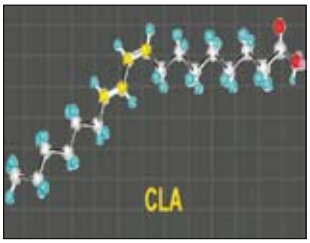
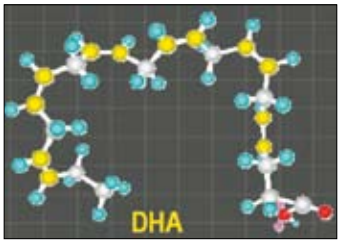
Egg-derived materials as a multifunctional bioreactor for biomedical purposes in health protection and for nutraceutical uses

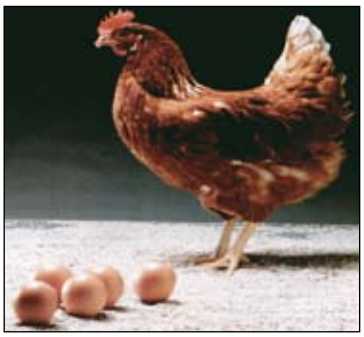
OVERVIEW OF RESEARCH FINDINGS

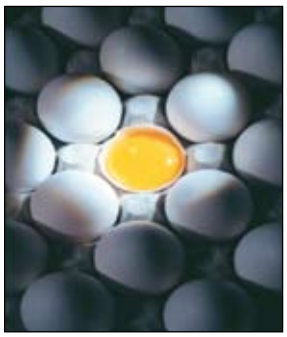
Being rich in compounds of high biological activity (both egg white and yolk), eggs are indispensable nutritional components and perfect materials to be used for biomedical purposes and as nutraceutics (medicinal foods). Eggs can be applied in the pharmaceutical, cosmetic, chemical, and fodder industries. Owing to sophisticated technologies, eggs may serve to isolate biologically active substances, including enzymes, inhibitors, antioxidants and bioactive peptides, which are used for treating humans and animals. The extraction of these compounds is made possible by very complex chemical technologies (ion exchange chromatography, affinity chromatography, membrane techniques) and biotechnological technologies using enzymatic hydrolysis. The research on egg uses also focuses on enriching eggs with polyunsaturated fatty acids (EPA, DHA, CLA) and other compounds. Such materials offer nutraceutical features and can be used for the production of "ideal lecithin" or as nutritional supplements. Substances of particularly important market significance that are isolated from eggs include: lysozyme, cystatin and biopeptides, all obtained from egg whites (mainly ovokinin) and immunoglobulin, phospholipids and phosvitin, lecithin, and biopeptides from these substances, all obtained from egg yolks. These compounds can be applied as nutraceutics, food-preserving agents or biomedical substances, generally as antimicrobiological agents, antioxidants, anti-cancer preparations and nourishing substances. Preparations obtained from shells in combination with phosphopeptide from egg yolks constitute excellent anti-osteoporosis agents. Parallel research will concentrate on the effects of enriching eggs with such compounds as polyunsaturated fatty acids and vitamins for culinary and processing purposes. So-called "designed eggs" of this type offer a perfect base material for producing dietary supplements, especially for children, teenagers and the elderly.

The Department of Animal Products Technology and Quality Management at the Wrocław University of Environmental and Life Sciences has made a number of significant achievements in this respect. It is now establishing a biotechnological company in the area of the Wrocław Technological Park, which will implement and produce new bioactive preparations based on egg-derived materials.









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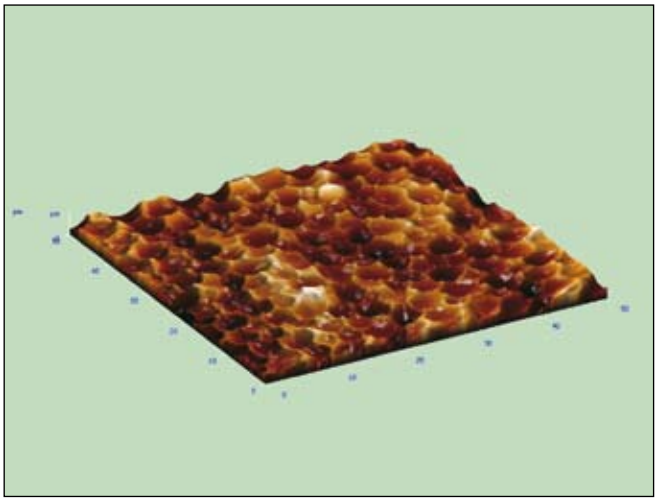
Molecularly surface-imprinted polymers for the identification of microorganisms

OVERVIEW OF RESEARCH FINDINGS

This 2x2 cm polymer matrix can be used in an ordinary optical microscope. Current microorganism tests require time-consuming laboratory investigations, professional staff, and specialized equipment. Using the polymer matrix, such a test lasts 20 minutes and is easy to conduct.

The scale of production in this case will depend on market demand from the food, pharmaceutical, and medical diagnostics industries. The polymer matrix has been presented at a number of conferences. It is covered by patent application P 380702. The anticipated price of the sets is EUR 30 per item. Aside from its low price, other advantages of the matrix include: its high efficiency and shorter time of detection (20 min), the ease of test performance, and reusability.



Surface imprinted polymer matrix – AFM image, scale of 0-50 μm

Instrumentation necessary for conducting control tests

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CelMat – a cellulose dressing material

OVERVIEW OF RESEARCH FINDINGS

This product developed by the Institute of Technical Biochemistry at the Technical University of Łódź is intended mainly for people with serious first- and second-degree burn injuries and those with non-healing trophic wounds.

CelMat is a medical product obtained via a biotechnological process which meets all modern standards for dressing materials.

The treatment of burn injuries using CelMat does not leave scars, enables self-purification of wounds, and prevents repeat infections. The material is characterized by a very high mechanical strength; it ideally fits different body shapes and can be produced in any size. Owing to its specific properties, CelMat is a material that may save the life of seriously burned individuals. No new-generation dressing material available on the market combines all of these features.

The Institute of Technical Biochemistry has developed the biosynthesis technology for producing the dressing material from bacterial cellulose, and owns its producer, i.e. the *Gluconacetobacter xylinus* E_{25} strain. CelMat is produced at the Institute of Technical Biochemistry in a technological facility equipped with a pilot production line specifically designed for the innovative technology.

