfaNI system and PCR procedures. M.SfaNI is encoded by a single gene, but amino acid sequence analysis of

the protein shows that it contains two separate conserved domains responsible for each strand methylation. Each domain contains its own set of the amino acid sequence motifs characteristic for N^6- adenine α-class methyltransferases. Sequence analysis of M.SfaNI revealed the presence of a structure carrying an additional domain that is not classed with the standard conserved motifs, and which may be important for DNA-modifying activity. Analysis of the amino acid sequence of SfaNI endonuclease suggests that it contains a PD-(D/E)XK nuclease motif common to many Mg^{2+}-dependent restriction endonucleases. The SfaNI R-M system is inserted between a transposae and a *ccr* gene complex. CcrA and CcrB are serine recombinases of the invertase/resolvase family catalyzing in a site-specific manner excision and integration of the SCC, a mobile genetic element that carries the central determinant for broad-spectrum beta lactam resistance encoded by the *mecA* gene in staphylococci. The presence of mobile genetic elements in the vicinity of the SfaNI R-M system and the noticeably lower G+C content of its genes may indicate that these gene clusters have been acquired through horizontal gene transfer end recombination

The most important discoveries of the publications presented that make up my scientific achievement are:

- demonstration of a high degree of structural and functional similarity between enzymes belonging to the MboII and NcuI R-M systems
- determination of the methylated base in the recognition sequence by M2.MboII and M2.NcuI; both enzymes belong to the small group of DNA methyltransferases able to modify single-stranded DNA
- determination of the genetic organization of the three studied R-M systems, together with their adjacent regions; within the MboII R-M system the *orf654* gene was identified whose product may be involved in the regulation of the expression and/or function of the MboII R-M system
- uncovering the differences in the G+C content of genes encoding enzymes belonging to the studied R-M systems in relation to genomic DNA; this suggests that their coding sequences have not evolved simultaneously with the host genome, and most likely have been adapted from the environment as a result of horizontal transfer
- demonstration of the fact that the natural $P_{mboIIM2}$ promoter is too weak to protect restriction sites against R.MboII digestion in an *E. coli* host while the expression of the *mboIIM1* gene from a natural promoter is fully sufficient to give protection to all cellular MboII sites; upon

the SOS-induced DNA repair in mitomycin C treated cells, containing only one out of two functional MboII methyltransferases, restriction significantly reduces cell viability

- detection of genes encoding serine recombinases in the vicinity of the SfaNI R-M system, which enable horizontal gene transfer as described previously only for SCCmec cassettes; location of the SfaNI R-M system may facilitate its spread among bacteria

The comparative analysis of enzymes forming isospecific R-M systems made it possible to find common structural and functional features, which in turn has led to a better understanding of the mechanisms responsible for the formation of proteins of the same specificity. These results indicate that horizontal gene transfer plays a significant role in shaping the structure of bacterial genomes.

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DESCRIPTION OF REMAINING SCIENTIFIC/RESEARCH ATTAINMENTS

In 1986 I graduated from Gdańsk University, Faculty of Biology. My MSc thesis titled "The influence of rifampicin on λ and pSC101 plasmids replication in dnaAts mutants of *Escherichia coli*" was completed in the Department of Molecular Biology, under the supervision of professor Karol Taylor. The results of the thesis were presented at the 9th Conference of the Polish Genetic Society in Gdansk in 1986. In the same year I started work in the Biochemistry Department in the research team headed by professor Alina Taylor. The aim of my work was to (i) develop an efficient method of purification of bacterial alkaline phosphatase (ii) obtain enzyme-conjugated anti-goat immunoglobin, and (iii) check the usefulness of the alkaline phosphatase in an ELISA immunoenzymatic assay. As a result, alkaline phosphatase was made commercially available, with the proceeds deriving from the sale of the enzyme by Dom Handlowy Nauki, supporting the Department of Biochemistry financially.

In 1990 I became a Doctoral student of Chemistry at the University of Gdansk and under the supervision of the head of the Department of Microbiology, professor Anna Podhajska started research on the staphylococcal bacteriocin - staphylococcin T produced by a strain of *Staphylococcus cohnii* T. Purified to homogeneity, the bacteriocin preparation was used to determine the properties, mechanisms of action and spectrum of antimicrobial activity. The results of these experiments were published **(publication 3, point IIA, appendix 5)** and presented in my doctoral thesis under the title "Characterisation of a bacteriocin from *Staphylococcus sp.*T", which I defended in April 1996 at the Faculty of Biology, Geography and Oceanology. The research conducted at that time, allowed me to gain knowledge and experience, and master the scientific methods of molecular biology, which resulted in two publications **(publication 1, publication 2, point IIA, appendix 5).** My research on the development of efficient methods for staphylococcin T purification continue to the present in cooperation with employees of the Department of Chemical and Process Engineering, the Technical University of Gdansk.

In 1998, I changed the subject of my scientific interests and started research on the isospecificity phenomenon of class IIS restriction-modification systems. In addition to mainstream research regarding the comparison of enzymes forming MboII and NcuI R-M systems, I also conducted research related to their genetic location. This is because genes of some R-M systems are located on mobile genetic elements such as natural plasmids, and although the data in the literature shows that most *M. bovis* strains have several plasmids there is little information available on the genetic information they encode. In the course of research on *M. bovis* ATCC 10900, meanwhile, it turned out that this strain has at least two plasmids; a

high molecular weight and a much smaller one, named pMbo4.6, which was found to be 4658 bp in size **(publication 5, point IIA, appendix 5)**. The data obtained from sequence analysis indicates the presence of two modules responsible for (i) replication and (ii) mobilization for conjugative transfer in pMbo4.6. These results suggest that plasmid pMbo4.6 can mobilize the conjugative transfer of the second plasmid present in the cells of *M. bovis* ATCC10900, whose structure is not yet known.

