DEPARTMENT OF BIOTECHNOLOGY
B-3

At the Department of Biotechnology we investigate biological molecules of microbiological, fungal, plant and animal origin using modern biotechnological methods. We would like to apply them for diagnostic and therapeutic purposes in human and veterinary medicine, for plant protection, the preparation of quality and safe food and for the protection of the environment, contributing to an improvement of peoples' health and the environment in which we live. Our research work is focused on the processes of cancer progression and immune response, neurodegenerative processes, the biology of fungi, plant stress response and in search for new biotechnological approaches and products.

Head (until 31. 10. 2019):
Prof. Janko Kos

Head (since 1. 11. 2019):
Prof. Boris Rogelj

In the field of research on bioactive proteins from mushrooms, we continued in 2019 to characterize L-amino acid oxidases (LAO) with antibacterial activity. These enzymes are widely distributed in various organisms and play a wide variety of biological functions, either in basal amino acid catabolism or in reactions related to hydrogen peroxide formation. The most studied are those from snake venoms, as LAOs in some represent the major toxic ingredient. We discovered an incredible diversity of LAOs in mushrooms that showed different substrate specificity, pH optima and molecular masses. We used model Gram-positive and Gram-negative bacteria to show the antimicrobial potential of LAOs from mushrooms and discovered that they completely inhibited the growth of *Escherichia coli*, while *Lactococcus lactis* had a longer lag phase. Higher fungi have been shown to represent a novel and readily available source of versatile enzymes with L-amino acid oxidase activity.

In the field of glycobiology, in 2019 we continued our research into active substances that influence the formation of biofilms of food-borne pathogenic and food-spoilage bacteria in collaboration with the Biotechnical Faculty of the University of Ljubljana. Bacterial biofilms enable the bacteria to survive even under adverse conditions, since the bacteria in them are hidden in the matrix of extracellular material. The prevention of biofilm formation can contribute to the reduced use of antibiotics.

The glycosylation profile is an important issue of cysteine peptidases inhibitor cystatin F, which was, in the previous year, one of the main targets we investigated in the field of antitumor immune response. Cystatin F can enter the lysosomes and cytotoxic granules of cytotoxic T lymphocytes and NK cells and inhibits cathepsins C and H, which are the main convertases of progranzymes, the triggers of cell death. In previous years, by using different cystatin F mutants, we have shown that the internalization of both dimeric and monomeric form of cystatin F leads to a reduction of the activity of cathepsins C and H in NK cells and consequently lower cytotoxicity in particular in NK cells. Last year we also demonstrated the same mechanism for cytotoxic T lymphocytes.

The studies of cystatin F and other effector molecules of anti-tumour immune response we extended to the tumour microenvironment. In this case tissue sections obtained from human brain tumours were used. Additionally, we developed a cell model of the tumour microenvironment with the interplay between cytotoxic cells and other immune cells, tumour stem cells and differentiated tumour cells. In this model cystatin F appeared as an important mediator, causing anergy of the cytotoxic cells and consequently a lower cancer-cell killing. On the other hand, cystatin F can increase the differentiation of cancer stem cells, which become more sensitive to convenient cancer therapy.

Tumour-cell invasion is impaired by organo-ruthenated nitroxoline derivatives through the inhibition of cathepsin-B activity

Besides the cystatin F exogenous synthetic peptidase inhibitors have been studied as potential anti-tumor compounds. In collaboration with the Faculty of Chemistry and Chemical technology at the University of Ljubljana we published a study on organo-ruthenated nitroxoline derivatives as promising inhibitors of cathepsin B.

