Questionnaire
Summary of the main activities of a research institute of the Slovak Academy of Sciences

Period: January 1, 2016 - December 31, 2021

1. Basic information on the institute:

1.1. Legal name and address

Ústav molekulárnej biológie Slovenskej akadémie vied, v. v. i. (Institute of Molecular Biology, Slovak Academy of Sciences)

Dúbravská cesta 21, 845 51 Bratislava 45, Slovakia

1.2. URL of the institute web site

http://www.imb.savba.sk

1.3. Executive body of the institute and its composition

Directoriat	Name	Year of birth	Years in the position, from - to
Director	Ing. Eva Kutejová, DrSc.	1952	from 2016
	RNDr. Ján Kormanec, DrSc.	1959	from 2012 to 2016
Deputy director	Ing.Daniela Krajčíková, CSc.	1960	from 2018
	RNDr. Marian Farkašovský, CSc.	1961	from 2016 to 2018
	RNDr. Gabriela Bukovská, CSc.	1957	from 2008 to 2016
- for economy	Ing. Anna Varcholová	1952	from 1984
Scientific secretary	RNDr. Lucia Bocánová, PhD.	1985	from 2022
	Mgr. Ľuboš Kľučár, PhD.	1970	from 2016 to 2022

1.4. Head of the Scientific Board

RNDr. Imrich Barák, DrSc. (2016 - 2022)

Mgr. Ľuboš Kľučár, PhD. (from 2022)

1.4.1 Composition of the International Advisory Board

prof. Anthony Joseph Wilkinson (chair)
Structural Biology Laboratory, Department of Chemistry, Biosciences Building, Wentworth Way, University of York, UK

prof. Birte Svensson (member)
Enzyme and Protein Chemistry, Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark

prof. Erik Bongcam-Rudloff (member)
Faculty of Veterinary Medicine and Animal Science, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Sweden

1.5. Basic information on the research personnel

1.5.1. Fulltime equivalent work capacity of all employees (FTE all), FTE of employees with university degrees engaged in research projects (FTE researchers)

2016		2017		2018		2019		2020		2021		2016-2021	
FTE all	FTE researchers	FTE all	FTE researchers	FTE all	FTE researchers	FTE all	FTE researchers	FTE all	FTE researchers	FTE all	FTE researchers	average FTE all per year	average FTE researchers per year
60,58	41,86	59,69	43,47	58,15	39,98	57,69	39,69	56,67	41,66	61,89	45,57	**59,11**	**42,04**

1.5.2. If applicable, add also a short information on the merger of the institute in the evaluation period. You can also add rows in the above table corresponding to the founding institutes

none

1.6. Basic information on the funding of the institute

1.6.1. Institutional salary budget, other salary budget[1], non-salary budget[2]

Salary budget	2016	2017	2018	2019	2020	2021	average
Institutional salary budget *[millions of EUR]*	0,974	0,957	1,029	1,161	1,299	1,290	**1,118**
Other salary budget *[millions of EUR]*	0,029	0,096	0,117	0,104	0,084	0,176	**0,101**
Total salary budget *[millions of EUR]*	1,003	1,053	1,146	1,265	1,383	1,466	**1,219**
Non-salary budget *[millions of EUR]*	0,482	0,583	0,577	0,543	0,498	0,539	**0,537**

*****2021: A difference in comparison with the Final Report 2021 was caused by a payment in advance provided by the cross-border/international Interreg project. The advanced payment was provided for a purchase of the scientific equipment in the amount of 0.619344 million EUR.

1.7. Mission Statement of the Institute as presented in the Foundation Charter indicating the years when it was adopted and revised

During the evaluation period the Institute has updated its Foundation Charter several times, each time in relation to a change in its legal form:

- on 1 July 2017 the Institute changed its legal form from “budget organisation” to “contributory organisation”,
- on 1 July 2018 the Institute unsuccessfully attempted to change its legal form to “public research institution”,
- on 1 January 2022 the Institute successfully changed its legal form from “contributory organisation” to “public research institution”.

The following text describes the main parts of the current Foundation Charter (January 2022), which to a large extent overlaps the content of previous versions.

The predominant activity of the Institute is research and it is focused on basic research on the molecular principles responsible for the functioning of living systems/organisms. Special emphasis

[1] Salary budget originating outside the regular budgetary resources of the organization, e.g. from the project funding.
[2] Includes Goods and Services and PhD fellowships

is given to the molecular biology of prokaryotic and eukaryotic microorganisms. The research interests cover a wide range of fields including the biological sciences (molecular biology, microbiology, genetics, cell biology, neuroscience, mycology and virology), chemical sciences (biochemistry and bioorganic chemistry), biotechnology (environmental, industrial, medical and agricultural), earth and environmental sciences (ecology, protection and exploration of the landscape), bioinformatics, biophysics, immunology and nanotechnology.

Other main activities include

- providing and administering research and development infrastructure,
- retrieving, processing and disseminating information in the fields of science and technology and knowledge from our own research and development,
- implementing PhD education in terms of generally valid regulations,
- cooperating in the field of science and technology with universities, other legal entities carrying out research and development and entrepreneurs

Other activities include development and innovation based on demand from the government, as business activities or in the form of projects.

The main governing bodies of the institute are Director, Management Board, Scientific Board and Supervisory Board.

1.8. Summary of R&D activity pursued by the institute during the evaluation period in both national and international contexts. Describe the scientific importance and societal impact of each important result/discovery. Explain on a general level – the information should be understandable for a non-specialist (recommended 5 pages, max. 10 pages for larger institutes with more than 50 average FTE researchers per year as per Table 1.5.1.)

The research activities at the Institute comprise basic and applied research mainly in the fields of Molecular Biology, Microbiology and Biochemistry and are covered by four research departments:

Department of Biochemistry and Protein Structure (headed by Dr. Eva Kutejová)
ATP-dependent proteases and proteins of mitochondrial nucleoid in mitochondria, structural and functional studies on human ryanodine receptor 2, septin-mediated cell division, neurotransmitter transporters, molecular immunology, *in silico* studies of amylolytic enzymes

Department of Genomics and Biotechnology (headed by Dr. Ján Kormanec)
soil bacteria *Streptomyces* as producers of important biologically active secondary metabolites; bacteriophages, their genomes and their lytic, replication and structural proteins; bioinformatics and computational genomics

Department of Microbial Ecology (headed by Dr. Domenico Pangallo)
microbial communities responsible for the deterioration of cultural heritage objects; genomics, transcriptomics and proteomics of responses to oxidative stress in prokaryotes and eukaryotes with a focus on antioxidant enzymes; defence mechanisms of non-sporulating bacteria against different environmental stress factors

Department of Microbial Genetics (headed by Dr. Imrich Barák)
cell division, sporulation and programmed cell death, apimedical research, assessment of the quality and authenticity of honeybee products

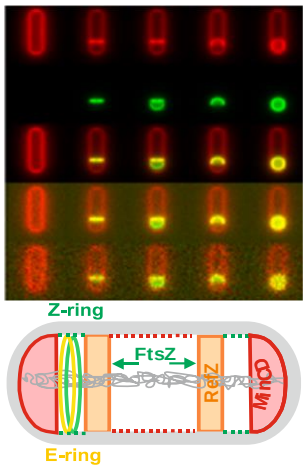
The key research areas where the most important scientific results were achieved are:

1. Bacterial cell division and differentiation
2. Protein structure-function relationships
3. Microbial ecology
4. Functional and computational genomics
5. Biotechnology
6. Molecular medicine

1. Bacterial cell division and differentiation

A *Bacillus subtilis* cell can measure its length and find the proper site of septation

(Department of Microbial Genetics)



Background and motivation: Probably the most controversial issue of *Bacillus subtilis* cell division concerns the mechanism that ensures the precise placement of the dividing septum in the centre of the cell during vegetative growth, but closer to one pole of the cell during sporulation (see figure at left) (Wollman et al., 2020). The molecular mechanism by which the cell locates the asymmetric septum site and the exact location of the asymmetric septum, have long been unknown.

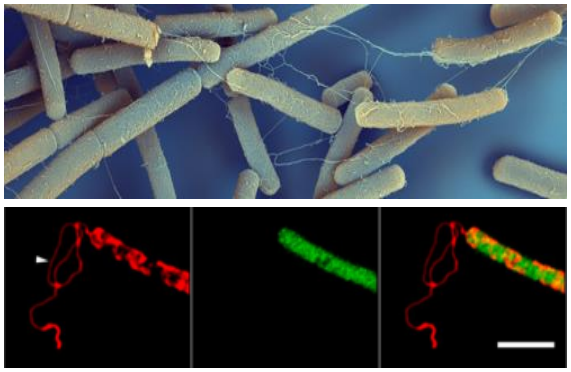
Main findings: In our work, we showed that an asymmetric septum forms at 1/6 of the cell length from the cell pole and that the accuracy of its localization is comparable to the accuracy of vegetative septum localization in the middle of the cell. We described the important role of the SpoIIE, RefZ and Min system proteins in determining the location of the asymmetric septum (see figure at left, bottom) (Barák and Muchová, 2018).

Significance: These results contribute to our understanding of how a cell can measure its length and find the specific septation site. Understanding the function of cell division proteins and their three-dimensional structure are crucial for developing new antibiotics against different pathogenic bacteria.

Bacterial nanotube formation as a "post mortem" manifestation of a stressed cell

(Department of Microbial Genetics)

Background and motivation: Bacterial nanotubes were discovered ten years ago. Their unique capabilities have suggested their involvement in processes such as DNA, RNA and protein transfer between the cells of various bacteria, as well as the "vampire-like" extraction of nutrients from eukaryotic cells.



Main findings: Our results are in stark contrast to previously published findings (Pospíšil et al., 2020). We showed that nanotubes, in principle, are formed from every cell when various stress factors are applied, such as pressure, or when the cells are exposed to antibiotics. The bacterial cell wall can maintain a pressure of up to twenty atmospheres inside the cell. However, if the wall is disturbed either mechanically or by the action of antibiotics, further maintenance of such high pressure is not possible. This pressure will cause the cytoplasmic membrane to be "fired out" in the form of a nanotube into the environment through the holes in the cell wall (see figure). An important finding was that just as the cell "fires out" these nanotubes, it dies.

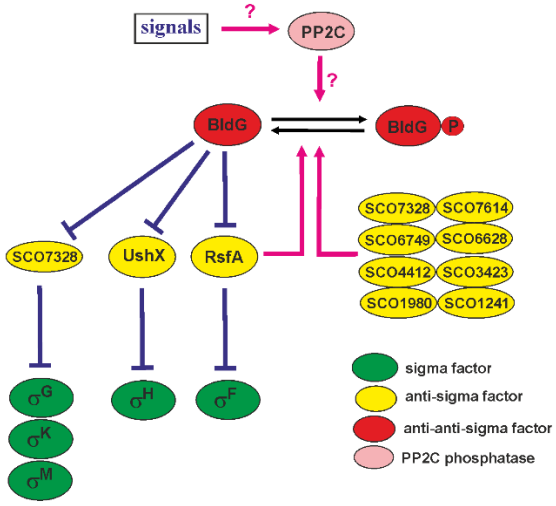
Significance: These results showed that the formation of nanotubes is not a controlled biological process, as previously believed, but a "post mortem" manifestation of a stressed cell. The findings of this research help us to understand the role of bacterial nanotubes in the spread of antibiotic resistance.

Exceptional cell differentiation regulation in *Streptomyces* by a complex cascade of RNA polymerase sigma factors

(Department of Genomics and Biotechnology)

Background and motivation: The *Streptomyces* gene expression program is extremely complex. The *S. coelicolor* genome contains genes for 65 RNA polymerase sigma factors. Unlike *B. subtilis*, which contains a single general stress response sigma factor SigB that is regulated by a partner-

switching phosphorylation mechanism involving anti-sigma factor RsbW, anti-anti-sigma factor RsbV, and two PP2C phosphatases, *S. coelicolor* contains nine SigB homologues with major roles in differentiation and response to osmotic stress, 45 RsbW homologues, 17 RsbV homologues, and 44 activating PP2C phosphatases.



Main findings: We identified and characterized the promoters recognized by these nine SigB homologues, several of which were cross-recognized by multiple sigma factors. However, immunoblot analysis revealed the presence of several SigB homologues at different developmental stages; this allows the recognition of specific promoters and the control of the expression of the corresponding genes only during these stages (Sevcikova et al., 2021). We also elucidated the signal transduction pathways that activate several SigB homologues, together with the roles of their corresponding anti-sigma factors and anti-anti-sigma factors. Interestingly, BldG, an anti-anti-sigma factor crucial for morpho-logical differentiation and antibiotic production, was found to have a pleiotropic role in the regulation of five SigB homologues: it interacts with 15 anti-sigma factors and is specifically phosphorylated by seven RsbW homologues (see the figure) (Sevcikova et al., 2020).

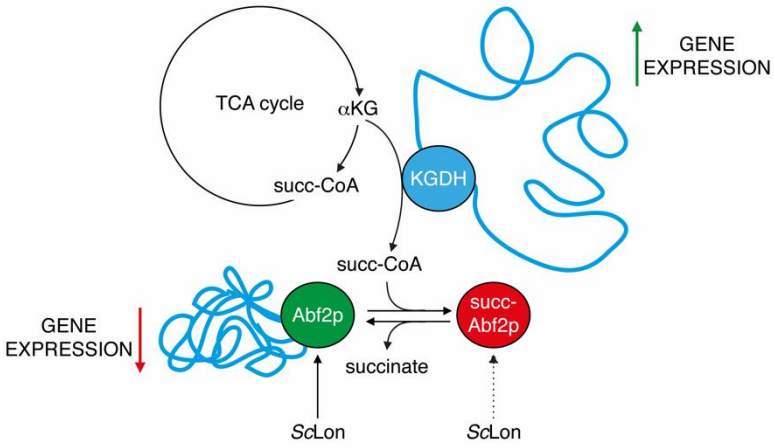
Significance: These results suggest that the anti-anti-sigma factor BldG uses a unique phosphorylation mechanism to activate five SigB homologues via multiple anti-sigma factors. This suggests that *S. coelicolor* A3(2) differentiation and stress response have a complex regulation mechanism. This is in contrast to the much simpler regulatory mechanisms known in nearly all Gram-positive bacteria.

2. Protein structure-function relationships

Biochemical and structural analysis of Lon, Abf2 and Mgm101 shed light on the mechanisms of mitochondrial nucleoid regulation and on their roles in nucleo-mitochondrial communication

(Department of Biochemistry and Protein Structure)

Background and motivation: Mitochondria are essential, semiautonomous organelles present in most eukaryotic cells. They possess their own genome, encoding mostly components of respiratory chain complexes. For proper functioning, the activity of the mitochondrial genome must be coordinated with its nuclear counterpart to provide all the components needed for the maintenance, expression and compaction of the mitochondrial DNA (mtDNA). mtDNA and its associated proteins form higher-order structures called mitochondrial nucleoids. In human cells, a failure in nuclear-mitochondrial commun-ication often results in fatal path-ologies; therefore, the investigation of the molecular mechanisms involved in mtDNA maintenance can have profound implications for human medicine.



Main findings: Lon protease is a major player in the regulation of mitochondrial homeostasis. This function of Lon was shown to be partly mediated by its regulation of mt nucleoid dynamics. Cryo-electron microscopy revealed that human Lon has a unique, highly flexible structure with its N-terminal domain playing an important role in the stability and activity of the enzyme. Exchanging ATP and ADP in its nucleotide-binding

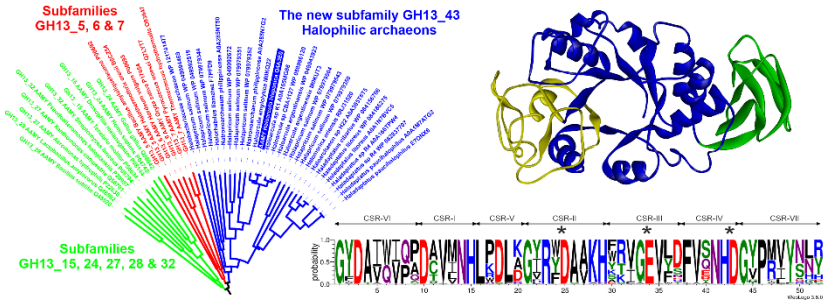
site resulted in a substantial rearrangement of its structure (Kereiche et al., 2016). This rearrangement is essential for the degradation of its substrates, which include Twinkle helicase and ribosomal subunit MrpL32 in human cells, and the mtDNA-packaging protein Abf2 and the mtDNA-maintenance factor Mgm101 in *S. cerevisiae*. These proteins become less susceptible to Lon degredation when bound to a nucleic acid, which affects mtDNA replication, transcription, and translation (Kunová et al., 2017). We also showed that Abf2 is modified by succinylation *in vivo* and that this affects not only its DNA-binding properties, but also its sensitivity to Lon (Frankovsky et al., 2021) (see figure). Moreover, we also found that Mgm101 in *C. parapsilosis*, which possesses a linear mitochondrial genome with telomeric ends, localizes near the tip of a model mitochondrial telomere, indicating its possible role in telomere maintenance (Pevala et al., 2016).

Significance: Pathological processes such as cancer and neurodegenerative disorders, like Parkinson's and Alzheimer's disease, are connected with significant changes in mitochondrial function. The results of our research revealed the function of essential mitochondrial homeostasis components, including the Lon protease and several mtDNA-binding proteins, and contributed to understanding how mitochondria react to the stress conditions caused by these pathologies. Moreover, knowledge of the Lon structure makes it possible to search for potential inhibitory agents that could modify its activity in cancer cells where it is upregulated.

In silico approaches to the study of amylolytic enzymes useful for their engineering and design

(Department of Biochemistry and Protein Structure)

Background and motivation: In protein chemistry, theoretical studies using *in silico* approaches represent, in general, the most reliable way of designing all kinds of experiments aimed at the rational characterization of protein structure, function and properties. Our main subject is various kinds of amylolytic enzymes that operate on starch and related alpha-glucans. These enzymes cover thousands of various sequences including tens of enzyme specificities from hydrolases, transferases and isomerases; many of them are industrially important enzymes. In the sequence-based classification of Carbohydrate-Active enZymes, the CAZy database (http://www.cazy.org/), they have been classified into several families of glycoside hydrolases (GHs).



New GH13 subfamily represented by the α-amylase from *Haloarcula hispanica*

Main findings: Our ongoing research involves comprehensive studies to describe the unique sequence-structural features of α-amylases, starch hydrolases and related enzymes (in their catalytic and non-catalytic domains) from both the main α-amylase family GH13 and the secondary α-amylase family GH57. Furthermore, and for the first time, a detailed bioinformatics analysis was performed on the GH126 family, identifying its conserved sequence regions and evolutionary relationships. In addition, several groups of more closely related amylolytic enzymes from families GH13 and GH57 were proposed to define novel GH13 and GH57 subfamilies and/or groups (see the figure). Finally, with respect to non-catalytic modules, i.e. the starch-binding domains classified in the CAZy database as the CBM families, several studies were devoted to the starch-binding domains from the families CBM20 (in the glucan phosphatase laforin involved in the Lafora-type of epilepsy), CBM41 (in industrially important pullulanases from family GH13) and we even predicted a potentially novel CBM (in amylomaltases from the family GH77).

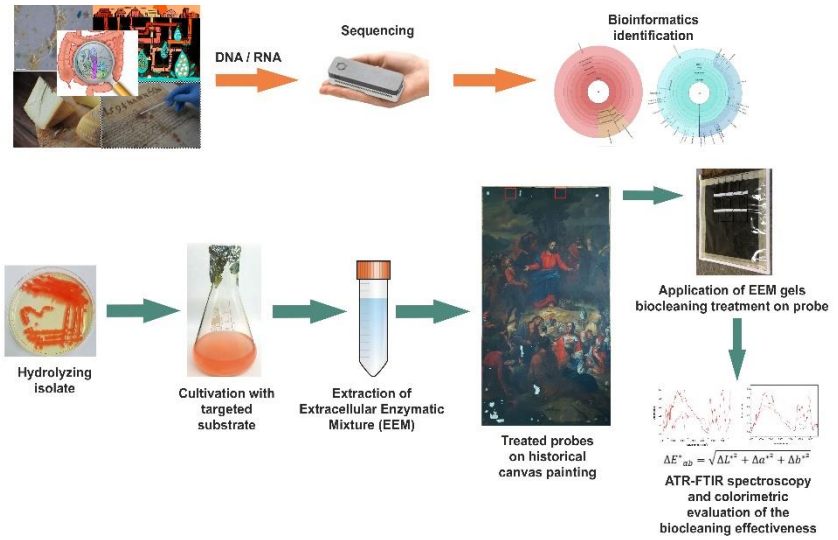
Significance: In addition to fundamentally contributing to basic knowledge on the structure-function relationships of amylolytic enzymes, the identification of their unique sequence-structural features may be used as sequence fingerprints for assigning the enzyme specificity of putative proteins obtained from genome sequencing as well as for their rational protein engineering and design. The information delivered by the *in silico* approaches is useful for wet-laboratory experimentalists in order for them to achieve their aims in a shorter time or by spending less money in their research.

3. Microbial ecology

High-throughput sequencing diagnostic and enzymatic bio-cleaning

(Department of Microbial Ecology)

Background and motivation: The study of microbial communities and the subsequent analyses of the metadata produced aids the understanding of the complex interactions between microbiota and various kinds of environments, including foods. The safeguarding of our health and cultural objects depends upon the safety and quality of our environment and foods. The microbial enzymatic potential of isolated microorganisms can be exploited for bio-cleaning processes oriented to cultural heritage bio-restoration.



Main findings: By analysing microbiota using high-throughput sequencing (see the related figure) we revealed: (i) the presence of dangerous microbial communities resistant to antibiotics, (ii) the microbial properties of food fermentation practices (Pangallo et al., 2019, Böhmer et al., 2020) and (iii) the micro-organisms responsible for the biodeterioration of cultural heritage objects (Kraková et al., 2018, Kisová et al., 2020). We are the authors of a new patent concerning the enzymatic bio-cleaning of proteinaceous residues present in various material surfaces, such as wood, paper and stone (Jeszeová et al., 2018). The lipolytic activity of a yeast enzymatic extract successfully removed the lipidic patina (procedure is showed in the figure) which affected the canvas of a historical painting (Kisová et al., 2021). Another enzymatic application regards the bio-removal of diverse synthetic and natural coloured stains occurring on historical books.

Significance: We are one of the few laboratories in Slovakia able to analyse the complete microbiota of different samples. To the best of our knowledge we are the only laboratory in Slovakia focused on the analysis of microbial communities in deteriorating cultural heritage items. The investigation of microbiota permits us to have a better view of the microorganisms present in a sample and therefore a precise diagnosis aids us in developing better solutions against pathogens, new potential therapies, safer and healthier food, valuable knowledge of particular environments and better preservation applications for our cultural and artistic heritage. The use of extracellular enzymatic extracts has a great potential to help restorers to eliminate synthetic and natural substrates, which form an unaesthetic patina on the surfaces of valuable cultural heritage objects, while avoiding the use of more dangerous chemical solvents.

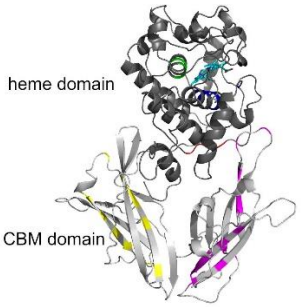
Highly efficient enzymatic antioxidants for environmental protection and molecular medicine

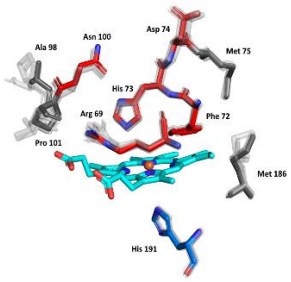
(Department of Microbial Ecology)

Background and motivation: Antioxidants are essential protective molecules for cellular oxidative metabolism and their significance for various areas of molecular medicine and biotechnology has been appreciated in recent years. Enzymatic antioxidants are oxidoreductases that specifically remove reactive oxygen species (ROS) which can damage the essential molecules of life (DNA, RNA, proteins and lipids) if not removed timely and efficiently. They include superoxide dismutases, catalases and peroxidises, which work together in a well-coordinated manner. We are investigating the genomics, transcriptomics, proteomics and secretomics of catalases and peroxidases originating from diverse microbial species, both prokaryotic and eukaryotic. Our aim is to find the best candidates among numerous peroxidase families for decomposing various toxic and recalcitrant compounds present in the environment. As ingredients of rational foods and cosmetics, these

enzymes can contribute to health care. Particular heme peroxidases were found to be essential components of innate immunity in humans.

Main findings: We discovered a completely new subfamily of Hybrid B heme peroxidases that are present in various fungi and have a protective antioxidant role in their metabolism. We optimized the heterologous expression of selected antioxidant enzymes in bacteria and methylotrophic yeast to get high yields that can be directly used in downstream applications. In this connection, we are the authors of a recent patent dealing with the rapid preparation and purification of huge amounts of a bifunctional catalase-peroxidase from a hyperthermophilic archaeon. Its proven thermostability and high catalytic performance are of great importance for direct application in removing harmful peroxides left from bleaching in the textile industry and for removing phenolic and other harmful compounds from wastewaters. We are also investigating the peroxidases serving as important components of innate immunity in invertebrates which share significant homology with mammalian thyroid peroxidases.





Wood fungus as a source of → Hybrid heme peroxidase → Insight in the active centre removing harmful peroxides

Significance: We are involved in an international consortium that maintains the bioinformatics database *RedoxiBase* (Savelli et al., 2019), which provides updated knowledge on thousands of antioxidant enzymes from dozens of divergent gene families. Collected data and the results obtained for oxidoreductase enzymes of interest (e.g. the hybrid heme peroxidase from a wood fungus seen in the figure above) can be directly used in various applications to remove dangerous peroxides (Chovanová et al., 2019) and phenolic substances from the environment by oxidizing them to harmless products and releasing molecular oxygen, which contributes to a clean atmosphere. Studying heme peroxidases and producing them recombinantly in significant amounts will allow the preparation of rational foods and cosmetics and their involvement in future medical studies to replace generic antibiotics, which have become less efficient due to increasing microbial resistance.

4. Functional and computational genomics

Efficient genome editing system in *Streptomyces* and the utility of recombinant strains in drug and enzyme production

(Department of Genomics and Biotechnology)

Background and motivation: Our Institute was involved in the large EU FP7 collaborative project STREPSYNTH, which aimed to develop a new industrial platform based on streptomycetes for commercially interesting biomolecules by creating an effective system for the targeted modification of the *Streptomyces lividans* genome to prepare a collection of strains with improved biotechnological properties for the production of small biologically active substances and biotechnologically and clinically relevant proteins.

Main findings: We developed a simple and efficient genome editing system that allows for large deletions and variable markerless insertions of foreign genes under the control of heterologous strong and regulated promoters. Using this system, we deleted several genes and gene clusters for secondary metabolites in the *S. lividans* genome and prepared a final collection of 27 *S. lividans* RedStrep strains where the gene clusters responsible for the production of interfering secondary metabolites and other relevant genes were progressively deleted. In collaboration with the Spanish biotechnology company EntreChem, a project partner, we demonstrated a dramatic increase in the heterologous production of the antitumor agent mithramycin A in these genetically engineered RedStrep strains (up to 3 g/L medium) (Novakova et al., 2018). Several strains had dramatically increased model protein secretion compared to the wild-type S*. lividans* strain

(Rezuchova et al., 2018). Based on these successful results, we were contacted by an Irish biotechnology company and signed a contract to improve the heterologous secretion of their product-of-interest in these strains. These strains were further manipulated and used for the high heterologous secretion of their protein. These results were included in a joint foreign patent application submitted in July 2021.

Significance: Our new genome editing system will allow simple and efficient markerless modifications of *Streptomyces* genomes, as verified by the above results. It makes it possible to prepare modified strains usable for biotechnological applications for the production of clinically important drugs and enzymes.

5. Biotechnology

Approaches to cope with the resistance of pathogenic bacteria

(Department of Genomics and Biotechnology)

Background and motivation: Resistance to antibiotics is currently a serious problem, not only in hospitals. There is, therefore, an urgent need to prepare new bioactive compounds. The use of molecular biology techniques to manipulate the genes and genomes of *Streptomyces* bacteria, which are the main producers of bioactive natural products, opens up exciting possibilities for the discovery of new biologically active substances. In addition to antibiotics, bacteriophages and their lytic proteins (endolysins) represent a new tool for countering the alarming rise of antibiotic resistant bacteria. We also sought to elucidate the all the mechanisms by which honeybee products exert antibacterial activities, including those outside of the classical ligand-target paradigm, and to characterise their additional therapeutic activities.

Main findings: We identified a biosynthetic gene cluster (BGC) in *Streptomyces lavendulae* subsp. *lavendulae* CCM 3239 containing the biosynthetic genes for the angucycline and pyro naphthoquinone classes of polyketides. This cluster was responsible for the production of the antibiotic auricin, active against Gram-positive bacteria, and it displays modest cytotoxicity against several human tumour cell lines. Auricin is transiently produced during a narrow time interval and this unusual production is due to the strict and complex regulation of its biosynthesis by several pathway-specific regulators. Structural analysis of auricin revealed that it possesses intriguing structural features that distinguish it from all other known angucyclines. Together with the interesting structure of the auricin BGC, this suggests that auricin is biosynthesized through a unique and as yet undescribed mechanism using two overlapping biosynthetic pathways, one for angucyclines and another for pyronaphthoquinones (Matulova et al., 2019). We determined the complete genomic sequence of *S. lavendulae* subsp. *lavendulae* CCM 3239, as well as its transcriptome. In addition to the auricin BGC, a bioinformatics analysis identified 31 other BGCs for potentially novel secondary metabolites, which were silent under laboratory conditions (Busche et al., 2018). We prepared and characterized several new recombinant endolysins derived from prophages of human clinical isolates of *Streptococcus agalactiae*, which is a major neonatal pathogen, causing sepsis and meningitis in newborns. In adults, this opportunistic pathogen colonizes the gastrointestinal and genitourinary tracts of up to 50% of healthy adults (Lichvarikova et al., 2020). The exolytic activity of these endolysins was demonstrated on different *Streptococcus agalactiae* serotypes, *Bacillus subtilis*, *Lactobacillus jensenii*, and *Escherichia coli*. The best candidate, EN534-C, lysed streptococci, but not beneficial vaginal lactobacilli. This recombinant endolysin could potentially serve as an antimicrobial agent against *S. agalactiae* urogenital infections and also in the prophylactic decolonization of pregnant women in the prevention of neonatal diseases. The production and application of endolysin EN534-C are part of two patent applications (PP 50075-2020 and PCT/SK2021/050016).

