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# Bio-Resources of the Future
## – Using Unconventional Biotechnology

***Iudith Ipate - Maria Duca - Alexandru T. Bogdan - Janos Seregi - Agnes Kovacs:***
***A jövő bio erőforrásai- a nem hagyományos biotechnológia alkalmazása***

***A speciális orvosi, összehasonlító biotechnológiai eljárások használata nagyban hozzájárul az állategészség fejlődéséhez, az állati termékek, nyersanyagok termelékenységének a növeléséhez, számos állatfaj megmentéséhez és nem utolsó sorban a humán egészség fejlesztéséhez. A transzgenikus állati szerveket ma már számos alapkutatásban felhasználják (gének meghatározása, a gének funkciójának megállapítása, a gének kontrollált módosítása); továbbá felhasználják farmakológiai vizsgálatokban, főleg olyan komponensek előállításában, amelyek a rákos sejteken fejtik ki hatásukat, valamint rekombinált fehérjék és bioreaktorok előállítására, élelmiszer biotechnológiai kutatásokban, stb. A bio-élelmiszerek a jövő nemzedékek és jövőbeni gazdaság alternatív élelmiszerei lehetnek. Az ezen a területeken munkálkodó tudósoknak előrelátóan és elővigyázatosan kell eljárniuk, figyelembe véve a mindig aktuális bio-élelmiszer előállítás lehetőségeit és méreteit a piaci igények függvényében, új élelmiszer források kutatásával, kifejlesztésével és megtartásával.***

***Kulcsszavak: bio-nyersanyagok és források, biotechnológia, összehasonlító orvostudomány***

**Abstract**

Using specific bio-techniques of compared medicine contribute to the improvement of animal health, to increase the productivity of animal products, to the conservation of various animal species and also advances in human health. Transgenic animal organisms can be used for fundamental research (identifying genes, elucidating the functions of genes, controlled modification of genes); for pharmacological studies, especially chemicals that can act on tumour cells, to obtain recombinant proteins; bioreactors; food biotechnology etc. Bio-food is an alternative source for the future's generation and economy. All professionals and specialists in this field must have a prevision, they must take into account the current bio-food possibilities and sizes depending on the market needs, to conserve and develop new resources of food.
Keywords: bio-resources, biotechnology, comparative medicine

**1. Introduction**

Bio-resources are the future of economy for all generations. Bio-food traceability is necessary to ensure food security. Food security is the primary goal of each state, nation, and the whole human community. We live in a changing world and we want to know what we eat and how long we will have bio-resources to ensure a worry-free future; but do we have to worry about?

Assurance and food supply are primary demand to be met as a result of food consumption and population growth in the world. According to UN statistics, in the last 30 years, the world population have increased by 1 billion people every decade. The world population in 2000 was 6.115 billion people, and a sharp continuous increase is observed. Nowadays this no. is estimated to be 7.123 billion. According to projections, the world population will be 8.309 in 2030, and will increase to 9.15 billion in 2050. FAO predictions

are based on estimating the total food supply of a country and the determination of food consumption per capita average. Issue of food security is part of a national security, and global security is a complex issue that should be further examined taking into account the past and current situation in order to predict and suggest solutions for the future. Solution may be the use of modern biotechnology obtaining food.

## 2. Food obtained through biotechnology

The "in vitro" meat, type of food biotechnology is a meat derived from animal tissues, cultivated on a synthetic substrate and collected in a laboratory. According to researchers, "in vitro" meat may have the potential to help global health in a variety of ways, including e.g. the creation a "perfect burger", which can help to prevent heart attacks. There are many benefits of this type of meat, ranging from properties of certainty lack of antibiotics, avoiding cruelty to animals, and also environmental benefits, including less emission of greenhouse gases. While "in vitro" meat is still in the research phase, there is hope for commercial production and manufacturing in the next 5-10 years. This type of modified food does not contain hormones and antibiotics. Meat obtained through biotechnology could have a positive effect on the veterinary control, e.g. by excluding diseases such as scrapie, avian flu, swine flu; so it may reduce the risk of transmission of zoonoses, assuring a better food safety. However, there is a concern that "in-vitro" meat will face the same initial criticisms and problems that genetically modified foods have faced when they were introduced to the food market. However, we can say that yogurt is made using biotechnology from ancient times, and witch became a food appreciated and accepted all over the world.

## 3. Areas of use of transgenic animals

Using genomics to obtain transgenic animals may be involved in basic research by identifying genes, elucidating the functions of genes, modifying genes controlled trials, realizing typically the transgenic mice. Transgenic animals are a model for analysing the role of gene regulatory elements. Their use in pharmacological studies is essential, especially chemical compounds that can act on tumour cells. Firstly oncogenes are cloned in a tissue, resulting lines in tumour cell whose development and function is than examined under different conditions. It can be also studied other features, such as processes of viral infections caused by virus-specific human hosts, such as mice. For this reason they, their genes are firstly cloned to determine their awareness of infection with viruses to which they manifest natural resistance. Trans-genesis can be used to obtain bioreactors, high quality food resources, animals resistant to diseases and sources of organs for transplantations.

