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Legislation of biotechnology in Poland

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Food and feed as the products of modern biotechnology are hot topics of social debate, but industrial enzymes and biopharmaceuticals as well as tailored medicine are nevertheless economically the most effective. The aim of this presentation is a review of current biotechnology legislation in Poland with focus on genetically modified resources of food and feed (GM plants and microorganisms) and the final products (poultry, meat, eggs, milk, cheese etc.) and some others selected products, most representative for modern biotechnology (like biopharmaceuticals). The intellectual property rights (IPR) have been included.

The public opinion and the perception of innovative technologies are important for legislators and politicians. Genetic engineering is of particular interest of public interest. The common opinion is critically important for the formation of a legislative system and it affects economy on a global scale. Moreover, public acceptance is of highest importance for producers who are dependent on the choices of consumers. It must therefore be considered as a value added chain:

Market = producer + legislator + consumer

Biotechnology is critically important for environment as well as environment is the key factor for future development of biotechnology. However, in this case we keep in mind the social environment (legislation, public acceptance, media, experts' opinion, interactions between industry and lay people, ownership of new inventions and willingness of politicians and business partners to invest in new ideas and technologies) is fundamental for further progress. This unique social environment is formed by all of us and the entire society (next generation) will be affected by the decisions made today.

Scientific and technological achievements have always been a discussion topic among selected groups of specialists, political and economic leaders. Particularly affected is the legislation including the rules of intellectual property rights protection. Consequently, legislation affects the economy at the national and regional level, as well as globally.

Polish biotechnology has great potential in highly qualified scientific staff and advanced research. The potential of Polish biotechnology lies in highly qualified scientific staff and advanced research. However, the results of the research (even if patented) are rarely implemented into production. In other words, the realization of research projects (which generally assume economic importance of the proposed solutions) does not usually end up in industrial deployments but remains merely at the stage of scientific publications.

The Polish legislation should be science-based and complementary to EU rules. The dynamics of changes and different applications of modern biotechnology, new perspectives of biotechnology development for political and economic decision-makers complicate the legislative system. Poland is currently in a difficult period of rapid changes in legislation.

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Bioeconomy – one of the ways of developing of the old continent

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Currently we are witnessing a trend of prospecting for novel technological solutions enabling the replacement of an old-fashion economy based on fossil fuels with a new economy based on renewable feedstocks. These attempts require a new approach to production, consumption, processing, storage, recycling and disposal of biological resources which are substantial elements of bioeconomy. Bioeconomy is defined by the EU Commission as "the sustainable production of renewable biological resources and their conversion and that of waste streams into food, feed bio-based products". Agriculture, forestry, fishing, food and paper production and energy industries are the sectors that should be subjected to sustainable bioeconomy development rules. In this presentation the challenges of bioeconomy from both the global and the Polish perspective are reviewed. A particular attention will be given to some biotechnological processes transferred to the Polish industry, like biosynthesis of microbial bionanocellulose. A wound dressing material called CelMat, exploits all the properties of bionanocelulose for healing severe injuries, such as extensive burns. There are two certified CelMat products: native cellulose and cellulose saturated with glycerol and methylnicotinamide, which has more intensive protective properties and its use assures the reduction of scar formation. Recently, a project concerning the application of bionanocelulose neurotubes for damaged peripheral nerves regeneration has been finalized.

Bionanocelulose based neurotubes showed several advantages over chemical polymers in *in vivo* tests. Other bionanocelulose based products for internal medical uses are artificial cartilages for trachea reconstruction and other types of scaffolds. The bionanocelulose which is the raw material for aforementioned medical devices can be produced on cheap media which contain waste substances. In the presentation, a few of the potential routes to follow starting at basic production or existing agro-processes for green energy, more enhanced, optimized, "greener" existing products, "green" wastes treatment processes and at last but not least advanced, new bioproducts will be discussed.