We have also identified several biologically active bee-derived compounds possessing strong antibacterial and antibiofilm effects and characterised their mechanisms of action. A recombinantly-prepared form of the bee antibacterial peptide defensin-1 was successfully pre-clinically tested in an excisional wound animal model (Bucekova et al., 2017). Apart from their therapeutic potential, bee-derived biologically active compounds have been successfully tested as a marker of honey quality and authenticity. To this end, a novel competitive enzyme-linked immunosorbent assay for quantifying defensin-1 in honey has been developed.

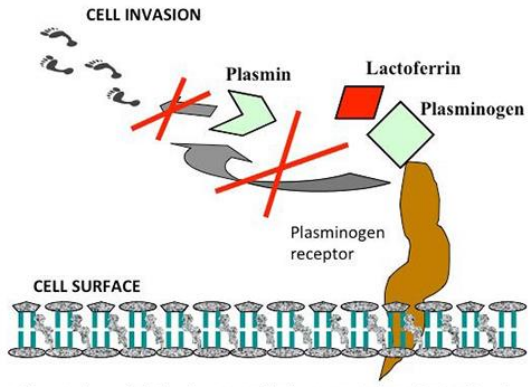
Significance: (i) We identified and characterized a new and unique antibiotic, auricin, with anti-tumor activity and an unusual biosynthetic pathway. Standard molecular biology approaches should allow modified forms with improved properties to be prepared. (ii) *S. lavendulae* subsp. *lavendulae* CCM 3239 has great potential for producing new biologically active compounds. (iii) A new recombinant endolysin was prepared for the treatment of *Streptococcus agalactiae* urogenital infections and also for the prophylactic decolonization of pregnant women in the prevention of neonatal diseases. (iv) *In vitro* and pre-clinical studies clearly showed that the bee-derived peptide, defesnin-1, possesses dual action in wound healing: antibacterial (antibiofilm) and re-epithelisation. These results increase the likelihood of translating its use to clinical practice.

6. Molecular medicine

Physiological and pathophysiological processes associated with the immune system at the molecular and cellular level

(Department of Biochemistry and Protein Structure)

Background and motivation: Physiological and pathophysiological processes associated with the immune system at the molecular and cellular level require manifold interactions between soluble factors, cell surface receptors and intracellular signalling molecules, and their spatial and temporal organisation. The loss of control over these interactions leads to various impairments and pathological conditions, e.g. tumorigenesis and inflammatory disorders.



Schematic model: The human milk glycoprotein lactoferrin directly binds to the protease plasminogen, blocks its conversion to the active form plasmin, which results in the inhibition of cell invasion.

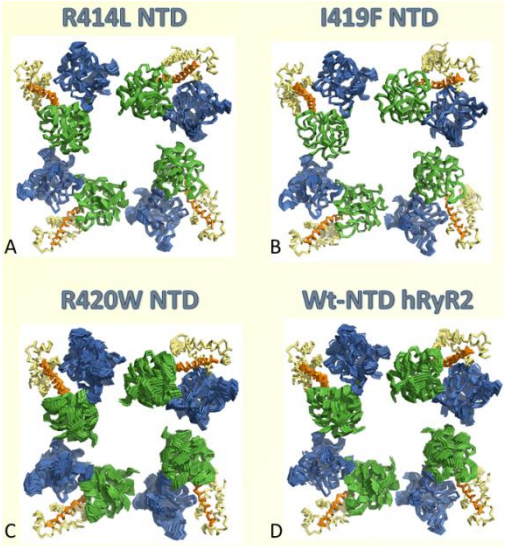
Main findings: Our research has focused on the molecular devices controlling pericellular proteolysis and protein transport. To date, our data strongly indicate that the mannose 6-phosphate/insulin-like growth factor 2 receptor (CD222) has a regulatory role in several cellular functions, including proteolysis, cell migration, signal transduction, and endocytosis. We have found that CD222 plays a crucial role in down-regulating plasmin activity (Leksa et al., 2017), in transporting the primary T cell kinase, Lck (Pfisterer et al., 2014), and in efferocytosis (Ohradanova-Repic et al., 2019), a process for removing apoptotic cells. We also identified a natural inhibitor of pericellular proteolysis in lactoferrin (Zwirzitz et al., 2018), a human milk glycoprotein with antimicrobial activities.

Significance: We have two overall objectives: (i) to understand the molecular mechanisms and determinants controlling the pericellular proteolysis and intracellular protein trafficking involved either in maintaining homeostasis or in disease progression; and (ii) to modulate pharmacologically their imbalance in human pathologies, namely, in infectious diseases, e.g. SARS-CoV-2, but also in inflammatory and cancer diseases. Thus, our research is of significant interest not only for scientists in basic research, but also for medical doctors and pharmacological companies.

Alteration of the dynamic motion of the N-terminal domain of the human ryanodine receptor 2: a possible cause of several cardiac arrhythmias

(Department of Biochemistry and Protein Structure)

Background and motivation: The human cardiac ryanodine receptor (hRyR2) plays an essential role in cardiac muscle contraction and is primarily responsible for regular heartbeat. Mutations of this channel are associated with several inherited cardiac arrhythmias (CPVT1, ARVC/D2, syncopes of unknown origin, sudden cardiac death and sudden infant death syndrome). These mutations appear to cluster in three main parts of the hRyR2 channel: the N-terminal, central and C-terminal. Our goal was to investigate the impact of three mutations: R414L, I419F and R420W, which are associated with CPVT1 and ARVD2, on the dynamics of the hRyR2, in particular its N-terminal domain (NTD) with the aim to understand better the role of these mutations in the gating of hRyR, by molecular dynamics.



Main findings: We found that the R414L and I419F mutations diminish the overall amplitude of motion without greatly changing the direction of motion of the individual domains (see figure, A, B), whereas R420W both enhances the amplitude and changes the direction of motion (C). Based on these results, we propose that R414L and I419F hinder channel closing, whereas R420W may enhance channel opening. Overall, we hypothesize that the wild-type hRyR2 (D) possesses a moderate level of flexibility which allows the gate to close and not easily open without an opening signal. These three mutations, however, disrupt this balance by making the gate either too rigid or too loose, causing closing to become either more difficult or less effective (Bauer et al., 2020).

Significance: Cardiac arrhythmias are one of the most serious illnesses, decreasing quality of life or, in some cases, causing death. Among the most malignant and difficult to treat of these is CPVT, which is often induced by stress during adolescence or early adulthood. Understanding the molecular causes and mechanisms of this illness will substantially help its individual therapy and improve predictions for those who may have inherited a disposition for this illness.

2. Partial indicators of main activities:

2.1. Research output

2.1.1. Principal types of research output of the institute: basic research/applied research, international/regional (in percentage)

During the evaluation period, the majority of the research carried out at the Institute was basic research although recent years have seen a significant increase in applied research outputs. This is partly due to our initiation of several collaboration projects which finished in applications for patents. In addition, in 2016 our research portfolio was extended by the new working group of Dr. Majtán and his team, who work on several applications from apimedical research. Therefore, the distribution of our main activities has partially shifted in favour of applied research from 10 to 20% of all research activities at the Institute during the evaluation period.

basic research: 80%
applied research: 20%

Research at the Institute is mostly based on well-established and internationally focused areas of Molecular Biology, Microbiology and Biochemistry. A small part of our work targets the local environment (apimedical research, conservation and preservation of cultural heritage)

international research: 90%
regional research: 10%

2.1.2 List of selected publications documenting the most important results of basic research. The total number of publications should not exceed the number of average FTE researchers per year. The principal research outputs (max. 10% of the total number of selected publications, including Digital Object Identifier – DOI if available) should be underlined / in bold. Authors from the evaluated organizations should be underlined.

Bacterial cell division and differentiation

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Microbial ecology

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5. BUČEKOVÁ, Marcela - JURICOVÁ, Valéria - DI MARCO, G. - GISMONDI, A. - LEONARDI, D. - CANINI, A. - MAJTÁN, Juraj**. Effect of thermal liquefying of crystallised honeys on their antibacterial activities. In *Food chemistry*, 2018, vol. 269, p. 335-341. (2017: 4.946 - IF, Q1 - JCR, 1.793 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0308-8146. https://doi.org/10.1016/j.foodchem.2018.07.012
6. BEKEOVÁ, Carmen - REHÁKOVÁ, Alena - FECKOVÁ, Ľubomíra - VLČKOVÁ, Silvia - NOVÁKOVÁ, Renáta - MINGYAR, Erik - KORMANEC, Ján. Characterisation of the genes involved in the biosynthesis and attachment of the aminodeoxysugar D-forosamine in the auricin gene cluster of Streptomyces aureofaciens CCM3239. In *Applied Microbiology and Biotechnology*, 2016, vol. 100, p 3177-3195. (2015: 3.376 - IF, Q2 - JCR, 1.256 - SJR, Q1 - SJR, CCC). (2016 - Current Contents). ISSN 0175-7598. https://doi.org/10.1007/s00253-015-7214-9
7. HALGAŠOVÁ, Nora - KRAJČÍKOVÁ, Daniela - KRAUS, Daniel - BUKOVSKÁ, Gabriela**. The helicase core accessory regions of the phage BFK20 DnaB-like helicase gp43 significantly affect its activity, oligomeric state and DNA binding properties. In *Virology*, 2021, vol. 558, p. 96-109. (2020: 3.616 - IF, Q3 - JCR, 1.389 - SJR, Q2 - SJR, CCC). (2021 - Current Contents). ISSN 0042-6822. https://doi.org/10.1016/j.virol.2021.02.016
8. BUČEKOVÁ, Marcela - BURIOVÁ, Monika - PEKÁRIK, Ladislav - MAJTÁN, Viktor - MAJTÁN, Juraj**. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. In *Scientific Reports*, 2018, vol. 8, art. no. 9061. (2017: 4.122 - IF, Q1 - JCR, 1.533 - SJR, Q1 - SJR, CCC). (2018 - Current Contents, WOS, SCOPUS). ISSN 2045-2322. https://doi.org/10.1038/s41598-018-27449-3

Molecular medicine

1. ZWIRZITZ, A. - REITER, M. - ŠKRABANA, Rostislav - OHRADANOVA-REPIC, A. - MAJDIC, O. - GUTEKOVÁ, Marianna - CEHLÁR, Ondrej - PETROVČÍKOVÁ, Eva - KUTEJOVÁ, Eva - STANEK, G. - STOCKINGER, H. - LEKSA, Vladimír**. Lactoferrin is a natural inhibitor of plasminogen activation. In *Journal of Biological Chemistry*, 2018, vol. 293, p. 8600-8613. (2017: 4.011 - IF, Q2 - JCR, 2.672 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0021-9258. https://doi.org/10.1074/jbc.RA118.003145
2. GACEK-MATTHEWS, A. - CHROMÍKOVÁ, Zuzana - SULYOK, Michael - LÜCKING, G. - BARÁK, Imrich** - EHLING-SCHULZ, M.**. Beyond toxin transport: novel role of ABC transporter for enzymatic machinery of cereulide NRPS assembly line. In *mBio*, 2020, vol. 11, no. e01577. (2019: 6.784 - IF, Q1 - JCR, 3.876 - SJR, Q1 - SJR). ISSN 2150-7511. https://doi.org/10.1128/mBio.01577-20
3. OHRADANOVA-REPIC, A. - MACHACEK, C. - DONNER, C. - MÜHLGRABNER, Vanessa - PETROVČÍKOVÁ, Eva - ZAHRADNÍKOVÁ, Alexandra, ml. - VIČÍKOVÁ, Kristína - HOŘEJŠÍ, Václav - STOCKINGER, H. - LEKSA, Vladimír**. The mannose 6-phosphate/insulin-like growth factor 2 receptor mediates plasminogen-induced efferocytosis. In *Journal of Leukocyte Biology*, 2019, vol. 105, no. 3, p. 519-530. (2018: 4.012 - IF, Q2 - JCR, 1.929 - SJR, Q1 - SJR, CCC). (2019 - Current Contents). ISSN 0741-5400. https://doi.org/10.1002/JLB.1AB0417-160RR

2.1.3 List of monographs/books published abroad

1. ABC01 BAUER, Jacob** - BAUEROVÁ-HLINKOVÁ, Vladena. Normal mode analysis: a tool for better understanding protein flexibility and dynamics with application to homology models. In *Homology molecular modeling: perspectives*

and applications. - London: IntechOpen, 2021, p. 13-30. ISBN 978-1-83962-805-4. http://dx.doi.org/10.5772/intechopen.94139

2. ABC02 BUČEKOVÁ, Marcela - VALACHOVÁ, Ivana - MAJTÁN, Juraj. Enzým glukózo oxidáza - kľúčový faktor podmieňujúci antibakteriálne vlastnosti včelieho medu a jeho úloha v ochrane včelstva. In *Ekologie chovu včel*. - Nakladatelství Pavel Mervart, 2016, s. 97-115. ISBN 978-80-7465-215-8.

3. ABC03 FURTMULLER, P.G. - ZÁMOCKÝ, Marcel - HOFBAUER, S. - OBINGER, C. Evolution, structure and biochemistry of human peroxidases. In *Mammalian heme peroxidases: diverse roles in health and disease*. - London: CRC Press, 2021, p. 3-20. ISBN 978-1-0032-1228-7.

4. ABC04 JANEČEK, Štefan. Alpha-amylases from Archaea: sequences, structures and evolution. In *Biotechnology of Extremophiles: advances and challenges*. - Springer Inter. Publ., 2016, p. 505-524. ISBN 978-3-319-13521-2. https://doi.org/10.1007/978-3-319-13521-2

5. ABC05 KORMANEC, Ján - ŠEVČÍKOVÁ, Beatrica - NOVÁKOVÁ, Renáta - HOMEROVÁ, Dagmar - REŽUCHOVÁ, Bronislava - MINGYAR, Erik. The complex regulatory network in the regulation of stress-response sigma factors in Streptomyces coelicolor A3(2). In *Stress and environmental regulation of gene expression and adaptation in bacteria*. - John Wiley & Sons, Inc., 2016, p. 328-343. ISBN 978-1-119-00488-2. https://doi.org/10.1002/9781119004813.ch29

6. ABC06 KORMANEC, Ján** - REŽUCHOVÁ, Bronislava - NOVÁKOVÁ, Renáta. Screening systems for stable markerless genomic deletions/integrations in Streptomyces species. In *Antimicrobial therapies : methods and protocols*. - New York : Springer-Verlag, Humana Press, 2021, p. 91-141. ISBN 978-1-0716-1358-0. https://doi.org/10.1007/978-1-0716-1358-0_6

7. ABC07 LEKSA, Vladimír** - SCHILLER, H.B. - STOCKINGER, H. Biotin-chasing assay to evaluate uPAR stability and cleavage on the surface of cells. In *Proteases and Cancer : methods and protocols*. - New York : Humana Press, 2018, p. 39-47. ISBN 978-1-4939-7595-2. https://doi.org/10.1007/978-1-4939-7595-2_4

8. ABC08 OTLEWSKA, A. - KRAKOVÁ, Lucia - PANGALLO, Domenico. Potential of culture-independent and molecular methods in the biodeterioraton study of historical paper-based materials. In *A modern approach to biodeterioration assessment and the disinfection of historical book collections*. - Lodz : Lodz Univ. Technology, 2016, p. 43-55. ISBN 978-83-63929-01-5.

9. ABC09 PIETRZAK, K. - KOZIROG, A. - BUČKOVÁ, Mária - PUŠKÁROVÁ, Andrea - SCHOLTZ, V. Disinfection methods for paper. In *A modern approach to biodeterioration assessment and the disinfection of historical book collections*. - Lodz: Lodz Univ. Technology, 2016, p. 56-80. ISBN 978-83-63929-01-5

10 ABC10 PIETRZAK, K. - OTLEWSKA, A. - DYBKA, K. - DANIELEWICZ, D. - PANGALLO, Domenico - DEMNEROVA, K. - ĐUROVIĆ, Momir - KRAKOVÁ, Lucia - SCHOLTZ, V. - BUČKOVÁ, Mária. A modern approach to biodeterioration assessment and disinfection of historical book. In *A modern approach to biodeterioration assessment and the disinfection of historical book collections*. - Lodz: Lodz Univ. Technology, 2016, p. 81-125. ISBN 978-83-63929-01-5.

11. ABC11 PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - PANGALLO, Domenico. Biodeterioration of photographic and cinematographic materials: methods of investigation. In *Biodeterioration and preservation in art, archaeology and architecture*. - London: Archetype, 2018, p. 57-70. ISBN 978-1-9094-9264-6.

12. ABC12 REBETS, Y. - KORMANEC, Ján - LUZHETSKYY, A. - BERNAERTS, K. - ANNÉ, J. Cloning and expression of metagenomic DNA in Streptomyces lividans and subsequent fermentation for optimized production. In *Metagenomics : methods and Protocols*. - New York : Springer, 2017, p. 99-144. (2016: 0.585 - SJR, Q3 - SJR). (2017 - SCOPUS). ISBN 978-1-4939-6689-9. https://doi.org/10.1007/978-1-4939-6691-2_8

13. ABC13 ROBERTS, M. - ROWLEY, G. - KORMANEC, Ján - ZALM, M.E. The role of alternative sigma factors in pathogen virulence. In *Foodborne pathogens: virulence factors and host susceptibility*. - Springer Inter. Publ., 2017, p. 229-303. ISBN 978-3-319-56834-8. https://doi.org/10.1007/978-3-319-56836-2_9

14. ABC14 SOJKA, Martin - HORNIAČKOVÁ, Miroslava - BUČEKOVÁ, Marcela - MAJTÁN, Viktor - MAJTÁN, Juraj**. Antibiofilm efficacy of honeybee products against wound biofilm. In *Biofilm, pilonidal cysts and sinuses*. - Springer, Cham, 2020, p. 89-108. ISBN 978-3-030-03076-6.

2.1.4. List of monographs/books published in Slovakia

1. AEDA01 DAROLOVÁ, Alžbeta** - KRIŠTOFÍK, Ján - MAJTÁN, Juraj - ZEMAN, Michal - OKULIAROVÁ, Monika - KNAUER, Felix - RUBÁČOVÁ, Lucia - HOI, Herbert. Does breeding environment affect eggshell bacteria load and female antibacterial defence investment? In *Tichodroma: ornitologický časopis*, 2018, roč. 30, s. 35-47. (2018 - Zoological Record). ISSN 1337-026X.

2.1.5. List of other scientific outputs specifically important for the institute, max. 10 items for institute with less than 50 average FTE researchers per year, 20 for institutes with 50 – 100 average FTE researchers per year and so on

- The Institute has developed and maintains several unique biological databases: www.phisite.org (2011), www.phibiotics.org (2013) www.virusite.org (2016) and www.bombase.org (work in progress). The popularity of these resources is reflected by their high number of unique visitors. During the evaluation period 2016–2021 *phiBIOTICS* had 3.2K visitors, *phiSITE* 61K visitors, and *viruSITE* 11K visitors; we noticed an increased use of *viruSITE* from spring 2020 in relation to its use as a tool for COVID-19-related research. The paper describing viruSITE (Stano at al., 2016) is among our most cited papers.

- The Institute has participated in the assembly of several new genomes:
  - the complete genomic sequence of our long-studied model strain *Streptomyces lavendulae* subsp. *lavendulae* CCM 3239, which produces the unique angucycline antibiotic auricin; the strain consists of a linear chromosome of 8,691,831 bp and the large linear plasmid pSA3239 of 241,081 bp (BioProject PRJNA407779)
  - the complete and fully annotated genomic sequence of the bacterium *Lactiplantibacillus plantarum* strain:LS/07, consisting of a circular chromosome (3,182,330 bp) and five plasmids (17–82 kbp) (BioProject PRJNA513985)
  - the full genome of the ascomycete fungi *Chaetomium cochliodes* strain CCM F-232, 34,745,808 bp in size (BioProject PRJNA309375)
  - the whole genome shotgun project for the ascomycete fungi *Thermochaetoides dissita* strain:CBS 180.67 genome (BioProject PRJNA595853)

- The Institute has participated in determining several important 3D protein structures deposited in the Protein Data Bank (PDB):
  - 5OHX: GIMÉNEZ-MASCARELL, P. - MAJTÁN, T. - OYENARTE, I. - EREÑO-ORBEA, J. - MAJTÁN, Juraj - KLAUDINY, Jaroslav - KRAUS, J.P. - MARTÍNEZ-CRUZ, L.A. Crystal structure of cystathionine β-synthase from honeybee Apis mellifera. In *Journal of Structural Biology,* 2018, vol. 202, p. 82-93. (3.433 - IF2017). (2018 - Current Contents). ISSN 1047-8477. https://doi.org/10.1016/j.jsb.2017.12.008
  - 6WEB, 6WEC: HOLMES S, et al. “Megahertz pulse trains enable multi-hit serial crystallography experiments at XFELs.” (To be published.)
  - 6FTR, 6GTH: WIEDORN, M.O. - OBERTHÜR, D. - BEAN, R. - SCHUBERT, R. - WERNER, N. - BARÁK, Imrich. Megahertz serial crystallography. In *Nature Communications,* 2018, vol. 9, no. 4025. (12.353 - IF2017). (2018 - Current

Contents, WOS, SCOPUS). ISSN 2041-1723. https://doi.org/10.1038/s41467-018-06156-7

- 5JHX, 5JHY, 5JHZ: GASSELHUBER, B. - GRAF, M.M. - JAKOPITSCH, C. - ZÁMOCKÝ, Marcel - NICOLUSSI, A. - FURTMULLER, P.G. - OOSTENBRINK, C. - CARPENA, X. - OBINGER, C. Interaction with the Redox Cofactor MYW and functional role of a mobile arginine in eukaryotic catalase-peroxidase. In *Biochemistry,* 2016, vol. 55, p. 3528–3541. (2.876 - IF2015). (2016 - Current Contents). ISSN 0006-2960. https://doi.org/10.1021/acs.biochem.6b00436
- 6ERC: NICOLUSSI, A. - DUNN, J.-D. - MLYNEK, G. - BELLEI, M. - ZÁMOCKÝ, Marcel - BATTISTUZZI, G. - DJINOVIC-CARUGO, K. - FURTMULLER, P.G. - SOLDATI, T. - OBINGER, C. Secreted heme peroxidase from Dictyostelium discoideum: Insights into catalysis, structure, and biological role. In *Journal of Biological Chemistry,* 2018, vol. 293, p. 1330-1345. (4.011 - IF2017). (2018 - Current Contents). ISSN 0021-9258. https://doi.org/10.1074/jbc.ra117.000463

- The Institute has participated in reconstructing the cryo-EM density maps of the hLon S855A mutant incubated with AMP-PNP and ADP. The hLon structures are deposited in the Electron Microscopy Data Bank under accession codes EMD-3275 and EMD-3274.

2.1.6. List of patents, patent applications, and other intellectual property rights registered abroad

PCT

ANTIMICROBIAL PROTEIN, ANTIMICROBIAL RECOMBINANT PROTEIN WITH LYTIC PROPERTIES, EXPRESSION VECTOR, METHOD OF THEIR PREPARATION AND USE
Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Slovakia; Comenius University Faculty of Natural Sciences; *WO*: 16.12.2021; PCT/SK2021/050016; *Status*: in proceeding *MPT*: C12N 9/00; Patent family in 152 countries.

Graphical symbols/Logos

Three Quality marks based on honey antibacterial activity designed and granted by the Institue

- *EUIPO No*.:008130330-0001, registered 19/08/2020, *Legal Status*: Legal entity, *Design status*: registered and fully published (A.1)
- *EUIPO No*.:008130330-0002, registered 19/08/2020, *Legal Status*: Legal entity, *Design status*: registered and fully published (A.1)
- *EUIPO No*.:008130330-0003, registered 19/08/2020, *Legal Status*: Legal entity, *Design status*: registered and fully published (A.1)

  Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Slovakia

2.1.7. List of patents, patent applications, and other intellectual property rights registered in Slovakia

Patent application

1. POLYPEPTID REKOMBINANTNEJ KATALÁZY-PEROXIDÁZY, SPÔSOB JEJ PRODUKCIE V BUNKÁCH ESCHERICHIA COLI A JEJ POUŽITIE

POLYPEPTIDE OF A RECOMBINANT CATALASE-PEROXIDASE, MODE OF ITS PRODUCTION IN THE CELLS OF *ESCHERICHIA COLI* AND ITS APPLICATIONS

Application No.: PP50015-2019/15.3.2019, *Status*: published MPT: C12N 9/08, C12N 15/53, C12N 1/21
Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Comenius University Faculty of Natural Sciences, Slovakia

2. ANTIMIKROBIÁLNY PROTEÍN, ANTIMIKROBIÁLNY REKOMBINANTNÝ PROTEÍN S LYTICKÝMI VLASTNOSŤAMI, EXPRESNÝ VEKTOR, SPÔSOB ICH PRÍPRAVY A POUŽITIE

ANTIMICROBIAL PROTEIN, ANTIMICROBIAL RECOMBINANT PROTEIN WITH LYTIC PROPERTIES, EXPRESSION VECTOR, METHOD OF THEIR PREPARATION AND USE

Application No.: PP 50075-2020/17.12.2020, *Status*: in proceeding MPT: C12N 9/78, C12N 15/63, A61K 38/50, A61P 31/04

Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Comenius University Faculty of Natural Sciences, Slovakia

Patent

BIOPREPARÁT Z EXIGUOBACTERIUM UNDAE, SPÔSOB JEHO VÝROBY A JEHO POUŽITIE

BIOPREPARATE FROM EXIGUOBACTERIUM UNDAE, METHOD OF ITS PRODUCTION AND APPLICATION

Application no: 50012-2018; *No*.: 288915 / 6.3.2018; *Legal status*: valid MPT: C08L 89/00, C12N 9/52, C09J 189/00

Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Institute of Musicology of the Slovak Academy of Sciences, Institute of Chemistry of the Slovak Academy of Science; Faculty of Wood Science and Technology, Technical University in Zvolen, Slovakia

Utility model

MEDOVÝ PRÍPRAVOK NA POUŽITIE V MEDICÍNE NA LOKÁLNU LIEČBU DLHODOBO NEHOJACICH SA RÁN ASOCIOVANÝCH S BAKTERIÁLNOU INFEKCIOU, SPÔSOB JEHO VÝROBY A NÁPLASŤ ALEBO KRYTIE

HONEY PREPARATION FOR USE IN MEDICINE FOR LOCAL TREATMENT OF LONG-TERM NON-HEALING WOUNDS ASSOCIATED WITH BACTERIAL INFECTION, METHOD OF ITS PRODUCTION AND PLASTER OR COVERING

Application No.: 50009-2018; *Registration No*.: 8435 / 6.5.2019, *Legal status*: valid MPT: A61F 13/02, A61K 31/375, A61K 35/644, A61L 15/44, A61P 17/02

Owner: Institute of Molecular Biology of the Slovak Academy of Science; Slovak Medical University, Bratislava, Slovakia

2.1.8. Narrative on the most important research outputs of the institute – especially focused on their importance for society (3-5 pages)

This section contains five narratives related to our five principal research outputs highlighted in bold in the section 2.1.2.

What is the deal with bacterial nanotubes?

