Biotechnology: Dr Jekyll or Mr Hyde?

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In 1886 the Scottish writer Robert Louis Stevenson wrote his famous novel *Strange Case of Dr Jekyll and Mr Hyde*. The main character of this book became the synonym of split personality, and indicated that there are both good and bad aspects of every human activity. The phrase was widely used to describe politicians, actors, councillors, or even whole football teams.

Biotechnology is supposed to be one of those domains that will determine further economic development during this century. It constitutes a very extensive area of human technical activity, and its development depends on interactions between various disciplines of science and technology. Chemistry plays a major role in the development of biotechnology. Biotechnology, on the one hand, is seen as an important tool for improving our life, and on the other hand it brings about fears of the effects of its application in creating a new reality. It is therefore a Dr Jekyll and Mr Hyde of contemporary science.

The term "biotechnology" is derived from three Greek words: *bios* - **life**, *technos* - **technology** and *logos* - **thinking**. However, the term "biotechnology" aroused controversy from the very beginning. This was due to the fact that there was no agreement on a uniform definition of this discipline of science and technology. We can exemplify this on the definition devised for the Ministry of Science and Higher Education and the Ministry of Economy for research and development purposes. It comprises two parts, of which the first reads like this: *Biotechnology is an interdisciplinary field of science and technology dealing with the transformation of living and inanimate matter with the use of living organisms, parts thereof, or products derived therefrom, and models of biological processes in order to create knowledge, goods and services* [1]. The second part of this definition is descriptive and constitutes a list of techniques (techniques of genetic engineering mainly) applied in research. Thus on the one hand, the stress is placed on the utilitarian aspect of this discipline, and on the other hand, on the methodologies applied. This definition is an attempt to distinguish biotechnology from traditional techniques of species breeding and crossing, or from food chemistry. It seems that a good definition is the one adopted by the UN: *Biotechnology - any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.* A more simple still is the one given in the Polish version of Wikipedia: *Biotechnology is a discipline that uses biological processes on an industrial scale* [2]. If we adopt the last definition, then we have to admit that biotechnology has been applied for centuries, for instance for producing beer, cheese, cosmetics, etc. It is interesting that the European Union does not define biotechnology, assuming that the term is understood intuitively.

Since the dawn of history, the biotechnological activities of man have been associated with the obtaining and processing of food. Today this discipline is called *green biotechnology* (the "coloured" classification of biotechnology was introduced by the Organization for Economic Co-operation and Development with the participation of the European Union). It is assumed to be the subsequent stage of the *green revolution*, which turned out to be a breakthrough in agriculture and saved the lives of millions. Its goal is to satisfy the nutrition needs of the growing population of the Earth in a manner more environmentally friendly and safe for human health. This goal is attained by the application of three basic techniques. One of them is *in vitro* (laboratory) breeding of plant cells, enabling the reproduction of whole plants or of their organelles, to help maintain genetic uniformity of plants and promote breeding processes. These techniques are commonly used today in flower growing when we want them to bloom on a particular day.

The second technique arouses the most heated discussion and grave concerns: application of genetic engineering to obtain plants (and animals, to a lesser extent) with required features (GMO – *Genetically Modified Organisms*). Thus, an improved organism retains its traits and is provided with an additional feature of great importance from the point of view of economics, health or environment protection. Plants of great economic significance are those that are modified genetically in the first place. Altering their genome is designed to provide them with traits desired by man, such as: higher persistence, resistance to disease, parasites or herbicides (enabling safe weed control), resistance to abiotic stress (e.g. frost, salinity) or improvement of qualitative features (one example is modified rice – *Golden Rice* – with an increased provitamin A content, which may prove useful in combating blindness of children in Asia, caused by deficiency of that provitamin). Ornamental plants are also modified to improve their persistence or intensify their colours. The fact is that most of the plants that are important for humans have been genetically modified. The plants most often modified include: maize, tomatoes, soybean, potatoes, cotton, melons and tobacco. The most often modified plants in Europe include: maize, rape, sugar beet and potatoes. In most cases (80%) these plants have a gene or genes of resistance to herbicides, 12% of genetically modified plants is provided with an artificially introduced gene of resistance to *Lepidoptera* caterpillars, marked with a Bt symbol (codes a protein of the *Bacillus thuringiensis* bacteria that is toxic to these insects). 8% of GMO plants is both resistant to herbicides and contains the Bt gene. This trend in green biotechnology development reduces environmental pollution by decreasing the use of crop protection chemicals.