One of the major directions of trans-genesis in animals is improving productive performance and production quality of domestic animals having economic importance. Gene transfer was performed for HGH human growth hormone (HGH - Human Growth Hormone) in different species of farm animals (pigs, sheep, rabbits). HGH gene transgenic animals have a better ability to convert feed and they grow faster. Transgenic birds have a high efficiency of assimilation of food, low fat and cholesterol content in eggs and high quality meat. Transgene expression in germ cells of the birds: they secrete a large amount of ovalbumin; this product may cause accumulation of protein in the eggs, where from it can then be than extracted.

Transgenic pigs having growth-hormone gene were found to have a faster growth and early production of meat, a better commercial value, and the animal's ability to a better conversion of the feed. However it was observed that transgenic animals presents some physiological difficulties because the permanent growth-hormone; normally the growth-hormone is produced only in the first two months of their life.

**4. Transgenic animals in the dairy industry**

One of the objectives of trans-genesis is to farm milk producing animals, improving their quality by changing the content of milk components. The composition of milk proteins: 80% caseins (α-S1 casein, α-S2 casein, β-casein, κ-casein and others). The quality and quantity of cheese produced depends on the casein content. They are synthesized by multiple alleles (about 20). It was established that the curd producing time of the milk κ-casein BB variant is lower, the amount of cheese produced is higher and the cheese is more palatable. In England a national project was launched to create a transgenic κ-casein BB gene that could produce milk for cheese production. In Russia transgenic sheep chymosin gene have been created (enzyme necessary to produce hard cheese with rennet) from cattle. From a single sheep in a lactation period, it can be get 30 grams of yeast, amount required to precipitate the casein in 300,000 kg of milk, sufficient for 30 tons of cheese. Another perspective for the dairy industry would be the obtainment of transgenic milk producing animals with lactose free milk. It is estimated that lactose free milk could be a very popular product on the market for lactic acid allergic people. Trans-genesis is achieved by lactase gene expression or gene inactivation of α-lactalbumin yeast (lactose synthesis catalyst) in mammary gland cells in animals.

Transgenic animals could be a source of milk for infants. Many mothers do not have enough milk to feed their children, and cow or goat milk differs in terms of quality of the human milk. B-lactoglobulin content in cow's milk is much higher than in human milk and may cause allergies in infants. By transgenic cows (for β-lactoglobulin) can be obtained milk where this protein is not secreted.

Another, dependent protein nutritional value of milk is lactoferrin. Lactoferrin is produced as the base of various drugs, dietary supplements and is traded on the world market worth 5 billion USD. Human gene for lactoferrin was transferred into cows. A transgenic cow can produce 1gr of lactoferrin per 1 litre of milk.

Cloning genes for human proteins in bacterial cells is a process unstable due to mutations that occur in bacterial cells, and is also very expensive. In addition, microbiological synthesis of human protein has also other disadvantages [Cornea, 1998], for example:

- Certain proteins, due to their complexity are synthesized in bacteria;
- Bacterial cells do not possess enzymatic equipment (specific to eukaryotes) for posttranslational modifications (phosphorylation, glycolysis) of the proteins synthesized;
- Some bacterial strains used as hosts for cloning produce large amounts of proteases that destroy the synthesized proteins;
- In trans-genetic experiments have been taken a number of measures for the efficient expression of eukaryotic genes in bacteria.

This is why obtaining transgenic animals, is one of the major concerns of specialists in genetic engineering. The technology is ecologically clean and does not require high expenses and energy need.

Thus, transgenic animals can serve as some continuous bioreactors producing the desired protein in milk. Medicine needs one-two tons of α1-antitrypsin (α1 - AT) for the 100,000 European and North American children with a deficiency of this enzyme. It has been calculated that to meet the global needs, 85 kg of factor IX, about 7 kg of factor VIII is necessary. A transgenic cow whose milk contains 1 mg/1 ml of milk and produces 6,000 litres of milk per year can give about 6 kg of protein. Therefore, two transgenic cows can provide yearly the world with factor VIII; and a herd of 15-20 cows - with factor IX. A medical need of fibrinogen is

3 tons per year. This quantity may be provided by a number of 500 transgenic cows. One of the transgenic sheep that get produced 35 g/ litre of milk, can produced in two and half years 5 kg of α1-antitrypsin [Maximilian, 2001].

## 5. Production of medicines based on transgenic proteins

The production of drugs based on human proteins is achieved by the following steps:

- Construction of genetic human genes under the control of mammary gland-specific promoters and insertion into the genome of animals;
- Obtaining the transgenic animals;
- Human proteins are extracted from milk produced by transgenic animals and pharmaceuticals produced.