POSPÍŠIL, J. - VÍTOVSKÁ, D. - KOFROŇOVÁ, Olga - MUCHOVÁ, Katarína - ŠANDEROVÁ, H. - HUBÁLEK, M. - ŠIKOVÁ, M. - MODRÁK, M. - BENADA, O.** - BARÁK, Imrich - KRÁSNY, L. Bacterial nanotubes as a manifestation of cell death. In *Nature Communications*, 2020, vol. 11, no. 4963. (2019: 12.121 - IF, Q1 - JCR, 5.569 - SJR, Q1 - SJR, CCC). (2020 - Current Contents). ISSN 2041-1723. https://doi.org/10.1038/s41467-020-18800-2

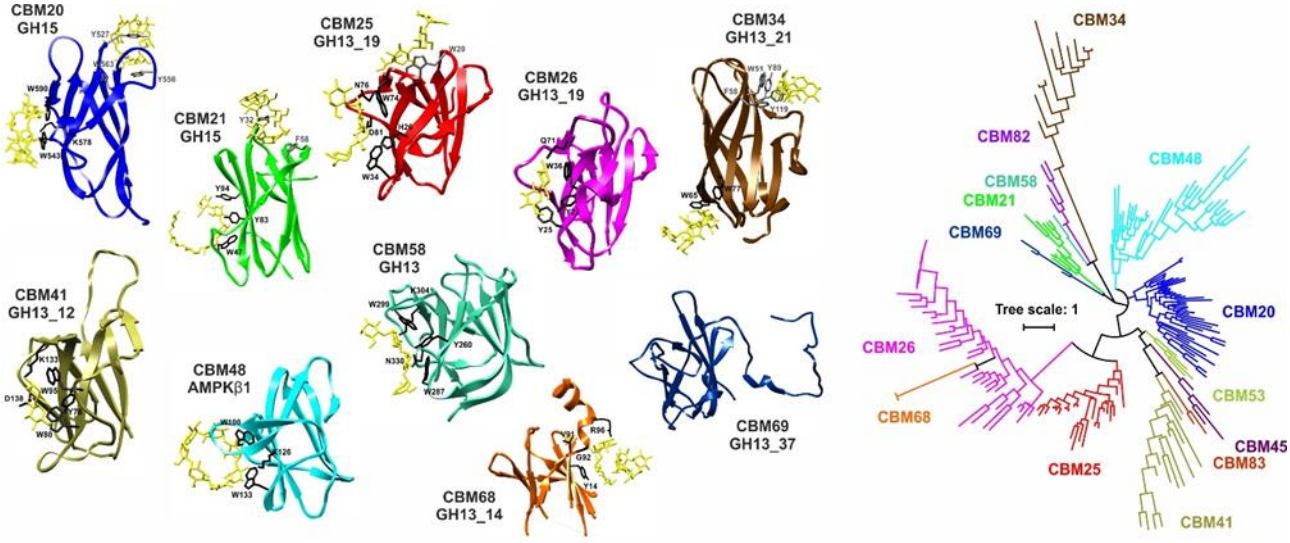
The group of Dr. Imrich Barak discovered the mechanism of how bacterial nanotubes form (Pospíšil et al., 2020). These formations have been characterized as lipid tubes emanating from bacterial cells into space, sometimes 50 times longer than the length of the bacterial cell itself. These long and narrow tubes have been described as a way by which a bacterial cell can connect to another cell of either the same species or a completely different species, even using them to connect to a eukaryotic cell (Dubey et al., *Developmental Cell,* Vol. 36, 2016). Processes such as DNA, RNA, and protein transfer between the cells of various bacteria as well as “vampire” nutrient extraction from human cells have been attributed to the unique capabilities of these nanotubes (Dubey and Yehuda, *Cell*,

Vol. 144, 2011; Pal et al., *Cell*, Vol. 177, 2019). Bacterial nanotubes have been considered the greatest discovery in microbiology in the last decade. The various roles of nanotubes have begun to be widely accepted and could already be referred to as textbook knowledge. During many different experiments with *Bacillus subtilis*, we were able to find nanotubes only in very rare cases. When we monitored the cells, and if we pressed the coverslip, nanotubes ran out of many cells. The greater the pressure we applied, the more nanotubes formed, many extraordinarily long (see the figure from section 1.8.1., page 4). We also noticed that the cells lose contrast and become translucent in phase contrast microscopy, which indicates that they are dying and forming so-called ghost cells. We observed that the formation of nanotubes also depends on the cell life cycle. In dividing cells, during exponential growth under a given stress, under pressure, nanotubes grow from all cells. In contrast, we did not observe these nanotubes in cells during stationary growth or sporulation. We have shown that these nanotubes are in principle formed from every cell when we use different stress factors such as pressure or expose them to different antibiotics. The bacterial cell wall can maintain a pressure of up to twenty atmospheres inside the cell. However, if the wall is disturbed, either mechanically or by antibiotics, it is not possible to maintain such high pressure. This pressure then literally causes the cytoplasmic membrane to be “fired out” in the form of a nanotube into the external environment through the holes created in the cell wall. An important finding was that just as the cell fired the nanotube, the cell died. Our findings show that the nanotube is most likely not used by bacteria to exchange various biological substances, but that it is only a “post mortem” manifestation of a stressed and dying cell. It is also important that we could observe such nanotubes in a wide variety of different bacteria, and thus these findings have a broad impact on the whole field of microbiology. These discoveries are groundbreaking, especially in that we do not have to worry about bacterial nanotubes as a path for transmitting antibiotic resistance between different bacterial species, as had been thought. Our results are in stark contrast to previously published findings, and this has caused the story of bacterial nanotubes to take on a whole new turn and was discussed worldwide by scientists on Twitter and in *TheScientist* (Issue June 2021). Our results suggest that the importance of nanotubes during the last decade has been greatly overestimated. On the other hand, many similar formations in bacteria may have a real function e.g. extracellular vesicles, or so-called nanowires, that are used for long-distance extracellular electron transport. Membranous nanotubes seem to be more interesting in higher organisms, where they can play an important role, for example in cancer invasion, or in the immune system. Lipid nanotubes have also been shown to have many similar properties to nerve synapses and are even considered to be evolutionary precursors of neurons.

The story of amylolytic enzymes

JANEČEK, Štefan** - MAREČEK, Filip - MACGREGOR, E.A. - SVENSSON, B. Starch-binding domains as CBM families – history, occurrence, structure, function and evolution. In *Biotechnology Advances*, 2019, vol. 37, iss. 873, no. 107451. (2018: 12.831 - IF, Q1 - JCR, 3.179 - SJR, Q1 - SJR, CCC). (2019 - Current Contents). ISSN 0734-9750. https://doi.org/10.1016/j.biotechadv.2019.107451

For several decades, the research group of the Laboratory of Protein Evolution has been engaged in bioinformatics approaches to study the structure, function and evolution of proteins. The Head of the Laboratory, Prof. Štefan Janeček is well known from his pioneering *in silico* studies of amylolytic enzymes. Since the beginning of the 1990s, the enzyme α-amylase (EC 3.2.1.1) has been known as the founding member of a quickly expanding, polyspecific protein family, which – in the established sequence-based classification of glycoside hydrolases (GHs) – has been assigned the number GH13. In the CAZy database (http://www.cazy.org/), α-amylase family GH13 presently numbers around 130 thousand sequences with more than 30 different enzyme specificities (e.g. isoamylase, cyclodextrin glucanotransferase, α-glucosidase, pullulanase, neopullulanase, etc.). In addition to GH13, there are probably three more α-amylase families in CAZy, families GH57, GH119 and GH126. At a higher level of hierarchy, GH13 is a member of the clan GH-H (with families GH70 and GH77), while at a lower level, it has been divided into more than 40 subfamilies. It is worth mentioning that amylolytic enzymes are multidomain proteins and their non-catalytic starch-binding domains (SBDs) – designated carbohydrate-binding modules (CBMs) – have been classified into 15 different CBM families in CAZy. These SBDs help the amylolytic enzymes – by binding – to degrade raw, i.e. thermally untreated starch and related α-glucans. Interestingly, they are also present in non-amylolytic enzymes and proteins, such as the glucan phosphatases laforin and SEX4, the β-subunit of AMP-activated kinase and many others.



Tertiary structures and evolutionary tree of starch-binding domains CBM families

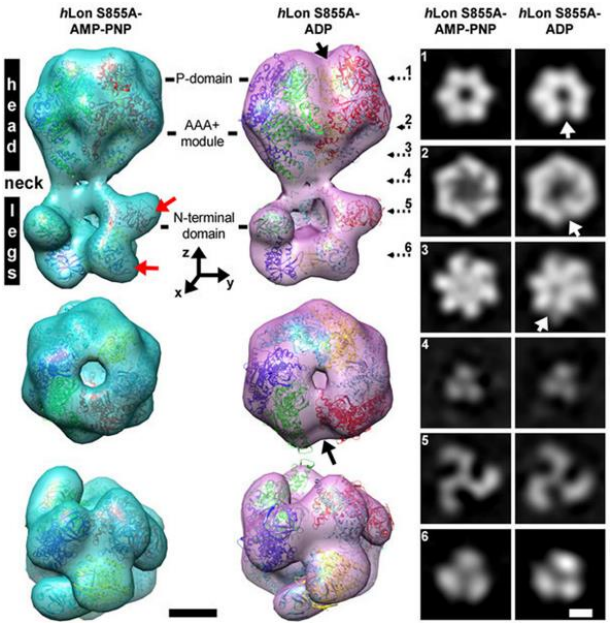
In addition to numerous fundamental contributions to both the structure-function and evolutionary relationships of amylolytic enzymes with respect to their catalytic domains, the laboratory led by Štefan Janeček has also identified several aspects of their SBDs, for example: (i) pointing out that the SBDs of the CBM20 family reflect the evolution of species more than the evolution of individual amylases; (ii) suggesting that the SBDs from families CBM20, CBM21, CBM48 and CBM53 are closely related; (iii) discovering the presence of a typical microbial SBD of the family CBM20 in the human protein genethonin-1; (iv) describing the evolution of the SBD of the family CBM20 in laforin; (v) elucidating the subgroups in the SBDs of the family CBM41 in pullulanases; (vi) sheding light on domain evolution aimed at SBDs of the family CBM34 in neopullulanases; and (vii) predicting an SBD function for several heretofore unassigned CBM-like domains. These long-term efforts, appreciated by the scientific community, resulted in the 2019 publication of a comprehensive Review (Janecek et al., 2019) on the history, occurrence, structure, function and evolution of SBDs as CBM families (see the figure). The international recognition of the devotion of Štefan Janeček to amylolytic enzymes is demonstrated by the fact that, closely supported by his colleagues and friends who represent the leading scientists in the field throughout the world, he established the journal *Amylase* (https://www.degruyter.com/journal/key/amylase/html), and, moreover, he is the founder and main organizer of a prestigious international symposium on enzymes from the α-amylase family, the ALAMYs (http://imb.savba.sk/~janecek/Alamys/) which has been held in Slovakia every three years since 2001.

The mitochondrial ATP-dependent protease LON and the proteins of mitochondrial nucleoids as crucial components of mitochondrial homeostasis

PEVALA, Vladimír - TRUBAN, Dominika - BAUER, Jacob - KOŠTAN, J. - KUNOVÁ, Nina - BELLOVÁ, Jana - BRANDSTETTER, M. - MARINI, V. - KREJČÍ, L. - TOMÁŠKA, L. - NOSEK, J. - KUTEJOVÁ, Eva. The structure and DNA-binding properties of Mgm101 from a yeast with a linear mitochondrial genome. In *Nucleic acids research*, 2016, vol. 44, no. 5, p. 2227-2239. (2015: 9.202 - IF, Q1 - JCR, 7.358 - SJR, Q1 - SJR, CCC). (2016 - Current Contents). ISSN 0305-1048. https://doi.org/10.1093/nar/gkv1529

Mitochondria are endosymbiotic organelles of eukaryotic cells that are essential for several metabolic processes. They supply cells with energy (ATP), participate in apoptosis and in the biosynthesis of cholesterol, cardiolipin, heme and Fe-S clusters. Proper mitochondrial function depends on mitochondrial homeostasis, which, in turn, requires a balance between the proteins synthesized in the cytosol and imported into the mitochondria and those synthesized endogenously. This balance is preserved by two processes: retrograde signaling from the mitochondria to the nucleus and the degradation of unnecessary proteins within the mitochondria. In mitochondria, endogenously synthesized proteins, such as several subunits of the respiratory chain complexes, are synthetized based on instructions from their own DNA (mtDNA), which is packed in large protein-DNA complexes called nucleoids. In humans, the degradation of mitochondrial proteins is carried out by the ATP-dependent proteases LON and CLPXP. Disruption of mitochondrial homeostasis seems to be a general characteristic of tumorigenic transformation; several studies suggest that mutations in

mtDNA play a role in the development of metastasis and resistance to chemotherapeutic drugs. LON is upregulated in colorectal and skin carcinomas, and high levels of LON are often associated with poor patient outcomes. LON participates in the remodeling of mitochondrial function and enables the reconfiguration of metabolic pathways in tumor cells. Mouse embryos lacking LON exhibit mtDNA loss and growth arrest during gastrulation, while LON-heterozygous mice develop normally and are protected against chemically induced colorectal and skin tumors. We studied the structure and function of the ATP-dependent protease LON and proteins involved in mitochondrial nucleoid formation and dynamics in human and yeast cells. We presented the first two structures of a full-length human LON protease, as determined by cryo-electron microscopy, and demonstrated that ATP hydrolysis by human mitochondrial LON protease induces conformational changes to the whole hexameric complex (see the figure). Furthermore, we showed that the N-terminal gate to its catalytic chamber appears to be closed by the axial pore loops when human LON (hLON) is incubated with AMP-PNP (a non-hydrolysable ATP analog), but opens following ADP binding. The proper assembly and functioning of the hLON complex are guaranteed only if the first N-terminal 156 amino acids are present (Kereiche et al., 2016).



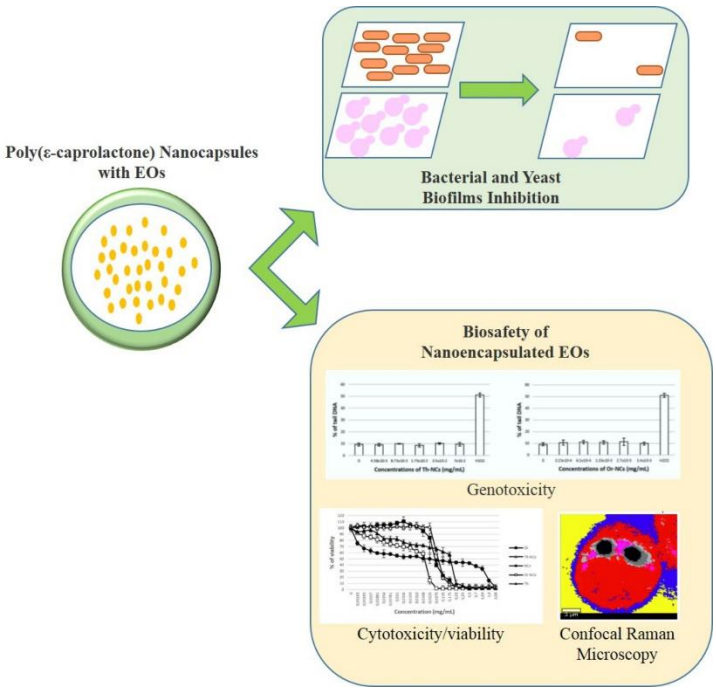
We have shown that LON protease is responsible for regulating the level of crucial components of the mitochondrial nucleoid, including the mtDNA-packaging protein TFAM, Twinkle helicase and ribosomal subunit MrpL32 in human cells and the mtDNA-packaging protein Abf2 and the mtDNA-maintenance factor Mgm101 in the yeast *Saccharomyces cerevisiae*. Importantly, the susceptibility of these proteins to LON protease was altered by their binding to nucleic acids, thus affecting the replication, transcription, and translation of mitochondrial DNA (Kunová et al., 2017). The structure and function of the mtDNA-maintenance factor Mgm101 partially differs in the yeast *Candida parapsilosis,* which possesses a linear mitochondrial DNA with defined ends – mitochondrial telomeres. Our biochemical and structural analyses revealed that *Cp*Mgm101 is able to form a ring-like structure and localize near the tip of a model mitochondrial telomere, indicating its possible role in telomere maintenance (Pevala et al., 2016). We also found that succinylation is an important post-ranslational modification (PTM) of the mtDNA-packaging protein Abf2 that substantially influences its DNA binding activity and susceptibility to LON protease, thus representing an important mechanism involved in the stabilization and maintenance of mtDNA (Frankovsky et al., 2021) (see the figure in “2. Protein structure-function relationships”, page 5).

Bactericidal, mycocidal and antibiofilm effect of essential oils and their nanoencapsulation

KAPUSTOVÁ, Magdaléna* - PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - GRANATA, Giuseppe* - NAPOLI, Edoardo - ANNUŠOVÁ, Adriana - MESÁROŠOVÁ, Monika - KOZICS, Katarína - PANGALLO, Domenico** - GERACI, Coradda**. Biofilm inhibition by biocompatible poly(epsilon-caprolactone) nanocapsules loaded with essential oils and their cyto/genotoxicity to human keratinocyte cell line. In *International Journal of Pharmaceutics*, 2021, vol. 606, no. 12, art. no. 120846. (2020: 5.875 - IF, Q1 - JCR, 1.153 - SJR, Q1 - SJR, CCC). (2021 - Current Contents). ISSN 0378-5173. https://doi.org/10.1016/j.ijpharm.2021.120846

It has been known for a long time that antibiotics are not as effective as in the past. Moreover, the abuse of their use has lead to the emergence of dangerous bacteria resistant to a large number of antibiotics. A possible alternative to antibiotics is provided by a number of natural compounds, and among the most studied are the essential oils (EOs). These are volatile compounds, produced by many plants, which have interesting and efficient antimicrobial properties. The characteristic aroma of many herbs, such as oregano and thyme, is provided by these substances, which are also involved in protecting the plants themselves against microbial pathogens and parasites. Our research has shown that EOs have antibacterial and antifungal properties at low concentrations (Puškárová et al., 2017). We tested different types of EOs with a large number of pathogenic bacteria, (both Gram$^+$

and Gram$^-$), molds and yeasts (Kozics et al., 2019; Kapustová et al., 2021). The EOs of many plants were highly efficient in eradicating all microorganisms tested. But when microorganisms are in a community or form biofilms, do EOs still have powerful antimicrobial properties? Concerning the EOs of thyme and oregano the answer is yes. We have demonstrated the antibiofilm ability of these two EOs by their encapsulation in Poly(ε-caprolactone) (PCL) nanocapsules. PCL is a biodegradable and biocompatible polyester polymer; due to the presence of a hydrolysis-unstable aliphatic ester linkage, PCL is biodegraded *in vivo* to low molecular weight non-toxic substances and completely metabolized in the human body. Nanoencapsulation of EOs represents a viable and efficient approach to increase their physical stability, water solubility, and antimicrobial potential and to decrease their toxicity while improving their bioaccessibility and bioavailability. Compared to large capsules, nanocapsules have higher surface area to volume ratios, which represents an important factor for reactivity. In fact, their subcellular size could favor the enhancement of EOs in water-rich phases or at liquid-solid interfaces, where microorganisms are frequently located. In particular, the improved penetrative ability of antimicrobial EOs by encapsulation could allow the microbial biofilm barrier to be overcome and the biofilm to be eradicated. Biofilm-associated microorganisms are a serious problem not only in hospitals but also in food industries and various indoor and outdoor environments. With this in mind, we prepared two PCL-based nanocapsules (NCs) loaded with thyme (Th-NCs) and oregano (Or-NCs) EOs and their antibacterial, antifungal and antibiofilm activities were assayed.



The concentrations of nanoencapsulated EOs able to prevent the growth and biofilm formation of *Staphylococcus aureus, Escherichia coli* and *Candida albicans* were determined. This was the first study to assess the cytotoxic and genotoxic activities of Th-NCs and Or-NCs in the human keratinocyte cell line HaCaT (T0020001). Label-free live cell Raman imaging found morphological alterations in cell cultures in high concentrations of EOs, NCs, and EO-NCs. The data showed that the nanoencapsulation of the oregano and thyme essential oils increases their antimicrobial and antibiofilm effects. Th-NC and Or-NC nanosuspensions showed antibiofilm activity against *E. coli* at low concentrations and no cytotoxic activity against HaCaT human keratinocyte cells at the same concentrations. These ecofriendly nanosystems could be an ecological alternative in the development of new antimicrobial and antibiofilm strategies for applications in the medical, food and environmental industries.

Genetically manipulated *Streptomyces* strains for the efficient production of proteins and biologically active compounds.

NOVÁKOVÁ, Renáta* - NÚÑEZ, L.E. - HOMEROVÁ, Dagmar - KNIRSCHOVÁ, Renáta - FECKOVÁ, Ľubomíra - REŽUCHOVÁ, Bronislava - ŠEVČÍKOVÁ, Beatrica - MENÉNDEZ, N. - MORÍS, F. - CORTÉS, J. - KORMANEC, Ján**. Increased heterologous production of the antitumoral polyketide mithramycin A by engineered Streptomyces lividans TK24 strains. In *Applied Microbiology and Biotechnology*, 2018, vol. 102, p. 857–869. (2017: 3.340 - IF, Q2 - JCR, 1.182 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0175-7598. https://doi.org/10.1007/s00253-017-8642-5

Based on many years of experience, the group of Dr. Ján Kormanec was invited to join the large collaborative 7FP project STREPSYNTH “Rewiring the *Streptomyces* cell factory for cost-effective production of biomolecules” as experts on genomic manipulations in *Streptomyces*. The aim of this project was to develop a novel streptomycete-based industrial platform for commercially interesting biomolecules. The task of our group within the consortium was to prepare an efficient system for the targeted genome editing of *Streptomyces lividans* and to prepare a collection of *S. lividans* RedStrep

strains with improved biotechnological properties for the production of two categories of products of biotechnological interest (PoI): (i) small biologically active compounds (antibiotics, cytostatics) and (ii) biotechnologically and clinically relevant proteins (enzymes, antibodies, cytokines). We developed an efficient and simple genome editing system that allows large-scale deletions of up to 500 kb in size and variable insertions of foreign genes under the control of heterologous strong and regulated promoters. Using this system, we prepared eight *S. lividans* RedStrep 1.0 to 1.8 strains in which the gene clusters responsible for producing interfering secondary metabolites were sequentially deleted. In collaboration with our project partner, the Spanish biotechnology company EntreChem, we introduced a gene cluster for mithramycin into these RedStrep strains and demonstrated a dramatic increase in the heterologous production of the antitumor agent mithramycin A, which was their commercial product. This increase (up to 3 g/L of medium) was six times higher than that of *S. argillaceus,* their usual mutant strain for overproducing mitramycin (Novakova et al., 2018). To further improve the biotechnological properties of the RedStrep strains for heterologous protein production, we deleted the *matAB* genes for mycelial aggregation in these strains. This deletion had a significant positive effect on the growth of these strains. This manipulation significantly improved the biotechnological properties of the RedStrep strains (Rezuchova et al., 2018). All these gene manipulations resulted in a final collection of 27 RedStrep strains, RedStrep 1.0 to 1.26. We characterized the heterologous secretion of red fluorescent protein mRFP as a model protein in these RedStrep strains. Several strains had dramatically increased mRFP secretion compared to the wild-type S*. lividans* strain (Hamed et al., 2018). Based on these results, we can say that we have prepared a collection of biotechnologically useful *S. lividans* RedStrep 1 strains for the heterologous production of both small biologically active compounds as well as proteins. The entire collection has been provided to biotechnology companies, as project partners, for the production of their commercial products of interest. In addition, these results were also appreciated by SAS and the group received an Award in the field of international scientific and technical cooperation in 2019.

2.1.9. Table of research outputs

Papers from international collaborations in large-scale scientific projects (Dwarf team, ALICE Collaboration, ATLAS collaboration, CD Collaboration, H1 Collaboration, HADES Collaboration, and STAR Collaboration) have to be listed separately

Scientific publications	2016			2017			2018			2019			2020			2021			total			
	number	No. / FTE researches	No. / one million total salary budget	number	No. / FTE researches	No. / one million total salary budget	number	No. / FTE researches	No. / one million total salary budget	number	No. / FTE researches	No. / one million total salary budget	number	No. / FTE researches	No. / one million total salary budget	number	No. / FTE researches	No. /1 million total salary budget	number	averaged number per year	av. No. / FTE researches	av. No. / one million total salary budget
Scientific monographs and monographic studies in journals and proceedings published abroad *(AAA, ABA)*	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**0**	**0,000**	**0,000**	**0,000**
Scientific monographs and monographic studies in journals and proceedings published in Slovakia *(AAB, ABB)*	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**0**	**0,000**	**0,000**	**0,000**
Chapters in scientific monographs published abroad *(ABC)*	6	0,143	5,983	2	0,046	1,899	**2**	0,050	1,745	0	0,000	0,000	1	0,024	0,723	3	0,066	2,046	**14**	**2,333**	**0,056**	**1,914**
Chapters in scientific monographs published in Slovakia *(ABD)*	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**0**	**0,000**	**0,000**	**0,000**
Scientific papers published in journals registered in Current Contents Connect *(ADCA, ADCB, ADDA, ADDB)*	31	0,741	30,914	16	0,368	15,195	32	0,800	27,923	24	0,605	18,972	30	0,720	21,692	34	0,746	23,192	**167**	**27,833**	**0,662**	**22,827**
Scientific papers published in journals registered in Web of Science Core Collection and SCOPUS not listed above *(ADMA, ADMB, ADNA, ADNB)*	9	0,215	8,975	4	0,092	3,799	11	0,275	9,599	5	0,126	3,953	11	0,264	7,954	10	0,219	6,821	**50**	**8,333**	**0,198**	**6,835**
Scientific papers published in other foreign journals (not listed above) *(ADEA, ADEB)*	0	0,000	0,000	1	0,023	0,950	1	0,025	0,873	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**2**	**0,333**	**0,008**	**0,273**
Scientific papers published in other domestic journals (not listed above) *(ADFA, ADFB)*	0	0,000	0,000	7	0,161	6,648	0	0,000	0,000	0	0,000	0,000	1	0,024	0,723	0	0,000	0,000	**8**	**1,333**	**0,032**	**1,094**
Scientific papers published in foreign peer-reviewed proceedings *(AECA)*	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**0**	**0,000**	**0,000**	**0,000**
Scientific papers published in domestic peer-reviewed proceedings *(AEDA)*	0	0,000	0,000	0	0,000	0,000	1	0,025	0,873	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**1**	**0,167**	**0,004**	**0,137**
Published papers (full text) from foreign scientific conferences *(AFA, AFC)*	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	0	0,000	0,000	**0**	**0,000**	**0,000**	**0,000**
Published papers (full text) from domestic scientific conferences *(AFB, AFD)*	4	0,096	3,989	6	0,138	5,698	4	0,100	3,490	2	0,050	1,581	0	0,000	0,000	3	0,066	2,046	**19**	**3,167**	**0,075**	**2,597**

2.2. Measures of research outputs (citations, etc.)

2.2.1. Table with citations per annum (without self-citations)

Citations of papers from international collaborations in large-scale scientific projects (Dwarf team, ALICE Collaboration, ATLAS collaboration, CD Collaboration, H1 Collaboration, HADES Collaboration, and STAR Collaboration) are listed separately

Citations, reviews	2015		2016		2017		2018		2019		2020		total		
	number	No. / FTE researchers	number	No. / FTE researchers	number	No. / FTE researchers	number	No. / FTE researchers	number	No. / FTE researchers	number	No. / FTE researchers	number	averaged number per year	av. No. / FTE researchers
Citations in Web of Science Core Collection (1.1, 2.1)	913	21,81	1 041	23,95	1 035	25,89	1 124	28,32	1 221	29,31	1 288	28,26	**6 622**	**1 103,67**	**26,25**
Citations in SCOPUS (1.2, 2.2) if not listed above	101	2,41	89	2,05	112	2,80	78	1,97	78	1,87	107	2,35	**565**	**94,17**	**2,24**
Citations in other citation indexes and databases (not listed above) (3.2,4.2)	0	0,00	15	0,35	27	0,68	48	1,21	0	0,00	0	0,00	**90**	**15,00**	**0,36**
Other citations (not listed above) (3.1, 4.1)	5	0,12	0	0,00	1	0,03	6	0,15	1	0,02	0	0,00	**13**	**2,17**	**0,05**
Reviews (5,6)	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	**0**	**0,00**	**0,00**

2.2.2. List of 10 most-cited publications published any time with the address of the institute, with number of citations in the assessment period (2015 – 2020)

1. ADCA01 WEINSTOCK, G.M. - ROBINSON, G.E. - BÍLIKOVÁ, Katarína - ŠIMÚTH, Jozef. Insights into social insects from the genome of the honeybee Apis mellifera. In *Nature*, 2006, vol. 443, no. 7114, p. 931. (2005: 29.273 - IF, Q1 - JCR, 10.333 - SJR, Q1 - SJR, CCC). (2006 - Current Contents, WOS, SCOPUS). ISSN 0028-0836. https://doi.org/10.1038/nature05260 **339 citations**
2. ADCA02 MEDEMA, M.H. - KOTTMANN, R. - YILMAZ, P. - CUMMINGS, M. - BIGGINS, J.B. - KORMANEC, Ján. Minimum information about a biosynthetic gene cluster. In *Nature Chemical Biology*, 2015, vol. 11, p. 625-631. (2014: 12.996 - IF, Q1 - JCR, 7.184 - SJR, Q1 - SJR, CCC). (2015 - Current Contents). ISSN 1552-4450. https://doi.org/10.1038/nchembio.1890 **190 citations**
3. ADCA03 MACGREGOR, E.A. - JANEČEK, Štefan - SVENSSON, B. Relationship of sequence and structure to specificity in the alpha-amylase family of enzymes. In *Biochimica et Biophysica Acta*, 2001, vol. 1546, p. 1-20. ISSN 0006-3002. . https://doi.org/10.1016/S0167-4838(00)00302-2 **150 citations**
4. ADCA04 ZÁMOCKÝ, Marcel - FURTMULLER, P.G. - OBINGER, C. Evolution of catalases from bacteria to humans. In *Antioxidants & Redox Signaling*, 2008, vol. 10, p. 1527-1547. (2007: 5.484 - IF, Q1 - JCR, 2.690 - SJR, Q1 - SJR, CCC). (2008 - Current Contents). ISSN 1523-0864. https://doi.org/10.1089/ars.2008.2046 **132 citations**
5. ADCA05 PACE, C.N. - FU, H. - FRYAR, K.L. - LANDUA, J. - TREVINO, S.R. - SCHELL, D. - THURLKILL, R.L. - IMURA, S. - SCHOLTZ, J.M. - GAJIWALA, K. - ŠEVČÍK, Jozef - URBÁNIKOVÁ, Ľubica - MYERS, J.K. - TAKANO, K. - HEBERT, E.J. - SHIRLEY, B.A. - GRIMSLEY, G.R. Contribution of hydrogen bonds to protein stability. In *Protein Science*, 2014, vol. 23, p. 652–661. (2013: 2.861 - IF, Q3 - JCR, 1.982 - SJR, CCC). (2014 - Current Contents). ISSN 0961-8368. https://doi.org/10.1002/pro.2449 **125 citations**
6. ADCA06 SIRAJUDDIN, M. - FARKAŠOVSKÝ, Marian - HAUER, F. - KUHLMANN, D. - MACARA, I.G. - WEYAND, M. - STARK, H. - WITTINGHOFER, A. Structural insight into filament formation by mammalian septins. In *Nature*, 2007, vol. 449, p. 311-315. (2006: 26.681 - IF, Q1 - JCR, 9.702 - SJR, Q1 - SJR, CCC). (2007 - Current Contents, WOS, SCOPUS). ISSN 0028-0836. https://doi.org/10.1038/nature06052 **122 citations**
7. ADCA07 JANEČEK, Štefan - SVENSSON, B. - MACGREGOR, E.A. α-Amylase: an enzyme specificity found in various families of glycoside hydrolases. In *Cellular and Molecular Life Sciences*, 2014, vol. 71, p. 1149–1170. (2013: 5.856 - IF, Q1 - JCR, 3.301 - SJR, CCC). (2014 - Current Contents). ISSN 1420-682X. https://doi.org/10.1007/s00018-013-1388-z. **119 citations**
8. ADCA08 MARKOVIČ, Oskar - JANEČEK, Štefan. Pectin methylesterases: sequence-structural features and phylogenetic relationships. In *Carbohydrate Research*, 2004, vol. 339, p. 2281-2295. (2003: 1.533 - IF, CCC). (2004 - Current Contents). ISSN 0008-6215. https://doi.org/10.1016/j.carres.2004.06.023 **115 citations**
9. ADCA09 MINÁRIK, P. - TOMASKOVA, N. - KOLLÁROVÁ, M - ANTALIK, M. Malate dehydrogenases - structure and function. In *General Physiology and Biophysics*, 2002, vol. 21, p. 257-265. (2001: 0.932 - IF, CCC). (2002 - Current Contents). ISSN 0231-5882. **110 citations**
10. ADCA10 GLORIEUX, Ch. - ZÁMOCKÝ, Marcel - SANDOVAL, J.M. - VERRAX, J. - CALDERON, P.B. Regulation of catalase expression in healthy and cancerous cells. In *Free Radical Biology and Medicine*, 2015, vol. 87, p. 84-97. (2014: 5.736 - IF, Q1 - JCR, 2.469 - SJR, Q1 - SJR, CCC). (2015 - Current Contents). ISSN 0891-5849. https://doi.org/10.1016/j.freeradbiomed.2015.06.017 **97 citations**