Research related to the gene cloning of the MboII R-M system has enabled the development of a cloning method by means of which it is possible to distinguish the resulting recombinant colonies from pseudorecombinants which have been restored to their original form **(publication 7, point IIA, appendix 5)**. This involves placing the *mboIIM2* gene, whose expression is lethal to bacteria, in the multiple cloning site. Methyltransferase M2.MboII activates methyl-induced autorestriction of the host DNA in Mcr-proficient *Escherichia coli* strains. In this method the *mboIIM2* gene is not fully expressed causing a sublethal effect. As a result, bacteria grow at a much slower rate and form colonies smaller than bacteria containing plasmid molecules with the target DNA. This method has been successfully applied in several gene clonings in our laboratory.

During our research on the MboII R-M system we obtained a deletion mutant in the gene encoding methyltransferase M2.MboII **(publication 9, point IIA, appendix 5**). Expression of the *mboIIM2ΔA356* gene gave rise to an expected premature translation stop and the production of a truncated (14.5 kDa) inactive protein, as well as a full-length active methyltransferases M2.MboII (32 kDa). We found that the T7 RNA polymerase displays a relatively high level of template-dependent transcriptional infidelity which leads to a shift in the reading of the mRNA. This involves multiple insertions or deletions of the nucleotide in the homopolymeric sequences rich in A/U in mRNA, leading to the synthesis of modified proteins. We identified four non-uniformly distributed erroneous hot spots in the *mboIIM2* gene in which a high level of insertion/deletion (InDel) type errors were introduced. The resulting phenotypically heterogeneous mixture of functional and non-functional proteins enhances the evolutionary potential of the bacterial host. We have shown that the repair of accidental frameshift mutations also applies to *mboIIM1* and *ncuIM2* genes.

Parallel to the research mentioned above, I also collaborated with the research team of the Laboratory of Biochemistry of Microorganisms in the Department of Biochemistry at the University of Gdansk, where I was involved in the study of oxidative stress induction in bacterial cells upon exposure to many chemically unrelated antibiotics. Trimethoprim, nalidixic acid, rifampicin, kanamycin and streptomycin at sublethal concentrations inhibit

Escherichia coli biofilm formation, and induce oxidative stress which leads to overproduction of tryptophanase and increased synthesis of indole **(publication 4, point IIA, appendix 5)**. I was also involved in research on bacterial persistence. Persister cells constitute a small part of an antibiotic-treated population of bacteria that is refractory to antibiotic killing without becoming genetically resistant. It was found that the increase in the number of surviving cells correlates with the appearance of improperly folded proteins and their aggregation (**(publication 6, point. IIA, appendix 5)**.

In addition to research aimed at uncovering the characteristics the R-M systems, I also started to search for restriction enzymes of previously unknown specificities, which could serve as a new tool in molecular biology **(publication 8, point IIA, appendix 5)**. As a result of the analyzes of water samples taken from the river Nile, the bacterial strain *Aeromonas hydrophila* was isolated. This bacterium is resistant to most β-lactam antibiotics, and produces five virulence factors which promote the colonization of the host bacteria and survival in a changing environment. In the bacterial cells of *A. hydrophila* a small plasmid, which was named pAhy2.5, was detected. Due to the fact that plasmids have accessory genes that encode functions beneficial to the host cell pAhy2.5 was cloned and sequenced. The study showed that *A. hydrophila* is the source of two restriction endonucleases named AehI and AehII and that these enzymes are isoschizomers of XhoI and StuI respectively.

RESEARCH PLANS

Currently, I am continuing my study within the framework of the research project "The new mechanism regulating the expression of genes encoding restriction-modification system MboII from *Moraxella bovis*" (2012/07 / BNZ2 / 01782). In relation to this I have conducted research on the degree of polymorphism genetic determinants of the MboII R-M system. My study shows that the 684 base pair length intergenic region between the *mboIIM2* and *mboIIM1* genes, contains an open reading frame capable of encoding a polypeptide of approximately 25 kDa. The deduced amino acid sequence was confirmed by comparison with the N-terminal part of the protein determined by automated Edman degradation. In the proximal part of the orf654 gene a tertiary structure resembling a hairpin loop preceded by the sequence 5'-GAAGA-3'/5'-TCTTC-3' was identified. Usually the pin structure plays an important role in the regulation of the expression of genetic information so we believe that the location of the gene *orf654* is not accidental. Moreover, we have detected the presence of the *orf654* gene in over one hundred *Moraxella bovis* strains coming from Argentina, Australia, Japan, New Zealand, the United States and Thailand, all possessing the MboII R-M system.

The location of the gene *orf654* and above all, its translational product suggests that this protein may be involved in regulation of the expression and/or functioning of genes of the MboII R-M system. As part of the project we have investigated whether and how protein ORF654 affects the achievement of equilibrium between the modifying activity which serve to protects cell, and DNA restriction. We hope to get to know the mechanism of action and function of protein ORF654 and understand the regulation of this process at the molecular level. The significance of this mechanism goes beyond the study of *M. bovis* genetics as the discovery of a new protein may provide insight into new, previously unknown aspects of gene expression regulation.

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