Strong movements of opponents of agricultural application of genetic engineering demand strict regulations on marking food products containing GMOs and derivatives thereof, and markings indicating that the given product has been obtained without the use of the technology mentioned. At the same time they are of the opinion that *in contrast to the stance of GMO advocates, "creating" these organisms has nothing in common with the selection made by breeders with the help of the forces of nature. This is an evolutionary experiment of unknown consequences*" [3]. This is a strong social movement, which often resorts to emotional arguments.

GMO advocates argue that agriculture is in its essence an activity that in a deliberate manner constrains biodiversity and applies laborious methods of genetic modification of organisms in order to select plants of the same traits as those obtained faster by means of methods of genetic engineering. What's more, the process of transgenesis is associated with other type of risk than that associated with traditional methods of generating genetic variability (remote cross breeding, mutagenesis) [4].

It's hard not to agree with the opinion, presented by the European Union, which adopted regulations based on two fundamental principles. One of them is the Precautionary Principle, which prescribes the use of means aimed at preventing undesired effects. The other principle states that each case of GMO should be decided on a case-by-case basis. According to that principle allegations that the use of GMOs is in general safe or unsafe are groundless. A good example is the case of genetically modified salmons, which arouses strong emotions. The fast-growing and requiring less food fish (and therefore easier to breed) has not been accepted in the USA, whereas the Irish are looking kindly on the efforts aimed at creating a genetically modified salmon, resistant to parasitic diseases. It is also important, what traits are to be introduced by genetic modification and whether these traits are important for the consumer.

There is much less controversy over genetically modified plants, which are used as biofactories producing compounds for various sectors of economy. Plants with a genetically modified route of fatty acid synthesis find use in the manufacture of industrial oils and biofuels. Modified plants may also be able to produce readily degradable biopolymers [5]. Some modifications find complete acceptance, like the lettuce devised in the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań, which produces a vaccine against hepatitis B (the vaccine will be taken by eating the vegetable) [6].

Much less mastered is the third technique of green biotechnology that is breeding with the use of molecular markers: a combination of traditional selective breeding and genetic engineering, where the molecular markers comprise "fragments" of DNA, attached to the gene that codes the desired trait, which the breeder wants to obtain by cross breeding and selecting the new variety.

The *red biotechnology* is more advanced and arouses less emotions. It is directed to fill the needs of medicine. Nearly 85% of biotechnological companies are active in this field. Biotechnological medicinal preparations constitute now ca. 20% of all drugs on the market and ca. 50% of drugs under clinical trials. An obvious step forward, in relation to preparation of some medicines by fermentation, is that most biopharmaceuticals are produced by recombined bacteria (mainly by *Escherichia coli)* or yeast (mainly baker's yeast - *Saccharomyces cerevisiae)*. Recombination is also applied to cell lines of higher organisms and hybrid interspecies cultures are created, as is the case with monoclonal antibodies, which are a hybrid of lymphocyte B and myeloma cells rendering them immortality. This method is used to obtain such groups of pharmaceuticals as: interferons, interleukins, hematopoetic growth factors, tumour necrosis factors, thrombolytic drugs, blood clotting factors, recombined hormones, or the monoclonal antibodies already mentioned.

Recombined drugs do not arouse such emotions as the genetically modified plants do, because the community is more willing to assume a higher risk when it comes to medical treatment methods. These drugs are cheaper than those derived from natural sources and carry no danger of infecting with hepatitis B, HIV or Creuztfeldt-Jakob disease. Therefore they are safer. Drugs obtained in this way can today be used to treat or prevent diabetes, stroke, congestive heart failure, hepatitis, anaemia, asthma, Crohn's disease, leukaemia and many types of neoplastic diseases. An example of a Polish recombined protein is *Gensulin* manufactured by *Bioton*. Apart from *Bioton*, only three companies: American *Eli Lilly*, Dutch *Novo Nordisk* and French *Aventis* (formerly *Hoechst*), manufacture human recombined insulin.