2.2.3. List of 10 most-cited publications published any time with the address of the institute, with number of citations obtained until 2020

1. ADCA01 WEINSTOCK, G.M. - ROBINSON, G.E. - BÍLIKOVÁ, Katarína - ŠIMÚTH, Jozef. Insights into social insects from the genome of the honeybee Apis mellifera. In *Nature,* 2006, vol. 443, no. 7114, p. 931. (2005: 29.273 - IF, Q1 - JCR, 10.333 - SJR, Q1 - SJR, CCC). (2006 - Current Contents, WOS, SCOPUS). ISSN 0028-0836. https://doi.org/10.1038/nature05260 **855 citations**
2. ADCA02 MACGREGOR, E.A. - JANEČEK, Štefan - SVENSSON, B. Relationship of sequence and structure to specificity in the alpha-amylase family of enzymes. In *Biochimica et Biophysica Acta*, 2001, vol. 1546, p. 1-20. ISSN 0006-3002. https://doi.org/10.1016/S0167-4838(00)00302-2 **413 citations**
3. ADCA03 SIRAJUDDIN, M. - FARKAŠOVSKÝ, Marian - HAUER, F. - KUHLMANN, D. - MACARA, I.G. - WEYAND, M. - STARK, H. - WITTINGHOFER, A. Structural insight into filament formation by mammalian septins. In *Nature*, 2007, vol. 449, p. 311-315. (2006: 26.681 - IF, Q1 - JCR, 9.702 - SJR, Q1 - SJR, CCC). (2007 - Current Contents, WOS, SCOPUS). ISSN 0028-0836. https://doi.org/10.1038/nature06052 **266 citations**
4. ADCA04 BISCHOFF, M. - DUNMAN, P. - KORMANEC, Ján - MACAPAGAL, D. - MURPHY, E. - MOUNTS, W. - BERGER-BACHI, B. - PROJAN, S. Microarray-based analysis of the Staphylococcus aureus sigmaB regulon. In *Journal of Bacteriology*, 2004, vol. 186, p. 4085-4099. (2004 - Current Contents). ISSN 0021-9193. https://doi.org/10.1128/jb.186.13.4085-4099.2004 **247 citations**
5. ADCA05 ZÁMOCKÝ, Marcel - FURTMULLER, P.G. - OBINGER, C. Evolution of catalases from bacteria to humans. In *Antioxidants & Redox Signaling*, 2008, vol. 10, p. 1527-1547. (2007: 5.484 - IF, Q1 - JCR, 2.690 - SJR, Q1 - SJR, CCC). (2008 - Current Contents). ISSN 1523-0864. https://doi.org/10.1089/ars.2008.2046 **245 citations**
6. ADCA06 ROWLEY, G. - SPECTOR, M. - KORMANEC, Ján - ROBERTS, M. Pushing the envelope: extracytoplasmic stress responses in bacterial pathogens. In *Nature Reviews Microbiology*, 2006, vol. 4, p. 383-394. (2005: 13.989 - IF, Q1 - JCR, 5.965 - SJR, Q1 - SJR). ISSN 1740-1526. https://doi.org/10.1038/nrmicro1394 **210 citations**
7. ADCA07 MEDEMA, M.H. - KOTTMANN, R. - YILMAZ, P. - CUMMINGS, M. - BIGGINS, J.B. - KORMANEC, Ján. Minimum information about a biosynthetic gene cluster. In *Nature Chemical Biology*, 2015, vol. 11, p. 625-631. (2014: 12.996 - IF, Q1 - JCR, 7.184 - SJR, Q1 - SJR, CCC). (2015 - Current Contents). ISSN 1552-4450. https://doi.org/10.1038/nchembio.1890 **190 citations**
8. ADCA08 MINÁRIK, P. - TOMASKOVA, N. - KOLLÁROVÁ, M - ANTALIK, M. Malate dehydrogenases - structure and function. In *General Physiology and Biophysics*, 2002, vol. 21, p. 257-265. (2001: 0.932 - IF, CCC). (2002 - Current Contents). ISSN 0231-5882. **180 citations**
9. ADCA09 MARKOVIČ, Oskar - JANEČEK, Štefan. Pectin methylesterases: sequence-structural features and phylogenetic relationships. In *Carbohydrate Research*, 2004, vol. 339, p. 2281-2295. (2003: 1.533 - IF, CCC). (2004 - Current Contents). ISSN 0008-6215. https://doi.org/10.1016/j.carres.2004.06.023 **164 citations**
10. ADCA10 JANEČEK, Štefan. Alpha-amylase family: molecular biology and evolution. In *Progress in Biophysics & Molecular Biology*, 1997, vol. 67, p. 67-97. ISSN 0079-6107. https://doi.org/10.1016/S0079-6107(97)00015-1 **159 citations**

1.2.4. List of 10 most-cited publications published during the evaluation period (2016-2021) with the address of the Institute, with number of citations obtained until 2021

1. ADCA01 PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - KRAKOVÁ, Lucia - PANGALLO, Domenico - KOZICS, Katarína. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. In *Scientific Reports*, 2017, vol. 7, no. 1, art. no. 8211. (2016: 4.259 - IF, Q1 - JCR, 1.692 - SJR, Q1 - SJR, CCC). (2017 - Current Contents). ISSN 2045-2322. https://doi.org/10.1038/s41598-017-08673-9 **71 citations**
2. ADCA02 BUČEKOVÁ, Marcela - JARDEKOVÁ, Lucia - JURICOVÁ, Valéria - BUGÁROVÁ, Veronika - DI MARCO, Gabriele - GISMONDI, Angelo - LEONARDI, Donatella - FARKAŠOVSKÁ, Jarmila - GODOČÍKOVÁ, Jana - LAHO, Maroš - KLAUDINY, Jaroslav - MAJTÁN, Viktor - CANINI, Antonella - MAJTÁN, Juraj**. Antibacterial activity of different blossom honeys: New findings. In *Molecules*, 2019, vol. 24, no. 8, no. 1573. (2018: 3.060 - IF, Q2 - JCR, 0.757 - SJR, Q1 - SJR, CCC). (2019 - Current Contents, WOS, SCOPUS). ISSN 1420-3049. https://doi.org/10.3390/molecules24081573 **48 citations**
3. ADCA03 ABBOTT, W. - ALBER, O. - BAYER, E. - BERRIN, J.G. - BORASTON, A. - JANEČEK, Štefan. Ten years of CAZypedia: a living encyclopedia of carbohydrate-active enzymes. In *Glycobiology*, 2018, vol. 28, p. 3-8. (2017: 3.664 - IF, Q2 - JCR, 1.493 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0959-6658. https://doi.org/10.1093/glycob/cwx089 **44 citations**
4. ADCA04 JANEČEK, Štefan - GABRIŠKO, Marek. Remarkable evolutionary relatedness among the enzymes and proteins from the α-amylase family. In *Cellular and Molecular Life Sciences*, 2016, vol. 73, p. 2707–2725. (2015: 5.694 - IF, Q1 - JCR, 3.400 - SJR, Q1 - SJR, CCC). (2016 - Current Contents). ISSN 1420-682X. https://doi.org/10.1007/s00018-016-2246-6 **42 citations**
5. ADC05 URZI, C. - DE LEO, F. - KRAKOVÁ, Lucia - PANGALLO, Domenico - BRUNO, L. Effects of biocide treatments on the biofilm community in Domitilla's catacombs in Rome. In *Science of the Total Environment*, 2016, vol. 572, p. 252–262. (2015: 3.976 - IF, Q1 - JCR, 1.653 - SJR, Q1 - SJR, CCC). (2016 - Current Contents). ISSN 0048-9697. https://doi.org/10.1016/j.scitotenv.2016.07.195 **35 citations**
6. ADCA06 MAJTÁN, Juraj** - JASENAK, M. β-Glucans: multi-functional modulator of wound healing. In *Molecules*, 2018, vol. 23, art. no. 806. (2017: 3.098 - IF, Q2 - JCR, 0.855 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 1420-3049. https://doi.org/10.3390/molecules23040806 **34 citations**
7. ADCA07 BERTELLI, M. - KAINI, A.K. - PAOLACCI, S.** - MANARA, E. - KURTI, D. - DHULI, K. - BUSHATI, V. - MIERTUS, J. - PANGALLO, Domenico - BAGLIVO, M. - BECCARI, T. - MICHELINI, S. Hydroxytyrosol: A natural compound with promising pharmacological activities. In *Journal of Biotechnology*, 2020, vol. 309, p. 29-33. (2019: 3.503 - IF, Q2 - JCR, 0.992 - SJR, Q1 - SJR, CCC). (2020 - Current Contents). ISSN 0168-1656. https://doi.org/10.1016/j.jbiotec.2019.12.016 **34 citations**
8. ADCA08 KRAKOVÁ, Lucia - ŠOLTÝS, K. - OTLEWSKA, A. - PIETRZAK, K. - PURKRTOVÁ, S. - SAVICKÁ, D. - PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - SZEMES, Tomáš - BUDIŠ, J. - DEMNEROVA, K. - GUTAROWSKA, B. - PANGALLO, Domenico**. Comparison of methods for identification of microbial communities in book collections: Culture-dependent (sequencing and MALDI-TOF MS) and culture-independent (Illumina MiSeq). In *International Biodeterioration & Biodegradation*, 2018, vol. 131, p. 51-59. (2017: 3.562 - IF, Q1 - JCR, 1.086 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0964-8305. https://doi.org/10.1016/j.ibiod.2017.02.015 **33 citations**
9. ADCA09 WIEDORN, M.O. - OBERTHÜR, D. - BEAN, R. - SCHUBERT, R. - WERNER, N. - BARÁK, Imrich. Megahertz serial crystallography. In *Nature Communications*, 2018, vol. 9, no. 4025. (2017: 12.353 - IF, Q1 - JCR, 6.582 - SJR,

Q1 - SJR, CCC). (2018 - Current Contents, WOS, SCOPUS). ISSN 2041-1723. https://doi.org/10.1038/s41467-018-06156-7 **31 citations**

10. ADCA10 BUČEKOVÁ, Marcela - BURIOVÁ, Monika - PEKÁRIK, Ladislav - MAJTÁN, Viktor - MAJTÁN, Juraj**. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. In *Scientific Reports*, 2018, vol. 8, art. no. 9061. (2017: 4.122 - IF, Q1 - JCR, 1.533 - SJR, Q1 - SJR, CCC). (2018 - Current Contents, WOS, SCOPUS). ISSN 2045-2322. https://doi.org/10.1038/s41598-018-27449-3 **28 citations**

1.2.5. List of most-cited authors from the Institute (at most 10 % of average FTE researchers per year) and their number of citations in the assessment period (2015– 2020). The cited papers must bear the address of the institute

1. Štefan Janeček (1276 citations)
2. Ján Kormanec (923 citations)
3. Domenico Pangallo (874 citations)
4. Marcel Zámocký (833 citations)
5. Imrich Barák (576 citations)

1.2.6. List of most-cited authors from the Institute (at most 10 % of average FTE researchers per year) and their number of citations obtained until 2020. The cited papers must bear the address of the Institute

1. Štefan Janeček (2844 citations)
2. Ján Kormanec (2227 citations)
3. Imrich Barák (1439 citations)
4. Marcel Zámocky (1426 citations)
5. Domenico Pangallo (1353 citations)

2.2.7. List of most-cited authors from the Institute (at most 10 % of average FTE researchers per year) and their number of citations obtained until 2021 of their papers published during the evaluation period (2016– 2021). The cited papers must bear the address of the Institute

1. Domenico Pangallo (483 citations)
2. Lucia Kraková (375 citations)
3. Andrea Puškárová (284 citations)
4. Juraj Majtán (278 citations)
5. Marcela Bučeková (190 citations)

2.3. Research status of the institute in international and national context

- **International/European position of the institute**

2.3.1. List of the most important research activities demonstrating the international relevance of the research performed by the institute, incl. major projects (details of projects should be supplied under Indicator 2.4). Max. 10 items for institute with less than 50 average FTE researchers per year, max. 20 for institutes with 50 – 100 average FTE researchers per year and so on

1. Project 7th FP Rewiring the Streptomyces cell factory for cost-effective production of biomolecules (Acronym: STREPSYNTH); registration number: 613877; duration of the

project: 01.12.2013 – 30.11.2018; Ján Kormanec - principal investigator from the the Institute of Molecular Biology SAS; coordinator: Katholieke Universiteit Leuven, Belgium

2. Project Interreg Building learning and research capacities in the structure and functional analysis of biomolecules for the needs of biomedicine and biotechnology**;** (Acronym StruBioMol); registration number: 305011X666; duration of the project:01.05.2019 - 30.04.2022, Eva Kutejová – principal investigator and coordinator from the Institute of Molecular Biology SAS
3. The project SNSF The role of metal homeostasis, reduction and sporulation in the metal resistance of Gram-positive bacteria **(**Acronym: Scopes – SNF); registration number: IZ73Z0_152527 / 1; duration of the project: 01.04.2014 – 31.03.2017; Imrich Barák - principal investigator from the Institute of Molecular Biology SAS; coordinator: EPFL Lausanne, Schwitzerland
4. The project TUBITAK Sustainable Water Reclamation Based on Ceramic Membrane Filtration (Acronym: SuWaCer); registration number: EIG_JC2019-058; duration of the project:01.04.2020 – 31.03.2023; Domenico Pangallo - principal investigator from the Institute of Molecular Biology SAS; coordinator: TUBITAK, Marmara Research Center (TUBITAK MRC)
5. Project JRP Exploring Microbial Diversity and Functionality in Thermophilic Bioreactors for Innovation in Biotechnology; registration number: SAS-MOST JRP 2014/3; duration of the project: 01.01.2015 – 31.12.2017; Domenic Pangallo – coordinator and principal investigator from the Institute of Molecular Biology SAS;
6. The project MOST Water/Wastewater epidemiology: Development of robust and reliable molecular detection systems for surveillance of disease outbreaks (Acronym: PathogenTracker); registration number: MOST 108-2221-E-006 -160 -MY3; duration of the project:01.01.2021 – 31.12.2023; Domenico Pangallo - principal investigator and coordinator from the Institute of Molecular Biology SAS
7. Project COST Understanding Movement and Mechanism in Molecular Machines; registration number: COST Action CM1306; duration of the project:06.2014 – 06.2018; Ľubica Urbániková - principal investigator from the Institute of Molecular Biology SAS; coordinator: University of East Anglia, Norwich, UK
8. Numerous international collaborations, either long-term or often *ad hoc*, not covered by specific grant contracts; Stefan Janecek – principal investigator from the Institute of Molecular Biology SAS, including, e.g., the Technical University of Denmark (Denmark), University of Groningen (The Netherlands), Lund University (Sweden), University of Kentucky (KY, USA), University of Michigan (MI, USA), Institut Teknologi Bandung (Indonesia), CSIRO Canberra (ACT, Australia).
9. Since 1999 the Institute has been hosting the National EMBnet node (The Global Bioinformatics Network). Cooperation with EU and worldwide bioinformatics groups has facilitated our cooperation in several international projects (5FP, COST) and mediated our cooperation in the organisation of several international scientific events.
10. Erasmus and Young Fellowship Program - during the last six years we hosted students from EU countries (medium and long stay – more than 30 days):
    - *Erasmus*: Valentina Petanjek, Martina Radic (Croatia); Athina Harito, Enriqueo Monton, Pierluca Nuccceteli (Italy); Gatien Tielemans (Belgium)
    - *Other young fellowship programs* (SAIA, Royal Society, Scope and other): Adam Hughes (Great Britain); Dragana Cucak (Serbia); Kristina Tesonovic (Serbia); Edyta Niska, Karolina Pelka (Poland); Radoslava Rechtoriková (Great Britain); Viktoria Ostapenko (Ukraine); Filip Opaterný (Nederland)

2.3.2. List of international conferences (co)organised by the institute

Conference	Place	Date	Participants
"EMBL opportunities for Slovak research community"	Bratislava, Slovakia	25 Feb 2016	90
Methodologies to identify the microflora responsible of biodeterioration of archival documents	Bratislava, Slovakia	31 Mar - 1 Apr 2016	40
The Sixth Symposium on the Alpha-Amylase Family - ALAMY_6	Smolenice, Slovakia	11 - 15 Sep 2016	80
Meeting of researchers of the trilateral cooperation project	Bratislava, Slovakia	9 - 10 October 2017	14
Exposition "CRISTALES: a world to discover"	Bratislava, Slovakia	27 Feb - 28 Mar 2018	N/A
Meeting of project developers EU 7FP StrepSynth (project 613877): Rewiring the Streptomyces cell factory for cost-effective production of biomolecules	Bratislava, Slovakia	8 - 9 Nov 2018	31
The Seventh Symposium on the Alpha-Amylase Family - ALAMY_7	Smolenice, Slovakia	29 Sep - 3 Oct 2019	70
1st StruBioMol Conference Structural biology for medicine and biotechnology	Bratislava, Slovakia	6 Dec 2021	133
12th Webinar of Molecules – Recent Advances in Carbohydrate-Active Enzymes; https://molecules-12.sciforum.net/	on-line	23 Jun 2021	66

2.3.3. List of edited proceedings from international scientific conferences

1. The Sixth Symposium on the Alpha-Amylase Family - ALAMY_6
   Smolenice Castle, Slovakia, 11-15 SEP 2016
   Janeček, Štefan (editor): Programme and Abstracts; 120 pp.
   ISBN 978-80-968364-5-1
2. The Seventh Symposium on the Alpha-Amylase Family - ALAMY_7
   Smolenice Castle, Slovakia, 29 SEP - 3 OCT 2019
   Janeček, Štefan (editor): Programme and Abstracts; 112 pp.
   ISBN 978-80-971617-2-9

2.3.4. List of journals edited/published by the institute and information on their indexing in WOS, SCOPUS, other database or no database, incl. impact factor and other metrics of journals in each year of the assessment period

1. Biologia
   section Cellular and Molecular Biology
   Managing Editor: Janeček, Štefan
   ISSN: 0006-3088
   EISSN: 1336-9563
   https://www.springer.com/journal/11756
   https://www.editorialmanager.com/biol/
   Impact Factor: 1.653 (2021); 1.350 (2020); 0.811 (2019); 0.728 (2018); 0.696 (2017); 0.759 (2016); 0.719 (2015). WOS, Scopus, Scimago

- **National position of the institute**

2.3.5. List of selected activities of national importance

- The Institute built the first unique specialized crystallisation laboratory in Slovakia fully equipped with Formulatrix instruments; it is open for students, postdocs and researchers in Slovakia. To promote this project several workshops and conferences were organised, which were of great interest to students and researchers. This laboratory was funded by the Interreg SK-AT project.
- The Institute participated in a research program of national importance to investigate the incidence of Lynch syndrome among the Slovak population and to identify the factors and tools to more efficiently prevent the cancer associated with it. The Institute's role in the strategic program was to identify the specific composition of the microbiome in patients with colorectal cancer using next generation sequencing. This research was funded by EU Structural funds.
- Our study of microbial communities led to a better understanding of which microorganisms are involved in the biodeterioration of cultural heritage objects. In this connection, we contributed to the microbial diagnostics of many Slovak cultural heritage objects and also proposed proper countermeasures for mitigating the presence of microbiota on object surfaces and in the environments where the objects are stored and exhibited. Our expertise permitted us to solve various problems for the Slovak National Archives (Bratislava), the University Library in Bratislava, the Academy of Fine Arts and Design (Bratislava), the Betliar Chateau (Slovak National Museum), the Saint Gorazd Seminary (Nitra), and the Evangelical Church (Spišská Nová Ves). Moreover, the study of the properties of the microbiota in Slovak traditional foods, such as bryndza cheese and wines, is important for improving their characteristics. In this field we cooperated with several companies, including Salaš Oľga Apoleníková (Pružina), Agrospol Hradová (Tisovec), Zväz vinohradníkov a vinárov Slovenska (Bratislava; Union of winegrowers and winemakers of Slovakia) and Night sky, s.r.o. (Bratislava).
- The development and optimisation of laboratory methods for determining the anti-bacterial, antibiofilm and immunomodulatory effects of natural products (e.g. honeybee products, beta-glucans, cannabidiol) allowed us to offer commercial laboratory services for private Slovak biotechnology companies, including Pleuran s.r.o., Immunoglukan, s.r.o. and Fresh & Fit, spol. s r.o. In addition, the research activities of the Laboratory of Apidology and Apitherapy are solving various research tasks for the Slovak beekeeping association as well as the Ministry of Health (working group on pharmacoeconomics, clinical outcomes and health technology assessment).
- The bioinformatics group at the Institute is involved in cooperative research projects investigating biomarkers in dozens of Slovak oncological patients. This includes two APVV projects supervised by prof. Mego from the National Cancer Instiute and it covers gene expression, miRNA and sequence-variant profiling in patients with breast cancer (with a focus on processes related to the circulating of tumour cells and the epithelial to mesenchymal transition) and testicular germ cell tumours (with a focus on cisplatin resistance).

2.3.6. List of journals (published only in the Slovak language) edited/published by the institute and information on their indexing in WOS, SCOPUS, other database or no database, incl. impact factor and other metrics of journals in each year of the assessment period

none

- **Position of individual researchers in the international context**

2.3.7. List of invited/keynote presentations at international conferences, as documented by programme or invitation letter

1. Barak, I.: *Bacillus subtilis* as a tool in basic science and applied research. XXII Biotechnology Summer School, Wielimowo, Poland, 5-9 Jul 2016. (invited lecture)
2. Janeček, Š.: How wide and flexible is the armful of the alpha-amylase family? The 6th Symposium on the Alpha-Amylase Family, Smolenice, Slovakia, 11-15 Sep 2016. (invited lecture)
3. Janeček, Š.: Starch hydrolases and related enzymes from Archaea, Bacteria and Eucarya - unique sequence-specificity fingerprints and evolutionary stories. Starch Round Table EU 2016, Lille, France, 17-18 Nov 2016. (key-note lecture)
4. Leksa, V.: Protein trafficking: a prequel to adaptive and innate immune responses. 12th EFIS-EJI Tatra Immunology Conference, Strbske Pleso, Slovakia, 3-7 Sep 2016. (invited lecture)
5. Urbániková, Ľ.: Protein as the main variable in crystallization. FEBS Practical Course Advanced Methods in Macromolecular Crystallization VII, Nove Hrady, Czech Republic, 27 Jun - 2 Jul 2016. (invited lecture)
6. Barák, I.: *Bacillus subtilis* as a tool in basic science and applied research. Workshop of Slovak Academy of Sciences and Smolenice with National University of Singapore, The Nanyang Technology University and the Agency for Science, Technology and Research, Smolenice, Slovakia, 16-17 Mar 2017. (invited lecture)
7. Barák, I.: Protein structure studies - the way to understand the mechanisms of basic cell processes in model organism Bacillus subtilis. School of Free Electron Laser and Synchrotron Radiation, Liptovsky Jan, Slovakia, 9-11 May 2017. (invited lecture)
8. Barák, I., Muchová, K., Chromíková, Z., Makroczyová, J., Krascsenitsová, E., Pavlendová, N., Valenčíková, R.: How the bacterial cells recognize the proper sites of cell division? XI. Serbian Microbiologists Congress, Beograd, Serbia, 11-13 May 2017. (invited lecture)
9. Bauer, J., Kutejová, E., Bauerová-Hlinková, V.: The Molecular Dynamics and Stability of the N-terminal domain of the human Ryanodine Receptor 2. 10th International conference Structure and stability of biomacromolecules, Košice, Slovakia, 4-7 Sep 2017. (invited lecture)
10. Bauerová-Hlinková, V., Bauer, J., Benko, M., Mačaková, K., Borko, L., Ševčík, J., Kutejová, E.: Identification, characterization and structural analysis of the N-terminal domain of human ryanodine receptor 2. Instruct Biennial Structural Biology Conference, Brno, Czech Republic, 24-26 May 2017. (invited lecture)
11. Janeček, Š.: Alpha-amylase family GH57 revisited: an *in silico* study focused on updating the old sequence fingerprints and suggesting the new enzyme specificities. Satellite Meeting of CBM12, Copenhagen, Denmark, 20-21 Apr 2017. (invited lecture)
12. Urbániková, Ľ.: What to do if protein "does not cooperate". Proteins in action, biophysical techniques for protein research, Ceske Budejovice, Czech Republic, 26-28 Jun 2017. (invited lecture)
13. Urbániková, Ľ.: A summary of RNase Sa structure - function studies. 10th International conference Structure and stability of biomacromolecules, Košice, Slovakia, 4-7 Sep 2017. (invited lecture)
14. Bauerová-Hlinková, V., Mačaková, L., Beck, K., Benko, M., Čierna, D., Kutejová, E., Bauer, J.: Structure-functional insights into the N-terminal part of the human cardiac ryanodine receptor: towards a deeper understanding of heart arrhythmias. The 43rd FEBS congress, Prague, Czech Republic, 7-12 Jul 2018. (invited lecture)

15. Urbániková,Ľ.: Proteins as the main variable in crystallization. The 8th FEBS practical crystallization course: Advanced methods in macromolecular crystallization VIII, Nové Hrady, Czech Republic, 10-16 Jun 2018. (invited lecture)
16. Urbániková, Ľ., Gavira, J.A., Mesters, J.: Conventional techniques and crystallization of own crystals. The 8th FEBS practical crystallization course: Advanced methods in macromolecular crystallization VIII, Nové Hrady, Czech Republic, 10-16 Jun 2018. (invited lecture)
17. Barák, I.: How the cell knows where to divide? New Trends in Bioscience, České Budějovice, Czech Republic, 10-11 Oct 2019. (invited lecture)
18. Bauer, J.: How Arrhytmia-Associated Mutations Alter the Dynamics of the Human Cardiac Ryanodine Receptor N-terminal Domain. 11th International Conference Structure and Stability of Biomacromolecules, Košice, Slovakia, 3-6 Sep 2019. (invited lecture)
19. Janeček, Š., Mareček, F., MacGregor, E.A., Svensson, B.: Occurrence, structure, function and evolution of starch-binding domains. New Horizons in Biotechnology - NHBT_2019, Thiruvananthapuram, India, 20-24 Nov 2019. (invited lecture)
20. Kutejová, E.: Michondrial proteases and proteins of the mitochondrial nucleoid - structure, function and role in mitochondrial homeostasis. 7th Summer School in Molecular Biophysics and Systems Biology 2019, Nové Hrady, Czech Republic, 8 Jul - 3 Aug 2019. (invited lecture)
21. Kutejová, E.: Introduction to protein purification. CAPSID Workshop an Introduction to Recombinant Protein Production, Purification, and Quality Assessment, Vienna, Austria, 3-4 Dec 2019. (invited lecture)
22. Kutejová, E.: ATP-dependent proteases and mitochondrial homeostasis. 11th International Conference Structure and Stability of Biomacromolecules, Košice, Slovakia, 3-6 Sep 2019. (invited lecture)
23. Urbániková, Ľ., Janeček, S.: Bioinformatic analysis of CE16 acetylesterases. New Trends in Bioscience, České Budějovice, Czech Republic, 10-11 Oct 2019. (invited lecture)
24. Bauer, J.: Interpreting Single-Molecule Force Spectroscopy Experiments with Normal Mode Analysis. Webinar #13 Instruct Slovakia - 12 Oct 2021 Structure meets function, Oxford, United Kingdom, 12 Oct 2021. (invited lecture)
25. Bauerová-Hlinková, V.: The Development of Structural Biology in Slovakia: Present and Future Perspectives. Strategy for future EMBL research infrastructures in the Life Sciences in Hamburg, Hamburg, Germany, 29-30 Mar 2021. (invited lecture)
26. Urbániková, Ľ.: Protein as the main variable in crystallization. 9th FEBS practical crystallization course - Advanced methods in macromolecular crystallization IX, České Budějovice, Czech Republic, 9-14 Aug 2021. (invited lecture)
27. Janeček, Š.: Alpha-amylase GH families and starch-binding domain CBM families in CAZy: in silico studies. Third Meeting & First Workshop of the Argentine Network of Enzymatic Technology, 8 Sep 2021, Rosario, Argentina (key-note lecture).
28. Janeček, Š.: Alpha-Amylase Families in CAZy: Sequences, Structures, Specificities and Evolution - a Bioinformatician's View. 12th Webinar of Molecules – Recent Advances in Carbohydrate-Active Enzymes, 23 June 2021, 15:00-16:45; online – zoom, https://molecules-12.sciforum.net/ (invited lecture)