Tackling diseases through new tools of red biotechnology

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Medical biotechnology continues to expand rapidly, proposing new diagnostic and therapeutic tools. Basic studies on the molecular foundations of diseases allowed development of more specific ways of combating illnesses than ever before. This knowledge permits novel approaches to the treatment of diseases with drugs acting on previously unknown targets resulting in, among others, increased survival rates in cancer patients. Moreover, building on the molecular diagnostics, treatments can be tailored based on patient profile rather than the "one hat fits all" approach.

Modern medical biotechnology provides therapeutic approaches with higher target selectivity and specificity, and with improved drug safety profile. The strategies range from the production of recombinant proteins to gene and cell therapies. Importantly, the direct correcting of genetic disorders through gene therapy might lead to cures for these diseases rathen than only treatments. The effectiveness of such an approach has been evidenced in X-SCID and ADA-SCID patients.

The crucial point, however, is understanding the molecular mechanisms of pathogenesis, resulting from comprehensive basic studies. Both in the gene and cell therapies the pathway from bench to bedside has shown a quick adoption into clinical trials. For example, a great effort is being undertaken to find out universal strategies applied to pluripotent stem cells differentiated to distinct cell types. The major unresolved problem is obtaining the sufficient number of autologous pluripotent cells and the inherited association of pluripotency with the risk of teratoma formation. The other obstacle, easier to be solved, is a massive death of progenitor cells after transplantation.

Cell survival can be improved by overexpression of cytoprotective genes. One of the candidates is heme oxygenase-1 (HO-1). We have shown that HO-1 improved the survival of murine proangiogenic progenitors (PPC) transplanted to the wounded skin. Moreover, expression of HO-1 significantly improved the angiogenic response of PPC and mature endothelial cells to VEGF and SDF-1α, acting mostly through facilitating the phosphorylation of VASP-1 protein. Accordingly, overexpression of HO-1 after adenoviral or AAV-mediated gene transfer, improved the wound healing in diabetic mice, enhanced revascularization of murine ischemic limbs, and enhanced therapeutic effects of proangiogenic cells in infarcted porcine heart. Thus, HO-1 overexpression can be beneficial in endothelial progenitors. However, the same strategy can lead to unexpected side-effects in muscle precursors. We found that HO-1-derived CO inhibits the nuclear translocation of cEBPδ, decreases its binding to myoD promoter, and thereby blocks the expression of myoD, the master regulator of myogenesis. In consequence, HO-1 disturbs myoblast maturation and development of myotubes. Furthermore, intramuscular transplantation of HO-1 overexpressing myoblasts to the may lead to the formation of hyperplastic, undifferentiated tumors.

Thus, red biotechnology is creating powerful new tools that are revolutionizing medicine and changing the ways diseases are diagnosed and possibly cured. The example of effects of HO-1 overexpression illustrates, however, the importance of choosing the cell-specific approaches and indicates that therapies should be based on knowledge on cell-specific regulatory pathways.