The future of red biotechnology lies in gene therapy. It comprises introduction of alien DNA or RNA into the patient's cells, usually by means of a modified virus, in order to force the cell to produce a protein coded by the new gene. Despite the fact that this technique has been clinically applied since the 1980s, only one spectacular success of this therapy has been reported. In 1990 in the National Cancer Institute a three-years old girl, whose organism was not able to produce an enzyme, adenosine deaminase, was subjected to this therapy. The deficiency occurs once in every 100,000 children and leads to loss of immunity. The therapy comprised infecting leukocytes in the blood taken from the girl with a virus containing the gene, followed by reinjecting the cells into the girl. The girl lives up to this day, but requires continuous treatment using just the enzyme protected by polyethylene glycol linked to it.

A spectacular achievement of biotechnology, or rather of nanobiotechnology, was the development of a prototype of an artificial human-implantable kidney. This artificial kidney is a complex system based on live cells, alien to the patient, placed in membrane systems. The use of such artificial organ may eliminate the need to dialyze patients.

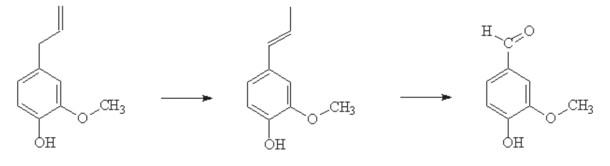
The third, future-oriented, trend in red biotechnology is molecular diagnostics. The present most advanced techniques of analysing genetic material are applied to identify particular types of tumours, detect infectious diseases, test histocompatibility in transplant donors and recipients, identify criminals or establish fatherhood. The main subject of research is the detection of changes within the patient's genetic material when the disease is characterized by DNA fragments altered by mutation or the detection of genetic material of the pathogen. Such material may be the genome of the pathogenic microorganism. Other widely applied methods include immunochemical methods comprising antigen – antibody reactions.

Future of red biochemistry may rely on diagnostic chips for checking patient's health state at his or her home, like it is done today in the case of blood glucose level test. Today health self-control tests have no great significance for an average patient, as the availability of such tests is relatively limited yet. However, mass production of such devices as molecular biochips is at hand, as every year several hundred prototypes of these devices appear on the market. A good example of that is "Serum Biomarker Chip" by *Whatman, Schleicher & Schuell®*, which identifies a dozen or so specific markers of particular diseases in human blood serum. The chip is designed to define risk groups and monitor selected disease conditions [7].

Colour number three in biotechnology is white. This colour is assigned to industrial biotechnology [4]. One of its goals is replacing conventional chemical processes with processes that harness enzymes and microorganisms as catalysts. This method enables manufacture of a diversity of products: specific chemicals, chiral precursors of pharmaceuticals, antibiotics, vitamins, food and fodder components, odorants and flavorants, sweetening agents, cosmetics, detergents, foodstuffs and biofuels. Industrial biotechnology brings about environmental benefits: it does not induce the greenhouse effect and is based mainly on renewable resources. Industrial processes in which biocatalysts are used have been recognized by the Organization for Economic Cooperation and Development (OECD) as an important element of the so-called strategy of sustainable development.

Biocatalysis is slowly becoming a standard process for the manufacture of organic compounds, and the number of its applications is growing steadily. This may be illustrated by the fact that in 1960 only 5 industrial biocatalytic plants with a capacity of more than 100 kg/year were built, in 1990 the number of new processes of this type slightly exceeded 60, while in 2002 as much as 134 organic compound manufacturing processes were implemented by the industry. A review of these processes indicates that their main use is obtaining natural compounds or analogues thereof. The consumer of substances obtained in processes catalysed by enzymes is the pharmaceutical industry, the largest beneficent among other sectors is the food industry. This is no surprise, if we consider the output of both these industries. If we made a ranking based on the production volume, rather than the number of substances, then the food industry would be the undisputed leader. For instance, nearly all organic substances manufactured on a mass scale (more than 20 thousand tonnes per annum) are carbohydrates used by that industry. Most industrial processes where enzymes are used are low-volume processes. Nevertheless, the use of enzymes in large-scale processes is not a rarity. The most widespread is conversion of glucose into fructose catalysed by glucose isomerase in the manufacture of glucose-fructose syrup from starch. More than 1 million tonnes of fructose is obtained every year using this process. Other example is the use of β-galactosidase in the hydrolysis of lactose to glucose and galactose in the process of obtaining low-lactose milk. This method is used to process ca. 250,000 litres of milk daily. The third process of this type is the manufacture of acrylamide from acrylonitrile using nitrile hydratase, which is applied at Mitsubishi Rayon plants at a scale of 30,000 tonnes a year.

