BIOTECHNOLOGY AND BIOENGINEERING UNDER THE LOOP: CREATIVITY OR DANGER

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Abstract: *No achievements of modern mankind was aroused so much disaprrovals and caused so much debate as the phenomenon of biotechnology. Human intervention for the improvement of crops, trees and livestock is nothing new: for millennia, humans have bred, crossed and selected those varieties, ecotypes and breeds that were more productive, and better adapted, but conventional breeding practices can now be complemented by a number of new and powerful techniques. Modern biotechnology represent a new aspect of biological and agricultural science which provides new tools and strategies. New techniques of molecular biology, including recombinant DNA and cell fusion, have became a powerful research tools in biology. As the foundations of biotechnology, these techniques hold great promise for the development of new products derived from plants, animals and microorganisms to be employed in industry and agriculture. Molecular biotechnology, including recombinant DNA, transplastomic plants, projectile insertion, cloning, nuclear microinjection, and cell fusion, offers the potential for reaching many of the same goals pursued by traditional methods of plant and animal breeding and by mutation and selection of microorganisms, but in a more precise and efficient manner. Thanks to that biotechnology managed to penetrate and find application in all branches of industry and basic researches. The first large-scale industrial applications of modern biotechnology have been the areas of agriculture and of pharmaceuticals. Nowadays biotech methods play an increasing role in environmental protection and biofuel production. However, when we talk about biotechnology inevitably question is whether are its achievements in accordance with nature and whether can cause undesirable consequences. Genetically engineered organisms should be evaluated and regulated according to their biological properties and phenotypes, rather than according to the genetic techniques used to produce them. Nonetheless, because many novel combinations of properties can be achieved only by molecular and cellular techniques, achievements of these techniques may often be subjected to greater scrutiny than the achievements of traditional techniques.*

Key words: *biotechnology,research areas, application*

INTRODUCTION TO BIOTECHNOLOGY

No achievements of modern mankind was aroused so much spit and caused so much debate as the phenomenon of biotechnology. Although often cell and tissue culture and genetic engineering are generalized as biotechnology, this includes a much broader phenomenon, unique, complex and multidisciplinary biological activity that uses biological systems, living organisms or their derivatives, and in order to achieve or change products and processes for specific use. The complexity of biotechnology is reflected in the fact that it combines knowledge and achievements in the field of genetics, molecular biology, biochemistry, embryology, cytology and physiology, which are supplemented by practical disciplines such as chemical engineering, information technology and biorobotics. Actually, this term should be used in a much broader context, to describe the whole spectrum of methods used to manipulate with organic matter.

DISCIPLINES OF BIOTECHNOLOGY

If the concept of biotechnology is viewed in a broader sense, biotechnology can be divided into conventional and modern or molecular. Conventional or traditional biotechnology includes both breeding animals and plants, and also use of organisms, primarily microorganisms, in processes of production. To begining the era of modern biotechnology application is taken 1972, when was constructed the first recombinant DNA molecule. This proof, until then only theoretical assumptions, has caused great excitement in scientific circles. The consequence was that the expectations and predictions for the possibility of applying this phenomenon became a bit unrealistic. Turned out to be premature, it was started with planning a wide range of research, development of therapies for incurable diseases to the cloning of organisms. The initial results were disappointing, primarily because then the level of technology development and techniques could not follow the objectives. Decisive role in the advancement of biotechnology research was the development of information technology and modernization of laboratory equipment. Achievement of technical and technological fields have caused development and improvement of laboratory techniques for biological systems manipulation, such as method of recombinant DNA, tissue culture techniques, cloning techniques, the specific transfer of genes from one organism to another, the so-called horizontal gene transfer, and use organisms-vectors for the DNA transfer into the host organism. (GLIK and PASTERNAK, 1998).

Division of biotechnology on traditional and molecular is rough. Since methods of bitechnology have many uses, the terms were developed with the intent to identify and differentiate basic biotechnological sphere of activity. Bioinformatics is an interdisciplinary discipline that is in solving biological problems relies on computer technology and information systems. This area is used as a supplementary method in analyzing the function and structure of genomes and proteins, and its significant contribution to the pharmaceutical sector. Blue biotechnology is a term used to describe biotechnology research in the field of ocean and water in general. The area of biotechnology is relatively rarely applied. Green biotechnology is synonymous with the methods applied in agriculture. This area has the widest application, and some of its examples can be: selection and domestication plants by micropropagation, the design of transgenic plants in order to grow and develop in specific environment conditions (MÜLLER and KAYSER, 2004; www.biotehnologija).