In the field of neurobiology we continued investigations of the molecular mechanisms of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) with four published articles. We have shown that the RNA from the GGGGCC expanded repeat mutation has properties of paraspeckles, which has important consequences for the regulation of this RNA. We show that the accumulation of GGGGCC RNA can be regulated with a paraspeckle protein SFPQ. In collaboration with the Francis Crick Institute in London and the Helmholtz Center Munich we have shown that Neat1 is also regulated with TDP-43 and vice versa, and that the cross-regulation has important implications for the transition from pluripotency to differentiation. Cytoplasmic accumulation and the aggregation of TDP-43 is the main pathological sign of ALS and FTD. Thus, the involvement of TDP-43 in embryonal development also has a significant impact for neurodegeneration. In collaboration with the King's College London we published a study

Cross-regulation between TDP-43 and Neat1, the non-coding RNA scaffold of paraspeckles, promotes transition from pluripotency to differentiation

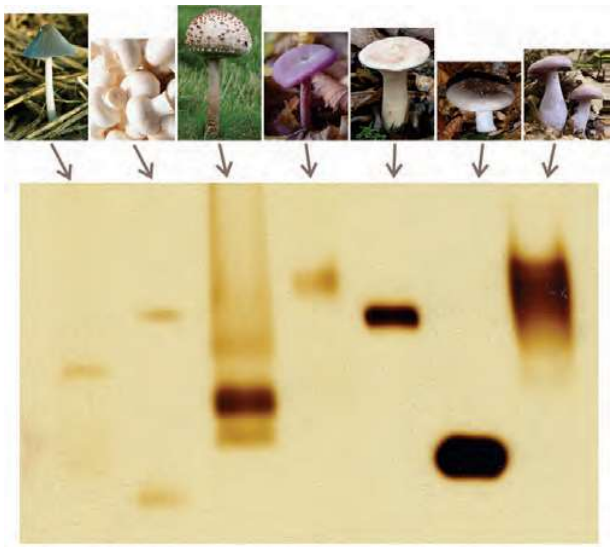

Figure 1: L-amino acid oxidase activity (LAO) in mushrooms; from left: grey shag (Coprinopsis cinerea), portobello mushroom (Agaricus bisporus), parasol mushroom (Macrolepiota procera), amethyst deceiver (Laccaria amtethystina), trooping funnel (Infundibulicybe geotropa), clouded agaric (Clitocybe nebularis) and wood blewit (Lepista nuda).

Development of electrospun poly(ethylene oxide) nanofibres that enable the long-term viability and high loading of probiotics, such as lactic acid bacteria.

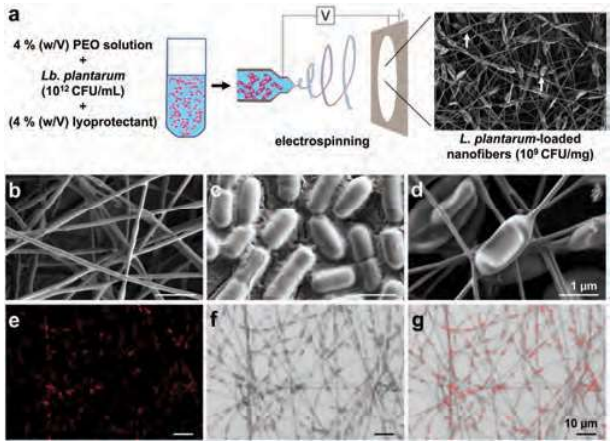

Figure 2: Schematic representation of electrospinning for the preparation of L. plantarum-loaded nanofibres. Scanning electron microscopy images are shown for (b) pure PEO nanofibres, (c) L. plantarum cells and (d) L. plantarum-loaded PEO nanofibres. Confocal microscopy images are also shown for PEO nanofibres with incorporated L. plantarum cells that express mCherry, as (e) fluorescence, (f) bright-field, and (g) merged images.

showing that the heterogeneous nuclear ribonucleoprotein E2 (hnRNP E2) is a component of TDP-43 aggregates in the A and C pathological subtypes of frontotemporal lobar degeneration. This interaction with TDP-43 in specific FTLD subtypes suggests different underlying neurodegenerative pathways. As a part of the MinE consortium for the determination of genetic causes of ALS we published about the project MinE databrowser, which brings large-scale whole-genome sequencing in ALS to researchers and the public and allows users to query a transcript and immediately access a unique combination of detailed data, annotations and association statistics that would otherwise require analytic expertise and visits to scattered resources. We also published a review of noncanonical functions of snoRNAs in the reputable journal Nucleic Acids Research (IF=11.1).

