DEVELOPMENT OF NEW GENERATION OF BIOLOGICAL CONTROL AGENTS AND BIOFERTILIZERS FOR SUSTAINABLE AGRICULTURE

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The **constant demand** for new, effective agents has triggered intensive research in the field of diverse antimicrobials of natural origin. These compounds are synthesized by all forms of life and have important biomedical and biotechnological properties, and are thus widely considered a **potential solution to the growing problem of resistance to conventional pesticides, fungal infection and plant diseases**. Our isolates belong to Risk 1 category and fulfil the criteria as biocontrol agents since they synthesize lipopeptide compounds with strong antibacterial and antifungal activity in situ, in vitro and in planta experiments. The control of plant diseases, which are responsible for the **loss of at least 10% of produced food worldwide**, is necessary to maintain the production and quality of crops around the world. During last decades, the **increase of agricultural production** was based mainly on **heavy use of chemical fertilizers and pesticides**. High effectiveness and easy use of chemical pesticides impact on **environmental contamination** and the accumulation of residues in the food chain, to the emergence of resistant strains of pathogens, and in addition onto **social, economic and health problems. Biological control** using natural antagonist microorganisms has been **highlighted as a promising alternative**.

The first objective of the project will be focused on **development of appropriate formulation of the selected isolates to achieve optimal performances on plants in vivo** and potentially increase the yield. The second objective will be the **development of the best formulations related to seed coating/treatment**, as well as granule that can be distributed together with seeds during the sowing. The last objective will be the development of formulations and strategies for treatment of crops during the most sensitive growth phases for attack of pathogens, when therefore the plant needs an efficient and reliable protection.

Potential market will cover **all small farms** and **agricultural crops fields** and **fruit orchard growers in Serbia** and neighbor countries, and in particular the market for producers who want to grow only organically produced food. Target is in the retail and manufacturing sector of the agricultural industry. The stores themselves will have many different products based on our technology related to seed coating, biofertilizers, plant disease controlling products, as well as for control of postharvest diseases.

We believe that the results would make a good starting point for **developing** and **patent** a **product** that would be utilized for plant protection on the large scale production. The potential of such **industrially manufactured products** is enormous and would certainly enable competition on the market. At the end we should give an answer to the following question: has the time for the use of biopesticides come? Certainly, some progress has been made through the development of many new biopesticides within continuous technological upgrade. The predictions indicate that biopesticides will overtake all other options in pest control in terms of market share that will constantly grow in the near future.

Процена утицаја микропластике на медитеранску дагњу (*Mytilus galloprovincialis*) праћењем различитих биомаркера (MEDIPLAST)

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Микропластика представља честице пластике које су присутне у повшинским водама или се депонују у седименту у зависности од механизама транспорта који могу бити биолошки или физичко-хемијски (Van Cauwenberghe et al., 2015). Овај вид загађивача представља биолошку опасност са неколико аспеката: приликом ингестије може доћи до отпуштања пластификатора као и загађивача који су адсорбовани на микропластику; честице микропластике могу послужити као вектор за пренос аутохтоних и алохтоних врста у животној средини (Teuten et al., 2009). Честице микропластике се прво везују са организмима примарног трофичког нивоа (фитопланктон и зоопланктон) а затим преносе даље кроз ланац исхране (уношењем ингестијом или респирацијом (Watts et al., 2014).
Студија у којој се пратио утицај микропластике на исхрану школьке *Mytilus edulis* у контролисаним условима, указује да приликом ингестије микропластике долази до акумулације и транслокације између ткива; честице величине 3 до 9,6 μм се акумулирају у дигестивној жлезди а касније премештају у хемолимфу (Browne et al., 2008). Штетна дејства микропластике по организме се испољавају путем инфламаторног одговора, дестабилизације лизозомне мембране, генотоксичних ефеката, смањењем репродукције, дисбалансом у синтези хормона (Cole et al., 2011). Такође, долази до диференцијалне експресије гена одговорних за метаболизам у лизозомима, имунолошке функције, антиоксидативну одбрану, затим гена одговорних за транскрипцију као и поправку ДНК (Avio et al., 2017).