Red biotechnology is biotechnology means the application in medicine. Some examples of this are are: designing organisms that synthesize antibiotics and the creation of drugs compatible with personal patient genetic code. White biotechnology is known under the term industrial biotechnology. Its application was made in industrial processes. The most commonly used to create organisms that synthesize useful substances. Another example is the use of enzymes as industrial catalysts to produce valuable chemical compounds or to break down toxic chemicals or potential polluters of the environment. Risk assessment of investment and degree of any money to get deals bioeconomy (WALSH, 2003; KAYSER and MÜLLER, 2004, TANG and ZHAO, 2009).

METHODS OF GENETIC MANIPULATION

In the biotechnological innovations have been developed methods based on direct manipulation of genes. These methods are based on the same or similar principles, and therefore are partly similar. In addition to that they pervade each other, and it is difficult to clearly mark off. Genetic engineering, recombinant DNA technology and genetic modification are terms related to the manipulation at the level of genes (SOUTH, 2001). Modern biotechnology is based on the principle of recombinant DNA. This precious molecule itself conceals information for the protein synthesis through which specifies and regulates

every physiological and biochemical process. Life features, growth and unique characteristics of individuals depend on the results hidden within the DNA molecule. Since that carries hereditary information in all organisms, the DNA molecule, built from the same components, makes possible that some of its parts, or genes, may be transferred from one organism to another. If a molecule of DNA is treated with restriction enzymes from the group of endonucleases, it is possible to cut out individual genes and transferre them from the genome of one into genome of other individuals. On this, artificial, way is created a qualitatively new DNA molecule, called recombinant DNA.

Genetic engineering is a complex laboratory technique that combines cloning on molecular level with the transformations, all with the goal of direct gene changes. In general this technique is based on five steps: isolation genes of interest, entering the desired genes into the body for the transfer, so-called vector, transfer vector in the body that wants to be modified, transform cells in the body and selection genetically modified organisms. The first step is the identification and isolation genes of interest. This stage is based on existing knowledge about the functions of individual genes. Genes can be isolated from genomic or complementary DNA. If necessary, as in case of insertion prokaryote genomic DNA in the eucariote organism, it is possible to modify the DNA additionally, by intron removal or changing prokaryote promotor region. Once the gene of interest is isolated, it can be entered into the vector. For this purpose most commonly are used plasmids, although and viruses proved as successful vectors. Recently attention have been caused so-called nonprokariotic vectors, such as liposomes. The most sofisticated method for input DNA is actually without vectors, when the specialized gun "shoot" desired gene into DNA. Process shooting DNA eventually become a routine procedure, in which the DNA molecule binds to precious metals such as gold or platinum, after which it is inserted directly into the cell. Molecular biologists have discovered many enzymes that can cut DNA at two positions, or connect chains interrupted. Once entered into the vector DNA donor can be used for tranformation of recipient organism. Depending on the vector transformation can be more or less complex. Although methodological the simplest, technical input of hereditary material by guns proved the least assertive and effective method. Other more complex methods, such as bacterial or viral vectors have been approved as more effective. By entering recombinant DNA and its instertion in the host genome, starts expression of this new gene. As a final result host synthesizes proteins characteristic for the donors. After the transformation it is necessary to choose the organisms that carry the transformation of those whodo not have them. In practice two methods are used for selection of organisms with transformation, one is screening test using DNA that binds to the gene of interest, another method is applied in cases when transfered gene synthesizes specific chemical substances, such as antibiotics or herbicides. These substances are then added, which provides that only organisms with a built-in vector in genome survive (WU, 2004, GRIFFITHS et al., 2005). This technology has found application in various fields, from agriculture to pharmaceutical industry. The most interesting results have been achieved in the field of pharmaceutical engineering, where they obtained the application of engineering strains of bacteria that synthesize human hormones such as insulin. By biotechnological manipulations is also created human growth hormone used for therapeutic purposes. The fact that before the biotech revolution this hormone was axtracted from human cadavers, after which followed a mandatory step purification indicates how much is accelerated and simplified the process in its synthesis. True revolution in the field of immunology has caused a synthesis of the first vaccine by genetic engineering. In 1987 in biotechnological laboratories have been produced a vaccine for hepatitis B, followed by a synthesis of many drugs, hormones and vaccines. Significant progress the introduction of genetic engineering has been achieved in the field of medicine. Manipulations at the level of gene created a new, experimental type of mouse, called

onkomous, which carries genes for the cancer, what significantly makes easier testing of potential anti-tumor drugs (KAYSER & MULLER, 2004; WALSH, 2003).