White biochemistry makes wide use of genetically modified organisms. A good example of this is the production of vanillin, which is responsible for the characteristic fragrance of vanilla. In 2007 Prof. Mayu Yamamoto was awarded the Ig Nobel Prize for developing a method of extracting vanillin from cow dung.



eugenol isoeugenol vanillin

Fig. 1. Route of microbial production of vanillin

The price of 1 kilogram of this substance isolated from vanilla pods is up to 3 thousand US$, while the same amount of vanillin obtained by chemical methods from guaiacol costs only 15 US$. Consumer and environmental organisations object against the use of chemically produced food additives. Therefore vanillin is also manufactured using biotechnological methods, often with the help of cells of genetically modified microorganisms (principally in order to block the process of vanillin transformation into vanillic acid). The starting substance is either eugenol (main component of clove oil) or ferulic acid (waste product in paper manufacture). The compound obtained in this way is called a *nature identical flavour*, assuming that it is almost natural, and therefore better than that obtained by means of a purely chemical method. A somewhat different procedure is applied to produce menthol, wherein the initial steps include two simple chemical reactions: alkylation of toluene and reduction of thymol. All of the eight menthol stereoisomers are obtained. In this mixture only the proper menthol is acylated by an appropriate lipase and separated from the other isomers. These isomers are racemised and the resultant mixture again contains the right menthol, which is separated in the form of an acetate by means of an enzymatic reaction. The thus obtained menthol is classified as a nature identical flavour, as it was formed by biotransformation.

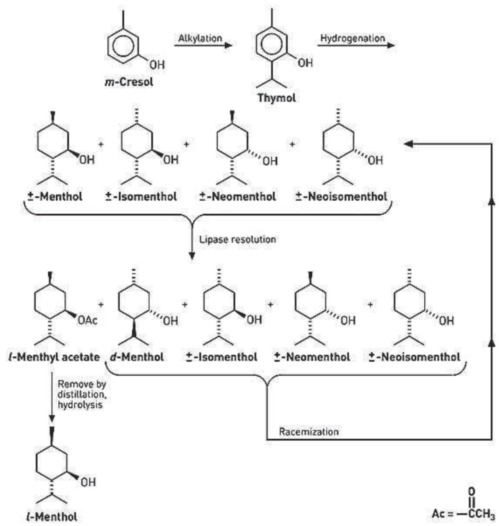


Fig. 2. Chemoenzymatic process for methol production

There is no difference between the compound obtained by chemical route, and the same compound obtained in a biocatalytic process. However, to the consumer, the word *chemical* carries negative connotations, while the word *natural* carries positive connotations.

Biotechnological land reclamation and environmental protection are sometimes classified as white biotechnology, or are grouped in a separate category of *grey biotechnology*. In this case biotechnological processes are applied to aid soil purification, treatment of wastewater, of flue and exhaust gases, as well as waste processing. An example of this is bioremediation of soil and ground water contaminated with hydrocarbons. In Poland this problem occurs particularly in areas where the Red Army had once been stationed. These processes can be stimulated by delivering to the contaminated site appropriate nutrient media to augment the microorganisms that are present in soil and water and are able to decompose hydrocarbons (autochthonous organisms), or by aerating the ground subjected to bioremediation, intensifying thereby the growth of these microorganisms. Soil may also be supplemented with bacteria or fungi, designedly selected to efficiently degrade defined type of pollutants. Genetically modified bacteria are sometimes used in this process. Bioremediation has this particular advantage, that it is applied at the site and does not require the use of intricate equipment. What's more, such methods of soil cleaning gain high public approval [9].

An important issue is the study of molecular mechanisms of pollutant degradation to facilitate the design of new processes for treating industrial and municipal waste, of genetically modified organisms able to degrade specific pollutants, or to introduce new materials safer for the environment. An example of such materials are biodispersant polymers. Plastics are generally very resistant to environmental conditions. Knowing that, researchers introduced into the structure of these polymers readily degradable materials, such as cellulose or starch. Degradation of cellulose or starch, usually playing the role of a filler, causes dispergation of the difficult to degrade components in the environment and significantly mitigates their impact on the environment.

Understanding molecular mechanisms of the degradation of pollutants, particularly the persistent pollutants, is not easy, as one type of microorganism is usually not sufficient to ensure complete digestion of the pollutants. Thus, these processes are often performed by bacterial consortia, and this joint breaking down of a compound into smaller fragments is called co-metabolism.