Some of the most important pharmaceutical proteins produced this way in large quantities (up to 25 g/l) in transgenic mice are: factor IX (mg/ml), interleukin-2 (mg/ml), α1-antitrypsin (mg/ml), interferon (ng/ml), trophoblastin (mg/ml), superoxide (mg/ml), urokinase (mg/ml), some immunoglobulin and many other proteins.

Some products created in transgenic domestic animals that secrete human proteins in milk are as follows: rabbit-interleukin (ng/ml), plasminogen activator tissue (tPA mg/ml), human hepatitis B virus antigen, somatotropin, γ-interferon, fibroblast interferon, sheep-α1-antitrypsin (mg/ml), factor IX (ng/ml), somatostatin, pig-human hepatitis B virus antigen, somatotropin, goat-plasminogen activator tissue (tPA mg/ml). Construction of transgenic human genes under the control of promoters specific to the mammary glands and vessels are also considered.

By micro-injections, the gene is inserted into sheep embryos that are then implanted into recipient females. The transgenic animals secrete the desired protein in milk, which then can be purified.

Recombinant DNA technology aims to equip transgenic animal with protection mechanisms that would avoid animal vaccination. Transgenic animal will produce antibiotics that block replication of pathogens or synthesize enzymes that will destroy the protective capsule of bacteria. In this context, some analysed genes involved in immune function are: histocompatibility complex genes, T-cell receptor genes, limfokinin genes. The introduction of genes into recipient body for antibodies is associated with specific antigens, named for immunization "in vivo". There were obtained transgenic cows that produce β-interferon, a citokinin giving resistance to the virus that causes animal diarrhoea.

The Env gene in chickens is to transfer glycoprotein encodes of avian leucosis virus (ALV). Viral glycoprotein is associated with the ALV cell receptor, and thus blocking viral infection of the cell. Chicken egg nucleus cannot be injected with DNA, because it is very small and is protected by egg albumin. Therefore we used another technique: we achieved an infection of fertilized eggs with DNA from a recombinant retrovirus. There were obtained transgenic chickens, each containing DNA provirus of avian virus (ALV) integrated at different positions in the genome. Most transgenic hens had viral infections, because there was a mutation in DNA provirus. Immunological tests showed that due to the viral protein present, these chickens are resistant to the virus infection.

Another perspective is to obtaine transgenic bulls with a gene for lactoferrin, a protein absent in cattle. Protein prevents mammary gland infections, iron set free from circulation, which prevents pathogenic bacteria.

Also transgenic rabbits were obtained with antisense RNA genes from human adenovirus Adh5, rabbits with antisense RNA genes in bovine leukaemia virus, rabbits with a high resistance to leukaemia.

Xeno-transplantations are transplants of animal and represents a new stage in the development of medicine. Worldwide is a shortage of organs for transplantation, the demand far outstripping supply. Only in USA 3,000 patients die annually due to lack of organs for transplantation; and the human transplant market worth more than 6 billion USD annually [Maximilian, 2001].
Therefore, the organs of other species can be used, where structural and functional differences are not particularly high. Thus, in 1963 was made a kidney transplant from chimpanzees to humans, stable for nine months [Calne, 1970]. Later heart and liver transplantation was tried in primates to humans [Starzl et. al., 1993]. Baboon kidneys were transplanted to patients who survived for months, and in 1984 a child lived 20 days with the heart of a baboon [Maximilian, 2001]. But the results of these experiments were still disappointing.

The best animal donors of xeno-transplantation are pigs [Cooper, et. all., 1991]. Thus, pig organs resemble the human in form, size, and number of biochemical parameters. In 1995 researchers at Duke University Medical Center transplanted hearts from genetically modified pigs to monkeys, who survived a few hours. For many surgeons, xeno-transplantation could be a temporary solution until the human organ will be available. Thus, pork liver transformed allowed a patient with acute liver failure to survive for several days until receiving an adequate human liver. However, successful transplantation of organs from animals to people shows difficulties because the patient's immune system is considerably reduced and eliminated the transplanted organ. To avoid rejection of transplants, a solution could be the creation of transgenic pigs in which the rejections are inactivated by histocompatibility genes because human genes are inserted.

Unfortunately in pig organ tissues after transplantation it was found a retrovirus that could infect human cells and cause cancer [Patience et. al, 1997]. Therefore, it requires a thorough donor animal in order to avoid the risk of viral infections. In this context, the opinions are divided. Although some of the scientists believe that xeno-transplantation should be prohibited. But the research in this field develops both theoretically and practically. Thus, companies like Diacrin and Genzyme Tissue Repair in the USA have developed and applied in medical practice input methods of pig nerve cells for patients suffering from degenerative diseases such as Parkinson's and Huntington [Zawada et. al., 1998]. The companies Neocrin and VivoRx achieved the treatment of diabetes by introducing pig pancreas cells into apes and humans. The companies Circe Biomedical, Nextran in USA and Immutran in England transferred pork liver cells into people.

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