From the moment of its the introduction cloning technique caused the greatest attention, and anxiety. Ethical aspects of implementation of this methodological procedure were and even today represents the subject of considerable controversy in scientific circles, or everyday life. Cloning is actually a process of creating genetically identical copies of an organism. The method of cloning is extremely complex and involves a series of successive processes. After choosing the donor and recipient organisms from the donor organism is isolated somatic cells. From non-fertiliyed eggs taken from the body of the recipient is removed nucleus, after which they are hybridized with the selected somatic cell of donor. As a result of fertilization, hybridized cells form the embryo, which contains only the donor genes, as previously was removed egg nucleus. In the next step, the embryo is implanted in the surrogate-mother's womb, where it continues to develop normally. As a result of such a complex manipulations obtained is offspring genetically identical to donor. Although the theoretical basis for cloning elaborated to the smallest details in the practical work in the encountering numerous problems. Each stage of this technique requires specifically equipped laboratories and conditions, and highly trained personnel. Nonetheless the results do not always meet expectations: in the project of cloning sheep Dolly from 277 attempts, only one was succesful. Cloning method is until now applied to various organisms, from bacteria to mammals, and apparently the man. This caused a storm protest, opened the discussion on the topic of ethics in biotehnology, and genetics itself, suggested the legislation that limited or even banned the use of this technique. However, besides the negative aspects, cloning may have significant application. There are two main types of cloning: reproductive and therapeutic. While reproductive cloning aims to create new organisms, therapeutic cloning produces cells that could be used for the treatment of various diseases (WU, 2003).

As a special kind of manipulation on the level of genes and genetic engineering can be mentioned and transgenese. Transgenese indicates the transfer of one or more genes from the genome of an organism in the genome of another, whereby recipients and donors of genetic material belonging to different taxa. Related to transgenic manipulation phenomena are used for many years, where they can be divided into two generations or stages. In the first stage the emphasis is placed on resistance, while the second stage is characterized by work on improving quality. The best known examples are the first generation of transgenic plants resistant to herbicides or insects. It is interesting that the genome of host plants introduced gene, isolated from most bacteria, which allows plants to synthesize substances, usually toxic, effective against insects. The second-generation plants are characterized by improved quality: enriched (vitamins, for these non-specific protein), or they changed the composition (fat and protein and the like; WU, 2004; SOMERWILLE and SOMERWILLE, 1999).

The beginning of the twentieth century was marked by discoveries in the field of microbiology. The new findings have contributed to the growing use of organisms for industrial purposes. Pioneer in this research was Chaim Njeizmann. Namely, it is the first pure microbial cultures used for industrial purposes and in the weapons industry. Njeizmann used bacterial culture for a wide range, from the production of corn starch using *Clostridium acetobutzlicum* to synthesis of acetone, for which Britain desperately searching for the production of explosives during the First World War (BRINK et al. 1998).

In recent years, special attention is paid to biotechnology and the creation of chloroplast transplastomic plants. Transplastomic plants are genetically modified plants, in which the new gene is not inserted in neclear DNA but in chloroplast DNA (ptDNK). This technology shows many advantages compared with conventional transgenic technologies, such as high expression of recombinant protein caused by a large number of chloroplasts in the cell,

