Capacity Building for Interdisciplinary Biosafety Research at the University of Latvia

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Summary

Biotechnologies provide solutions to many problems of the modern society in areas of food and energy shortage or health; However, with an increased ability to manipulate living organisms new risks and responsibilities emerge. Biosafety refers to safe use of biotechnology products in a manner that does not harm humans or environment. Biosafety includes both the technical side of safe use of biotechnology products, but also social and legal aspects of biotechnology use including science communication and public participation in technology assessment. New Member States including Latvia that acceded to EU in 2004 potentially are facing the same biosafety issues as the EU15 States, but they have limited human and financial resources, therefore it is essential to increase the capacity of human resources in biosafety. University of Latvia, Riga, carries out a three-year research project co-funded by European Social Fund „Capacity building for interdisciplinary biosafety research". Project employs over 20 people with expertise in different aspects of biology and medicine, as well as in social sciences - anthropology and law. Several of them have returned to Latvia from different foreign universities and research institutions bringing new skills and experiences in conducting biotechnology research. Specifically, biosafety research is carried out in the following areas: a) human somatic stem cells; b) virulence factors in multidrug resistant and biofilm forming bacteria; c) genetically modified organisms; d) governance of biotechnologies. Here we present the structure and the research activities of the project and discuss those in relation to wider biosafety issues.
http://www.biodrosiba.lu.lv/eng/

GMO biosafety

Cultivation of GM crops is a controversial issue in European Union, although the worldwide area under GM crops increases each year. However, arguably the most important use of GMOs is in basic science for studying gene function and in biotechnology to obtain recombinant proteins.

Transgenic model organisms for studying gene function in crop plants

We are using GM plants to study function of plant cell death related genes from *Arabidopsis thaliana* and barley (*Hordeum vulgare*). Apart from the improved understanding of the basic cellular processes that lead to programmed cell death in plants, this study may provide insight into the mechanisms of plant disease resistance, which often involves programmed cell death, and the signals that plant cells use during the pathogenesis. *Arabidopsis* mutant *lsd1* is hypersensitive to various cell death signals, and develops runaway cell death symptoms in response to them. While *Arabidopsis* as a model organism has extensive mutant collections and well established transformation protocols, as well as a wealth of knowledge about function of many genes, not all this information and resources are easily translated into applied knowledge for crop plants. Several homologues of the *Arabidopsis LSD1* exist in barley, but the functional orthologue remains to be discovered. Increased knowledge of mechanisms of plant cell death and hypersensitive response may lead to development of crop varieties resistant to various plant pathogens. Complementation of the *Arabidopsis lsd1* mutant with the barley homologues may help to identify the functional barley orthologue. Currently, T-DNA constructions with the barley genes under native *Arabidopsis LSD1* gene and CaMV 35S promoters have been constructed and *Agrobacterium tumefaciens*-mediated transformation of the *Arabidopsis lsd1* line is under way. Recently, primary transformants have been selected and confirmed using analysis of genomic DNA and cDNA.
Apart from the scientific objective, the research also provides us with the opportunity to increase the capacity in GMO biosafety by training the students in safe handling of recombinant DNA, bacterial strains of *Escherichia coli* (DH5α) and *Agrobacterium tumefaciens* (AGL1), as well as transgenic *Arabidopsis thaliana* lines.

Biotechnology and biosafety

Biosafety relates to the necessity to use products of modern biotechnology in a safe, responsible and sustainable manner. Genetically modified crops may be the most controversial biotechnology product, but there are other current trends related to use of stem cell therapies or increased use of antimicrobial drugs leading to multidrug resistant bacteria. While technical aspects of safe use of biotechnology products are clearly important, biosafety is not just a biological issue - there are social and legal aspects of biosafety researchy including public awareness, perception of biotechnology products and bioethics.

Public perception of biotechnology products in Latvia

Biotechnology and its various products are perceived differentially in public domain, because of diverse approaches to the way they are integrated into existing perception systems of various society groups. The attitude towards certain biotechnology products depends on many different factors. The attitude to biotechnology product could reveal or overlap, for example, with national or family values. At the moment one of the essential aspects that affects perception of biotechnology is the way scientists communicate with society, and the manner in which the authority to express their point of view is divided among communication participants. The study reveals that the notions and values of specialists and lay people are not complementary in understanding what is safety and what is risk in the context of development, implementation and application of biotechnology.
We have carried out a preliminary study, i.e., discourse analyses of printed and electronic media on social aspects and positions of all parties involved in the four biotechnologies that have been already implemented into daily practice: GMOs, stem cells, artificial insemination, and antimicrobial drugs. Although the study is still in progress, some conclusions regarding public perception of biotechnology products can be made.