At the department we continued our work on probiotic lactic acid bacteria. In collaboration with the Chair of Pharmaceutical Technology from the Faculty of Pharmacy, we were among the first to develop a procedure for the incorporation of model lactic acid bacterium Lactobacillus plantarum ATCC 8014 into nanofibres by using the electrospinning technique. Nanofibres are considered an innovative delivery system, appropriate for local topical administration, e.g., for vaginal probiotic delivery. The incorporation of probiotic bacteria in nanofibres merges two technological steps into one by concomitant drying of the bacteria and the formation of dosage form. Bacteria were incorporated into poly(ethylene oxide) polymer, and high loading of L. plantarum cells (up to 7.6 × 108 CFU/mg) was achieved. The long-term storage (6 months) of nanofibres was tested, and the viability of L. plantarum was improved considerably when amorphous lyoprotectant trehalose was included in the formulation. The release of bacteria from the nanofibres was relatively fast, with almost all of the L. plantarum cells released over 30 min, which is advantageous for some applications. In a further study, we expanded this work by effective incorporation of ten species of lactic acid bacteria with markedly different properties (morphology, zeta-potential, hydrophobicity, average cell mass, growth characteristics) into nanofibres. Again, all the species were viable upon release from nanofibres and the viability was shown to correlate with cell hydrophobicity.

We also continued our work on the genetic engineering of lactic acid bacteria. Subject to invitation, we prepared a thorough review of applications of engineered lactic acid bacteria for the delivery of proteins and therapeutic peptides for Applied Microbiology & Biotechnology. We developed new anti-inflammatory lactic acid bacteria capable of targeting the IL-23 receptor, and assessed TNF -binding lactococci in inflammatory disease patients' mucosa, with both cytokine pathways important for the disease pathogenesis. To improve the technique of surface display that is crucial in this process, we screened a collection of lactococcal and phage surface anchoring domains. We have identified a new ChW-containing anchoring domain in AM12 phage endolysin that is capable of surface display comparable to the currently established approach using the cAcmA anchor. However, its mode of anchoring was shown to be different, which enabled the concomitant use of both anchors, suggesting considerable biotechnological potential.

The results of the research at the Department of Biotechnology in 2019 were published in 34 scientific papers in journals with an impact factor. We received two new research grants from the Slovenian Research Agency. Prof. Boris Rogelj received the national Zois recognition for scientific achievements in molecular basis of neurodegeneration, Nika Kruljec and Katja Škrlec received Krka awards and Abida Zahirović received the Deans award from the Faculty of Pharmacy. Members of the department were also very active in pedagogical work as lecturers and mentors to students

preparing diploma and doctoral thesis at the universities in Slovenia and abroad. In 2019 two doctoral theses were completed at the department.

Some outstanding publications in the past year

1. Modic, Miha, Rot, Gregor, Lepko, Tjaša, Rogelj, Boris, Ule, Jernej, et al. Cross-regulation between TDP-43 and paraspeckles promotes pluripotency- differentiation transition. *Molecular cell*, ISSN 1097-2765. [Print ed.], 2019, vol. 74, no. 5, str. 951-965, IF 14.5
2. Schmieder, Stefanie S., Stanley, Claire E., Rzepiela, Andrzej, Swaay, Dirk Van, Sabotič, Jerica, Nørrelykke, Simon F., Demello, Andrew J., Aebi, Markus, Künzler, Markus. Bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae. *Current biology*, ISSN 0960-9822. [Print ed.], 2019, vol. 29, issue 2, str. 217-228, IF 9.2