2.3.8. List of researchers who served as members of the organising and/or programme committees

1. Barák, Imrich
   - chairperson of the programme and organizing committee of 7th European Spores Conference April 2016, Royal Holloway, University of London, United Kingdom

- chairperson of the programme and organizing committee of 8th European Spores Conference April 2018, Royal Holloway, University of London, United Kingdom
- chairperson of the programme and organizing committee 9th European Spores Conference April 2020, Naples, Italy, - cancelled due to coronavirus pandemic

2. Kutejová, Eva
   - member of the organizing committee of “EMBL opportunities for Slovak Research Community” - IMB SAS in cooperation with EMBL Hamburg, February 2016, Bratislava, Slovakia
   - chairperson of the programme and organizing committee of ATP-dependent proteases and mitochondrial homeostasis. 11th International Conference Structure and Stability of Biomacromolecules, Sep 2019, Košice, Slovakia.
   - member of the programme and organizing committee of 1st StruBioMol Conference Structural biology for medicine and biotechnology, December 2021, Bratislava, Slovakia
   - chairperson of the programme committee of XXVI. Annual Congress of Czech and Slovak Societies for Biochemistry and Molecular Biology with cooperation of Austrian and German Biochemical Section, 2021, České Budějovice, Czech Republic
3. Kľučár, Ľuboš
   - member of the programme and organizing committee of EMBnet Conference 2020, Bioinformatics Approaches to Precision Research, videoconference/on-line
   - chairperson of the programme and organizing committee of GOBLET & EMBnet AGM 2021, on-line
4. Janeček, Štefan
   - main organizer and chairman of the programme committee of The Sixth Symposium on the Alpha-Amylase Family - ALAMY_7, September 2016, Smolenice, Slovakia; http://imb.savba.sk/~janecek/Alamys/Alamy_6/
   - main organizer and chairman of the programme committee of The Seventh Symposium on the Alpha-Amylase Family - ALAMY_7, September-October 2019, Smolenice, Slovakia; http://imb.savba.sk/~janecek/Alamys/Alamy_7/
5. Urbániková, Ľubica
   - member of the programme committee of 16th International Conference on the Crystallization of Biological Macromolecules, July 2016, Prague, Czech Republic. http://www.xray.cz/iccbm/
6. Bauerová, Vladena
   - member of the organizing committee of "EMBL opportunities for Slovak Research Community" - IMB SAS in cooperation with EMBL Hamburg, February 2016, Bratislava, Slovakia
7. Pevala, Vladimír
   - chairperson of the programme committee of 3rd Structural biology meeting, INSTRUCT ULTRA, November 2019, Bratislava
   - member of the programme and organizing committee of 1st StruBioMol Conference Structural biology for medicine and biotechnology, December 2021, Bratislava, Slovakia

2.3.9. List of researchers who received an international scientific award

1. Majtán, Juraj: The prize World Expert in Honey Research - awards by the Expertscape platform (USA)

- **Position of individual researchers in the national context**

2.3.10. List of invited/keynote presentations at national conferences, as documented by programme or invitation letter

1. Faltinova, A., Sevcik, J., Zahradnikova, A.: Interaction of the RyR2 channel with its domain peptide. 7th Slovak Biophysical Symposium, Nový Smokovec, Slovakia, 6-8 Apr 2016. (key-note lecture)

2.3.11. List of researchers who served as members of organising (O) and programme (P) committees of national conferences

1. Beke, Gábor
   - member of the programme and organizing committee of Bioinformatic workshop 2021, on-line
2. Bauerová, Vladena
   - member of the programme and organizing committee of Exposition "CRISTALES: a world to discover", February – March 2018, Bratislava, Slovakia
3. Urbániková, Ľubica
   - member of the programme and organizing committee of Our proteins 2016 – Structure and Function
   - member of the programme and organizing committee of "302nd Discussions of Czech and Slovak Crystallographic Association", October 2017, Bratislava, Slovakia
   - member of the programme and organizing committee of Exposition "CRISTALES: a world to discover", February – March 2018, Bratislava, Slovakia
4. Kľučár, Ľuboš
   - member of the programme and organizing committee of Bioinformatic workshop 2021, on-line

2.3.12. List of researchers who received a national scientific award

Awards by the Slovak Academy of Sciences

1. Pangallo, Domenico
   - The Slovak Academy of Sciences Award 2016 for the top scientific team of SAS; the award for the exceptional results of the scientific research in the field of Cultural heritage deterioration and the quality of traditional Slovak food: the study of microbial communities.
2. Leksa, Vladimír
   - The Slovak Academy of Sciences Award 2018 for the scientific publication in Nature Index; the award for publishing the scientific paper in Nature Index: *Lactoferrin is a natural inhibitor of plasminogen activation*. ZWIRZITZ, A. - REITER, M. - ŠKRABANA, Rostislav - OHRADANOVA-REPIC, A. - MAJDIC, O. - GUTEKOVÁ, Marianna - CEHLÁR, Ondrej - PETROVČÍKOVÁ, Eva - KUTEJOVÁ, Eva - STANEK, G. - STOCKINGER, H. - LEKSA, Vladimír**. Lactoferrin is a natural inhibitor of plasminogen activation. *In Journal of Biological Chemistry*, 2018, vol. 293, p. 8600-8613. (2017: 4.011 - IF, Q2 - JCR, 2.672 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0021-9258. https://doi.org/10.1074/jbc.RA118.003145
3. Majtán, Juraj
   - The Slovak Academy of Sciences Award 2018 for popularization activities; The Presidium of Slovak Academy of Sciences, SAS
4. Ševčík, Jozef
   - The Outstanding Personality of SAS Award 2018; The Presidium of Slovak Academy of Sciences, SAS

5. Zámocký, Marcel

   - The Slovak Academy of Sciences Award 2018 for the scientific publication in Nature Index; the award for publishing the scientific paper in Nature Index: NICOLUSSI, A. - DUNN, J.-D. - MLYNEK, G. - BELLEI, M. - ZÁMOCKÝ, Marcel - BATTISTUZZI, G. - DJINOVIC-CARUGO, K. - FURTMULLER, P.G. - SOLDATI, T. - OBINGER, C. Secreted heme peroxidase from Dictyostelium discoideum: Insights into catalysis, structure, and biological role. In *Journal of Biological Chemistry*, 2018, vol. 293, p. 1330-1345. (2017: 4.011 - IF, Q2 - JCR, 2.672 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0021-9258. https://doi.org/10.1074/jbc.RA117.000463

6. Gašperík, Juraj

   - The Significant Personalities of SAS Award 2019; the award for the pioneering work in the implementation and application of gene manipulation techniques in basic scientific research and the development of molecular biology, gene engineering and inherited disorders diagnosis in CSSR.

7. Hostinová, Eva

   - The Significant Personalities of SAS Award 2019; the award for the pioneering work in the implementation and application of gene manipulation techniques in basic scientific research and the development of molecular biology, gene engineering and inherited disorders diagnosis in CSSR.

8. Kormanec, Ján

   - The Slovak Academy of Sciences Award 2019 for the Best Project in the Field of the International Scientific and Technological Cooperation; the award of the project: Genetically manipulated *Streptomyces* strains in the effective production of biotechnologically relevant biomolecules.

Other national awards

1. Barák, Imrich

   - The Personality of Science and Technology 2016; the award of The Ministry of Education, Science, Research and Sport of the Slovak Republic, for outstanding results in finding answers for questions of basic research: i) How is a bacterial cell able to recognize the precise site of its division? ii) How is a bacterial cell able to recognize the polar site where the division septum in sporulation is formed? iii) Why does a single-cellular organism possess a system for committing a suicide?
   - The Award of Dr. Ludmila Sedlárová Rabanová 2020; the award for recognition of merits in the field of genetics and evolutionary biology and for work published in Nature Communications POSPÍŠIL, J. - VÍTOVSKÁ, D. - KOFROŇOVÁ, Olga - MUCHOVÁ, Katarína - ŠANDEROVÁ, H. - HUBÁLEK, M. - ŠIKOVÁ, M. - MODRÁK, M. - BENADA, O.** - BARÁK, Imrich - KRÁSNY, L. Bacterial nanotubes as a manifestation of cell death. In Nature Communications, 2020, vol. 11, no. 4963. (2019: 12.121 - IF, Q1 - JCR, 5.569 - SJR, Q1 - SJR, CCC). (2020 - Current Contents). ISSN 2041-1723. https://doi.org/10.1038/s41467-020-18800-2. * - corresponding authors.
   - ESET Science Award; The finalist of the ESET Science Award 2021, The Outstanding Scientist in Slovakia
   - The Scientist of the Year 2021, Slovakia; award Slovak Centre of Scientific and Technical Information, CVTI-SAV-ZSVS in the category of the Scientist of the Year 2021, the award for unique results in the field of bacterial physiology and the discovery of the origin of bacterial nanotubules and the manifestation of a dying cell.

2. Majtán, Juraj

   - The Premium for Three-year Scientific Response, the award by The Literature Fund 2020; the 1st place in the category of Biological and Medical Sciences

Young scientist awards

1. Kunová, Nina
   - The awards of young scientific researcher; 44th Annual Conference on Yeasts 2017, Smolenice, 2.-5. May 2017, Slovakia. The Best Poster Award in the category of Young Scientists.
   - The awards of young scientific researcher; 9th Drobnica Memorial 2017, Danišovce, 12.-14. September 2017, Slovakia. The 3rd place in the Competition of Young Scientific Researchers for the best work in Biochemistry and Molecular Biology.
2. Kotrasová, Veronika
   - The awards of the doctoral fellow; 9th Drobnica Memorial 2017, Danišovce, 12.-14. September 2017, Slovakia. The 1st place in the Competition of Young Scientific Researchers for the best poster in Biochemistry and Molecular Biology.
   - The Winner of the 10th Interactive Conference of Young Scientists 2018 by Civic Association Preveda
3. Bučeková, Marcela
   - Young scientist of the academic year 2017/2018 in the field of Natural Sciences, Chemistry; award by Junior Chamber International – Slovakia.
4. Vičíková, Kristína
   - The winner of the 9th Interactive Conference of Young Scientist 2017 by Civic Association Preveda
   - The awards of young scientific researcher; the UK Rector's Prize for the best diploma thesis 2016
   - The best presentation Prize, 3rd Black Sea International Immunology School, Lukovit, Bulgaria, 2016
5. Guteková, Marianna
   - The UK Rector's Prize for the best diploma thesis, 2017

2.4. Research grants and other funding resources

(List type of project, title, grant number, duration, total funding and funding for the institute, responsible person in the institute and his/her status in the project, e.g. coordinator "C", work package leader "W", investigator "I". Add information on the projects which are interdisciplinary, and also on the joint projects with several participating SAS institutes)

- ### International projects

2.4.1. List of major projects of Framework Programmes of the EU (which pilar), NATO, COST, etc.

Add information on your activities in international networks

1. Rewiring the Streptomyces cell factory for cost-effective production of biomolecules
   Grant agency: 7FP
   Grant registration number: 613877
   Duration: 12/2013 – 11/2018
   Total Funding: 8,691,070 EUR
   Funding for IMB SAS:183,680 EUR
   Responsible person at IMB SAS: Ján Kormanec /I
   Coordinator: Katholieke Universiteit Leuven, Progenus s.a., Q-Biologicals nv, Belgium
   Number of cooperating institutions: 10

2. Building learning and research capacities in the structure and functional analysis of biomolecules for the needs of biomedicine and biotechnology
   Grant agency: Interreg
   Grant registration number: 305011X666
   Duration:5/2019 – 4/2022
   Total Funding: 1,166,799 EUR
   Funding for IMB SAS: 821,133 EUR
   Responsible person at IMB SAS: Eva Kutejová /C
   Number of cooperating institutions: 2

3. The role of metal homeostasis, reduction and sporulation in the metal resistance of Gram-positive bacteria
   Grant agency: SNSF/Swiss national Science Foundation
   Grant registration number: IZ73Z0_152527 / 1
   Duration: 4/2014 – 3/2017
   Total Funding: 160,000 EUR
   Funding for IMB SAS: 62,000 EUR
   Responsible person at IMB SAS: Imrich Barák /I
   Coordinator: EPFL Lausanne, Switzerland
   Number of cooperating institutions: 3

4. Regulatory network of septin higher-order structures.
   Grant agency: Humboldt Foundation, Germany
   Grant registration number: 3.4 - 1006989 - SVK - IP
   Duration:1/2017 – 12/2019
   Total Funding: 54,995 EUR
   Funding for IMB SAS: 54,995 EUR
   Responsible person at IMB SAS: Marián Farkašovský /C
   Number of cooperating institutions: 2

5. Water/Wastewater epidemiology: Development of robust and reliable molecular detection systems for surveillance of disease outbreaks
   Grant agency: MOST
   Grant registration number: MOST 108-2221-E-006 -160 -MY3
   Duration:1/2021 – 12/2023
   Total Funding: 74,988 EUR
   Funding for IMB SAS: 74,988 EUR
   Responsible person at IMB SAS: Domenico Pangallo /C
   Number of cooperating institutions: 2

6. Sustainable Water Reclamation Based on Ceramic Membrane Filtration
   Grant agency: TUBITAK
   Grant registration number: EIG_JC2019-058
   Duration: 4/2020 – 3/2023
   Total Funding: 50,000 EUR
   Funding for IMB SAS: 50,000 EUR
   Responsible person at IMB SAS**:** Domenico Pangallo /I
   Coordinator: TUBITAK, Marmara Research Center (TUBITAK MRC)
   Number of cooperating institutions:3

7. Single-molecule in vivo imaging to study sporulation in Bacillus subtilis
   Grant agency: The Royal Socienty
   Grant registration number: The Royal Society - IES - 2014/R3
   Duration:3/2015 – 2/2017
   Total Funding: 12,000 EUR
   Funding for IMB SAS: 9,000 EUR
   Responsible person at IMB SAS: Imrich Barák /I
   Coordinator: Department of Physics and Biology, University of York, York, UK
   Number of cooperating institutions: 2

8. Modern approach for biodeterioration assessment and disinfection of historical book collections
   Grant agency: IVF Small Grants
   Grant registration number: 11530002
   Duration: 1/2016 – 6/2016
   Total Funding: 6,000 EUR
   Funding for IMB SAS: 2,000 EUR
   Responsible person at IMB SAS: Domenico Pangallo /I
   Coordinator: Lodz University of Technology, Poland
   Number of cooperating institutions:3
9. Understanding Movement and Mechanism in Molecular Machines
   Grant agency: COST
   Grant registration number: COST Action CM1306
   Duration: 6/2014 – 6/2018
   Total Funding: 0 EUR
   Funding for IMB SAS:0 EUR
   Responsible person at IMB SAS: Ľubica Urbániková /I
   Coordinator: University of East Anglia, Norwich, UK
   Number of cooperating institutions: 26
10. Exploring Microbial Diversity and Functionality in Thermophilic Bioreactors for Innovation in Biotechnology
    Grant agency: MOST
    Grant registration number: SAS-MOST JRP 2014/3
    Duration:1/2015 – 12/2017
    Total Funding: 189,623 EUR
    Funding for IMB SAS: 74,566 EUR
    Responsible person at IMB SAS**:** Domenico Pangallo /C
    Number of cooperating institutions: 2

- **National projects, incl. international projects with only national funding**

2.4.2. List of ERA-NET projects funded from SAS budget

none

2.4.3. List of projects of the Slovak Research and Development Agency, APVV

1. Development of structural methods to the study of pharmacologically active enzymes
   Grant agency: APVV
   Grant registration number: APVV DS-2016-0050
   Duration: 1/2017 – 12/2018
   Funding for IMB SAS: 10,000 EUR
   Responsible person at IMB SAS: Ľubica Urbániková /C
   Number of cooperating institutions: 3
2. Asymmetric cell division during bacterial endospore formation (Acronym: CELLDIV)
   Grant agency: APVV
   Grant registration number: APVV-18-0104
   Duration: 01.07.2019 – 30.06.2023
   Total Funding: 170,000 EUR
   Funding for IMB SAS: 170,000 EUR
   Responsible person at IMB SAS: Imrich Barák /C
3. The double-edged sword of the plasminogen system: From homeostasis maintenance to COVID-19 (Acronym: PLASARS)
   Grant agency: APVV
   Grant registration number: APVV-20-0513

Duration: 01.08.2021 – 30.06.2025
Total Funding: 160,000 EUR
Funding for IMB SAS: 135,280 EUR
Responsible person at IMB SAS: Vladimír Leksa /C
Responsible person at SAV: Rostislav Škrabana
Number of cooperating institutions:2

4. Colored stains on historical papers: biological and chemical characterization coupled with removal solutions (Acronym: StainsAway)
   Grant agency: APVV
   Grant registration number: APVV-19-0059
   Duration: 01.07.2020 - 30.06.2023
   Total Funding: 150,000 EUR
   Funding for IMB SAS: 72,500 EUR
   Responsible person at IMB SAS: Mária Bučková /C
   Number of cooperating institutions: 3

5. Fungal Hybrid Heme Peroxidases from Primeval Forest with Application in Environmental Biotechnologies (Acronym: FUNPOXHYB)

   Grant agency: APVV
   Grant registration number: APVV-20-0284
   Duration: 01.07.2021 - 30.06.2025
   Total Funding: 180,000 EUR
   Funding for IMB SAS: 69,039 EUR
   Responsible person at IMB SAS: Marcel Zámocký /C
   Number of cooperating institutions: 2

6. Identification of new treatment options in refractory testicular germ cell tumors (Acronym: REZTEST)
   Grant agency: APVV
   Grant registration number: APVV-20-0158
   Duration: 01.07.2021 - 30.06.2025
   Funding for IMB SAS: 16,908 EUR
   Responsible person at IMB SAS: Ľuboš Kľučár /I
   C Coordinator: Faculty of Medicine, Comenius University Bratislava
   Number of cooperating institutions:2

7. Microbial contaminants in traditional Slovakian cheeses: their elimination by scientific tools based on quantitative analysis and mathematical modelling (Acronym: SafeCHeese)
   Grant agency: APVV
   Grant registration number: APVV-19-0031
   Duration: 01.07.2020 - 30.06.2023
   Total Funding: 250,000 EUR
   Funding for IMB SAS: 70,000 EUR
   Responsible person at IMB SAS: Domenico Pangallo /I
   Coordinator: Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava
   Number of cooperating institutions:3

8. Cancer immunoediting in multiple myeloma: immune checkpoints and clinical significance (iMMunoedit)
   Grant agency: APVV
   Grant registration number: APVV-20-0183
   Duration: 01.08.2021 - 30.06.2025
   Funding for IMB SAS: 28,222 EUR
   Responsible person at IMB SAS: Ľuboš Kľučár /I
   Coordinator: Biomedical Research Center of the SAS
   Number of cooperating institutions:2

9. Preparation of new antibiotics and antitumor agents by manipulations of secondary metabolite genes and synthetic biology methods
   Grant agency: APVV
   Grant registration number: APVV-19-0009
   Duration: 01.07.2020 - 30.06.2024
   Total Funding: 200,000 EUR
   Funding for IMB SAS: 143,754 EUR
   Responsible person at IMB SAS: Ján Kormanec /C
   Number of cooperating institutions:2
10. Synthetic biology for the production of new biologically active compounds in streptomycetes
    Grant agency: APVV
    Grant registration number: APVV-15-0410
    Duration: 01.07.2016 - 30.06.2019
    Total Funding: 199,544 EUR
    Funding for IMB SAS: 159,557 EUR
    Responsible person at IMB SAS: Ján Kormanec /C
    Number of cooperating institutions:2
11. Tree and country - influence of trees on diversity of soil microorganisms in agricultural land (Acronym: STRAKA)
    Grant agency: APVV
    Grant registration number: APVV-20-0257
    Duration: 01.07.2021 - 30.06.2025
    Total Funding: 210,000 EUR
    Funding for IMB SAS: 21,078 EUR
    Responsible person at IMB SAS: Marcel Zámocký /I
    Coordinator: Institute of Botany SAS, Bratislava
    Number of cooperating institutions: 2
12. Microbial starters and adjunct cultures for production of Slovakian bryndza cheese with traditional organoleptic properties (Acronym: BryndzaStart)
    Grant agency: APVV
    Grant registration number: APVV-20-0001
    Duration: 01.07.2021 - 30.06.2024
    Total Funding: 250,000 EUR
    Funding for IMB SAS: 60,000 EUR
    Responsible person at IMB SAS: Domenico Pangallo /I
    Coordinator: Food research Institute, National Agricultural and Food Centre (NPPC)
    Number of cooperating institutions: 2
13. Research & development of effective processes for the preparation of vanillin and other natural flavors using the oxidative and protective effect of recomtinant catalase and peroxidase (Acronym: Vannote)
    Grant agency: APVV
    Grant registration number: APVV-17-0333
    Duration: 01.08.2018 - 30.06.2022
    Total Funding: 239,766 EUR
    Funding for IMB SAS: 28,003 EUR
    Responsible person at IMB SAS: Marcel Zámocký /I
    Coordinator: Faculty of Natural Science, Comenius University in Bratislava
    Number of cooperating institutions:2
14. Harnessing the immunological mechanisms in various subtypes of B cell lymphoma
    Grant agency: APVV
    Grant registration number: APVV-19-0212
    Duration: 01.07.2020 - 30.06.2024
    Total Funding: 249,424 EUR
    Funding for IMB SAS: 30,847 EUR

Responsible person at IMB SAS: Ľuboš Kľučár /I
Coordinator: Biomedical Research Center of the SAS
Number of cooperating institutions: 3

15. Interaction between proteases, chaperones and kinases in stress condition cause by pathological conditions
Grant agency: APVV
Grant registration number: APVV-19-0298
Duration: 01.07.2020 - 30.06.2024
Total Funding: 244,000 EUR
Funding for IMB SAS: 160,000 EUR
Responsible person at IMB SAS: Eva Kutejová /C
Number of cooperating institutions:2

16. Characterization of bacterial communities of Slovakian wine by molecular-biological methods (Acronym: BAKTVIN)
Grant agency: APVV
Grant registration number: APVV-0344-12
Duration: 01.10.2013 - 30.09.2016
Total Funding: 248,615 EUR
Funding for IMB SAS: 75,000 EUR
Responsible person at IMB SAS: Domenico Pangallo /I
Coordinator: Food research Institute
Number of cooperating institutions:2

17. Identification and validation of signalling pathways associated with circulating tumor cells in breast cancer (Acronym: CTC)
Grant agency:
Grant registration number: APVV-16-0010
Duration: 01.07.2017 - 30.06.2021
Total Funding: 249,624 EUR
Funding for IMB SAS: 14,938 EUR
Responsible person at IMB SAS: Ľuboš Kľučár /I
Coordinator: Faculty of Medicine, Comenius University Bratislava
Number of cooperating institutions:2

18. Metatranscriptome of ewes' lump cheese: An RNA-based approach to determine the contribution of microorganisms to organoleptic quality of bryndza cheese (Acronym: BryndzaRNA)
Grant agency:
Grant registration number: APVV-14-0025
Duration: 01.07.2015 - 30.06.2018
Total Funding: 248,992 EUR
Funding for IMB SAS: 70,000 EUR
Responsible person at IMB SAS: Domenico Pangallo /I
Coordinator: Food research Institute, National Agricultural and Food Centre (NPPC)
Number of cooperating institutions: 2

19. Modified polymers from renewable resources and their degradation (Acronym: MOPODEG)
Grant agency: APVV
Grant registration number: APVV-15-0528
Duration: 01.07.2016 - 30.06.2020
Total Funding: 246,002 EUR
Funding for IMB SAS: 246,002 EUR
Responsible person at IMB SAS: Domenico Pangallo /I
Coordinator: Institute of Polymers
Number of cooperating institutions:2

20. Immune modulation by cytomegalovirus and its immunotherapeutic potential Acronym: IMMUNOMOD)
    Grant agency: APVV
    Grant registration number: APVV-14-0839
    Duration: 01.07.2015 - 30.06.2019
    Funding for IMB SAS: 56,279 EUR
    Responsible person at IMB SAS: Eva Kutejová /I
    Coordinator: Institute of Virology - Biomedical Research Center of the SAS
    Number of cooperating institutions: 2
21. Post-translation modifications in mitochondria and their role in pathological processes (Acronym: PoMoMiPaPro)
    Grant agency: APVV
    Grant registration number: APVV-14-0839
    Duration: 01.07.2016 - 30.06.2020
    Total Funding: 246,000 EUR
    Funding for IMB SAS: 160,000 EUR
    Responsible person at IMB SAS: Eva Kutejová /C
    Number of cooperating institutions: 2
22. Bacteriophage preparations for therapy of vaginal and urinary infection (Acronym: Terafag)
    Grant agency: APVV
    Grant registration number: APVV-16-0168
    Duration: 01.07.2017 - 30.06.2021
    Total Funding: 235,000 EUR
    Funding for IMB SAS: 70,000 EUR
    Responsible person at IMB SAS: Gabriela Bukovská /I
    Coordinator: Faculty of Natural Science, Comenius University, Bratislava
    Number of cooperating institutions: 3
23. Regulation of Pericellular Proteolysis: From Molecular Mechanisms To Novel Immune Cell Subsets and Therapeutic tools (Acronym: PPIS)
    Grant agency: APVV
    Grant registration number: APVV-16-0452
    Duration: 01.07.2017 - 30.06.2021
    Total Funding: 193,000 EUR
    Funding for IMB SAS: 160,888 EUR
    Responsible person at IMB SAS: Vladimír Leksa /C
    Number of cooperating institutions:3
24. Cytoarchitecture of calcium signalling in cardiac myocytes in the development of myocardial hypertrophy
    Grant agency: APVV
    Grant registration number: APVV-15-0302
    Duration: 01.07.2016 - 30.06.2019
    Total Funding: 250,000 EUR
    Funding for IMB SAS: 10,500 EUR
    Responsible person at IMB SAS: Vladimír Leksa /I
    Coordinator: Institute of Molecular Physiology and Genetics CBS SAS
    Number of cooperating institutions:2
25. Gut microbiota and diabetic peripheral neuropathy: effect of cemtirestat in rat models of diabetes
    Grant agency: APVV
    Grant registration number: APVV-20-0411
    Duration: 01.08.2021 - 30.06.2024
    Total Funding: 220,570 EUR
    Funding for IMB SAS: 220,570 EUR
    Responsible person at IMB SAS: Domenico Pangallo /I

Coordinator: The Centre of Experimental Medicine (CEM) of the Slovak Academy of Sciences (SAS) – Institute of Experimental Pharmacology and Toxicology
Number of cooperating institutions:2

26. Structure, properties and biotechnological potential of novel microbial enzymes degrading plant biomass (Acronym: NOVOBIOZYM)
Grant agency: APVV
Grant registration number: APVV-0602-12
Duration: 01.10.2013 - 30.06.2017
Total Funding: 250,000 EUR
Funding for IMB SAS: 70,000 EUR
Responsible person at IMB SAS: Ľubica Urbániková /C
Coordinator: Institute of Chemistry Slovak Academy of Sciences (SAS)
Number of cooperating institutions:2

2.4.4. List of projects of the Scientific Grant Agency of the Slovak Academy of Sciences and the Ministry of Education, VEGA (for funding specify only total sum obtained from all VEGA grants in particular year)

VEGA	2016	2017	2018	2019	2020	2021
Number	18	19	17	18	16	14
Funding in the year (EUR)	130,607	132,261	130,313	127,456	124,983	121,026

1. How the cell finds the asymetric site of septation during sporulation of Bacillus subtilis. Imrich Barák /C
2. Amylolytic enzymes – thousands of sequences, hundreds of structures, dozens of specificities – and what about evolution...? Štefan Janeček /C
3. Bio-cleaning of colored stains on historical documents: microbial, enzymatic, and chemical approaches. Domenico Pangallo /C
4. Lactoferrin and lactoferricin as natural plasmin inhibitors: From the structure resolution to therapeutic. Vladimír Leksa /C
5. Regulation of the interaction specificity of muti-PDZ proteins. Martina Baliová /C
6. The role of N-terminal phosphorylation and protein disorder in regulation of neurotransmitter transporters stability. František Jurský /C
7. Signal cascades of regulation of sigma factors of RNA polymerase in response to stress, cell and physiological differentiation in soil bacteria of the genus Streptomyces. Ján Kormanec /C
8. Study of the effect of cardiac arrhythmia-associated mutations on the structure and function of the human ryanodine receptor 2. Vladena Bauerová /C
9. Assembly of Septin Complex to Higher Order Structures. Marian Farkašovský /C
10. Effect of honeybee glucose oxidase on honey antibacterial properties and characterisation its production and activity in hypopharyngeal glands of honeybee (Apis mellifera). Juraj Majtán /C
11. Alpha-amylase, starch hydrolases and related enzymes - clarifying the relationships among their primary and tertiary structures, catalytic machinery, functions, properties and evolution. Štefan Janeček /C
12. Analysis of the Receptor Binding Proteins of Streptococcal, Staphylococcal, Streptomycetal and Enterococcal phages. Zuzana Šramková /C
13. Antibacterial and immunomodulatory properties of bee defensin-1 in chronic wound healing process. Juraj Majtán /C
14. ATP-dependent proteases and mitochondrial homeostasis. Eva Kutejová /C
15. Evolution of amylolytic enzymes. Štefan Janeček /C
16. Factors that influence mitochondrial nucleoid dynamics. Eva Kutejová /C