The technology used in producing the cellulose material is cheap and environmentally-friendly. The bacterial strain used is not pathogenic, comes from the Institute's collection, and is completely safe for both human life and the environment.

The technological process is not highly energy-consuming and the possibility of applying recirculation gives rise to considerable savings of water and culture media. There is another possibility of using food industry wastes as a source of substrate in the process of biosynthesis for the production of cellulose. Any production waste is biodegradable.

The developed technology enables the production of the following materials:

- dressing materials for various types of wounds: burns, surgical wounds, ulceration and varicose vein wounds, bedsores and chronic wounds, etc.;
- bioactive dressing materials enriched with drugs (antiseptics, antibiotics, pain-killing preparations), hormones, enzymes, etc., depending on specific needs;
- dressing materials of various sizes and shapes depending on anatomical needs;
- tubes of various diameters used as prostheses of blood vessels and other organs,
- matrixes for tissue cultures (e.g. artificial skin);
- biological dressings used after the removal of

necrotic tissues and to prepare the patient for autotransplantation.

The products may be used by:

- surgical (burn injury, oncology, plastic surgery, etc.) and dermatological wards at hospitals;
- outpatient clinics;
- all sorts of emergency-reaction units (medical rescue units, police, army, ambulances, fire engines, etc.), which should be equipped with "water dressing coats" from bacterial cellulose for immediate application to cool wounds, alleviate pain, and protect wounds against excessive extrarenal loss of liquid or infection; immediate application to a seriously burned patient may furthermore potentially save their life;
- veterinary units, as a dressing material for animals.

The sizes of CelMat are: 10x10, 10x15, 20x20, 40x60 +/- 0.5 cm; the substance of the wet product is 0.25 +/- 2 g/cm^2; its thickness: 4 +/- 1 mm; mechanical strength: 284 +/- 15 N; color: white/transparent; sterility/durability – 1 year (minimum).

The scale of production and forms of promotion of the product have yet to be established. Recent research of the Polish market shows that demand for the materials constitutes 15-20% of the demand for dressing materials.

The technology is protected by the following patents:

1. Krystynowicz A., Galas E. (1997) "A method of producing bacterial cellulose", Patent PL 171952B1.
2. Krystynowicz A., Galas E., Rzyska M., Antczak T., Pawlak E., Błaszki B.(2003) "A method of producing bacterial cellulose", Patent PL 185337.
3. Krystynowicz A., Czaja W., Bielecki S.: "A method of producing bacterial cellulose, a method of immobilizing bacteria, a method of producing immobilized biocatalysts, the application of bacterial cellulose, a method of cellulose membranes modification" (patent application recorded under the number P 361067).
4. Krystynowicz A., Czaja W., Bielecki S.: "A method for the production of bacterial cellulose, a method of immobilizing bacteria, a method for the production of immobilized biocatalysts, and application of bacterial cellulose, a method for the modification of cellulose membranes" (patent application dated 2 July 2004 recorded under the number PCT/PL 2004/000051).
5. Kielecki S., Krystynowicz A., Kołodziejczyk M., Bigda J., Śmietański M., Jankau J.: "A biomaterial from microbiological cellulose for internal uses, a method of producing the biomaterial and the application of the biomaterial from microbiological cellulose in soft-tissue surgery" (patent application number P-381 3882, dated 4 December 2006).

The material has an interdisciplinary and original character. It is a highly biological material. It is also an excellent example of the success of integrated activities in the fields of molecular biotechnology, industrial biotechnology, and medicine in developing new, modern approaches to solving very serious social problems.

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Computer system for monitoring and analyzing the intracranial pressure signal during infusion testing

OVERVIEW OF RESEARCH FINDINGS

This innovative method is for monitoring and analyzing the infusion test, the most frequently applied non-imaging, albeit invasive, diagnostic test for assessing the state of intracranial compensation mechanisms in patients with presumed hydrocephalus. The test is conducted by making two lumbar punctures (Fig.). One needle is used to infuse physiological saline solution, another is connected to the pressure sensor and the ICP monitoring system. The model tests of cerebrospinal fluid (CSF) circulation help to determine the values of intracranial compensation parameters. The most significant of them – outflow resistance (RCSF) and intracranial elasticity (E), along with the evaluation of clinical symptoms and imaging test results, constitute a basis for choosing a treatment therapy for patients with presumed communicating hydrocephalus. The method of analyzing the infusion test focuses on ICP at all test stages – before, during and after infusion – and takes into consideration the analysis of intracranial pressure components, including slow waves and pulse-related amplitudes.

The testing method is characterized by the following innovative solutions:

a) analysis of ICP decrease after infusion, which in turn enables:

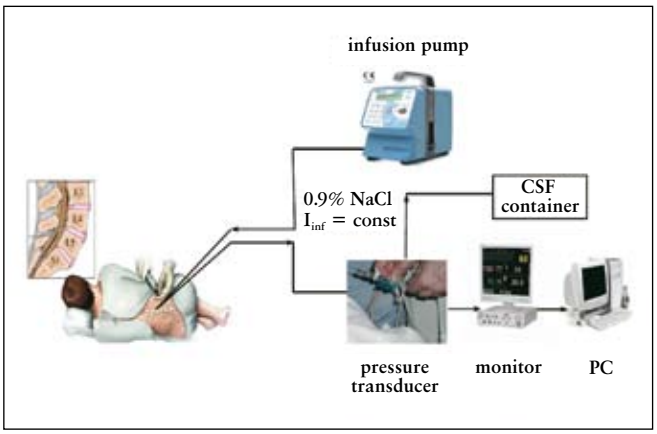
- the identification of the phenomenon of increased ICP after infusion,
- the introduction of new parameters determined by the iterative method into hydrocephalus diagnostics,
- the introduction of a description of ICP changes after infusion into the mathematical model describing cerebrospinal fluid circulation,

b) analysis of whether the test may be halted (thus reducing infusion time) without losing information about compensation parameter values – currently undergoing tests,

c) analysis of slow waves in the course of the infusion test – currently undergoing tests.

The method is covered by a patent application submitted to the Patent Office of the Republic of Poland: Juniewicz H., Cieślicki K., Kasprowicz M. "Method of determining intracranial compensation parameters during infusion testing" No. P 382 725, 22 June 2007.