also a new gene is integrated into plastid genome by homologous recombinations, without manifesting the positioning effects and inactivation of genes in a transkriptional unit, it can be achieved expression of several transgenes due to chloroplast prokariotic origin, and expression of foreign proteins without methionine on N'terminal end. A significant aspect of this technology is the drastic reduction of transgenic pollen, since the majority of plants showing the effect of maternal inheritance of chloroplast, which prevents gene flow from genetically modified plants in the offspring. Plastid DNA of higher plants is the size of 120-150bp and in the cell can be present with 1000 to 10000 copies. As a vector for manipulation of ptDNK is used *Echerichia colli* plasmid, and entry is achieved through the gold particles that are incorporated transformed DNA. To obtain stable transplastomic plants all ptDNK copies must be replaced. Transformation of chloroplasts were first realized in *Chlamydomonas reinharbdti* 1988, and later in tobacco and *Arabidopsis thaliana.* However, for many plant species, application of this method is still not routine. Due to these advantages transplastomic plants are often used in practice, where the main areas of application are engineering of important agronomic traits, the application in the pharmaceutical industry and metabolic engineering (BOZARTH et al., 2009; KERRY and MALIGA, 2007).

THE MAIN RESEARCH DIRECTIONS IN BIOTECHNOLOGY

Achievements in the field of biotechnology are used in various areas of science and everyday life. In accordance with this directions of research in modern biotechnology are extremely diverse. However, at this point the research that the most attractive and attract the most attention in research primarily genome, and proteins. Scientists primarily attract dogmas concerning the human genome and proteins, as well as the possibility of applying the results obtained in medicine with the aim of developing new drugs and therapies. This study can be conducted on several levels: the whole genome research, protein research and finding a protein target for drugs, and finally a practical application in this manner results achieved through the design of drugs (KAYSER and MÜLLER, 2004).

According to the basic principles of genetics, phenotypic traits are caused by multiple gene action and depend on their interaction. It is therefore not sufficient to study the genes individually, but they should be seen as part of a complex whole. This approach to studying DNA is drastically different from the former that is mostly based on observation of individual genes. To access the general application of biotechnological methods to a body it is necessary to comprehensively study the genome of the organism. Genomics is a specific area of genetics, which is characterized by a special approach to studying DNA. The term genomics was first used by geneticist Tom Roderick 1987 in order to described approach to studying DNA at the level of chromosomes, entire genomes or large groups of genes. Within these investigations are carried out systematic investigations of the genome, or complete chromosomal sets specific organism. This comprehensive research require sophisticated and sensitive laboratory equipment and create powerful database, and consequently a large investment. Genome can be examined from two aspects: structural and functional. Structural analysis dealing with the mapping of genes and determining DNA sequences, while functional studies aimed at examining the role and activities of DNA sequences. These two ways of studying the genome are closely linked and conditioned, as the finishing reading the genome of individual organisms, so the focus of work shifts from structural to functional aspects of research, aimed specifically applicable results in practice (SOMERWILLE and SOMERWILLE, 1999; SOUTH, 2001; WU, 2004).

Identification of proteins present in cells and analysis of their interactions are currently one of the most attractive tasks in the field of biotechnology. To characterized the set of all proteins of an organism Mark Njillkins in 1994 used term proteom. Proteins are driving,

work force each organism and the target for protein drugs and thus is of huge importance to thoroughly investigate the structure and interrelation of all the proteins in proteom, but also to identify and sequence genes that encoding protein synthesis. Based on these postulates is developed a new area of research - Proteomics. It is expected that in the near future biomedical research in the fully diverted from genomic to proteomičc research as a key technology for the transformation of knowledge in pharmaceutical products. The primary guiding principle in these studies will be need for improving the efficiency of finding new drugs, but the factor of time play a significant role. Currently applied gradual and chemically optimized methods have a long and expensive. Negative effect is high percentage of failure during clinical testing due to toxicity or low-efficiency potential of new drugs, resulting in a growing interest in biomarkers suitable for use in therapeutic cloning and designing drugs (NORTH, 2001; WU, 2004).

During the following years is expected progress in the pharmaceutical industry on the basis of information obtained in the genomic and proteomic research. The basic idea is to make its contribution to Proteomics pharmaceutical industry so that will determine targets for drugs, which caused the creation of a new generation of drugs that block those proteins that true cause of the disease. Actually getting specific information from the genetic code and find the cellular mechanisms that lead to the pathological condition or disease could be a theoretical basis for the design of new, highly specific drugs. A special feature of these drugs that are synthesized in accordance with the individual's genetic code and thus much more efficient compared with existing drugs (KAYSER and MÜLLER, 2004).