17. Phylogenomic and physiological comparison of oxidative stress responses in thermophilic and mesophilic microbes (Acronym: Fylox). Marcel Zámocký /C
18. Hybrid, lignolytic and versatile heme peroxidases from Ascomycetes and Basidiomycetes (Acronym: FUNPOX). Marcel Zámocký /C
19. Characterizations of de novo isolated bacteria and its heavy-metal-resistance determinant from soil contaminated by high nickel content. Peter Ferianc /C
20. Inhibition of neurotransmitter transporters by benzophenanthridine alkaloids. František Jurský /C
21. Innovative disinfection strategies: the essential oils effect on microflora and materials of cultural heritage objects. Domenico Pangallo /C
22. Isolation and advanced characterization of new probiotic microorganisms with potential for use in biomedicine and biotechnology. Responsible person at IMB SAS: Vladimír Pevala /I, Faculty of Medicine, UPJS, Košice /C
23. Combination of nanoparticles and essential oils for mitigating the biodeterioration on various types of building materials. Responsible person at IMB SAS: Mária Bučková /I; Institute of Physics SAS /C
24. Complex regulation of the stress response and cell differentiation by alternative sigma factors of RNA polymerase in soil Gram-positive bacteria of the genus Streptomyces. Ján Kormanec /C
25. Human milk bioactive glycoprotein lactoferrin as a regulator of homeostasis. Vladimír Leksa /C
26. Mechanisms of asymmetric cell division during sporulation of Bacillus subtilis. Imrich Barák /C
27. Protecting our memories: investigation into the biodeterioration of photographic and cinematographic materials. Domenico Pangallo /C
28. PDZ interactions of GABA transporter GAT1. Martina Baliová /C
29. Peptidoglycan hydrolases in streptomycete genomes and their phages. Jarmila Farkašovská /C
30. Proteolytic system on the surface of apoptotic cells as a component of the inflammatory microenvironment. Vladimír Leksa /C
31. The replisome of corynephage BFK20 - study of phage replication proteins. Gabriela Bukovská /C
32. Bacillus subtilis spore coat – study of formation and self-assembling properties of spore coat proteins. Daniela Krajčíková /C
33. Structure-function study of CE16 acetylesterase from *Hypocrea jecorina*. Ľubica Urbániková /C
34. Structure and function of proteins involved in regulation of basic cell processes in Bacillus subtilis. Imrich Barák /C
35. Structural and functional studies on human ryanodine receptor 2 RIH domains together with ligands for the development of new drugs to treat cardiac arrhythmias. Jacob Bauer /C
36. The study of model bacteriophages' replication proteins in system bacteriophage – host. Gabriela Bukovská /C
37. Formation and regulation of higher-order septin structures. Marian Farkašovský /C
38. Formation of proteinaceous shell of *Bacillus subtilis* spores– studies of protein-protein interactions and self-assembly of coat proteins. Daniela Krajčíková /C

2.4.5. List of projects supported by EU Structural Funds

- Long strategy for research and development oriented to incidence of Lynch syndrome in the Slovak republic population and the possibility for the prevention of cancer connected to this syndrome (Acronym: PreveLynch)

  Dlhodobý strategický výskum a vývoj zameraný na výskyt Lynchovho syndrómu v populácii SR a možnosti prevencie nádorov spojených s týmto syndrómom

  Grant agency: EU Structural Funds
  Grant registration number:
  Duration: 01.01.2020 - 30.06.2023
  Total Funding: 9,205,045 EUR
  Funding for IMB SAS: 400,000 EUR
  Responsible person at IMB SAS: Domenico Pangallo /I
  Coordinator: Comenius University in Bratislava, Science Park Comenius University Bratislava
  Number of cooperating institutions: 7

2.4.6. List of other projects funded from national resources

1. The influence of wound proteolytic environment on the antibacterial effects of honey

Vplyv proteolytického prostredia rany na antibakteriálne účinky medu

Grant agency: DOKTOGRAND SAV
Grant registration number: APP0035
Duration: 01.01.2021 - 31.12.2021
Total Funding: 2000, - EUR
Funding for IMB SAS: 2,000 EUR
PhD student: Veronika Bugárová

2.4.7. List of projects funded from private funds

1. New biologic approach into the eradication of honeybee parasite Varroa destructor

   Grant agency: private person - Juraj Krchňák
   Grant registration number: APP0035
   Duration: 01.07.2017 - 01.03.2018
   Total Funding: 7,190 EUR
   Funding for IMB SAS: 7,190 EUR
   Responsible person at IMB SAS: Juraj Majtán /C

2. Assessment of antibacterial activity of Slovak honeys: a pilot study

   Grant agency: SZV
   Grant registration number:
   Duration: 01.08.2018 - 15.07.2019
   Total Funding: 2,547 EUR
   Funding for IMB SAS: 2,547 EUR
   Responsible person at IMB SAS: Juraj Majtán /C

3. Determination of novel qualitative parameters taking into account biological properties of honey

   Grant agency: SZV
   Grant registration number:
   Duration: 01.08.2018 - 15.07.2019
   Total Funding: 3,013 EUR
   Funding for IMB SAS: 3,013 EUR
   Responsible person at IMB SAS: Juraj Majtán /C

2.4.8. List of projects funded from other competitive funds

none

2.5. PhD studies and educational activities

2.5.1. List of accredited programmes of doctoral studies, period of validity, source of funding

Study Program (SP)	Study Field (SF)	No. SF	University/Faculty
Biology (1536)	Genetics	4.2.4.	Faculty of Natural Sciences, Comenius University, Bratislava (2021 – present). (Institutional Guarantor**:** Imrich Barák
	Microbiology	4.2.7.	Faculty of Natural Sciences, Comenius University, Bratislava (2005 – present). Institutional Guarantor: Imrich Barák
	Molecular Biology	4.2.3.	Faculty of Natural Sciences, Comenius University, Bratislava. (2005 – present) Institutional Guarantor: Imrich Barák
Chemistry 1420	Biochemistry	4.1.22.	Faculty of Natural Sciences, Comenius University, Bratislava (2006 – present). Institutional Guarantor: Štefan Janeček

Head of the Institutional PhD training centre: Eva Kutejová

The Institute strongly encourages applicants for PhD study from the following research fields: molecular biology, microbiology, genetics, biochemistry, biophysics, and bioinformatics. Possible supervisors are evaluated by the Institute's Scientific Board; as of 31 December 2021, there were 29 possible PhD supervisors.

2.5.2. Summary table on doctoral studies (number of internal/external PhD students at the end of the year; number of foreign PhD students, number of students who successfully completed their theses during the year, number of PhD students who quit the programme during the year*)*

PhD study	2016			2017			2018			2019			2020			2021		
Number of potential PhD supervisors																		
PhD students	number, end of year	defended thesis	students quitted	number, end of year	defended thesis	students quitted	unumber, end of yea	defended thesis	students quitted	number, end of year	defended thesis	students quitted	number, end of year	defended thesis	students quitted	number, end of year	defended thesis	students quitted
Internal total	12	5		11	3		14	2		13	3		16	1		14	4	1
from which foreign citizens							1			2			2			2		
External	2			3			2		1	2			1	1		2		
Other supervised by the research employees of the institute	2			4			2			4			4			3		

2.5.3. PhD career path – Information on the next career steps of the PhD graduates who received their degree from the institute

In the period from 2016 till the end of 2021, twenty PhD students successfully defended their PhD thesis. Most of them, fifteen PhD graduates, continued their work in the field of science and research at the SAS or other research institutions. Five of these continue to work at our Institute as post docs. Five graduates work at foreign universities, two each in the USA and Germany, one in the Czech Republic. The rest work outside research but in

areas where they can apply their qualifications, only one graduate does not use her qualification in her job.

2.5.4. Summary table on educational activities

Teaching	2016	2017	2018	2019	2020	2021
Lectures (hours/year)*	368	170	154	140	124	131
Practicum courses (hours/year)*	462	440	436	437	344	301
Supervised diploma and bachelor thesis (in total)	56	27	31	19	15	14
Members in PhD committees (in total)	5	4	3	2	2	3
Members in DrSc. committees (in total)	1	1	1	2	0	3
Members in university/faculty councils (in total)	2	2	1	1	1	1
Members in habilitation/inauguration committees (in total)	0	0	0	0	1	4

2.5.5. List of published university textbooks

1. Proteins – Structure and Function Part 1: Protein Structure
   BAUEROVÁ-HLINKOVÁ, V., KABÁT, P., BAUER, J. PROTEÍNY - ŠTRUKTÚRA A FUNKCIA. 1. diel: ŠTRUKTÚRA PROTEÍNOV. Reviewers: J. Ševčík, H. Drahovská. 1st Edition. Publisher Comenius University, Bratislava, Slovakia. 2020-09, 109 p. ISBN: 978-80-223-4524-8
2. Proteins – Structure and Function Part 2: Protein Function
   KABÁT, Peter - BAUEROVÁ, Vladena - BAUER, Jacob. PROTEÍNY - ŠTRUKTÚRA A FUNKCIA. 2. diel: FUNKCIA PROTEÍNOV. Reviewers: H. Drahovská, M. Kúdelová. 1st Edition. Publisher Comenius University, Bratislava, Slovakia. 2021-12. 105 p. ISBN 978-80-223-5094-5

2.5.6. Number of published academic course books

none

2.5.7. List of joint research laboratories/facilities with universities

Crystallisation laboratory built together with Max Perutz Labs University of Vienna that is fully open for the following strategic partners:

- University of Vienna, Faculty of Life Sciences Department of Pharmaceutical Chemistry
- Medical University of Vienna, Institute of Pharmacology (Center for Physiology and Pharmacology)
- Medical University of Vienna Center for Anatomy and Cell Biology Division of Cell and Developmental Biology
- University of Vienna Department of Microbiology and Ecosystem Science
- University of South Bohemia, České Budějovice
- Comenius University, Faculty of Natural Sciences
- Slovak University of Technology, Faculty of Chemical and Food Technology
- Pavol Jozef Šafárik University in Košice, Faculty of Science
- University of Ss Cyril and Methodius in Trnava, Faculty of Natural Sciences

2.5.8. Supplementary information and/or comments on doctoral studies and educational activities – focused on what changes have occurred since the last evaluation in 2016

The Institute of Molecular Biology, SAS as an external organization for PhD studies educates PhD students in both internal and external forms of study. Our research employees have also supervised PhD students from other institutes. According to the rules of SAS our Institute can accept on average three applicants for PhD studies per year who are funded by the central budget of SAS. Every year, the number of registered candidates significantly exceeds the number of possible students admitted, therefore we asked the SAS several times for an exception from this limit.

Since 2020, the Institute has had a new and detailed internal quality assurance system for doctoral studies. The Institute has two guarantors of PhD study. Dr. Imrich Barák is the guarantor for the field of Biology, study programs molecular biology, microbiology and genetics, and Prof. Štefan Janeček is the guarantor for the study programs in the field of Chemistry and Biochemistry. The guarantors are obliged to meet with each doctoral student and their supervisors separately once a year and as necessary. These meetings are usually scheduled after seminars where the PhD students present their work. If necessary, the guarantors are supposed to contact the management of the Institute or the scientific council.

The Institute's research employees act as lecturers and educators at the Faculty of Natural Sciences, Comenius University in Bratislava, at the Faculty of Chemical and Food Technology, Slovak Technical University in Bratislava, and at the Faculty of Natural Sciences University of Ss Cyril and Methodius in Trnava. The Institute has highly qualified research staff capable of lecturing and educating in many different subjects (microbiology, molecular biology, protein structure and function, modelling and design of proteins, bioinformatics, genomics) at these universities.

In the years 2020 and 2021 we published a two-part University textbook entitled "Proteins – Structure and Function", which focuses on the tertiary and quaternary structure of proteins with an emphasis on viral proteins. It is the first textbook of this kind published in Slovak. The book arose from a long-term collaboration between our Institute and the Department of Microbiology and Virology, Comenius University Bratislava in teaching a subject entitled "Structure and function of bioactive proteins".

List of travel grants and fellowships

Nina Kunová

- Youth Travel Fund at EMBO/FEBS Lecture Course – Mitochondria in Life, Death and Disease in Selva di Fasano, Italy
- SFEL17 Grant at School of XFEL and Synchrotron Radiation Users "SFEL2017", Liptovský Ján, Slovakia
- INSTRUCT Biennal Structural Biology Meeting, 24 - 26 May 2017 in Brno, Czech Republic
- INSTRUCT Biennal Structural Biology Conference, 22 - 25 May 2019 in Parador de Alcalá, Colegios, Alcalá de Henares, Spain

Veronika Kotrasová

- 2017 Travel Grant INSTRUCT Biennal Structural Biology Meeting, 24 - 26 May 2017 in Brno, Czech Republic
- 2019 Travel Grant INSTRUCT Biennal Structural Biology Conference, 22 - 25 May 2019 in Parador de Alcalá, Colegios, Alcalá de Henares, Spain
- 2019 FEBS Travel Grant at 13th European Summer School – "Advanced Proteomics" 28 Jul – 3 Aug 2019 in Brixen/Bressanone, Italy

Barbora Stojkovičová

- 2018 Youth Travel Grant at 8th FEBS practical crystallization course: Advanced methods in macromolecular crystallization VIII, Nové Hrady 10 - 16 Jun 2018, Czech Republic
- 2019 EMBO Travel Grant at EMBO Workshop Protein quality control: From mechanisms to disease in Costa de la Calma, Spain
- 2019 Travel Grant INSTRUCT Biennal Structural Biology Conference, 22 - 25 May 2019 in Parador de Alcalá, Colegios, Alcalá de Henares, Spain

Kristína Vičíková

- Ernst Mach grant (OeAD) – Center for Pathophysiology, Infectology, Immunology, Medical University in Vienna, Sep 2017 - Mar 2018
- Slovak Academic Information Agency (SAIA) – Institute of Molecular Genetics, Czech Academy of Sciences, Sep 2018 - Mar 2019
- EMBO Short term fellowship, Sep - Dec 2019
- EFIS-IL Short-term fellowship, Jan - Jun 2020

Mariana Guteková

- Austria-Slovakia Scholarship for graduates - OeAD-GmbH - Agency for International Mobility and Cooperation in Education, Science and Research, 2016

Eva Petrovčíková

- She has been awarded an Ernst Mach Stipendium (OeAD), 2015

Dominik Hadžega

- Internship at SLU – Swedish University of Agricultural Sciences (Aug - Sep 2019), supported by *Erasmus +* and *Nadace pro rozvoj vzdělání*
- Workshop - *Managing single cell transcriptomics data course*, 3 - 5 Jul 2019, Hinxton, Cambridge

2.6. Societal impact

2.6.1. The most important case studies of the research with direct societal impact, max. 4 for institute with up to 50 average FTE researchers per year, 8 for institutes with 50 – 100 average FTE researchers per year and so on. Structure: Summary of the impact; Underpinning research; References to the research; Details of the impact; Sources to corroborate the impact. One page per one case study

1. Antibacterial activity as qualitative marker of honey determining its biological functionality

Summary of the impact

Our long-term research on honey antibacterial activity and elucidating its mechanism of action allowed us to develop a rapid, easy and reliable assay for evaluating the antibacterial potential of honey (Ref. 1, 2 and 3). This method determines the antibacterial activity of honey with the aim of its introduction as a functional food with health-promoting properties (Ref. 4). The method is currently accredited and commercially available for beekeepers and consumers worldwide. The Institute, together with the State Veterinary and Food Institute in Bratislava, provides certificates and quality marks for tested honey samples which has had a significant impact on the functional food market, one of the fastest-growing sectors in Europe, and provides certain advantages for the tested honeys. It is the first such commercial service available in Europe.

Underpinning research:

The method developed at the Institute is based on a broth microdilution assay performed in a 96-well microplate. It is superior to the more commonly used radial diffusion assay. The method was validated and is suitable for every type of honey, regardless of its origin and rheological properties. Furthermore, as our research results indicate, the method is also suitable for determining the negative effects of thermal processing and long-term storage on honey.

References to the research:

1. FARKAŠOVSKÁ, Jarmila - BUGÁROVÁ, Veronika - GODOČÍKOVÁ, Jana - MAJTAN, V. - MAJTÁN, Juraj**. The role of hydrogen peroxide in the antibacterial activity of different floral honeys. In *European Food Research and Technology*, 2019, vol. 245, p. 2739–2744. (2018: 2.056 - IF, Q2 - JCR, 0.704 - SJR, Q1 - SJR, CCC). (2019 - Current Contents). ISSN 1438-2377. https://doi.org/10.1007/s00217-019-03393-y
2. BUČEKOVÁ, Marcela - JARDEKOVÁ, Lucia - JURICOVÁ, Valéria - BUGÁROVÁ, Veronika - DI MARCO, Gabriele - GISMONDI, Angelo - LEONARDI, Donatella - FARKAŠOVSKÁ, Jarmila -

GODOČÍKOVÁ, Jana - LAHO, Maroš - KLAUDINY, Jaroslav - MAJTÁN, Viktor - CANINI, Antonella - MAJTÁN, Juraj**. Antibacterial activity of different blossom honeys: New findings. In *Molecules*, 2019, vol. 24, no. 8, no. 1573. (2018: 3.060 - IF, Q2 - JCR, 0.757 - SJR, Q1 - SJR, CCC). (2019 - Current Contents, WOS, SCOPUS). ISSN 1420-3049. https://doi.org/10.3390%2Fmolecules24081573

3. BUČEKOVÁ, Marcela - BUGÁROVÁ, Veronika - GODOČÍKOVÁ, Jana - MAJTÁN, Juraj**. Demanding new honey qualitative standard based on antibacterial activity. In *Foods*, 2020, vol. 9, no. 1263. (2019: 4.092 - IF, Q1 - JCR, 0.660 - SJR, Q2 - SJR, CCC). (2020 - Current Contents, WOS, SCOPUS). ISSN 2304-8158. https://doi.org/10.3390/foods9091263
4. MAJTÁN, Juraj** - BUČEKOVÁ, Marcela - KAFANTARIS, I. - SZWEDA, P. - HAMMER, K. - MOSSIALOS, D. Honey antibacterial activity: A neglected aspect of honey quality assurance as functional food. In *Trends in Food Science and Technology*, 2021, vol. 118, p. 870-886. (2020: 12.563 - IF, Q1 - JCR, 2.676 - SJR, Q1 - SJR, CCC). (2021 - Current Contents). ISSN 0924-2244. https://doi.org/10.1016/j.tifs.2021.11.012

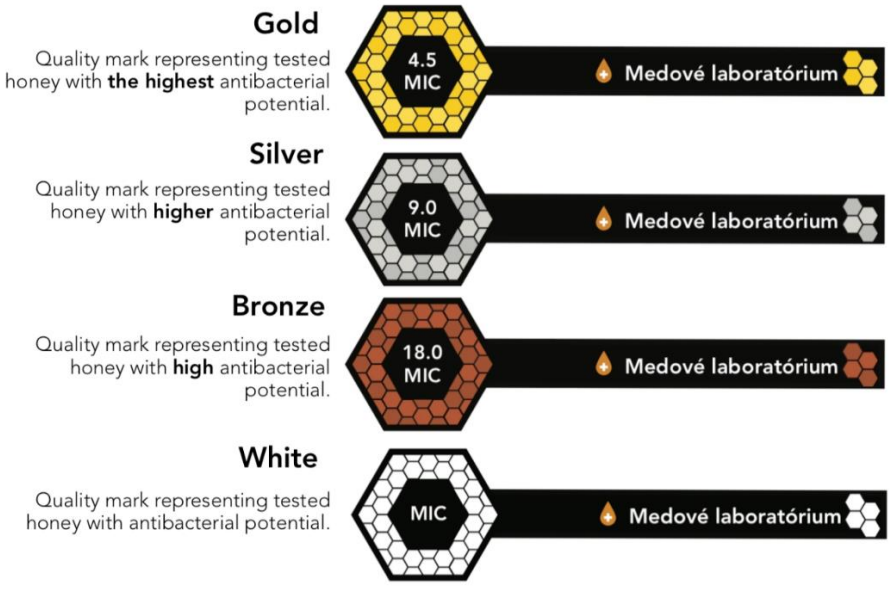
Details of the impact:

This method of determining the antibacterial activity of honey was commercially introduced and launched at the Institute in 2020 as part of the virtual project "Honey laboratory" (www.medovelaboratorium.sk/en/). The main goal of Honey laboratory is to strengthen the value of honey as a functional food through this method. Our method has direct impacts at different levels and areas of society:

- Increasing the value of honey both for beekeepers (financial aspect) and customers (functional food) via commercial testing of honey samples.
- Recognition of the most active honey recommended for medicinal use – strengthening the position of honey as a medical device in wound care. This accredited method will be offered to authorities at the Ministry of Health of the Slovak Republic as a suitable tool and criterion for selecting the most active medical-grade honey.
- The introduction of a new honey qualitative parameter into legislative norms at the national level with the collaboration of the Slovak Beekeepers' Association

Sources to corroborate the impact:

1. Annual report of the Institute for 2021 including financial statements for revenue expertise and services: https://www.sav.sk/php/download.php?inst_no=56&annual_year=2021
2. Popularisation in telecommunication, press and internet media including press release. For example: https://www.forbes.sk/vo-svojom-medovom-laboratoriu-skumaju-kvalitu-a-ucinky-medu-co-nim-mozeme-liecit/
3. Slovak Beekeepers' Association, www.vcelari.sk
4. State Veterinary and Food Institute, https://www.svuba.sk/



Quality marks based on the antibacterial activity of honey designed and granted by the Institute.

2. Detection of cultural heritage degrading microbiota by high-throughput sequencing

Summary of the impact

In our daily work, we contribute to preserving the beauty and knowledge of our artistic and cultural heritage objects from microbial colonization. Precious cultural heritage objects are exhibited or stored in various kinds of environments and can be influenced by outdoor and indoor conditions (temperature, humidity, light, weather events etc.). Our work concerns the identification of the microbiota responsible for the biodeterioration of cultural heritage items. We can perform reliable analyses of the microbiota present in the surrounding environment (such as air and surfaces) and on targeting cultural heritage objects using high-throughput sequencing approaches. All these analyses increase the knowledge about biodeteriogenic colonizers and such diagnosis allows a more specific and dedicated care of the cultural heritage health.

Underpinning research

It is necessary to consider the fragile characteristics of cultural heritage items and therefore we are able to apply non-invasive methods for sampling and investigation. Frequently, we can use only very small samples for analysis, and usually there is no second chance! These small samples should be enough for DNA / RNA extraction and the subsequent investigation of microbial communities using diverse high-throughput sequencing platforms. In particular, we have developed specific pipelines based on the Illumina MiSeq (Ref. 3 and 4) and on third-generation sequencing (MinION, Oxford Nanopore Technologies, Ref. 1 and 2). To the best of our knowledge we are among the first groups in the world to apply a MinION approach to study the deterioration-causing microbiota on cultural heritage objects. The identification of deteriorating microbiota is a crucial step, because precise diagnosis permits the best solutions for safeguarding our cultural and artistic heritage.

References to the research

1. PLANÝ, Matej - PINZARI, F.** - ŠOLTÝS, K. - KRAKOVÁ, Lucia - CORNISH, L. - PANGALLO, Domenico - JUNGBLUT, A.D. - LITTLE, B. Fungal-induced atmospheric iron corrosion in an indoor environment. In *International Biodeterioration & Biodegradation*, 2021, vol. 159, no. 105204. (2020: 4.320 - IF, Q2 - JCR, 1.103 - SJR, Q1 - SJR, CCC). (2021 - Current Contents). ISSN 0964-8305. https://doi.org/10.1016/j.ibiod.2021.105204
2. ŠOLTÝS, K. - PLANÝ, Matej - BIOCCA, P. - VIANELLO, V. - BUČKOVÁ, Mária - PUŠKÁROVÁ, Andrea - SCLOCCHI, M.C. - COLAIZZI, P. - BICCHIERI, M. - PANGALLO, Domenico** - PINZARI, F. Lead soaps formation and biodiversity in a XVIII Century wax seal coloured with minium. In *Environmental microbiology*, 2020, vol. 22, 1517–1534. (2019: 4.933 - IF, Q1 - JCR, 2.180 - SJR, Q1 - SJR, CCC). (2020 - Current Contents). ISSN 1462-2912. https://doi.org/10.1111/1462-2920.14735
3. KRAKOVÁ, Lucia - ŠOLTYS, Katarína - PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - JESZEOVÁ, Lenka - KUCHARÍK, Marcel - BUDIŠ, Jaroslav - OROVČÍK, Ľubomír - SZEMES, Tomáš - PANGALLO, Domenico**. The microbiomes of a XVIII century mummy from the castle of Krasna Horka (Slovakia) and its surrounding environment. In *Environmental microbiology*, 2018, vol. 20, iss. 9, p. 3294-3308. (2017: 4.974 - IF, Q1 - JCR, 2.209 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 1462-2912. https://doi.org/10.1111/1462-2920.14312
4. KRAKOVÁ, Lucia - ŠOLTÝS, K. - OTLEWSKA, A. - PIETRZAK, K. - PURKRTOVÁ, S. - SAVICKÁ, D. - PUŠKÁROVÁ, Andrea - BUČKOVÁ, Mária - SZEMES, Tomáš - BUDIŠ, J. - DEMNEROVA, K. - GUTAROWSKA, B. - PANGALLO, Domenico**. Comparison of methods for identification of microbial communities in book collections: Culture-dependent (sequencing and MALDI-TOF MS) and culture-independent (Illumina MiSeq). In *International Biodeterioration & Biodegradation*, 2018, vol. 131, p. 51-59. (2017: 3.562 - IF, Q1 - JCR, 1.086 - SJR, Q1 - SJR, CCC). (2018 - Current Contents). ISSN 0964-8305. https://doi.org/10.1016/j.ibiod.2017.02.015




Details of the impact

While we are, most likely, the only laboratory in Slovakia to focus on studying the biodeterioration of cultural heritage objects, we continuously collaborate with many Slovak institutions to address their particular requests. These include the University Library in Bratislava (bio-restoration applications, Jeszeová et al., 2019), the Academy of Fine Arts (bio-removal procedure, Kisová et al., 2021 https://doi.org/10.1016/j.jbiotec.2021.06.008), the Slovak National Archives (microbial colonization of photographs, Puškárová et al., 2016), and the Slovak National Museum (Ref. 3). We also cooperate with several scientific institutes of the Slovak Academy of Sciences. Our large international cooperation is reflected by our publication activity and includes Italy (Istituto centrale restauro conservazione patrimonio archivistico librario, Ref. 2, Sclocchi et al., 2017), Poland (Pietrzak et al., 2017), Czechia (Ref. 4), Austria, UK (Natural History Museum, London; biocorrosion of the skeleton of the whale Hope, Ref.1), USA (Harvard University, Center for the Environment; Book on Biodeterioration and Preservation, 2018, https://archetype.co.uk/our-titles/biodeterioration-and-preservation/?id=272), and Spain (recent cooperation with the Universities of Valencia and also of Santiago de Compostela). In conclusion, as the famous writer said: "*Beauty will save the world*". That's true, and we are proud to be a small yet valuable part of this social and cultural rescue operation.

Sources to corroborate the impact

1. Popularization activity 2018, 2019
   https://vedanadosah.cvtisr.sk/tag/domenico-pangallo/
   https://www.facebook.com/page/1206095982861418/search/?q=pangallo
   https://www.facebook.com/Vedicina/videos/329681907917972
2. Visegrad Fund – Small Grant (PL, CZ, SK): Modern approach for biodeterioration assessment and disinfection of historical book collections
   https://www.researchgate.net/publication/315685688_A_modern_approach_to_biodeterioration_assessment_and_the_disinfection_of_historical_book_collections
3. Cooperation with the Slovak National Museum
   https://www.ta3.com/clanok/87516/mumifikovane-telo-z-betliarskeho-muzea-skutocne-patri-zofii-seredyovej
4. Use of essential oils for the preservation of our cultural heritage
   https://svk.press/magazin/kultura-knihy-film-divadlo/domenico-pangallo-slovenske-pamiatky-zbytocne-chatraju/

3. Building education and research capacities in the structure and functional analysis of bio-molecules for the needs of biomedicine and biotechnology

Summary of the impact

Biomedicine and biotechnology are modern scientific disciplines whose development contributes to improving the health of the population and the environment. Structural biology identifies the nature of serious illnesses and provides a basis for the design of potential drugs. It also allows us to search for potentially harmful substances and to study their environmental impact. What was lacking in our region was a common educational and scientific research base to educate professionals capable of transferring modern knowledge in this area into practice.

Underpinning research

The Institute of Molecular Biology has a 30-year tradition in structural biology. More than 60 different macromolecular objects in total have been crystallized, and 37 structures determined, at the Institute.

Within the framework of this project, we set up a joint high-level educational and scientific research centre for structural biology for doctoral students, scientists and practitioners to build a broad scientific and technical base in order to make significant advances in research into biomedical and biotechnology applications for improving quality of life. We built on the experience of both partners – IMB SAS and the Max Perutz Laboratory University of Vienna – as well as their long-established international cooperation. Lectures and training seminars were organized alternately at both institutions for free and attracted a wide community and encouraged mutual contacts between PhD students and researchers with the possibility of further cooperation. The individual objectives of the project included (a) strengthening the existing cooperation and providing a long-term, sustainable basis for the development of structural biology, (b) educating university students, PhD students, researchers and practitioners, and (c) building a research centre of excellence in structural biology for the study of human diseases.