Phytoremediation: State of art and perspectives

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Environment being, as a side effect of antropopressure, very often highly polluted, with heavy metal, organic compounds or other xenobiotics appearing along with the implementation of new technologies, new types of pollutants (noble metals, pharmaceutical, cosmetics, contraceptives and sanitary products) requires elimination or lowering them to permissible limits. During evolutionary processes plants appeared in the already metal polluted world, and as organisms of sessile style of life developed additional defense mechanism(s), existing only in that group of organisms, that allow them to survive in very polluted sites and to tolerate high accumulation of toxic compounds. This suggests that it should be possible to detoxify contaminants using agricultural and biotechnological approaches, make plants very useful for environmental biotechnology – phytoremediation – and it opens up new ways of cleaning up and revitalizing the degraded/polluted environments. The idea of using plants to decrease the levels of pollutants in the environment has been known for quite a long time, but its origins is uncertain. Phenomenal discoveries of recent years, especially within the areas of physiological, biochemical and molecular basis of mechanisms of harmful substances uptake from the environment, together with knowledge on selection, directed breeding, including with the of tissue cultures selection as well as in plant cultivation have enabled elaboration of the environmental biotechnologies for cleaning the environment. There are significant genotypic interspecies and intraspecies differences in plant usefulness for phytoremediation, which depend on the levels and types of dominant pollutants. Industrial technologies usually remove only one group of pollutants or just move pollutants to other sites. The advantage of plants is their capability to take up a wide range pollutants at a time, such as heavy metals and organic compounds from the soil and the water and gaseous pollutants (NO, VOC, CO_2, CO and O_3) and particulate matter from the air. Plant biomass collected from contaminated sites contains pollutants and must be utilized with care, preferably *via* combustion in special furnaces (in incinerators, combined heat and power stations, cement plants), during which organic compounds are degraded to CO_2. Ashes (with heavy and noble metals) can be stored in old mines or might serve as a source for elements recovery. Cultivation, on contaminated sites, of plants with high phytoremediation potential, apart from its already well known functions, plays a very important role of a "green liver" and as such can also greatly reduce human health risk and improve the quality of life.

Posttranscriptional gene silencing strategy as a molecular tool in plant functional genomics

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Posttranscriptional gene silencing (PTGS) in plants is a mechanism based on RNA degradation processes, similar to RNA interference (RNAi) operating in animals. It is triggered by the presence of a long double-stranded RNA which is cleaved into short, ≅21 base pairs long RNA duplexes called small interfering RNAs (siRNAs). These are incorporated into RNA Induced Silencing Complexes (RISC), one siRNA strand is removed and the other one, serving as a guide, is involved in the degradation of target RNAs (e.g. viral RNA, transposon transcripts, retrotrasposons). PTGS represents an ancient mechanism responsible for plant protection against various invasive nucleic acids forms. However, PTGS is not the only mechanism of gene expression regulation operating in the cell at the posttranscriptional level. Based, in principle, on similar mechanisms in plants (as well as other eucariots) it has developed a system of genome encoded small RNAs, called microRNAs (miRNAs) which are involved in the regulation of endogenous gene expression by targeting cleavage of cognate mRNAs. MiRNAs are regarded as key posttranscriptional regulators of eukaryotic gene expression. The details of their biogenesis are now under intensive investigations. However, the key players involved in plant miRNA biogenesis are already known. This allowed to develop new technologies using artificial microRNAs that target a protein-coding gene of interest.

In nature, plants are exposed to a wide array of environmental stimuli and stresses which trigger various functional and/or structural responses. Drought is one of the main environmental factors affecting the yield and distribution of crop plants. Because of this crucial importance, understanding plant tolerance to water limitations is one of the major current research topic. The application of high throughput technologies such as genome-wide gene expression and proteomics has led to the identification of numerous genes with altered expression during drought stress. Recent studies have revealed that proteins involved in RNA processing affect ABA signal transduction operated in drought stressed plants. Among these, the cap-binding protein 80 (CBP80 also known as Abscisic Acid Hypersensitive 1, ABH1) gene in *Arabidopsis thaliana* was shown to be an important player in the ABA transduction pathway regulation and drought tolerance. Interestingly, its inactivation in *A. thaliana* leads to ABA-hypersensitive stomatal closing and reduced wilting during drought. The CBP80 protein forms a dimer with CBP20 which recognizes and binds the cap structure of RNA Pol II transcripts in the nucleus.