APPLICATION OF BIOTECHNOLOGY

Improving methods of modern biotechnology has opened up many opportunities for its application in various fields. How important is this technology says the fact that she found application in major industries: pharmaceutical industry, food production, chemical industry (eg production of biofuels, oil and biodiesel) and environmental protection. Certain strains of bacteria are also used in the mining industry for blasting rocks, so-called biomining. In recent decades increasing attention is paid to environmental protection. Biotechnology in this field has found its application which is tantamount to the creation of organisms, especially from a group of microorganisms, in order to find an effective way of purifying environment. Basic principles of application of biotechnology in environmental protection based on the fundamental biological laws, with special attention given to katabolic activities of microorganisms. In a recent past various methodological approaches based on the application of organisms for recycling have been developed, but also a special technique - bioremedation. Bioremedation is procedure of cleaning places and areas contaminated by industrial activities using living organisms. However, great discoveries are often abused and used for harmful purposes. Similar happened with biotechnological principles, on which more and more based design biological weapons (CRAWFORD et al., 1996). (SPILLANE, 1999, SERAGELDIN and PERSLEY, 1999, TANG and ZHAO, 2009; AWISE, 2004, CRAWFORD et al., 1996).

More growing need for new sources of fuel represents a new challenge for the biotechnology sector. Estimated to derivatives-based ethanol can significantly reduce the consumption of fossil fuels has encouraged biotechnology to improve the production of cultivars which can be successfully used forbiofuel production. In the last couple of years observed the rapid growth of new genotypes of corn and soybeans suitable for handling industrial purposes, which is next to a suitable chemical composition and are characterized by resistance to pests and drought.

Recently, genetically modified organisms caused special attention and frequent controversy. These organisms were made by controversial genetic manipulations, whereby they

enter into their genome a gene or genes from other organisms. Introduction of foreign genes is done to improve product quality, increase resistance to various diseases or parasites, etc.. A special aspect of the biological phitomedicine fight against weeds and pests (SEREGALDIN and PRESLEY, 1996).

In a time when less and less arable land available to breeding is increasing attention is paid to the stability and yield increase. Using biotechnological methods it is possible to transfer one or more genes in a highly developed crop to improve and introduce new features that can significantly improve the yield. These techniques produced fruits and vegetables resistant to bacterial infections and pesticides that a longer period remains fresh. Application agrochemical measures significantly expensive agricultural production, and raises the question to what extent the products are treated with chemicals suitable for consumption. One of the aspects of the commercial application of biotechnology in agriculture is based on the production and reduction of agrochemicals. The basic idea is to create organisms that are characterized by their ability to synthesize materials that have toxic effects on pathogens. For example, the *Bacilus thuringiensis* soil bacteria that synthesize a specific protein protoxin. What makes this unusual protein is the fact that it is characterized by insecticide action. Traditionally, these bacterial strains were used for the production of insecticides, as in the form of Bt toxins contains protoxin inactive form, which after the bacterial digestion becomes effective in the fight against insects. Biotechnological manipulation of organisms which are synthesized from the bacterial strains entered the gene responsible for synthesis of Bt toxins, which synthesize these transgenic plants in active form. When insects attack the plant genome enriched for the Bt toxin die after a few days since this matter is linked to the wall (SAXENA et al., 1999, JAMES, 2000).

One of the primary goals of biotechnology is to enter into the existing culture of the genes that coded non-specific synthesis of substances for a given culture. These products of metabolism are not only used as a source of food, but also for use in industry. Thus, the oil seed culture can be modified to synthesize fatty acids that are used in the manufacture of detergents in the petrochemical industry. Among other: potatoes, tomatoes, tobacco, rice and sunflowers as a result of modification trained to synthesize insulin and some vaccines.

CONCLUSION

Biotechnology and its achievements carry incredible opportunities, but also enter and fear of possible negative consequences. When we talk about biotechnology inevitably question is whether are its achievements in accordance with nature and whether can cause undesirable consequences. Genetically engineered organisms should be evaluated and regulated according to their biological values and phenotype, rather than according to the genetic techniques used to produce them. Nonetheless, because many novel combinations of properties can be achieved only by molecular and cellular techniques, achievements of these techniques may often be subjected to greater scrutiny than the achievements of traditional techniques. All in all, only controlled application of these methods, especially in combination with conventional methods, can provide progress in many fields.

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