Sometimes also *blue biotechnology* is distinguished. The term is applied to technical application of processes and microorganisms that are characteristic for the seas and oceans. It makes use of the biodiversity of the marine environment to obtain new products. It is interesting that ca. 80% of all living organisms of the world are found in aquatic ecosystems. Its task is also to maintain or improve the wealth of these water bodies, as to much extent all marine activities of man depend on that. This sector of biotechnology will have to face great challenges, as it is at its early stage of development. Nevertheless, more than 15,000 substances of diverse biological activity have up to now been isolated from marine organisms, including a number of promising antibiotics. These antibacterial agents are obtained not only from bacteria, but also from other marine organisms [10]. The dimer of malingolide, isolated recently from marine cyanobacteria *Lyngbya majuscula*, is a good example of the opportunities offered to biotechnology by the marine ecosystem. This compound demonstrates increased activity against the *Plasmosium falciparum* protozoan strain, which induces malaria.

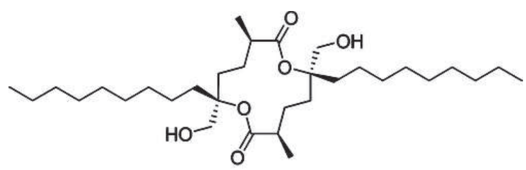


Fig. 3. The dimer of malingolide

The last in the list of biotechnology colours is the colour violet (or purple). This area is associated with social, legal, ethical and philosophical issues of biotechnology. If a company that has patented a genetically modified plant is pursuing its approval as a product of the European market, then the discussion referring to this plant falls into violet biotechnology. A good example of that is the attempt made in 2007 by BASF to introduce onto the European market two genetically modified varieties of potato: high starch content variety *Amflora* and a variety resistant to potato blight (which was responsible for the great famine in Ireland in the 19th century). The first variation has not been accepted by the European Union, although it was not an edible variety (fodder potato), while the other variety was accepted in the UK, despite being edible. The decision of the British government agency was criticised by environmental and consumer organizations.

Some circles suggest further colours: yellow (biotechnology of feeding and fodder), brown (biotechnology of arid and desert zones), gold (bioinformatics – biocomputers and biochips) and dark (bioterrorism and biological weapons) [11]. It is worth noting that orange and indigo are the rainbow colours that remain to be used.

The colour classification of biotechnology, however useful it is, is not unambiguous, because of the overlap of the individual domains. Into which biotechnology should patenting (violet biotechnology) of a new vaccine (red biotechnology) produced by a genetically modified plant (green biotechnology) be included? Results of surveys show that 70% of Poland's population is opposed to the use of genetically modified organisms. Consequently, the politicians also object to GMOs, and due to this the Polish legislation is one of the most restrictive in this respect. On the other hand, 90% of the experts are of the opinion that genetically modified organisms should be used [12]. This difference in opinions probably arises from the fact that there is a general public feeling that genetic modification is associated with the manipulation of the plant genome (new plant varieties) or, worse even, with the manipulation of the human genome (cloning, production of supersoldiers). Experts know that the issue has a much wider background and that in fact the community approves of it, particularly when it pertains to the problems of health protection. Thus the answer to the question whether biotechnology is Dr Jekyll or Mr Hyde is not straightforward, as it depends on each particular case. One thing is certain: countries that renounce development of biotechnology are sure to become retarded in the progress of civilization.

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Prof. Paweł KAFARSKI was born in 1949 in Gdańsk. He studied chemistry at the Chemical Faculty of the Wrocław University of Technology, where in 1977 he obtained his doctoral degree (thesis supervisor Prof. Przemysław Mastalerz), and in 1990 his postdoctoral degree. In 2000 he was awarded the title of a professor of chemical sciences. Since 1992 he has been the head of the Department of Bioorganic Chemistry at the Chemical Faculty of the Wrocław University of Technology. Since 1982 he has also been working for the University of Opole. He performs many functions typical for an academic worker. He is co-author of more than two hundred and fifty scientific and research papers, which have been cited more than 2000 times. He supervised over twenty doctoral dissertations. His scientific interests include the design, synthesis and testing of selected biological properties of aminophosphonic compounds and of derivatives thereof. Other research path is the chemistry of natural products. Among the many prizes and honors awarded to him, Prof. Kafarski has a particularly high regard for the Jan Hanus Medal (Czech Chemical Society) and Włodzimierz Trzebiatowski Medal (Technological University of Wroclaw).

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