References to the research

1. KOTRASOVÁ, Veronika* - KERESZTESOVÁ, Barbora* - ONDROVIČOVÁ, Gabriela - BAUER, Jacob - HAVALOVÁ, Henrieta - PEVALA, Vladimír - KUTEJOVÁ, Eva** - KUNOVÁ, Nina**. Mitochondrial kinases and the role of mitochondrial protein phosphorylation in health and disease. In *Life-Basel*, 2021, vol. 11, p. 82. (2020: 3.817 - IF, Q2 - JCR, 0.973 - SJR, Q1 - SJR, CCC). https://doi.org/10.3390/life11020082
2. HAVALOVÁ, Henrieta* - ONDROVIČOVÁ, Gabriela* - KERESZTESOVÁ, Barbora - BAUER, Jacob - PEVALA, Vladimír - KUTEJOVÁ, Eva** - KUNOVÁ, Nina**. Mitochondrial HSP70 chaperone system - the influence of post-translational modifications and involvement in human diseases. In *International Journal of Molecular Sciences*, 2021, vol. 22, no. 8077. (2020: 5.924 - IF, Q1 - JCR, 1.455 - SJR, Q1 - SJR, CCC). https://doi.org/10.3390/ijms22158077
3. BAUER, Jacob** - BORKO, Ľubomír - PAVLOVIĆ, Jelena - KUTEJOVÁ, Eva - BAUEROVÁ-HLINKOVÁ, Vladena. Disease-associated mutations alter the dynamic motion of the N-terminal domain of the human cardiac ryanodine receptor. In *Journal of Biomolecular Structure and Dynamics*, 2020, vol. 38, p. 1054-1070. (2019: 3.310 - IF, Q2 - JCR, 0.504 - SJR, Q2 - SJR, CCC). (2020 - Current Contents). ISSN 0739-1102. https://doi.org/10.1080/07391102.2019.1600027
4. BAUER, Jacob** - BAUEROVÁ-HLINKOVÁ, Vladena. Normal mode analysis: a tool for better understanding protein flexibility and dynamics with application to homology models. In *Homology molecular modeling: perspectives and applications*. - London: IntechOpen, 2021, p. 13-30. ISBN 978-1-83962-805-4. http://dx.doi.org/10.5772/intechopen.94139

Details of the impact

The teaching and research infrastructure that was built during the project period represents the first automated laboratory for structural biology in Slovakia, which has already started to offer crystallization services for the proteins of interest of the universities and 23 strategic partners. A conference, several workshops and lectures were organized for students, postdocs and partners. They introduced information about the infrastructure and the potential applications of structural biology in biomedicine and biotechnology. This is the first complex infrastructure of structural biology in Slovakia. Detailed information about the project, partners, technologies, events and the conference are available on the project web page and were also described in an interview on Rádio Devín.

Sources to corroborate the impact

1. In terms of the project, through an open access agreement, we collaborate closely with the Max Perutz Laboratory of the University of Vienna and with strategic partners and research institutes. The strategic partners signed a letter of intent to participate in the project.
2. Project webpage: http://www.imb.savba.sk/strubiomol/index.php?id=home&lang=en

2.6.2. List of the most important studies and/or other activities commissioned for the decision-making authorities, the government and NGOs, international and foreign institutes (title, name of institution, contract value, purpose (max 20 words))

1. REVIEWING THE ASSIGN ACCURACY OF GENETIC TECHNOLOGIES AND GENETICALLY MODIFIED ORGANISMS INTO APPROPRIATE SAFETY LEVEL.
   Dr. Peter Ferianc was a member of the advisory group to the Ministry of Environment in its assessment of the initiation activities of GMOs for classification into the appropriate risk classes.
2. NATIONAL ROADMAP FOR RESEARCH INFRASTRUCTURE USAGE AND DEVELOPEMENT - SK ROADMAP 2016
   Dr. Ľuboš Kľučár as a member of the advisory group for Research and Innovation Strategies at the Ministry of Education, Science, Research and Sports participated in the preparation and editing of the final document.
3. PROGRAM OF REFORMS IN THE FIELD OF SCIENCE AND RESEARCH BASED ON THE GOVERNMENT PROGRAM STATEMENT
   Dr. Imrich Barák was a member of the advisory group of Ľudovít Paulis, state secretary of the Ministry of Education, Youth and Sports for the preparation of the given document.

2.6.3. List of contracts and research projects with industrial and other commercial partners, incl. revenues (study title, name of institution, contract value, country of partner, purpose (max 20 words))

1. On 6 June 2020, the Institute signed a contract with an Irish biotechnology company to improve the production of their commercial protein of clinical relevance. The contract is valid until the end of 2022 and can be extended. The current income from the contract is 50,000 EUR. The results of this cooperation were included in a joint foreign patent application filed in July 2021.
2. BOHEMIAN BIOTECH: DEVELOPMENT OF BIOSURFACTANT MANUFACTURING, CHARACTERISATION AND APPLICATIONS. A consortium agreement was made on 31 July 2015 between three parties: Bohemian Biotech s.r.o. and the Slovak Technical University and the Institute of Molecular Biology, Slovak Academy of Sciences. The cooperation ended in November 2016.

2.6.4.1 List of intangible fixed assets (internally registered IP (confidential know-how), patent applications, patents granted, trademarks registered) denoting background IPR

One PCT registered abroad

ANTIMICROBIAL PROTEIN, ANTIMICROBIAL RECOMBINANT PROTEIN WITH LYTIC PROPERTIES, EXPRESSION VECTOR, METHOD OF THEIR PREPARATION AND USE. PCT/SK2021/050016

Background of IP: The pathogenic bacteria *Streptococcus agalactiae* group B (GBS) causes serious diseases in humans, mainly in pregnant women and children. One of the treatment options for infections caused by multidrug-resistant GBS is the application of phage endolysins. The endolysin protein EN534-C, prepared by our researchers, is the first endolysin derived from the human clinical isolate *Streptococcus agalactiae* KMB-534 (GBS). It has a unique arrangement of catalytic and binding domains and has been prepared as a recombinant protein. Our aim was to prepare a new product suitable for use in the prevention, diagnosis and treatment of vaginal and urinary tract infections caused by the pathogenic bacteria *Streptococcus agalactiae* (GBS). The application of this endolysin will expand the possibilities for treating mainly recurrent infections. Its application is also suitable for patients where the use of antibiotics is not recommended. Several different endolysins with therapeutic potential

in the treatment of group B streptococcal infections are currently known, but their use, however, is protected by international patents.

Graphical symbols/Logos registered abroad

Three Quality marks based on honey antibacterial activity were designed and granted by the Institute

- *EUIPO No*.:008130330-0001, *registered* 19/08/2020, *Legal Status*: Legal entity, *Design status*: *registered* and fully published (A.1)
- *EUIPO No*.:008130330-0002, *registered* 19/08/2020, *Legal Status*: Legal entity, *Design status*: *registered* and fully published (A.1)
- *EUIPO No*.:008130330-0003, *registered* 19/08/2020, *Legal Status*: Legal entity, *Design status*: *registered* and fully published (A.1)

Owner: Institute of Molecular Biology of the Slovak Academy of Sciences, Slovakia

Background of IP: The Institute's unique commercial laboratory service, which was developed by our researchers for determining the antibacterial activity of honey, is intended for consumers, beekeepers and industrial honey companies. Our aim was to distinguish those honey samples which have been tested by labelling with our in-house designed honey quality marks. Three different quality marks are based on the antibacterial potency of a particular honey sample showing the value of its minimal inhibitory concentration. To make them intelligible for ordinary consumers, graphical symbols were designed using the traditional gold, silver and bronze colours. These quality marks bear our mark "Medove laboratorium" which is currently being considered for trademark status.

Two Patent applications registered in Slovakia

1. POLYPEPTIDE OF A RECOMBINANT CATALASE-PEROXIDASE, MODE OF ITS PRODUCTION IN THE CELLS OF *ESCHERICHIA COLI* AND ITS APPLICATIONS. PP50015-2019/15.3.2019

   Background of IP: There is a demand for the production of large amounts of antioxidant enzymes like catalase and peroxidase, mainly for cosmetic and textile industries. In this case a bifunctional enzyme containing both these activities in one protein molecule was obtained from a hyperthermophilic archaeon and it could be produced in huge amounts using a rather simple procedure. The main potential use for this joint catalase-peroxidase recombinant protein is as an additive for microbial cultivation media to support the growth of sensitive microorganisms (e.g. Lactobacilli that do not possess their own catalase). Another application is in the removal of phenolic substances from wastewaters.

2. ANTIMICROBIAL PROTEIN, ANTIMICROBIAL RECOMBINANT PROTEIN WITH LYTIC PROPERTIES, EXPRESSION VECTOR, METHOD OF THEIR PREPARATION AND USE. PP 50075-2020/17.12.2020

   Background of IP: The pathogenic bacteria *Streptococcus agalactiae* group B (GBS) causes serious diseases in humans, mainly in pregnant women and children. One of the treatment options for infections caused by multidrug-resistant GBS is the application of phage endolysins. The endolysin protein EN534-C, prepared by our researchers, is the first endolysin derived from the human clinical isolate *Streptococcus agalactiae* KMB-534 (GBS). It has a unique arrangement of catalytic and binding domains and has been prepared as a recombinant protein. Our aim was to prepare a new product suitable for use in the prevention, diagnosis and treatment of vaginal and urinary tract infections caused by the pathogenic bacteria *Streptococcus agalactiae* (GBS). The application of this endolysin will expand the possibilities for treating mainly recurrent infections. Its application is also suitable for patients where the use of antibiotics is not recommended. Several different endolysins with therapeutic potential in the treatment of group B

streptococcal infections are currently known, but their use, however, is protected by international patents.

One Patent registered in Slovakia

BIOPREPARATE FROM EXIGUOBACTERIUM UNDAE, METHOD OF ITS PRODUCTION AND APPLICATION

Application no: 50012-2018; *No*.: 288915 / 6.3.2018

Background of IP: In the past, a number of different adhesives were used to join different parts of historical objects, especially furniture and musical instruments. In restoration work, it is often necessary to remove these old glues, but the methods normally used to do this can potentially damage the artefacts. This is especially an issue for restoring wooden musical instruments, whose tonal qualities would be adversely affected by the steam or chemical solvents employed in other contexts. We prepared a biopreparate from the gram-positive psychrophilic bacterium *Exiguobacterio undae,* which is able to degrade glue and other adhesives but without producing the harmful effects of the older restoration techniques. Perhaps the most important potential use of this application is in the restoration of organ pipes and wooden flutes, where its use would not adversely impact their acoustical properties.

One Utility model registered in Slovakia

HONEY PREPARATION FOR USE IN MEDICINE FOR LOCAL TREATMENT OF LONG-TERM NON-HEALING WOUNDS ASSOCIATED WITH BACTERIAL INFECTION, METHOD OF ITS PRODUCTION AND PLASTER OR COVERING

Application No.: 50009-2018; *Registration No*.: 8435 / 6.5.2019

Background of IP: The development of new strategies and products for the management of non-healing wounds is challenging and necessary. In order to improve and enhance the wound-healing properties of honey, it was fortified with ascorbic acid to a level high enough to significantly increase its antibacterial and antibiofilm activity. This novel approach of medical-grade honey fortification will help to eliminate resistant bacterial biofilm in chronic wounds and shorten healing time.

Open Access resources

Four biological databases and tools developed at and hosted by the Institute (www.phisite.org, www.phibiotics.org, www.virusite.org and www.bombase.org) are all non-profit services to the scientific community; they are freely available to any individual and for any purpose and are licensed under the Creative Commons Attribution-Share Alike 3.0 Unported License.

2.6.4.2 List of licences sold abroad and in Slovakia, incl. revenues (background IPR identification, name of institution, contract value, country of partner, purpose (max 20 words))

none

2.6.5. Summary of relevant activities, max. 300 words (describe the pipeline of valorization in terms of Number of disclosure, Number of registered IP internally, number of CCR/LIC contracts and their respective summary values, the support you are receiving in specific points internally at the institute, at SAS, externally – also the limitations and drawbacks

The Institute encourages activity in relation to IP and offers full financial support for inventors for preparing patent applications with the help of the SAS Technology Transfer Office. In addition, inventors receive a financial reward for successful application. Senior researchers, postdoctoral researchers and PhD students regularly attend meetings organised by the SAS

Technology Transfer Office. Moreover, the Institute has well-established collaborations with private companies which provide outstanding support and services in the field of IP.

In order to increase the visibility and value of our inventions it is necessary to register and protect all our inventions, designs and trademarks at the European and international level. However, covering the fees for the protection of our inventions at these levels is the main limitation. Currently, the Institute does not possess any CCR/LIC contracts.

2.7. Popularisation of Science (outreach activities)

2.7.1. List of the most important popularisation activities, max. 20 items

1. Article in the magazine *Diabetic*

   (July- August 2016, *Diabetic*, Bratislava)
   Juraj Majtan published an article entitled “Honey can also heal”.

2. Researcher’s Night 2016
   (30/9/2016, Old Market Hall, Bratislava)
   We presented the stand “Microbes – pathogenic or beneficial?” We showed how to use natural essential oils, which have antimicrobial effects and are often used in natural medicine, agriculture, food and pharmacy, to gently clean several years old photos from fungi and bacteria. Twelve researchers and PhD students from the Institute took part, and the presentation was greatly appreciated by the visitors.

3. Open Doors Day 2016
   (8/11/2016, IMB SAS, Bratislava)
   As part of Science and Technology Week in the Slovak Republic, we organised a popularisation seminar with two major talks: “From gene to the 3D protein structure” (V. Bauerová) and “Microorganisms like Art” (D. Pangallo) along with a practical presentation of microorganisms living in soil, water, food and the human body. This event, mainly intended for high school students, took place at our Institute.

4. Article in the magazine *Zdravie*
   (December 2016, *Zdravie*, Bratislava)
   Juraj Majtan published an article entitled “A teaspoon of honey daily”.

5. Researcher’s Night 2017
   (29/9/2017, Old Market Hall, Bratislava)
   We presented the stand “Bees to Scientists, Scientists to people”. Visitors learned about the use of bee products in modern medicine, as well as about their individual components and mechanisms of action. We showed them ways to detect counterfeit honeys and tasted different types of honey. The youngest visitors learned new information about the life of bees through interesting tasks.

6. Lectures “Science Hiding Behind Essential Oils”
   (30/9/2017, CVTI, Bratislava)
   A series of presentations on how natural essential oils, which have antimicrobial effects and are often used in natural medicine, agriculture, food and pharmacy, can be used to gently clean even several years old photos from fungi and bacteria. The presenters were Maria Buckova, Domenico Pangallo, and Andrea Puskarova.

7. Lecture “Apitherapy”
   (24/11/2017, Banska Stiavnica)
   Juraj Majtan gave a lecture to the Slovak Association of Apitherapy.

8. International exhibition - CRISTALES: a world to discover
   (27/2/2018, SAV Patronka, Bratislava)
   In the area of the SAS Bratislava campus, we organized the visit of an international exhibition entitled “Cristales – a world to discover”, which was hosted from 27. 02. to 28. 03. 2018. The posters - works of art - were designed by Spanish artists and architects on the occasion of the International Year of Crystallography (2014). Vladena Bauerova and Lubica Urbanikova contributed to the exhibition with posters that described the scientific results in the field of protein crystallization and crystallography obtained at the

Institute. These included, for example, the crystal structure of the N-terminal domain of the human cardiac ryanodine receptor, whose structure and function are directly related to cardiac arrhythmias, and the crystal structure of the structural protein plectin, which is associated with muscle contraction.

9. Excursion of students from Giraltovce High school
   (27/6/2018, IMB SAS, Bratislava)
   The visit of students from Giraltovce High school was connected with several lectures and practical presentations: R. Novakova “Drawing by soil bacteria and their role in the production of antibiotics”, M. Buckova and A. Puskarova “Microbes – pathogenic or beneficial?” and J. Majtan “Honey and bee products”.

10. Weekend with SAS 2018
    (7-8/9/2018, Primate's Square, Bratislava)
    W presented three stands. The first was "Bacteria also have viruses". The topic was devoted to bacteriophages. Practical presentations focused on the life cycle of bacteriophages and their use in medicine (phage therapy). The second was “Microbes – pathogenic or beneficial?” and the third was “Honey and bee products”. The topic was devoted to bee products and biotherapies in medicine. There was also a live book - lecture and open discussion with J. Majtan on the topic “Honey and bee products”.

11. Researcher’s Night 2018
    (28/9/2018, Old Market Hall, Bratislava)
    We presented the stand “Bacteria also have viruses”, showing that bacteriophages are an important tool in the treatment of bacterial infections, especially those where antibiotics cease to be effective.

12. Researcher’s Night 2019
    (27/9/2019, Old Market Hall, Bratislava)
    We presented the stand “Mitochondria the cellular powerhouses” showing that mitochondria have a bacterial origin and are unique in possessing their own DNA that is inherited from generation to generation exclusively after the maternal lineage and that we can use this to build a tree of life for all living organisms on Earth. Decreased mitochondrial functions are the cause of a number of serious human diseases such as Parkinson's and Alzheimer's disease, diabetes mellitus, cardiovascular problems, epilepsy, and dementia. Practical presentations focused on mitochondria and on using knowledge from medical research. The presentation was complemented by competitions for younger visitors. Twelve of us took part in the event and this activity met with a significantly positive response.

13. Be in, do it *in silico*!
    (7/8/2019, Dennik N, Bratislava)
    Interview with Lubos Klucar about bioinformatics and data analysis.

14. Weekend with SAS 2019
    (21-22/6/2019, Primate's Square, Bratislava)
    We presented two stands. The first was entitled “What do the crystals hide?” The topic was devoted to crystals and their use in industry and medicine. The second was “Honey and bee products”. The topic was devoted to bee products and biotherapies in medicine.

15. Mitochondria
    (9/5/2020, Radio Devin, Bratislava)
    Eva Kutejova took part in a talk on Slovak Radio *Devin* focused on mitochondria.

16. Our coexistence with bacteria
    (6/6/2020, Radio Devin, Bratislava)
    Jan Kormanec took part in a talk on Slovak Radio *Devin* focused on new trends in antibiotics development.

17. Coronavirus
    (23/6/2021, Radio Expres, Bratislava)
    An interview with Vladimir Leksa about the third wave of the coronavirus pandemic.

18. Slovak and Czech scientists have described the phenomenon that occurs when a bacterial cell dies
    (25/10/2020, *Pravda,* Bratislava)
    The research results were published in the prestigious scientific journal *Nature Communications*. A new study, created in collaboration with Slovak and Czech scientists (I. Barak and L. Krasny), describes the phenomenon that occurs when a bacterial cell dies.

19. Article in the science magazine *Quark*
    (December 2021, *Quark* No. 12, Bratislava)
    Gabriela Bukovska published an article entitled “The Phage therapy from Slovakia”.

20. Online Open Doors Day 2021
    (9/11/2021, IMB SAS, Bratislava)
    As part of The Science and Technology Week in the Slovak Republic, we organised a popularisation seminar with three online major talks: “Streptomycetes - the most important producers of antibiotics” (R. Javorova), “How to read the code of life?” (D. Hadzega) and “Do proteins have their own face?” (V. Bauerova). This event was mainly intended for high school students. Eight classes from seven Slovak high schools registered for the event. After the event, the lectures were evaluated very positively by the students and their teachers.

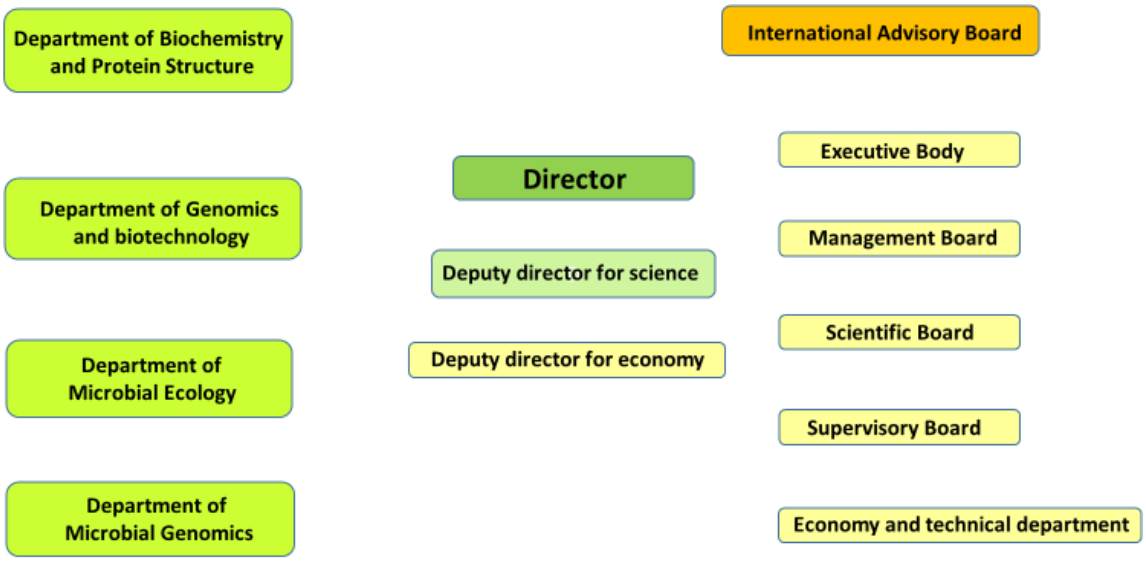
2.7.2. Table of outreach activities according to institute annual reports

Outreach activities	2016	2017	2018	2019	2020	2021	total
Articles in press media/internet popularising results of science, in particular those achieved by the Organization	5	5	12	9	27	27	85
Appearances in telecommunication media popularising results of science, in particular those achieved by the Organization	0	2	4	4	7	7	24
Public popularisation lectures	7	7	9	8	4	4	39

2.8. Background and management. Infrastructure and human resources, incl. support and incentives for young researchers

The Institute of Molecular Biology SAS has four departments with several working groups supported by their own grants. We reduced number of the department from six to four and abolished the projects and the entire previous Laboratory of Prokaryotic Biology.

The Institute created an International Advisory board that has brought new ideas to the management of the Institute and to its research program. The scientific board introduced a new system for evaluating employees’ research activity in order to stimulate motivation and satisfy researchers’ needs. We also continued to evaluate working groups using the system that was prepared during the previous assessment period. While transitioning the Institution to a new administrative form, we generated several documents that defined in detail the function of the supporting boards as well as the working conditions at the Institute including new documents concerning the policy for intellectual property. Meetings of our Institutional Board are held on a regular basis. In order to involve our youngest researchers in our decision-making processes, they also have representative as a member of this board.



Organization structure of the Institute of Molecular Biology SAS

A new *Laboratory devoted to honeybee and apimedical science* was established in 2016. This laboratory led by Dr. Juraj Majtan significantly accelerated the development of applied research at the Institute. Presently, the Institute provides a unique commercially available laboratory service for beekeepers and consumers worldwide, offering certificates and quality marks for beekeepers.

We motivated researchers to improve their qualifications. There are no limitations for PhD students, postdocs and young scientists for submitting applications to attend workshops and short international fellowships. In 2018 Eva Kutejová successfully defended her work, obtaining a DrSc (Doctor of Sciences) degree, the highest scientific degree in Slovakia, in Biochemistry and in 2019 the young scientist Juraj Majtán defended his work, obtaining a DrSc degree in Microbiology. In 2021 after a successful inaugural process Štefan Janeček was appointed a Professor of Molecular Biology by the President of the Slovak Republic. In addition, several of our young scientists successfully entered scientific category IIa, enabling them to become independent researchers and potential supervisors of PhD students. We used our foreign contacts to enable young researchers to work in collaborating workplaces and to obtain conference grants. From the Erasmus program as well as from our scientific collaborations, the following students and postdocs work or worked in our Institute: Valentina Petanjek and Martina Radic (Croatia); Athina Harito, Enriqueo Monton and Pierluca Nuccceteli (Italy); Gatien Tielemans (Belgium), Adam Hughes (Great Britain); Dragana Cucak and Kristina Tesonovic (Serbia); Edyta Niska and Karolina Pelka (Poland); Radoslava Rechtoriková (Great Britain); Viktoria Ostapenko (Ukraine); and Filip Opaterný (Nederland)

The response to COVID-19 substantially influenced all management and work in our Institute, which is mostly experimentally oriented. We had to reduce the number of people in a lab even for short periods or completely stop experimental work. Our collaborations and possibilities to attend workshops and conferences were also limited during this period. We used video meetings for better communication and combined lab experiments with home office.

2.8.1. Summary table of personnel

2.8.1.1. Professional qualification structure (as of 31 December 2021)

	Degree/rank				Research position		
	DrSc./DSc	CSc./PhD.	professor	docent/ assoc. prof.	I.	II.a.	II.b.
Male	6	12	1	0	6	7	4
Female	1	26	0	0	1	15	11

I. – director of research with a degree of doctor of science/DrSc.

II.a – Senior researcher

II.b – PhD holder/Postdoc

2.8.1.2. Age and gender structure of researchers (as of 31 December 2021)

Age structure of researchers	< 31		31-35		36-40		41-45		46-50		51-55		56-60		61-65		> 65	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Male	1,0	0,2	2,0	2,0	1,0	0,1	3,0	3,0	2,0	2,0	2,0	2,0	3,0	2,5	3,0	3,0	0,0	0,0
Female	2,0	2,0	4,0	4,0	2,0	2,0	6,0	6,0	1,0	1,0	1,0	1,0	7,0	7,0	6,0	6,0	3,0	3,0

A – number

B – FTE

2.8.2. Postdoctoral fellowships (list of positions with holder name, starting date, duration. Add brief information about each fellow's career path before and after receiving PhD degree, etc.)

2.8.2.1. MoRePro and SASPRO fellowships

none

2.8.2.2. Stefan Schwarz fellowships

1. Mgr. Marcela Bučeková, PhD (2020) - 1 Jun 2020 - 31 May 2023 (duration 2+1 years)
   Mgr. Marcela Bučeková was a PhD student at the Institute of Molecular Biology from 2016 to 2018. As a PhD student she was given a scientific internship funded by the National Grant Program at the Universita del Piemonte Orientale in Italy for 4 months. Thanks to this internship she obtained results for her thesis and founded a lasting collaboration with the laboratory of Dr. Ranzato. After receiving a PhD degree in 2018, she held a postdoctoral fellowship at the National University of Singapore in Singapore, where she worked in a protein science lab under supervision of Prof. Kini. She gained significant knowledge in protein science, recombinant techniques, skills in cell culture research, and experienced work in a multinational team. Thanks to her position in Singapore she was awarded with the Stefan Schwarz fellowship by the Slovak Academy of Sciences in 2020 for 2 years. She proved herself to be a successful and highly motivated research fellow due to her extensive publication and citation rate and the Stefan Schwarz fellowship was extended for one more year.
2. Mgr. Lucia Kraková, PhD (2013) - 1 May 2013 – 30 Apr 2017 (duration 4 years).
   Mgr. Lucia Kraková started as a PhD. student in the year 2008. The topic of her thesis was: "Cultural heritage preservation: assessment of microbial communities and their biodegradative characteristics". During her PhD. study she visited two important

laboratories in the field of cultural heritage protection: the laboratory prof. Clara Urzí (2 weeks, University of Messina, Italy) and a laboratory at BOKU University in Vienna (3 months) where she learned, under the supervision of prof. Guadalupe Piñar, to use denaturing gradient gel electrophoresis to study complex microbial communities. After her PhD study, she was employed by the Institute and applied for and received a Stefan Schwarz fellowship. This fellowship allowed her to focus her interest on the detection and identification of microbial communities using high-throughput sequencing approaches.

3. Mgr. Marek Gabriško, PhD (2011) 1 Jan 2013 - 31 Dec 2016 (duration 2+2 years).
   Mgr. Marek Gabriško started his PhD studies at the Institute of Molecular Biology in 2007. After receiving a PhD in 2011, he continued his postdoctoral research at the Laboratory of Protein Evolution of the Institute of Molecular Biology (2011 - 2013), studying the molecular evolution of amylolytic enzymes. The skills obtained and results achieved qualified him for the Stefan Schwarz fellowship, which was awarded to him by the Slovak Academy of Sciences in 2013 for 2 years. Exceptionally good results allowed this to be extended for 2 additional years. Since completing the Stefan Schwarz fellowship, he continues to work as a postdoctoral researcher at the Laboratory of Protein Evolution of the Institute of Molecular Biology (2016-present).

4. Mgr. Matej Stano, PhD (2012) 1 May 2012 - 30 Apr 2016 (duration 4 years)
   Mgr. Matej Stano, our previous master degree student, started his PhD studies at the Institute in 2007 and received his PhD in 2011. The topic of his thesis was “Study of gene expression regulation in bacteriophages – a bioinformatics approach”. During the study he acquired advanced knowledge in diverse bioinformatics techniques including database design and employment, construction of visual genome browsers and designing tools to search for sequence motifs and patterns. After finishing his study, he continued to work in our Bioinformatics group as a postdoc and was awarded a Stefan Schwarz fellowship for four years. In 2016 he started to work in the private sector but continues to work at the Institute part-time, updating and maintaining our biological database systems.

2.8.2.3. Postdoctoral positions from other resources (specify)

1. Zuzana Kisová, PhD. is fully paid through the European Structural and Investment Funds project (PreveLynch) from 23 Aug 2021 until 30 Jun 2023.
   Zuzana Kisová, PhD. was an active and very motivated student during her PhD study. Her microbiology knowledge is coupled with good skills in molecular biology methods, especially PCR-based methods, microarray analysis and high-throughput sequencing for the analysis of complex microbiota. During her PhD, despite the pandemic situation, she managed to get a two-month fellowship at the University of Reggio Calabria (Italy).

   She is employed at the Institute and is fully paid by the European Structural and Investment Funds project: “Long strategy for research and development oriented to incidence of Lynch syndrome in the Slovak republic population and the possibility for the prevention of cancer connected to this syndrome. AKRONYM: PreveLynch”. Her main scientific role the project is to study gut microbiota using high-throughput sequencing followed by bioinformatics processing.

2. Nina Kunová, PhD. is partly (80%) paid by the European Regional Development Fund Project: Interreg V-A Slovakia - Austria StruBioMol “Building learning and research capacities in the structure and functional analysis of biomolecules for the needs of biomedicine and biotechnology”, from 1 Jan 2020 until 30 Nov 2022.