Awards and Appointments

1. Prof. Boris Rogelj, Presented with the Zois Certificate of Recognition for outstanding achievements in the field of Molecular basis of neurodegeneration
2. Katja Škrlec: Krka Grand Prize for Research, Krka d.d., Novo mesto, Surface display of evasins and bepecin on bacteria *Lactococcus lactis* NZ9000 and *Lactobacillus salivarius* ATCC 11741 and evaluation of their anti-inflammatory action, Novo mesto, 18 October 2019
3. Abida Zahirović, Borut Štrukelj, Mojca Lunder (Faculty of Pharmacy, University of Ljubljana), Ana Koren, Peter Kopač, Peter Korošec (University Hospital, Golnik): Best research achievements in 2019, Ljubljana, University in Ljubljana, An important step towards more effective immunotherapy for allergy to bee venom, 18 December 2019
4. Abida Zahirović: Faculty of Pharmacy Dean´s Awards 2019, Ljubljana, Faculty of Pharmacy, Identification of epitopes of major bee venom allergen Api m 1 and characterisation of corresponding mimotopes for use in immunotherapy, 4 December 2019

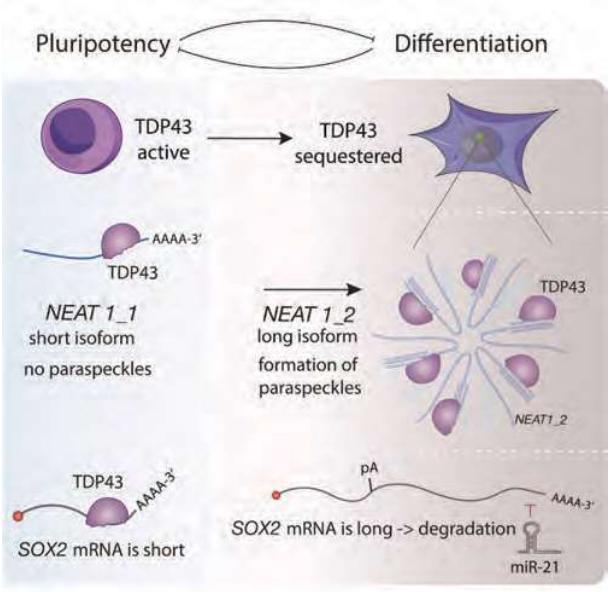

Figure 3: Reciprocal cross-regulation and activity of TDP-43 and the long isoform of the lncRNA Neat1 in pluripotent and differentiated cells. In pluripotent cells, TDP-43 represses the formation of paraspeckles, which form on the scaffold of long isoform of Neat1 by enhancing the polyadenylated short isoform of Neat1. TDP-43 also promotes pluripotency by regulating alternative polyadenylation of transcripts encoding pluripotency factors. One example is Sox2, where TDP-43 partially protects SOX2's 3' UTR from miR-21-mediated degradation. On the other hand, long isoform of Neat1 sequester TDP-43 and other RNA binding proteins and promote exit from pluripotency. (Modic et al., Molecular Cell 2019)

Organization of conferences and meetings

1. Minisymposium Tumor microenvironment: tumor-immune cell interactions, Jožef Stefan Institute and Nacional Institute of Biology, 24 September 2019
2. NanoTemper Workshop: Measure binding affinities and protein stability with NanoTemper Technologies, 15 October 2019
3. Annual meeting of co-workers of the research programme Pharmaceutical Biotechnology: Knowledge for Health, from the Department of Biotechnology at the Jožef Stefan Institute and the Chair of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Ljubljana, 21 November 2019

INTERNATIONAL PROJECTS

1. De-regulated expression of CodY controlled proteins in L. lactis for enhancing nisin production
   Prof. Aleš Berlec
   Fermentech Gsv Pvt Ltd.
2. COST CA18238: Ocean4Biotech - European Transdisciplinary Networking Platform for Marine Biotechnology
   Dr. Jerica Sabotič
   Cost Association Aisbl
3. Regulation of Cytotoxicity of „Super Charged" Natural Killer Cells with Cystatin F
   Prof. Janko Kos
   Slovenian Research Agency
4. ALS and FTD Relevant Characterization of In Vivo Protein Interactors of FUS
   Prof. Boris Rogelj
   Slovenian Research Agency