Public perception of GMOs in Latvia

In case of GMO, the perception of biotechnology is substantially affected by environmental protection NGOs that position themselves as experts struggling with the industry's orders. Their opinion in public space is very active, and NGOs are perceived very intensely, because NGO activists use various strategies to reduce the significance of local expert opinion, for instance, by searching for the foreign experts, who express their point of view. Lay people often adopt justification of risk and safety, and argumentation schemes from public media, which show attitude towards products, using these schemes to justify even those risks that were not mentioned in the media, transferring well-known schemes of substantiating logic from one biotechnology field to another. Rejection of using GMO biotechnology is closely related to perceived national values.

Stem cell therapies

In contrast to GMO products, stem cell (SC) therapies in public space are mostly presented as promising resource for future and present medicine; however the public perception of SC therapies, in general, is fragmented. Public is aware of local achievements in SC research and their application for treating different diseases (currently at the clinical trials' level) and they also perceive it as an opportunity for economic development of the country under the conditions of global competition. There is little discussion on risks from specialists, mostly highlighting the potential and present options of SC therapies. Lay people often consider ethical and moral issues with the status of embryo used for obtaining hESC, or the potential intervention to the God's or the Nature's established order which comes with unknown and uncontrolled risks in the context of research and development in SC therapies. On the other hand, preservation of umbilical cord SC in private banks is perceived in society as investment in family's safety and as carrying out parent's (or mother's) duty, although experts often emphasize the limited usage of umbilical cord SC between family members.

Antimicrobial drugs

The analysis shows that opinion of lay people and medical specialists in the case of antimicrobial drugs is not so polarized as in case of other biotechnologies. Lay people are included in the public space to discuss advantages and disadvantages of the technology. They also participate in public debate to discuss application of the technology. On one hand, it is related with more symmetrical power relationships between specialists and non-specialists, but it is also linked with lay people daily experience with antimicrobial drugs.

Artificial insemination

The technology has a relatively long history of application in Latvia, but there is lack of evaluation of ethical issues and governance. The current research shows that artificial insemination and the opportunities offered by the biotechnology are perceived relatively positively, because the technology is being positioned as beneficial for family. In general, artificial insemination in society is viewed as a whole and there is no identification of the different part of technology. In the public space donation and surrogate are not addressed as a component of the technology because of predominantly negative attitude in society. There is a lack of wider public debate about the risks including all stakeholders in Latvia, although there is support for improving the governance of technology.

Bioethics and biosafety

Bioethics and biosafety are research areas that help to create appropriate governance of technology application; reduce or avoid risks that are related to biotechnologies and may affect public health, safety and environment; and measure risks from social and ethical viewpoint. Ethical application of technologies is one of the aspects which affects stakeholders' perception of biotechnologies as safe. It also affects, whether the users of biotechnologies will integrate them in their daily experiences. At present, research in bioethics and biosafety in Latvia is at the beginning, and as the role of biotechnology becomes more prominent in everydays life, the bioethics and biosafety research will become more important.

Establishment of somatic stem cell genome stability and biosafety criteria for clinical studies

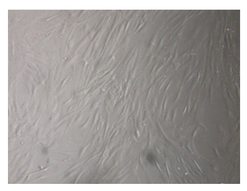
The aim of the study is to select appropriate biomarker panel to evaluate somatic stem cell stability during prolonged in vitro *propagation..*

Project has an approval from the local Ethical committee to collect human bone marrow and skin specimens. Mesenchymal stem cells are isolated, expanded and collected in cell bank for long term cyostorage. The cell bank consists of 57 bone marrow, 10 epidermal and 24 dermal human mesenchymal stem cell samples. We are currently developing two technologies that allow to monitor genomic and phenotypic stability of the mesenchymal stem cells from bone marrow, dermis and epidermis.