   Nina Kunová, PhD. finished her PhD studies at the Institute of Molecular Biology in 2016 in the Department of Biochemistry and Protein Structure. Here she gained valuable knowledge and skills in protein isolation, purification, determination of their enzymatic activities and other characterizations. Her experience in basic and several advanced molecular biology methods helps in all aspects of the project including harvesting the practical data, evaluating and presenting results and supervising undergraduate students.

Currently, her main focus remains on work with proteins and studying their function and structure, particularly the human mitochondrial nucleoid proteins TFAM and Lon protease, whose malfunctions are often connected with serious human disorders, including neurodegenerative diseases, cancer and aging.

3. Barbora Stojkovičová PhD. is partly (80%) paid by the European Regional Development Fund Project: Interreg V-A Slovakia - Austria StruBioMol "Building learning and research capacities in the structure and functional analysis of biomolecules for the needs of biomedicine and biotechnology", from 1 Sep 2021 until 20 May 2022.

   Barbora Stojkovičová, PhD. finished her PhD studies in 2021. Her doctoral thesis was focused on the proteomic and biochemical characterization of selected yeast mutants. During her studies, she participated in several international workshops and practical courses focusing on protein stability and structure. Barbora is well-experienced in all standard molecular biology methods (PCR-based methods, DNA cloning, protein isolation and purification, Western blotting) and in several advanced techniques (yeast mitochondria isolation, determining the activities of yeast oxidative complexes, immunoprecipitation), which she actively uses in fulfilling the aims of the project. From May 2022, Barbora is on maternity leave.

2.8.3. Important research infrastructure introduced during the evaluation period with the information about the sources of funding *(max. 2 pages)*

The research infrastructure of the Institute of Molecular Biology is adequate and sufficient for fulfilling its research and development mission. During the evaluation period, the research infrastructure was further updated and improved thanks to internal and external resources (*e.g.* Interreg SK-AT). In particular, a joint high-level educational and scientific research centre for structural biology was established within the Institute in order to provide education, services and analyses in the field of biomedicine and biotechnology. Several state-of-the-art instruments and equipment were purchased:

1. Nano-format of Differential Scanning Fluorimetry (NanoDSF) Prometheus

   NanoDSF is a fast, robust, high-quality, label-free and in-solution method for the analysis of protein stability, thermal protein unfolding and melting temperature. Funding: Interreg SK-AT.

2. Crystallization Imager (ROCK IMAGER 54)

   Routine and fully automated imaging of entire 96-well plates with autoexposure and EFI with visible light and cross polarization, can store and inspect up to 54 crystallization plates at a precisely controlled temperature over several weeks to months. Funding: Interreg SK-AT.

3. Drop Setter for protein crystallisation (NT8)

   Set up hanging drop, sitting drop, microbatch, additive and seeding experiments, aspirates + dispenses drops from 10 nL to 1.5 µL with active humidification and plate copy head with liquid sensing. It is used for setup crystallization screens. Funding: Interreg SK-AT.

4. Crystallography Screen Builder (FORMULATOR)

   Uses microfluidic technology to dispense up to 16 different ingredients down to 200 nL with no upper limit to all micro-plate types. It is used to optimize crystallization conditions and to build custom crystallization screens. All the crystallization equipment from Formulatrix will be is integrated into one software package (Rock maker). Funding: Interreg SK-AT.

5. BACTRON Anaerobic/Environmental Chamber

   The Bactron chamber is designed to control atmospheric conditions to protect materials sensitive to oxygen (*e.g.* environmental samples, cultivation of anaerobic bacteria). Funding: SAS.

6. QuantStudio 1 Real-Time PCR System - Applied Biosystems

   Funding: SAS.

7. Ultracentrifuge OPTIMA XPN - 90

   The ultracentrifuge is optimized for spinning a rotor at very high speeds and is capable of generating an acceleration of up to 694,000 *g.* It is used for the pelleting of fine particulate

fractions, such as cellular organelles (mitochondria, microsomes, ribosomes) and the isolation of DNA, proteins and viruses. It can also be used for gradient separations. Funding: Interreg SK-AT.

8. Computing server

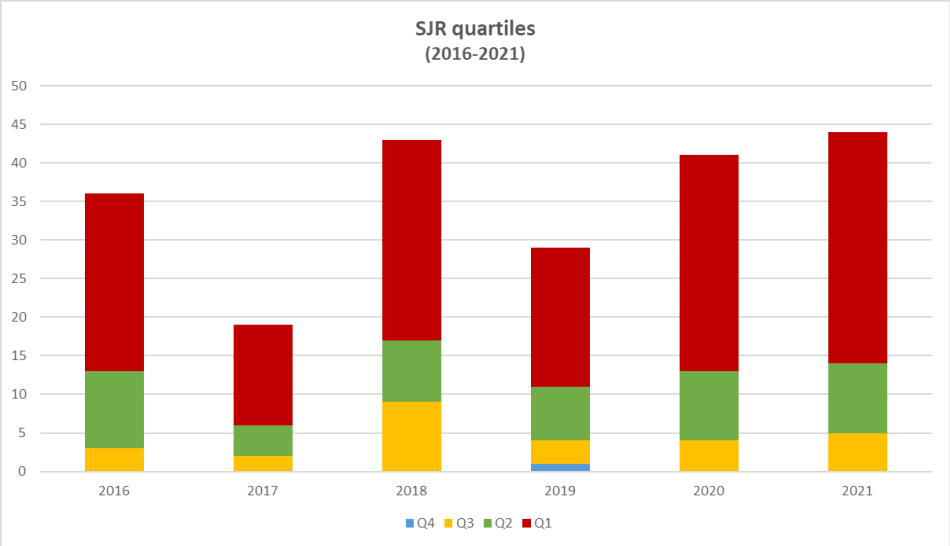
   A computational server primarily used for bioinformatics computing and serving as the main storage and application servers (64 CPU cores, 12,288 CUDA cores. 1 TB RAM and 0.1 PB disk storage). Apart from numerous individual bioinformatics tools the multipurpose analysis system Galaxy (https://galaxy20.embnet.sk) is also installed and is configured with hundreds of different bioinformatics tools. Funding: several national research projects.

Through international collaborations, the Institute has access to state-of-the-art equipment available to our European partners. These include the synchrotrons in Hamburg and Grenoble (thanks to our participation in INSTRUCT), crystallographic robots at the Department for Structural and Computational Biology, Max Perutz Laboratories in Vienna and at BIOCEV Prague, Electron microscops at Cryo-electron microscopy and tomography core facility (CEMCOF) at CEITEC Brno, and also to all EMBL core facilities.

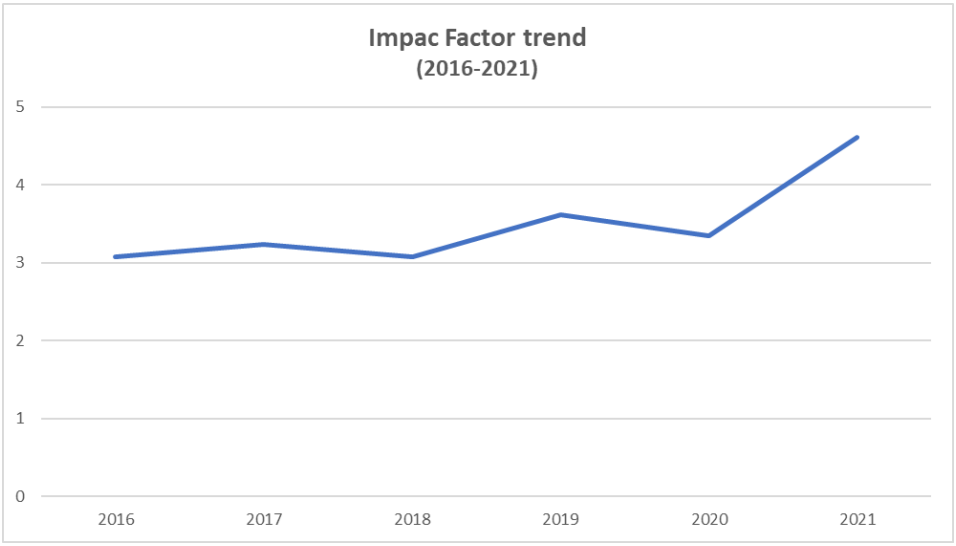
The Institute's real property has been also renovated. Several laboratories were reconstructed and newly equipped.

2.9. Supplementary information and/or comments on all items 2.1 – 2.8 (max. 2 pages in total for the whole section)

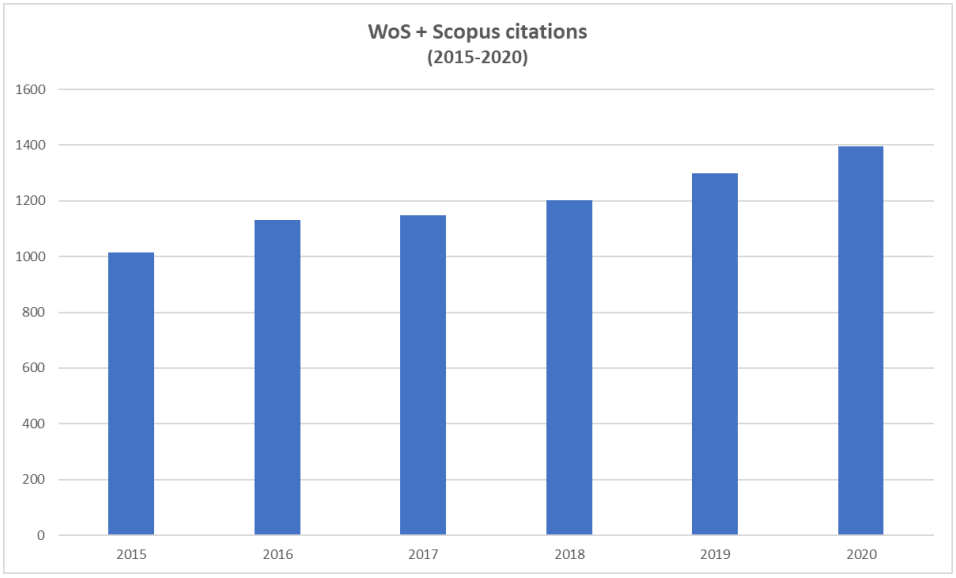
The quality of our research outputs is our primary goal. We are trying to publish our results in the best possible journals. This is reflected by the very high portion of our results that appear in the highest-ranking journals - almost 90% are in Q1+Q2 journals on average.



There is also a significant increase in the average impact factor of the journals in which we publish, from 3 in 2016 to 4.6 in 2021.



Additionally, the direct response of the scientific community to our work, i.e. citations, increased every year and, during the evaluation period, the total number of citations increased by more than one third.



The vast majority of our research outputs (e.g. research publications) are based on collaboration with national and especially international partners. The number of collaborative outputs during the evaluation period is not correlated with our secured European and international research grants. In fact, some of our research activities with huge international collaborations, such as bioinformatics projects, do not need to be supported financially. On the other hand, we actively participated as a valuable partner in several European Horizon consortiums which, unfortunately, were not successful in securing funds. The groups led by Dr. Barak and Dr. Kormanec participated in, respectively, three and two European consortiums. In addition, our long-term collaborations as well as newly established collaborations will result in securing significant research grants in the near future.

The 2020 COVID-19 pandemic affected the whole world. Several approaches were applied to overcome this serious problem. First, non-pharmacological approaches: The nationwide testing of COVID-19 was used to stop the widespread extent of viral infection at the beginning. There was also an urgent need of a systematic increase in public awareness of the viral disease itself, its manifestations as well as the importance of vaccination, namely via radio/mass media and the daily press. Secondly, in addition to emerging vaccines, there has been an urgent need of specific pharmacological approaches that would contribute to a blockade of SARS-CoV-2. Our institute was mainly involved in non-pharmacological approaches. Our young researchers participated in COVID-19 screening at their places of residence. We had several radio interviews and published several

articles in the press informing the public about the different aspects connected with COVID-19 (RTVS, Denník N, Noviny PLUS). Our colleagues took part in a collaboration group initiated by Slovak Academy of Sciences working on mathematical modelling of population infections and two of our labs also participated in detecting and identifying SARS-CoV-2 variants in Slovak waste waters.

Matej Stano and Imrich Barak from the Institute are two of the four founders of “Science wants to live!”, the independent initiative which aims to point out the problems of science, research and education in Slovakia and encourages positive changes in these areas. The founders of this initiative are researchers who realized that is necessary for working scientists to actively approach and engage the issues of science policy in Slovakia, which are not a long-term concern of either the political elite or the public. (https://www.vedachcezit.sk/, https://www.facebook.com/vedachcezit). Science and research in Slovakia continually suffer from insufficient funding. Slovakia ranks lowest among the countries of the EU and OECD in the financing of scientific and research projects as a proportion of GDP. The initiative has been actively appealing to the competent authorities for many years so that support for science and research will not be just an empty phrase, but will become a real priority, as it is in all developed countries throughout the world. Through the initiative “Science wants to live!” we have been informing policymakers and the public about the pitiful situation and distortion of the system in Slovak science, we have provided space for ideas and comments from the academic sector, we have been making constructive suggestions to the discussion of these topics, and have initiated systematic changes. We have been bringing the success stories of our scientists, popularizing and propagating science in public as well as in the media and reminding everyone of the importance of scientific exploration to the life of a society. This initiative also serves as a forum for those who are not ignorant of the fate of Slovak science and who are convinced that after almost 30 years of the independent existence of Slovakia, it is time for us to make education a priority.

3. ***Implementation of the recommendations from the previous evaluation period***

- The Institute needs clear future strategy of its research program to prioritize the numerous research projects and achieve more synergy between them.

  We decided to support the research program covered by national and international grants, which allows high impact and outstanding outcomes to be secured. Based on this strategy and perspective, two less effective projects were shuttered and the entire Laboratory of Prokaryotic Biology was terminated. This reorganization resulted in an increased publication activity over the evaluation period. We also supported joint research projects that included several working groups of our Institute in order to increase inter-group collaboration and to highlight the interdisciplinary aspect of the research (molecular biology, microbiology, biochemistry and structural biology). We increased our cooperation with laboratories at both the national and international levels, which brought about high quality outcomes, including publications. We supported several applied projects including making contracts with private companies which resulted in the preparation of two international patents.

- Structure of the Institute (6 departments and 11 laboratories) is very complicated for just 40 FTE and should be reconsidered.

  We have reduced the number of departments from 6 to 4 and created working groups that cooperate and support each other. The reduction was made after a detailed departmental productivity survey that was done based on the recommendation of the evaluation committee after the last evaluation at the beginning of 2017. The least productive Laboratory of prokaryotic biology was disbanded. Some of its members left the Institute while others joined the more effective research groups to bolster their research.

- The policy for Intellectual property is lacking and should be developed quickly.

A detailed directive on the application and protection of intellectual property rights has been in force at our Institute since March 2012. It was updated in May 2019 and all documents are available for all employees on the Institute's intranet and employees were informed via e-mail. We have closely cooperated with the SAS Technology Transfer Office (KTT SAV) in the development of a policy for intellectual property and we cooperate with the company MAJLINGOVÁ&PARTNERS, s. r. o. on applying for patents. We also organized a Seminar on knowledge transfer into practice "I have a good idea ?!" presented by Ing. Martin Grof, PhD. (KTT SAV) for all employees in April 2018. The originators of the patent application were awarded and received a one-time reward of 1000 EUR.

- More applied research projects, including international ones, would be beneficial.

  Although our Institute has been mostly oriented towards basic research, we have had several applied research projects.

  - We cooperate intensively with an Irish biotechnology company to improve the production of their commercial protein of clinical relevance. Our collaboration resulted in the filing of a foreign patent application in June 2021 involving two of our researchers (Jan Kormanec and Dagmar Homerova). The Developmental Service Agreement (DSA) between the company and our Institute was signed in June 2020 and is still in force. We have signed a non-disclosure agreement with the company, therefore, we cannot provide further details about the agreement and the results.
  - Our long-term research on bee products has led us to find that the current legislative standards that define the quality of honey in Slovakia and Europe are insufficient to detect all forms of adulteration or degradation of honey and do not reflect its biological potential. For this reason, we have developed and optimized new methods for evaluating the quality of honey with regard to its antibacterial effects. Our utility model "Medový prípravok na použitie v medicine na lokálnu liečbu dlhodobo nehojacich sa rán asociovaných s bakteriálnou infekciou, spôsob jeho výroby a náplasť alebo krytie" (Honey preparation for use in medicine for local treatment of long-term non-healing wounds associated with bacterial infection, method of its production and plaster or covering) has already received a Certificate of registration of the utility model c. UV 8435 to the register of utility models in March 2019. We also designed and registered quality marks for honey based on its antibacterial activity. More info can be found on the web site https://www.medovelaboratorium.sk/en/.
  - We developed a method for gently disassembling glued wooden objects. The method is based on the use of a biopreparation from *Exiguobacterium undae*. The patent No. 28815 was granted on the method of its preparation and use in practice in December 2021.
  - Research focusing on an alternative way of dealing with bacterial infections in women resulted in the patent application "Nový antibakteriálny rekombinantný protein EN534-C s lytickými vlastnosťami voči patogénnym kmeňom *Streptococcus agalactiae"* (Antibacterial recombinant protein with lytic properties, expression vector, method of their preparation and use) PP 50075-2020. An international patent application under PCT c.PCT/SK2021/050016 was filed in December 2021. As a result of this patent filing, a cooperation with the Swiss firm MICREOS GMBH began. A non-disclosure agreement and a supply agreement were signed and samples were sent for testing.
  - Research into a hyperthermostable catalase – peroxidase resulted in the patent application "Spôsob produkcie rekombinantnej hypertermostabilnej katalázy - peroxidazy v bunkách *Escherichia coli"* (Method of production of recombinant hypertermostable catalase - peroxidase in Escherichia coli cells) in January 2019.

  Some other projects have also had application potential. These include a project aimed at the studying the gut microbiome of the Slovak population and its possible impact on Lynch syndrome; lactoferin and lactofericin as potential COVID-19 protease inhibitors; and the search and preparation of new antibiotic compounds.

4. ***Research strategy and future development of the institute for the next five years*** (Recommended 3 pages, max. 5 pages)

Research strategy of the institute in the national and international contexts, objectives, and methods (including the information on when the strategy was adopted)

The research strategy and future development of the Institute were significantly modified based on the results and recommendations of the 2017 evaluation. Considering our conditions, possibilities and positions in our research areas we identified the following strategic priorities:

- create a supportive environment for all researchers, from early career researchers to research leaders, and make attractive working conditions for talented, world-class young researchers,
- spearhead innovative, high-impact and relevant basic research and increase research income by securing additional funding and contracts,
- continue the development of disciplinary and interdisciplinary research,
- increase collaborations with national and international research institutions, industries and universities,
- enhance the communication of our research activities and impact to the wider society at the local and international levels.

Personal management and career development of young researchers

Maintaining an optimal age distribution among the Institute's researchers is necessary for successfully advancing future research at the Institute. In upcoming years, many of our researchers (including several group leaders) will reach retirement age. It is essential to establish an efficient system for hiring young prospective scientists and new group leaders with international experience. We will be more assertive in seeking resources, advertising positions in international venues and recruiting candidates. We will be active in identifying young researchers who have the potential to successfully apply for support from European research schemes (Horizon Europe and ERC). We are already revitalising our internal postdoc support scheme, in which young colleagues are hired in a competitive manner once a year. This initiative was neglected in recent years, due mainly to a very tight and partially unpredictable salary budget. The increasing number of retiring colleagues should allow us to again accumulate sufficient funds for this initiative.

Our scientific board and director have adopted internal regulations defining the main principles of our personnel management policy. This document is updated regularly and the latest version of this document was approved in 2021.

Another important opportunity for young, talented and highly motivated researchers is in applying for postdoctoral grants at the Academy (e. g. SASPRO, IMPULZ). Although it requires co-financing from our salary budget, it does provide additional resources, allowing us to attract more excellent candidates. However, there do exist some administrative problems in the selection process (in particular, there is no timely selection process and insufficient communication with the candidates); these difficulties have already caused us to lose one candidate, who had found another position before receiving a final decision from our agency.

Attracting new, talented postdoctoral fellows and new prospective group leaders is essential for sustaining the Institute. The easiest way would be to hire the most talented of our own finishing PhD students; however, we are aware of the necessity for international mobility and gaining interdisciplinary experience, especially for younger researchers. For these reasons, we will prefer applicants after a long or at least mid-term stay in foreign research centres.

Finally, we are aware that career development is an important factor for determining the future leadership of our young researchers. The Institute's management board is ready to prepare suitable conditions for career development by supporting several candidates who will gain their management skills by completing MBA, MHA or MPH study. This approach would allow us to train excellent researchers with comprehensive management skills, which, we believe, are essential for future Institutional progress and development.

PhD studies and internationalisation of the PhD programme

At present, we have a contract with one university (Comenius University in Bratislava) in four study programmes (Molecular Biology, Microbiology, Genetics and Biochemistry). Concerning the number of study programmes, we consider this sufficient, but with regard to the number of universities, it is insufficient. We have immediate plans to expand our portfolio by adding at least the Slovak Technical University in Bratislava and the University of Ss Cyril and Methodius in Trnava. Although increasing the number of contracted universities will not allow us to educate more PhD students, it will certainly increase the number of potential candidates for PhD studies. This should result in a more stringent selection process, leading us to taking in students of higher quality. Luckily, to date, we have had more candidates each year than available PhD positions, allowing us to select the best possible candidates. In any case, we have the capacity to accommodate more PhD students than our present allowance (currently, only three new students per year). The only current option for hosting additional students is to use external funding sources, although there are practically no national funds covering a full PhD scholarship at present.

The Institute will promote the internationalisation of the PhD programme by collaborating with foreign Universities and Research centres, as well as through participation in international research projects and programmes. In this connection, we plan to participate in a consortium applying for Marie Sklodowska-Curie Action – Doctoral Networks where several PhD students would perform part of their study at our Institute. This would increase the Institute's internationalisation level by having foreign PhD students who will start their research career here.

Researcher assessment

We will continue the annual evaluation of working groups and individuals carried out by our Scientific Board. Our assessment methodology, which was optimised in recent years, is based on four main parameters: scientific outputs, citations, number of supervised PhD students and financial assets. The most important parameter is publishing research results in high-impact journals. It is summarised each year as an average over the last three years' data. Long-term consistent methodology allows both detailed analytics over time and the possibility for employees to plan their outputs to maximise possible benefits. The results of the evaluation are used, among other things, for selective financial rewards as well as during the renewal of the employees' contracts.

Research strategy

The research strategy of the Institute is based on excellence and impact in basic research and innovation in applied research. This long-term strategy was adopted after the former Institutional evaluation (2012-2015). Our fundamental research interests centre around microorganisms. The Institute is recognized as the leading research centre in SAS on research into human and environmental bacteria, bacteriophages and fungi. To study microorganisms at the molecular level, we will be supported by structural biology where the Institute is a pioneer and is well-recognized at national level due to its state-of-the-art equipment and facilities. In addition, we are open to active participation in immediately needed research in areas connected with public health (e.g. DNA testing during a pandemic situation). Our research strategy in particular research areas will be in line with the latest developments in each particular field.

Basic research:

The Institute deals with various research topics where the common factor is molecular biology, closely associated with other disciplines including microbiology, biochemistry, structural biology and genetics.

- The mechanisms of cell division, differentiation, and programmed cell death have been well-characterized in *Bacillus subtilis*. However, despite intensive research, there are still crucial molecular details missing in the complex mosaic of these processes. Our discoveries have led to new questions in these areas, the most interesting ones being (i) how does the cell recognize, with high precision, the site of septation at the cell pole during sporulation; (ii) how are these septa formed and what is the role of the protein complexes involved; (iii) how is the highly resistant spore coat formed; and (iv) what are the roles of nanotube-like structures as nanowires

and other bacterial membranous extracellular vesicles, are they formed mainly under stress conditions?

- The role of posttranslational modifications (PTMs) in mitochondrial homeostasis. PTMs play a crucial role in human pathological processes like cancer and neurodegenerative diseases. We plan to study the effect of phosphorylation on the structure, function and biochemical properties of crucial mitochondrial nucleoid components to better understand their involvement in the changes of mitochondrial function that occur in normal and cancerous tissues.
- In silico approaches for studying amylolytic enzymes. These are necessary because of the enormous and continuously increasing number of hypothetical proteins whose sequences are being generated by ongoing genome sequencing projects. Future research will therefore include deep and insightful studies focused on identifying the unique sequence-structural features of putative amylolytic enzymes that would enable one to utilize them in assigning enzyme specificity prior to detailed biochemical characterization.

Research with application potential:

Our strategy is to identify and support research topics which are attractive for the private sector and which have high application potential in biotechnology and medicine.

- Genomic manipulations (the introduction of strong promoters upstream of particular genes in the genome) and synthetic biology (combining various biosynthetic genes in new artificial gene clusters) are important approaches for preparing new and effective biologically active compounds. Extensive genomic sequencing and subsequent genome mining of many *Streptomyces* strains, which are some of the best producers of biologically active secondary metabolites, has revealed a tremendous potential for new secondary metabolites. These two approaches will be used in the future to activate these clusters in order to identify new biologically active compound that can be used in clinical practice.
- An attractive topic for the health-care sector is the preparation of new hybrid lytic proteins by combining the catalytic and binding domains of different endolysins. This new approach may help eradicate multi-antibiotic resistant bacterial pathogens. We plan to examine and characterise the effects on both free-swimming and biofilm-embedded forms.
- The characterisation of microbial communities in both environmental (especially in and around cultural heritage objects) and food samples by high-throughput sequencing is of great interest worldwide. We will therefore focus on i) identifying and characterising interesting culture starters (from dairy environments), ii) developing enzymatic bio-cleaning methods (bio-restoration) and finally iii) examining those communities responsible for the bio-geological cycles and those involved in environmental and also intestinal resistomes.
- We will investigate the molecular evolution and structure-function relationships in enzymatic antioxidants with great potential in future applications. We will especially investigate green algae, which has been insufficiently explored in this respect. We will also support emerging biotechnologies with up-to-date knowledge and expertise on relevant oxidoreductases that will hopefully find applications in the food and pharmaceutical industries.

Planed future strategic research areas at the Institute:

Institute's aim is to attract new experienced group leaders who would undertake leadership in selected research areas and setup new research groups at the Institute. The Institute has ambitions to introduce the following new research areas and strategic programmes related to microorganisms:

- Biofilm research with respect to nature/climate and the human environment
- Microbiome research with respect to health (gut, oral and wound microbiome) and disease conditions (e.g. cancer, immunodeficiencies)

These new strategic research areas fit into the Horizon Europe programme structure.

<u>International cooperation and recognition</u>

In order to strengthen the Institute's international position, it will be necessary to actively seek and participate in European and international research projects. Apart from co-operations that are more accessible (e.g. COST projects) more effort will be put into collaborations that would result in

applications for major EU projects (e.g. Horizon, ERC). Many of our research groups are involved in beneficial international collaborations, but not so many are involved in high-impact projects. We are already participating in the preparation of several European projects, including Marie Sklodowska-Curie Action – Doctoral Networks and others.

The Institute will undertake internationally excellent, world-leading research and will make certain that its research outputs are of the highest quality and are cited and recognised worldwide.

Structural biology represents is another open area that would support the projects previously listed as well as providing new possible national and international collaborations. The Institute is closely involved with the European X-ray Free Electron Laser (XFEL) in Hamburg, Germany. XFEL has been operational since 2018, and the Institute is a partner in setting up two consortia at XFEL: SFX/SPB (Serial Femtosecond X-ray crystallography) and XBI (XFEL Biology Infrastructure), and Dr. Barak, is a member of the SFX and XBI Consortia Management Boards (2014–present). The Institute will further promote the use of this state-of-the-art facility by Slovak scientists, especially structural biologists. The Institute has a tradition of and sufficient hardware resources for mid-size bioinformatics projects and is open to ongoing collaboration with other national and international research groups whose main emphasis is on human oncological research and diverse microbiota analysis. We also plan to join the ELIXIR ESFRI project, which would allow to further enhance our position in bioinformatics research and training on the national and international levels.

Research infrastructure

Our possibilities for acquiring new research infrastructure and instruments are quite limited. The most important and utilised national grant agencies (VEGA and APVV) do not provide this type of funding. The only possibility for renewing our research infrastructure is to obtain Structural Funds from the European Union. However, these are not always available and are usually limited to only certain types of research. A great help for solving this issue and making investments more effective and also improving inter-institutional cooperation within the Academy would be the creation of centralised research facilities. We strongly support the initiatives of the SAS Presidium together with other research institutes within SAS to establish a new multi-user core facility on the SAS campus on advanced research in proteomics (nano-HPLC, MALDI-TOF/TOF mass spectrometer) and microscopy (e.g. laser scanning confocal microscopy and scanning electron microscopy) and on other related disciplines which would us to address a great range of challenges in our research.

Societal impact and public engagement

To communicate the results of our work to society at large and to provide outputs directly visible and directly beneficial to the general public are other essential aspects of our work. We will continue to disseminate our results and will be very active in participating in public outreach activities, especially the annual Researchers' Night and Science Week Europe events. We are aware that it is important to present our outputs in a more acceptable way as basic research, the main focus of our research activities, is not as attractive to the public as are direct applications. In addition to this, our research areas that have immediate impact and application potential (e. g. the classification and assessment of the antimicrobial activity of honey, the preservation of cultural heritage objects damaged by microorganisms, and our very recent participation in COVID-19-related research) will continue to be advertised by our colleagues through different communication channels at the national and international levels. Last but not least, we aim to increase the attractiveness of science and technology in primary and secondary schools. We plan to share our knowledge and the importance of science in an understandable way to pupils by regularly visiting schools and creating exhibitions recommended for elementary schools and also actively participating e. g. in annual Summer School of Young Scientists organised by the NGO All4Science (www.all4science.sk).