Computer system for monitoring and analyzing the intracranial pressure (ICP) signal during the infusion test



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Microbiological soil remediation from petroleum derivatives

OVERVIEW OF RESEARCH FINDINGS

This technology is patented (under patent number PL 180141 B1) and it involves microbiological soil remediation from petroleum derivatives. After the identification and elimination of potential pathogens, autochthonous microorganisms isolated from polluted soil are multiplied and introduced into the polluted soil. The process requires microbiological and chemical monitoring.

The method can be applied for in-situ and ex-situ soil treatment. It enables the removal of petroleum derivatives such as diesel oil, petroleum, air-petroleum, lubricants, cooling fluids from soil. So far the method has been used to treat over 60 thousand tons of soil in more than 20 different areas (petrol stations, airports, military bases). Its effectiveness is 99.7%. The duration of the process depends on the type of pollutants and atmospheric conditions.

Bioremediation area

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Novel approaches in human joint replacement and regeneration

OVERVIEW OF RESEARCH FINDINGS

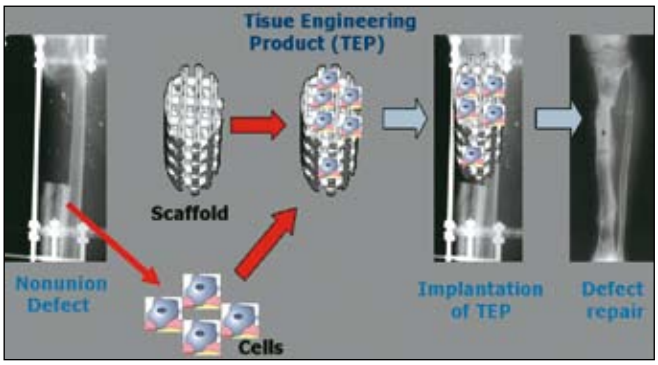
Arthritis is one of the most common diseases affecting human joints. To some degree, almost every person over 60 suffers from joint inflammation. In the case of arthritic joints, cartilage is degraded causing pain, loss of movement, and joint stiffness. Given the very limited potential for cartilage regeneration caused by the absence of blood circulation, the treatment of osteoarthritis (OA) is one of the most significant problems in orthopedic surgery.

The proposed method to be applied at an early stage of OA is a tissue engineering approach. This method involves using an advanced bioactive 3D scaffold to support the growth of a new tissue in the place of a joint defect (Fig. 1).

At a later stage of OA, a newly designed surface replacement implant can be applied. This method involves infiltrating composite hydrogenl, functioning as artificial cartilage, into a porous metal, which is then fixed to the underlying bone by the bone in growth fixation (Fig. 2).

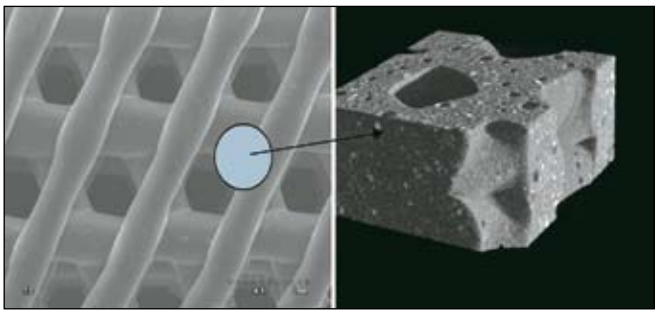
Fig. 1. The tissue engineering approach (a) and the 3D scaffold * (b)

a



* in cooperation with D. Hutmacher

b



a

b

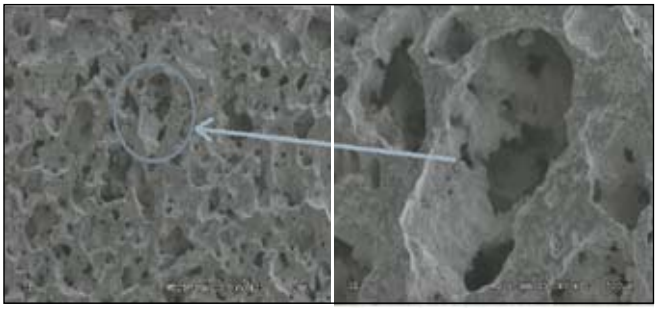


Fig. 2. Artificial cartilage made of hydrogel (a) and 3D porous titanium (b)

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SplB protease, target peptides, and their applications – an expression platform for industrial production of recombinant proteins

OVERVIEW OF RESEARCH FINDINGS

The outcome of this research is a method of obtaining SpIB protease and its application for specific hydrolysis of the polypeptide chains and amino acid sequences it recognizes.

Proteolytic enzymes (proteases, proteinases) of high substrate specificity (recognizing and cleaving only selected peptide bonds) are widely used in laboratories and the biotechnological industry for specific hydrolysis of polypeptides. These enzymes mainly serve the removal of so-called fusion tags – fragments of recombinant polypeptides.

The enzyme's high specificity, along with the efficiently recognized site introduced between the tag and the polypeptide fragment constituting the final product, enables precise removal of the tag without the risk of degrading the desired polypeptide.

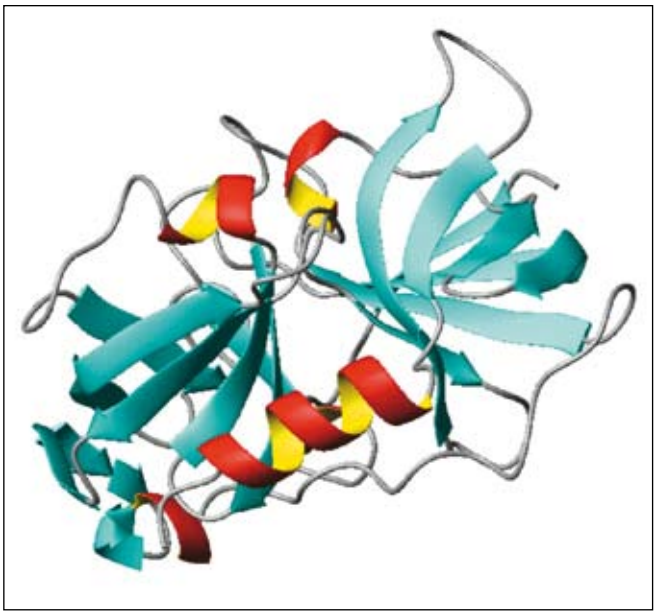
Such characteristics of the method help to create a fully-fledged commercial product – an expression platform used for the production of recombinant proteins.