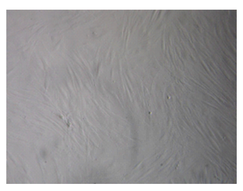
The genomic stability technology includes: 1) karyotyping by Giemsa staining method; 2) mycoplasma detection by PCR and fluorochemical methods; 3) telomerase (TERT) expression analysis by PCR.

The tumorigenesis screening technology involves analysis of several markers: 1) mesenchymal marker CD90, CD73, CD105 expression analysis by flow cytometry; 2) polyploidy analysis by flow cytometry; 3) proliferation marker Ki67 detection by immunofluorescence analysis; 4) TERT detection by immunofluorescence analysis.

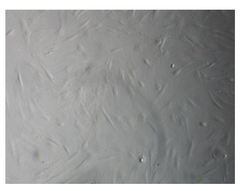
The results obtained so far indicate that human mesenchymal stem cells from dermis, epidermis and bone marrow have stable karyotype and do not express tumorigenesis markers during *in vitro* propagation.



Bone marrow MSCs



Dermal MSCs



Epidermal MSCs

Study on accumulation of antimicrobial drug resistance and on virulence factors in „innocent" bacteria

Drug resistant staphylococci are dangerous by their ability to colonize in healthy people and in medical personnel for a long time and to spread when appropriate conditions appear. The high prevalence of *Staphylococcus epidermidis* infections during the last years can be explained by the increased use of indwelling catheters and implanted devices. It is important to evaluate the risk of emerging resistant and virulent bacteria in the community and learn about mechanisms, which drive usually innocent bacteria from human microflora to become virulent. Importantly, the percentage of carriage of resistant staphylococci in Latvian community is not known yet.

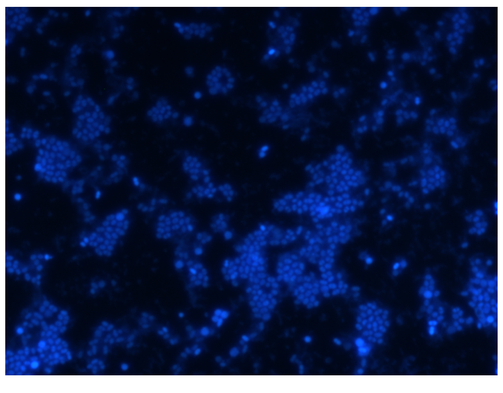
The aim of this study is to identify novel and to implement already known markers of resistance and virulence for staphylococci.

Methods

The selected markers of drug resistance and virulence were tested in two groups of samples, presented, firstly, by isolates from clinical cases and, secondly, by healthy people from the community (outward). All cultures were kept in cryobank system, and the corresponding data are stored in a database. DNA and RNA were isolated by magnetic MiniMag system (Biomeriux). In clinical group, 137 culture isolates were collected and 119 (86,86 %) were identified as *S.epidermidis* by BBL Crystal system (Becton Dickinson). Control group was presented by 74 *S.epidermidis* culture isolates. Methicillin susceptibility was tested phenotypically by cefoxitin disk and by methicillin resistance coding mecA gene. Biofilm formation, which is the main virulence factor, was explored by detection of several candidate genes and tested phenotypically by cultivation in biofilm forming conditions on microtiter plate.

Results

Results indicate that 106 (96,36%) of clinical samples were methicillin resistant, which means resistance to all beta-lactam antibiotics. In control group, methicillin resistance was detected in 70 samples. Of those, 60 (85,72%) were methicillin susceptible and 10 (14,28 %) were methicillin resistant.
Biofilm formation mechanism was extensively studied (see below). As the biofilm detector molecular marker, aap was found being the best candidate gene. Microtiter plate showed that 40.5% of tested clinical cultures are biofilm forming, while in contrast, only 25% of control group samples were biofilm positive.



Staphylococcus epidermidis forming biofilm on catheter surface, epifluorescent microscopy.

Conclusions

Preliminary results indicate on high prevalence and a risk to be infected by methicillin resistant staphylococci in hospital in Latvia. The percentage of resistance among healthy people is not high, however, it should be monitored during the next years. This study will help to determine new molecular criteria of biological risk rising from the mentioned bacteria. This project will also contribute to better knowledge about risk of resistance spread in Latvia outside hospitals.