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CONSTRUCTION OF STRAINS, PRODUCING RECOMBINANT DEOXYNUCLEOSIDE KINASE, CONTAINING IN THEIR SRTUCTURE CHITIN-BINDING DOMAIN

Enzyme immobilization technology is one of the key modern industrial biotechnologies. This technology is used to improve the enzyme catalytic functions such as activity, stability, and selectivity, it can be used repeatedly and continuously, which significantly reduces the cost of the process.

The commonly employed techniques for immobilization of enzymes are – chemical, physical and affine binding.

Chemical immobilization is based on formation of covalent bonds between matrix and enzyme. It makes impossible to desorb enzyme, but leads to multiple modification enzymes and, as a consequence, to change their properties and inactivation. Physical method of immobilization is based on adsorption without formation of covalent bonds, leading to enzymes desorption during enzymatic reactions. Affine method saves enzyme activity unchanged and provides reliable bonding with surface of the carrier.

Among the many affine carriers must highlight the chitin – one of the most common biopolymer, which have chemical resistance, a well-defined pore structure and low cost.

The literature describes chitin-binding domain (ChBD) of *Bacillus circulans* chitinase A1 (ChiA1). The properties of ChiA1 defines the robust and affinity bonds with the substrate.

In connection with the above, the aim of this work was the creation of strains of chimeric proteins containing in their structure ChBD of chitinase A1 *B. circulans.*

Using polymerase chain reaction method gene ChBD was isolated from A1 chitinase *B. circulans* and inserted with 5'- and 3'-ends of the target gene in plasmid pET42dnkDm. As a result, two new constructs were obtained: pET42dnkDm_ChBD (N) and pET42dnkDm_ChBD (C). These constructs were used to transform cells *Escherichia coli* BL21 (DE3).

Thus obtained two strains producing *deoxynucleoside kinase* (*E. coli* pdnkDm_ChBD (N) and *E. coli* pdnkDm_ChBD (C)) having ChBD at N- and C-ends.

It was *established* that a strain of *E. coli* pdnkDm_ChBD(N) produces the target protein in 5 times more than strain *E. coli* pdnkDm_ ChBD (C). D*eoxynucleoside kinase* amount in cell lysates was about 50 and 10% of total cellular protein in strains *E. coli* pdnkDm_ChBD (N) and *E. coli* pdnkDm_ChBD (C), respectively.

The results will be used in further research on the immobilization of proteins and heterogeneous biotechnological synthesis.

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MOLECULAR-BIOLOGICAL MARKERS OF COLORECTAL CANCER

The issue of the day of modern oncology and proctology is colorectal cancer (CRC), the increase of its morbidity is related to a great extent to worsening of ecological situation in the Republic of Belarus. 99% of colorectal cancers are well-known as adenocarcinomas, and nowadays the development of colonic adenocarcinomas is considered to be the well studied model of oncogenesis.

At the present time, the research of features of tumor biological behavior is one of the most crucial in oncology. The findings of structural and functional changes of oncogenes and genes-suppressors in the process of the tumor development and progression were the cause of the basis of the determination of clinically significant molecular factors .

A colorectal cancer is a multifactorial disease depending both on genetic and exogenous factors.

In the last few years of rapid development of molecular biology, it was found out that the genes possessing the ability to control the process of the disease development are of great significance.

So, the mutations of AIP gene (adenomatous intestinal polyps) are associated both with the inherited and sporadic cases of colorectal cancer. Gene damages result in the unlimited division of tumor cells and, as a result, lead to the increased distribution of the tumour process.

K – ras is a gene playing an important role in a receptor signaling system of the epidermal growth factor (EGFR). This gene damaging leads to the decline in the CRC patient's survival rate.

P53 is an albumen preventing the division of potentially tumorigenic cells. However, the insufficient functioning of this albumen makes a cellular division possible even at DNA damages. Its hyperexpression results in an unfavorable prognosis of the course of the disease and the early relapses.

DCC is a supressor gene, and its albumen is a surface glycoprotein, which is responsible for the processes of cellular adhesion. The decline in the expression of DCC gene results in the dispersion of tumor cells, and the prevalence of tumor process finally increases.

Also, a diagnostically important marker for determination a proliferative activity is the nuclear albumen Ki – 67. Its hyperexpression allows distinguishing tumor cells being in the active phase of a cellular cycle.