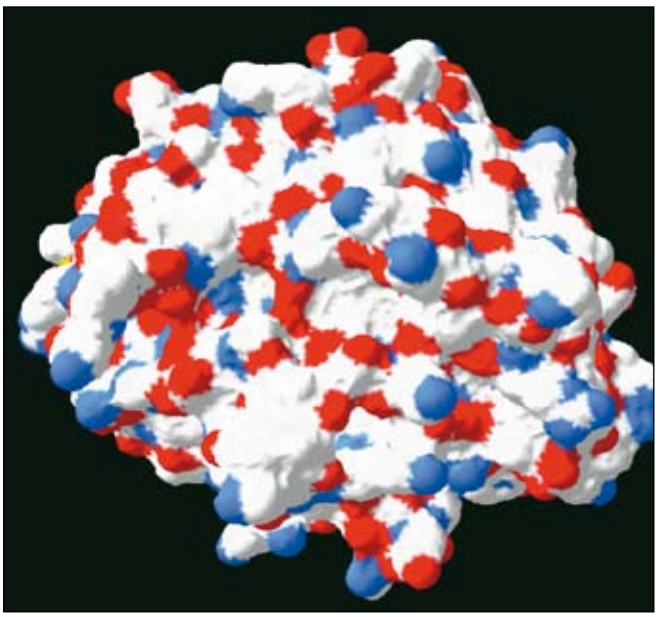
The expression systems for the production of recombinant proteins constitute a basis for producing new drugs and developing diagnostic techniques. They also have a direct therapeutic application (http://www.exzellenznetzwerk-biowissenschaften.uni-halle.de/content/view/43/89/).

The most significant drugs available on the market produced by means of similar methods include:

- recombinant hormones;
- interferons;
- interleukins;
- hematopoetic growth factors;
- tumor necrosis factor;
- blood coagulation factors;
- thrombolytic preparations;
- therapeutic enzymes;
- monoclonal antibodies;
- vaccines.

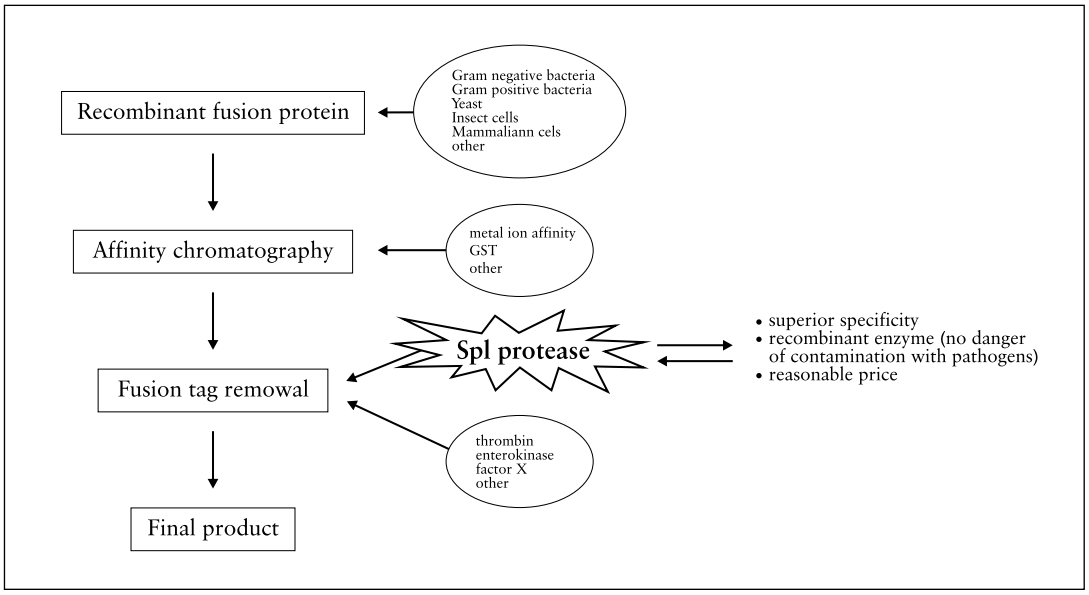
X-ray sturcture of SplB protease in a ribbon and surface representation models. Solving of the crystal structure of the interesting enzyme helped in the deterination of its substrate specificity.





The entities interested in such products include mostly companies from the chemical, pharmaceutical and biotechnological sectors.

Patent applications cover two different enzymes, SplB i SplA, of different substrate specificity, their application in the specific hydrolysis of the polypeptide chains and amino acid sequences recognized by them and their applications.



The use of SplB protease for recombinant protein purification

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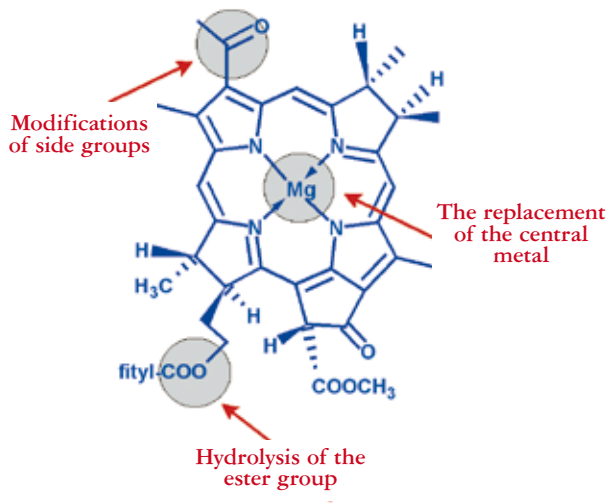
Photodynamic therapy of tumours: photosensitizers

OVERVIEW OF RESEARCH FINDINGS

This project is meant to develop a special type of pigment obtained via the chemical modification of chlorophylls – natural photosynthetic pigments. Chlorophylls are characterized by photosensitizing capacity: they are capable of activating other substances in their surroundings by directly transferring the energy of absorbed photons. Appropriately modified chlorophylls can be used as photosensitizers in the photodynamic therapy of tumours. Such pigments administered in darkness constitute a form of prodrug, which can be activated when light of an appropriate wavelength and energy is directed at a specific location (diseased organ tissue) in the patient's body. The light-activated photosensitizer induces the production of cytotoxic drug forms, whose activity concentrates on the irradiated tissue.