The cultivated potato (Solanum tuberosum ssp. *tuberosum*) is widely known to be very sensitive to soil water deficit. To learn more about the genetic factors that improve potato plant resistance to drought we decided to silence *CBP80* gene in the potato tetraploid cultivar Desiree. We designed artificial microRNAs targeting potato *CBP80* mRNA and obtained Desiree transgenic lines with the silenced *CBP80* gene. Our results show that this approach is successful in inactivating gene expression in polyploid plants. Moreover, potato plants with silenced *CBP80* gene, similarly to *Arabidopsis cbp80* mutants, show morphological, and physiological changes essential for improvement to drought tolerance: ABA-hypersensitive stomatal closing, the increase in leaf stomata and trichome density, compact cuticle structure with the lower number of microchannels. These results provide evidence of the evolutionary conservation of *CBP80* function in *Arabidopsis* and potato plants response to water deficit, and point to the *CBP80* gene as a good single target for mutagenesis aiming to obtain crop plants with improved tolerance to drought.

Modern methods in plant cytogenetics

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Chromosomes are not just part of the phenotype but also hereditary elements and units of mutation and transmission. They have been studied for over century for different reasons and using a variety of methods.

Traditional cytogenetics, based on the staining of chromosomes *in toto*, was applied successfully in the past to solve many fundamental problems in plant, animal and human biology.

Different chromosome banding methods, developed 40 years ago, opened the way to a better understanding of the eukaryotic genome organization. They allowed, for the first time, the identification of individual chromosomes on the basis of banding patterns, and stimulated research on chromosomal changes in the phylo- and ontogeny.

Both traditional and banding methods are also applicable today, often in combination with other methods, such as staining with base-specific fluorochromes, flow cytometry and computer imaging.

In the eighties of the 20th century completely new methods were developed based on the detection of nucleic acid sequences in fixed chromosomes by in situ hybridization (ISH). Shortly thereafter came into use different fluorescent modifications of this technology: FISH, GISH, Zoo-FISH, chromosome painting etc. The most powerful applications of FISH technology are physical mapping of eukaryotic genomes and studies of interphase chromatin. GISH, Zoo-FISH and chromosome painting rely on the use of larger (more complex) probes and are particularly useful in a comparative karyotype analysis and a cytogenetic diagnosis of chromosomal disorders. Only in the model species, the BAC-based painting probes enabled discrimination of all chromosome arms, which in turn allowed research of karyotype in a limited number of closely related species. The widespread utilization of FISH technology in routine studies of different taxonomic groups is still obstructed by considerable cost-per-slide and technical complexity of current protocols.

With the exceptions of 45S rDNA, 5S rDNA and telomere repeats, there are no highly conserved sequences that can be used as probes across most plant or animal species. Expanding the set of useful markers requires identification of repeats with a potential use as FISH probes in the genome under study. Solving of this problem may be due to the dissemination of the next-generation sequencing. The sensitivity of the hybridization method is also a serious limitation of FISH technology. It was shown that the smallest nucleic acid sequence detectable by FISH is a DNA fragment of a few hundred nucleotides. Practically, however, the efficiency of detecting single-copy DNA target of this size is too low for routine application. There are also problems with the penetration properties of probes and the stability of the resulting DNA/DNA duplexes.

Attempts are being made to increase the efficiency and applicability of the existing methods and to develop new approaches. The most promising include: peptide nucleic acid (PNA) oligonucleotide probes, FISH directed towards living cells, microchip-based FISH technologies and immunostaining of chromosome domains enriched with modified DNA or proteins.

Further development in plant cytogenetics depends not only on the sophisticated experimental methods, but also on the collection, storage and availability of data on the chromosome numbers and morphology, banding patterns, presence and localization of rDNA etc.

Confocal microscopy as a tool in plant genetics and physiology

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Olympus Polska Sp. z o.o.

The invention of confocal microscope has contributed to the development of modern biology especially in case of structure and spatial organization of tissues. The major advantage of confocal microscopy is that it enhances the quality of microscopy images by reduction of interferences caused by out-of-focus light. Therefore it is possible to capture series of high-resolution optical sections and reconstruct them in 3D. However, modern confocal microscopy is not only a device for structural studies, but also precise measuring tool as well. In combination with techniques of image analysis, confocal microscopy is a powerful tool by which molecules, molecular interactions, and cell components can be localized and studied.