RESEARCH PROGRAMME

1. Pharmaceutical Biotechnology: Knowledge for Health
   Prof. Janko Kos

R & D GRANTS AND CONTRACTS

1. Nucleart transport defect in neurodegenerative diseases
   Prof. Boris Rogelj
2. Cathepsin X inhibitors impair the resistence of tumor cells to antiprotease therapy
   Prof. Janko Kos
3. Pathogenic role of paraspeckle-like nuclear bodies in neurodegenerative diseases ALS and FTD
   Prof. Boris Rogelj
4. Inhibition of cathepsin X activity as a novel strategy for the treatment of Parkinson's

disease
Prof. Janko Kos
5. Targeting Campylobacter adhesion in the fight against antimicrobial resistance
Dr. Jerica Sabotič
6. Advanced surface finishing technologies for antibacterial properties of patient specific 3D printed implantable materials
Asst. Prof. Helena Motaln
7. Phase transitions in systems of nucleotide repeat expansions associated with neurodegenerative diseases
Prof. Boris Rogelj
8. New antimicrobial strategies in prevention of biofilm formation by using lectins that inhibit bacterial adhesion
Dr. Jerica Sabotič
9. Improvement of immunotherapeutic potential of NK cells through modulation of cystatin F
Prof. Janko Kos
10. Development of new, environment-friendly approaches for plant and human virus inactivation in waters
Asst. Prof. Helena Motaln
11. Innovative ECO plasma seed treatment (for sowing and for human and animal diet/ nutrition)
Prof. Boris Rogelj
Ministry of Education, Science and Sport

VISITORS FROM ABROAD

1. Dr. Luc Dupuis, French Institute of Health and Medical Research, INSERM, University of Strasbourg, Strasbourg, France, 19 September – 22 September 2019
2. Prof. Jürgen Dittmer, Martin Luther University, Halle – Wittenberg, Halle (Saale), Germany, 23 September – 27 September 2019
3. Jakub Nowak, Nanotemper Technologies GmbH, Munich, Germany, 15 October 2019

STAFF

Researchers
1. Prof. Aleš Berlec
2. Prof. Janko Kos*, Head, until 31. 10. 2019
3. Asst. Prof. Helena Motaln
4. Prof. Boris Rogelj, Head, since 1. 11. 2019
5. Dr. Jerica Sabotič
6. Prof. Borut Štrukelj*

Postdoctoral associates
7. Dr. Janja Božič
8. Dr. Nikolaja Janež
9. Dr. Ana Mitrović
10. Dr. Milica Perišić Nanut
11. Dr. Mateja Prunk
12. Dr. Anja Pucer Janež
13. Dr. Katja Rebolj
14. *Dr. Petra Zadravec, left 06.05.19*

Postgraduates
15. *Ana Bajc Česnik, B. Sc., left 01.05.19*
16. Mirjana Malnar, B. Sc.
17. Tina Vida Plavec, B. Sc.
18. Emanuela Senjor, B. Sc.
19. Abida Zahirović, B. Sc.

Technical officer
20. Eva Erzar, B. Sc.

Technical and administrative staff
21. Maja Šimaga, M. Sc.

Note:

* part-time JSI member

BIBLIOGRAPHY

ORIGINAL ARTICLE

1. Urša Pečar Fonović, Milica Perišić, Nace Zidar, Brigita Lenarčič, Janko Kos, "The carboxypeptidase activity of cathepsin X is not controlled by endogenous inhibitors", *Acta chimica slovenica*, 2019, **65**, 1, 58-61.
2. Tina Vida Plavec, Aleš Berlec, "Surface anchoring on Lactococcus lactis by covalent isopeptide bond", *Acta chimica slovenica*, 2019, **66**, 1, 18-27.
3. Jerica Sabotič, Miha Renko, Janko Kos, "Beta - trefoil protease inhibitors unique to higher fungi", *Acta chimica slovenica*, 2019, **66**, 1, 28-36.
4. Abida Zahirović, Borut Štrukelj, Peter Korošec, Mojca Lunder, "Epitope mapping of major ragweed allergen Amb a 1", *Acta chimica slovenica*, 2019, **66**, 1, 37-44.
5. Janja Božič, Iztok Dolenc, "Feedback regulation of cathepsin C by the Propeptide dipeptides of Granzymes A and B", *Acta chimica slovenica*, 2019, **66**, 2, 501-509.
6. Anže Meden, Damijan Knez, Marko Jukič, Xavier Brazzolotto, Marija Gršič, Anja Pišlar, Abida Zahirović, Janko Kos, Florian Nachon, Jurij Svete, Stanislav Gobec, Uroš Grošelj, "Tryptophan-derived butyrylcholinesterase inhibitors as promising leads against Alzheimer's disease", *Chemical communications*, 2019, **55**, 26, 3765-3768.
7. Damijan Knez, Izidor Sosič, Anja Pišlar, Ana Mitrović, Marko Jukič, Janko Kos, Stanislav Gobec, "Biological evaluation of 8-Hydroxyquinolines as multi-target directed ligands for treating Alzheimer's disease", *Current Alzheimer research*, 2019, **16**, 9, 801-814.
8. Stefanie S. Schmieder, Claire E. Stanley, Andrzej Rzepiela, Dirk van Swaay, Jerica Sabotič, Simon F. Nørrelykke, Andrew J. deMello, Markus Aebi, Markus Künzler, "Bidirectional propagation of signals and nutrients in fungal networks via specialized hyphae", *Current biology*, 2019, **29**, 2, 217-228.
9. Katja Škrlec, Špela Zupančič, Sonja Prpar Mihevc, Petra Kocbek, Julijana Kristl, Aleš Berlec, "Development of electrospun nanofibers that enable high loading and long-term viability of probiotics", *European journal of pharmaceutics and biopharmaceutics*, 2019, **136**, 108-119.
10. Tina Vida Plavec, Borut Štrukelj, Aleš Berlec, "Screening for new surface anchoring domains for Lactococcus lactis", *Frontiers in microbiology*, 2019, **10**, 1879.
11. W. Kattuah, Boris Rogelj, R. Andrew King, Christopher E. Shaw, Tibor Hortobágyi, Claire Troakes, "Heterogeneous nuclear ribonucleoprotein E2 (hnRNP E2) is a component of TDP-43 aggregates specifically in the A and C pathological subtypes of frontotemporal lobar degeneration", *Frontiers in neuroscience*, 2019, **13**, 551.
12. Ana Mitrović, Jakob Kljun, Izidor Sosič, Matija Uršič, Anton Meden, Stanislav Gobec, Janko Kos, Iztok Turel, "Organoruthenated nitroxoline derivatives impair tumor cell invasion through inhibition of cathepsin B activity", *Inorganic chemistry*, 2019, **58**, 18, 12334-12347.
13. Bogdan Zdravković, Tanja Prunk Zdravković, Marko Zdravković, Borut Štrukelj, Polonca Ferk, "The influence of nano-TiO_2 on metabolic activity, cytotoxicity and ABCB5 mRNA expression in WM-266-4 human metastatic melanoma cell line", *JBUON*, 2019, **24**, 1, 338-346.
14. Ana Bajc Česnik, Simona Darovic, Sonja Prpar Mihevc, Maja Štalekar, Mirjana Malnar, Helena Motaln, Youn-Bok Lee, Julija Mazej, Jure Pohleven, Markus Grosch, Miha Modic, Marko Fonović, Boris Turk, Micha Drukker, Christopher E. Shaw, Boris Rogelj, "Nuclear RNA foci from C9ORF72 expansion mutation form paraspeckle-like bodies", *Journal of cell science*, 2019, **132**, 5, 1-14.
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16. Biljana Božić, Goran Lončar, Tjaša Vižin, Zoran Radojičić, Vera Popović Brkić, Janko Kos, "Relationship of high circulating cystatin C to biochemical markers of bone turnover and bone mineral density in elderly males with a chronic heart failure", *Journal of Medical Biochemistry*, 2019, **38**, 1, 53-62.
17. Bosa Mirjanić-Azarić, Novak Vasic, Darko Černe, Janko Kos, Nataša Bogavac-Stanojević, "Plasma cathepsin S is associated with high-density lipoprotein cholesterol and bilirubin in patients with abdominal aortic aneurysms", *Journal of Medical Biochemistry*, 2019, **38**, 3, 268-275.
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19. Tina Vida Plavec, Milan Kuchař, Anja Benko, Veronika Lišková, Jiří Černý, Aleš Berlec, Petr Malý, "Engineered lactococcus lactis secreting IL23 receptor-targeted rex protein blockers for modulation of IL-23/Th17-mediated inflammation", *Microorganisms*, 2019, **7**, 5, 152.
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21. Camila Ramaho Bonturi *et al.* (10 authors), "A bifunctional molecule with lectin and protease inhibitor activities isolated from Crataeva tapia bark significantly affects cocultures of mesenchymal stem cells and glioblastoma cells", *Molecules*, 2019, **24**, 11, 2109.
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23. Mojca Lunder, Irena Roškar, Jan Hošek, Borut Štrukelj, "Silver fir (Abies alba) extracts inhibit enzymes involved in blood glucose management and protect against oxidative stress in high glucose environment", *Plant foods for human nutrition*, 2019, **74**, 1, 47-53.
24. Kristina Sepčić, Jerica Sabotič, Robin Ohm, Damjana Drobne, Anita Jemec Kokalj, "First evidence of cholinesterase-like activity in Basidiomycota", *PloS one*, 2019, **14**, 4, e0216077.
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26. Saša Simčič, Aleš Berlec, Sanja Stopinšek, Borut Štrukelj, Rok Orel, "Engineered and wild-type L. lactis promote anti-inflammatory cytokine signalling in inflammatory bowel disease patient's mucosa", *World journal of microbiology & biotechnology*, 2019, **35**, 3, 45.