Chemical modifications of chlorophylls



Scheme of photosensitizer activity in the photodynamic therapy of tumours

The aim of the project is to obtain photosensitizers of increased efficiency, achieved via the synergy of phototherapy and chemotherapy. This synergy leads to greater therapeutic effectiveness than when either phototherapy or chemotherapy are applied separately.

These methods of chlorophyll modification are protected by patents in Poland and abroad, and new methods (developed in cooperation with Prof. G. Stochel from the Faculty of Chemistry of Jagiellonian University and Dr. K. Urbańska from the Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University) are covered by further patent applications. The methods may be used in medicine and diagnostics, mostly in the photodynamic therapy of tumours.

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Application of carbon dioxide in a subcritical state (LCO_2) for the extraction of important substances from natural sources

OVERVIEW OF RESEARCH FINDINGS

Carbon dioxide is a valuable low cost solvent – it is safe and readily available. While the use of organic solvents poses limitations with regard to the obtaining of solvent-free extracts, extraction using LCO_2 may be applied for the isolation of important substances from natural sources in their non-contaminated state. What may serve as an example here is the isolation of squalene from *Amaranthus Cruentus* L. Squalene is a very important isoprenoid and appears to function in the skin as a quencher of singlet oxygen, protecting human skin against UV radiation and other sources of ionizing radiation. Squalene may also act as a "sink" for highly lipophilic xenobiotics. LCO_2 extracts of squalene are free of peroxidation products and could be used directly for the supplementation of foodstuffs. Another example of LCO_2 use is red pepper extracts containing unique pigments such as capsorubin or capsanthin fatty acid esters responsible for a deep red color, provitamins such as β-cryptoxanthin and β-carotene or capsaicinoids responsible for pungency. LCO_2 enables fractionation of extracts on the basis of increasing polarity of LCO_2 with the decrease of its temperature. Such a technique may be successfully applied for the extraction of natural fragrances from a relatively moist matrix because LCO_2 is miscible with water.

Fig. 1. Laboratory with a LCO_2 extraction unit

Fig. 2. Red pigment extracted from ground red paprika

The cost of food-grade CO_2 is very low. Relatively low pressure – max. 56 bars – enables the construction of quite cheap installations. The operating temperature in this case is down to –35°C.

Another important factor here, for instance, is that the extraction of squalene from plants may contribute to the protection of deep sea sharks, which are currently the only industrial source of squalene.

The world demand for squalene including pharmaceutical, cosmetic and technical applications is estimated to be approximately 120 000 metric tons/year.

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A new method of producing the anticancer drug Taxol (Paclitaxel)

OVERVIEW OF RESEARCH FINDINGS

The powerful anticancer drug Taxol, or paclitaxel, it is obtained through the process of semi-synthesis from 10-deacetyl-baccatine III by the attachment of the side chain (N- benzoylphenylisoserine). Researchers at the Institute of Organic Chemistry have developed and patented a new method for producing this compound, which has enabled the commencement of Taxol synthesis in Poland. Significantly for the whole process, the Polish company AGROPHARM Sp. z o.o. owns a yew-tree plantation (*Taxus baccata*) and produces 10-deacetyl-baccatine III.

Paclitaxel is an anticancer drug used mainly to treat breast cancer and ovarian carcinoma. It takes the form of a white powder, which after dissolution in water is applied intravenously in quantities of approximately 30 mg per day. The daily doses administered during the whole treatment period range from approx. 1.0 to 2.0 g. The original method of synthesis developed at the Institute of Organic Chemistry is protected by Polish Patent No. PL 193190, entitled "Method to produce paclitaxel and related compounds." The pharmaceutical company Polfa has developed a stable pharmaceutical form of this drug and begun its production (Patent application 343615, 31 October 2000).

The planned production rate of the drug is initially 5 kg per year, at an estimated price of USD 250 per gram (USD 1,250,000 a year), which is the quantity of the drug necessary to produce its pharmaceutical form.

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A new substrate for fluorescent and spectrophotometric detection and assays of lovastatin esterase, plus a method to immobilize lovastatin esterase and the use of immobilisates in the biotechnological synthesis of simvastatin

OVERVIEW OF RESEARCH FINDINGS

This patent pertains to the application of coumarin derivatives as new substrates for the fluorescent and spectrophotometric detection and assays of lovastatin esterase, the enzyme used in the biotechnological method of purifying simvastatin from lovastatin. Simvastatin is presently one of the most significant drugs used for lowering blood cholesterol levels.

The biotechnological purification of simvastatin from lovastatin requires the use of an enzyme which, for biotechnological purposes, has to be immobilized on a solid support. The Institute's researchers have developed a method to immobilize lovastatin esterase providing active biocatalysts for the biotechnological purification of simvastatin. In the course of the continuous process, the immobilized enzyme does not change its selectivity or high substrate activity.

During biotechnological purification of simvastatin from lovastatin, lovastatin esterase used in the process is isolated from biological material for technological purposes. The process of enzyme isolation requires control, which in its traditional form is complex and expensive. The new compound developed at the Institute simplifies this isolation process and enables the detection of the enzyme in very low concentrations. This result is covered by a patent application.

The application of immobilized lovastatin esterase in the process of simvastatin synthesis, one of the best selling drug on the international market, helps to significantly increase the efficiency of synthesis; this has been also covered by the patent application.

Fluorescence of solutions: a colored compound (left) and a colored compound with a lovastatin esterase concentration of < 1 ng (right)

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Lower Extremity Telescopic Orthosis LETOR for PARAPLEGIA (1981)

OVERVIEW OF RESEARCH FINDINGS

LETOR is a prefabricated device used to prevent hypokinesia after spinal cord injuries (SCI). It is functional at all stages of the rehabilitation process, from the moment the patient is first allowed to stand, through bipedal gait exercises and clinical assessment, to the stage it is used at home. It has the following advantages: a semi-elastic column enables adjustable stability level improving afferentation and accelerating rehabilitation; the lack of a knee-hinge plus the fact that the device is affixed to the trousers and sports shoes facilitates the process of don/doff and ensures seating comfort; the front slant of the column contributes to better trunk control when the person stands and facilitates the initiation of swing phases in the course of walking; and an easy and fast way of fitting to individual limb shapes enables its usefulness to be tested out, which eliminates the risk of disappointment (conventional orthoses can not be easily tried out and they can not be returned as they have to be first manufactured for a given individual). The modular LETOR is our contribution to the advancement of ortho-prosthetics, initiated in Copenhagen in 1963 by Prof. Marian Weiss and his pioneering concept of Immediate Post-Operative Fitting, which after 27 years of controversy and following the first ISPO Consensus Conference held in Glasgow in 1990 developed into Modular Prosthetics.

SCI L2, Nepal 2007

The device is made of carbon fibre, duralumin, rubber, Velcro, nylon/leather, steel screws, rivets. It is fabricated in one standard size which fits 88% of adults, weight approx. 1.2 kg. This device is original on international scale, functional and still competitive.

The company Zakłady Ortopedyczne (Orthopedic Workshop) in Konstancin delivers about 50 items a year; the total number of patients supplied with the device in Poland since 1984 stands at approximately 1350, total turnover on this simple solution at around 1 million euro.

Forms of promotion to be applied: specialist conferences, individual consultations, lectures and presentations, to promote the concept and establish a world distribution network.

Price and sale of the device: EUR 600 a pair, for clinics and for home use, with a subsidy of 20-30% from the NFZ (National Health Fund).

Patents: 1999 – trademark 158127 for the Institute of Biocybernetics and Biomedical Engineering copyrighted until 20 November 2009; 1983 – patent 140171 “Orthopedic apparatus”; 1983 – utility model W40145: “Stabilizing base for an orthopaedic apparatus”; 1982 – patent 135586: “Telescopic lower-limb orthosis.”

Further information:

- 2007 – available new-generation concept for the synthesis of a telescopic orthosis;
- 2006 – contribution to the educational programme at the University of Strathclyde, by adoption of the LETOR system at the National Centre for Training and Education in Prosthetics & Orthotics – a leading research and education centre in P&O, WHO Collaborating Centre for Research and Training in Prosthetics, Orthotics and Orthopaedic Technology, the think-tank of the International Society for Prosthetics and Ortho-tics (ISPO) with head office in Copenhagen;
- 2006 – invited presentation during the closed-circle ISPO-USAid-WHO Consensus Conference on Appropriate Lower Limb Orthotics for Developing Countries, Hanoi, Vietnam;
- 2004 – know-how transfer to Shandong Orthotics & Prosthetics Rehabilitation Centre, Jinan, China;
- 2002 – LETOR usefulness verified with randomly selected 57 users under grant 4T11E00823 awarded by the KBN (Committee for Scientific Research); some 88% of users reported their approval (against approx. 50% for conventional lower limb orthotics), and 67% reported benefits from standing and walking exercising;
- 2001 – mark of distinction for the dissertation by Dr. Maciej Pokora, supervised by Prof. Marek Darowski;
- 2001 – seminar at Otto Bock HealthCare GmbH, Duderstadt, Germany, and the sale of the evaluation set to the world leader of the orthopaedic industry;
- 2000 – affirmation of competitiveness: the unauthorized copy offered as LEVATE by ProWALK GmbH, Germany;
- 1989 – appointment to the International Scientific Committee, ISPO World Congress, Kobe, Japan;
- 1989 – monograph published by Limbird T. et al.: Lower extremity telescopic orthosis for im mediate fitting in paraplegia, Orthopedics, 12 (6), 851-4;
- 1987 – know-how transfer to 3D Orthopedics Inc (DeRoyal Inc), Dallas TX, USA, in 1987-1991 ca. 900 items manufactured;
- 1986 – a licence sold for cash for Svensk Handikappteknik AB, Stockholm, Sweden.

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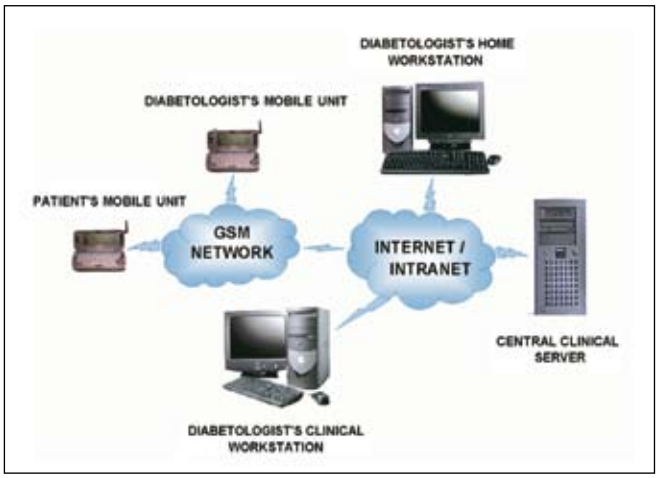
TeleMed – a mobile telecare system

OVERVIEW OF RESEARCH FINDINGS

The telematic system TeleMed was developed by the Institute of Biocybernetics and Biomedical Engineering of the Polish Academy of Sciences in 2004 and is designed for applications requiring intensive insulin treatment in patients suffering from diabetes. The system facilitates practically continuous patient-doctor contact during the day. The prototype was applied for the treatment of thirteen patients with newly diagnosed type 1 diabetes, each patient using the system for 3 weeks. Use of the TeleMed system reduced the hospitalization period to a minimum and enabled patients to continue their treatment at home, the most convenient form of treatment for patients. Very good results were obtained in treating newly diagnosed diabetes, and patient acceptance of the system was high. The information regarding the course of the insulin therapy was provided by the system in the form of automatically generated short text messages many times a day and did not hamper doctors in carrying out their duties during the day, yet enabled full evaluation of the treatment being applied and its necessary verification. The full visualisation of the treatment results on the doctor's work stations (at home and at the clinic) enabled the thorough assessment of how treatment was progressing.

The reduction in hospitalisation time resulting from the use of the TeleMed system is cost-effective. The system should be very efficient not only in the case of patients with newly diagnosed diabetes, but most of all in the cases of unstable, difficult to treat diabetes. The most important element of the system is an original software package implemented on Nokia mobile phones and doctor's work stations. The price of a single package depends on the scale of production and the selection of system elements.