Review Article

1. Ana Mitrović, Janko Kos, "Nitroxoline: repurposing its antimicrobial to antitumor application", *Acta Biochimica Polonica*, 2019, **66**, 4, 2904.
2. Tina Vida Plavec, Aleš Berlec, "Engineering of lactic acid bacteria for delivery of therapeutic proteins and peptides", *Applied microbiology and biotechnology*, 2019, **103**, 2053–2066.
3. Barbara Breznik, Ana Mitrović, Tamara Lah Turnšek, Janko Kos, "Cystatins in cancer progression: more than just cathepsin inhibitors", *Biochimie*, 2019, **166**, 233-250.
4. Tanja Jakoš, Anja Pišlar, Anahid Jewett, Janko Kos, "Cysteine cathepsins in tumor-associated immune cells", *Frontiers in immunology*, 2019, **10**, 2037.
5. Jerica Sabotič, Janko Kos, "CNL- *Clitocybe Nebularis* Lectin - the fungal GalNAcβ1-4GlcNAc-Binding Lectin", *Molecules*, 2109, **24**, 23, 4204.

Independent Component Part or a Chapter in a Monograph

1. Clemens Peterbauer, Stefan Heinl, Aleš Berlec, Reingard Grabherr, "Recombinant gene expression in lactobacilli: strategies and applications", In: *Lactobacillus genomics and metabolic engineering*, Caister Academic Press, 2019, 169-186.

Theses and Mentoring

1. Janko Ignjatović, *Detection and immunigenicity evaluation of recombinant monoclonal antibodies' structural variants:* doctoral dissertation, Ljubljana, 2019 (mentor Borut Štrukelj; co-mentor Urban Švajger).
2. Mateja Prunk, Role of cystatin F and cysteine cathepsins in the function of cytotoxic T lymphocytes: doctoral dissertation, Ljubljana, 2019 (mentor Janko Kos).