The system was developed within the framework of the research project No. 8T11E01519 (2001 0 2004) funded by the Ministry of Science and Higher Education, entitled "The design, development and assessment of the effectiveness of short-time applications of the telematic system supporting intensive insulin treatment of newly diagnosed diabetes, based on a mobile teletransmission module." The prototypical TeleMed system is original. Its authors have submitted patent application No. P359740 entitled: "Method of monitoring and registering the patient's condition during telesupervision, especially during intensive insulin treatment" (16 April 2003).



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The creation of Festulolium – a grass cultivar obtained from interspecific hybrids *Festuca pratensis* and *Lolium multiflorum*

OVERVIEW OF RESEARCH FINDINGS

Following many years of genetic experiments and plant husbandry work with fescue and ryegrass hybrids (referred to as Festulolium), the Institute of Plant Genetics in Poznań in cooperation with the Szalejewo Plantation has created the first Polish Festulolium cultivars – Felopa, Sulino, Rakopan and Agula – obtained from interspecific hybrids of Italian ryegrass *(Lolium multiflorum)* and meadow fescue *(Festuca pratensis)*. Festulolium cultivars have previously been produced only in Great Britain, Germany, and the Czech Republic.

The genotypes of Festulolium hybrids combine significant agricultural characteristics of both parent species – Italian ryegrass's high pasture quality and meadow fescue's resistance to environmental stresses. The created Festulolium cultivars match the best Italian ryegrass varieties in terms of crop yield and quality, while at the same time being significantly more durable, cold resistant, and drought tolerant. They can be cultivated in field monocultures and in mixtures with lucerne and clover.

Polish Festulolium cultivars, next to the Czech varieties, are the varieties most frequently cultivated in the European Union, USA, and Canada. In 2007, the area of seed plantations of Polish Festulolium cultivars in Poland and abroad will exceed 1000 ha. The crop yield of the Polish varieties amounts to 6-8dt/ha and is comparable to other cultivar crop yields.

a – general morphology of Festulolium plants, b – a field trial with Festulolium cultivars, c – a Festuloluim seed production plantation

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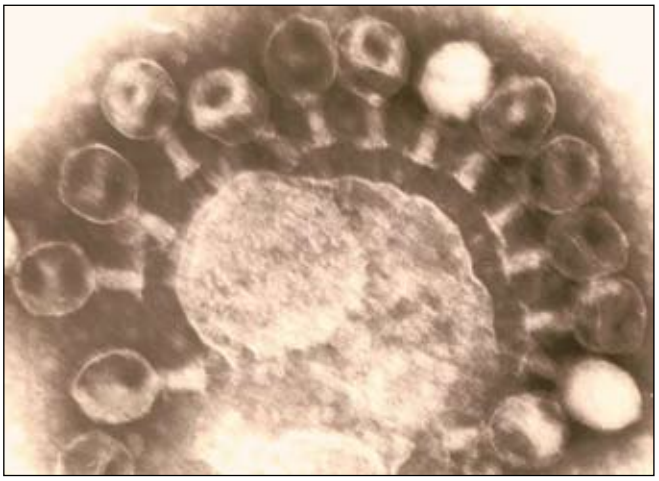
Bacteriophage therapy for treating drug-resistant bacterial infections

OVERVIEW OF RESEARCH FINDINGS

We are the only research center in the European Union offering a unique form of drug-resistant bacterial infection therapy. It is generally known that the problem of drug-resistant bacterial infections poses one of the greatest challenges of contemporary medicine. Aside from medical sciences, phages can also be used in veterinary sciences, for plant protection, and to prevent food contamination.

Our achievements are depicted by national and international media as well as leading scientific journals – notably, *Nature Medicine* and *Science* (for more information see:
www.iitd.pan.wroc.pl/phages/phages.html).

We have filed a patent application to protect our intellectual property rights to the applied phages. Our analyses show that the use of phages may be significantly cheaper than the use of antibiotics, which means that the implementation of our project could give rise to significant budget savings. Since investigations into phages' influence on the immunity system constitute a new and original domain of scientific inquiry, our project is of an important innovative significance.



Phages attacking a bacterial cell

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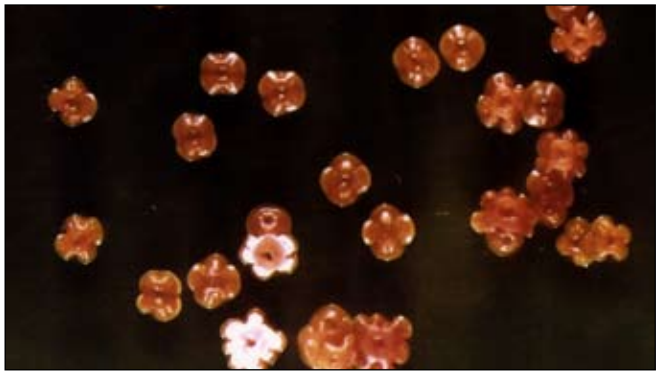
The Polish Collection of Microorganisms (PCM) at the Institute of Immunology and Experimental Therapy

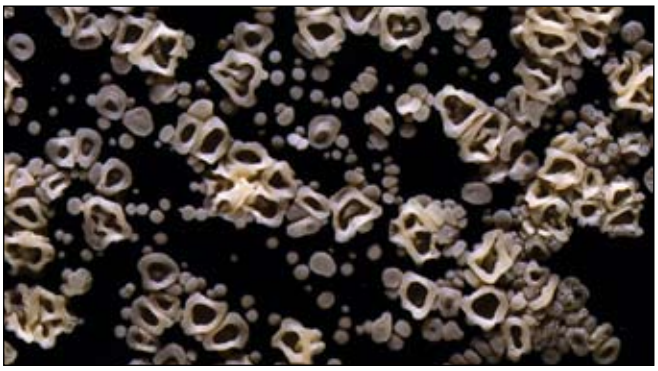
OVERVIEW OF RESEARCH FINDINGS

The Polish Collection of Microorganisms boasts approximately 3000 strains of bacteria. These mostly include typical strain varieties – obtained from world renowned collections (ATCC, NCTC, CIP, CCM, JCM, DSM, CNCC, CCUG, NRRL, and others). The strains are lyophilized, stored, and supplied at the request of national and foreign laboratories. Bacterial strains serve as a reference and research material. The Collection contains sets of strains for testing the activity of antibiotics, lyzozyme, phagocytes, antiseptic agents and for investigating carcinogens. Furthermore, it includes strains producing antibiotics, enzymes, A-protein, ethanol, and immunologically active substances, as well as strains for phenol, toluene or cyanide degradation. The Institute provides information on the sources, features, and necessary conditions that need to be ensured for specific microorganisms and offers access to a Catalogue of Microorganisms. The Institute's Collection is registered with the World Federation of Culture Collections (WFCC) under registration number 106 and the acronym PMC, and with the European Culture Collection Organization (ECCO). In 2000, the Collection acquired the status of an international depositary authority under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

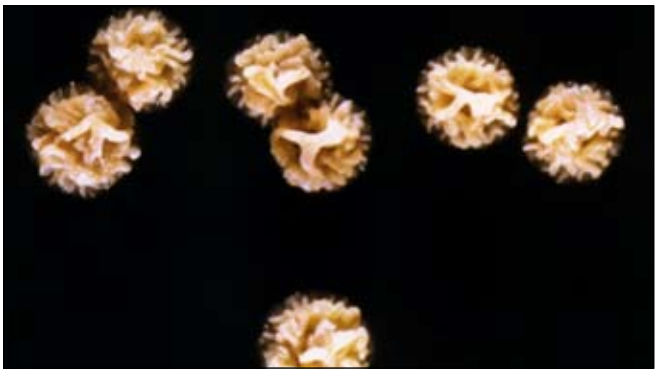
Actinomadura glauciflava



The PMC holds the status of International Depositary Authority (IDA) granted by the World Intellectual Property Organization (WIPO). The Polish Collection of Microorganisms is headed by Prof. Andrzej Gamian.



Gordonia amarae



Saccharopolyspora thermophila

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Cryopreservation of genetic diversity of endangered forest tree species producing difficult-to-store seeds

OVERVIEW OF RESEARCH FINDINGS

Cryogenic conservation techniques involve the storage of plant tissues at the boiling temperature of liquid nitrogen (–196°C) or its vapor (around –135°C) and very often use an in vitro technique to recover whole plants from cryopreserved explants.

The Institute of Dendrology of the Polish Academy of Sciences in Kórnik has developed successful cryopreservation techniques and cryopreserved the genetic diversity of the pedunculate oak (*Quercus robur* L.), an endangered species in Europe. This is the first experiment described in the world literature involving the cryopreservation of *Quercus robur* embryonic axes used to obtain plants. At present, small trees derived from embryonic axes frozen in liquid nitrogen are growing in the Institute's experimental forest.

The acorns of the pedunculate oak belong to the recalcitrant seed category, meaning that they are very sensitive to desiccation and low temperatures (below –10°C), making them very difficult to keep alive over a relatively short storage duration. In the course of the experiment, several millimeter-long embryonic axes isolated from acorns were first subjected to cryoprotection in sucrose and then glycerol solutions. Before they were frozen in liquid nitrogen, embryonic axes were dried down in control conditions to 25% water content, thawed from –196°C at 40°C, and then cultivated in vitro on the woody plant agar medium to produce properly growing seedlings (Fig.1).

Apart from recalcitrant seeds, researchers at the Institute also investigate the sensitivity of seeds from categories more resistant to drying, i.e. the intermediate and orthodox classes (Fig. 2). They have man-

Fig. 1. Pedunculate oak in vitro plantlets, obtained from embryonic axes frozen in liquid nitrogen

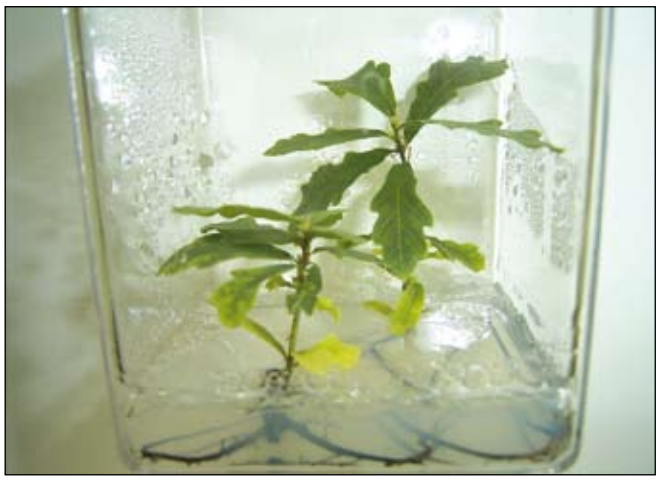

Fig. 2. From bottom: nuts of a common birch with husks, nuts of a black alder, spherical seeds of a small-leaved linden, winged nuts of a mountain elm, nuts of a European hornbeam, pits of a bird cherry, triangular nuts of a European beech, and samaras of a European ash

aged to identify safe moisture content limits for the freezing of seeds of more than 20 forest tree species. Both broadleaves and coniferous seeds of forest species were stored in liquid nitrogen at the Institute's laboratory for two years. The success of these experiments leads to the conclusion that the longevity of frozen seeds is significantly longer than that of seeds preserved in standard conditions. Similar conclusions are to be drawn from the research of other groups. For instance, researchers at the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado (with which the Institute of Dendrology collaborates, Fig. 3) extrapolated the results of 30-year storage (so far the longest in the world) of orthodox seeds in liquid nitrogen or its vapour – showing that the estimated storage lifespan should be reckoned in hundreds of years, rather than decades.

The results of the Institute's research have been used in the Kostrzyca Forest Gene Bank (http://www.lbg.jgora.pl) established in 1995 in the southern part of Poland, where an ecological disaster took place in the 1980s. The center presently preserves plant gene resources in standard conditions and it boasts a cryogenic unit (Fig. 4) created in 2007. In times of global climatic change, ecological disasters and fires, cryopreservation techniques will enable the most valuable gene resources of endangered Polish forest flora, which can not be stored by classical methods, to be safeguarded for the future.

Fig. 3. Storing seeds or isolated seed tissues in liquid nitrogen vapor (-135°C) is a technique enabling plant genetic resources to be preserved for even as long as hundreds of years. Paweł Chmielarz (Institute of Dendrology, Polish Academy of Sciences) develops methods to cryopreserve oak germplasm within the framework of a project funded by a competitive grant from the Kosiuszko Foundation at the National Center for Genetic Resources Preservation in Fort Collins, Colorado.

Fig. 4. The Cryogenic Laboratory at the Kostrzyca Forest Gene Bank, created in 2007 (Poland)

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