Главни циљ овог пројекта је проучавање утицаја микропластике и загађивача адсорбованих за површину честица микропластике на медитеранску дагњу (*M. galloprovincialis*). Прецизније, утицај се прати у контролисаним лабораторијским условима у којима се јединке излажу различитим типовима микропластике, загађујућих супстанци као и честицама микропластике које носе загађујуће супстанце. Ниво оштећења ДНК молекула у ћелијама хемолимфе дагње прати се комет тестомом (детектује оштећење ДНК молекула као биомаркера излагања) и микронуклеус тестом (ДНК оштећење се прати као бимаркер ефекта тј. трајно ДНК оштећење). Пројекат се остварује путем сарадње са Националним институтом за Биологију-Морска биолошка постаја Пиран у Словенији.

Survey for antimicrobials effective against carbapenem-resistant Gram - negative bacteria

Principal Investigator: Branko Jovčić, Associate professor, Faculty of Biology, University of Belgrade (2016-2019)
Funded by: International Centre for Genetic Engineering and Biotechnology (ICGEB)
Participants: Branko Jovčić, Associate professor, Faculty of Biology, University of Belgrade; Jelena Lozo, Associate professor, Faculty of Biology, University of Belgrade

Among all of the bacterial drug resistance problems, gram-negative pathogens are particularly worrisome, because they are becoming resistant to nearly all drugs that are being considered for the treatment. Causative agents of the most serious gram-negative infections (due to limited treatment options and a high mortality rates) are carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and Acinetobacter. Globally, urgent actions are recognized as necessary in order to find novel antimicrobials that will diminish the threat of multidrug-resistant Gram-negative bacteria to global public health. So far, novel antibiotic producers were screened among soil Actinomycetales. However, we are now aware that novel ecological niches must be exploited and screening should be expended towards other bacterial species as potential antibiotic producers. Sediments of glacial lakes from Western Balkans are unexplored natural treasure and represent a challenge regarding their microbial genomic and metabolic potential. Remote high mountain lakes, being far from habitation and located in extreme environments, receive less impact from human activities but magnify the effects of global climate changes, and can thus be taken as a mirror of natural environmental changes. Therefore, exploiting their microbial is of crucial importance.

During the project, we will analyze sediments of five lakes in Southern part of Serbia, Montenegro and Bosnia and Herzegovina. Lakes will be selected due to possible anthropogenic influences - ranging from lakes with minimal anthropogenic impact to those with significant anthropogenic impact. In order to get the full insight into these microbial communities, both microbiological and metagenomic approach will be used for analyses. Cultivable bacteria will be isolated from sediments and grown in aerobic and anaerobic conditions on various media. Cultivated bacteria will be then subjected to testing of antimicrobial compounds production. 16S rDNA metagenomic analysis will be performed in order to determine the microbial diversity in lake sediments. Functional metagenomic libraries will be constructed in order to fully exploit the genomic potential of microbial communities from sediments. Functional metagenomic libraries will be expressed in E. coli and tested for the production of compounds active against carbapenem- resistant, multidrug-resistant Gram-negatives in order to avoid selection of antimicrobials that are already known, and those that cannot overcome existing resistance mechanisms that are globally dispersed among Gram-negative pathogens. Also, functional metagenomic libraries will be screened for the presence of various antibiotic-resistance genetic determinants. After the selection of cosmid that encompasses potentially novel antimicrobial compound, it will be subjected to sequencing in order to reveal gene(s) involved in biosynthesis of this compound. Also, we will determine biochemical properties of a novel antimicrobial compound, perform its chemical purification, as well as analyze the interactions between the compound and potential target molecules.

However, since sampling of glacial lake sediments is possible only in the summer period due to high altitudes of lake localities we will, during first six months of the project, analyze laboratory collection of Lactic Acid Bacteria (LAB) for the producers of antimicrobial peptides active against multidrug-resistant Gram-negative bacteria. This

unique collection includes (LAB) isolated from fermented milk products produced in households of Western Balkan according to traditional recipes as well as strains of human origin. Screening will be performed by means of agar well diffusion assay, and compounds active against tested bacteria will be subjected to further biochemical characterization.

Assessment of ecological status according to the Water Framework Directive - intercalibration among W-Balkan countries

Principal investigator: dr Susanne Claudia Schneider, Norwegian Institute for Water Research (NIVA)
Funded by: The Norwegian Ministry of Foreign Affairs (2015 – 2018)

Participants:

1. Norwegian Institute for Water Research (NIVA), Norway
2. Hydrobiological Institute, Ohrid, Macedonia
3. Faculty of Agriculture and Environment, Agricultural University Tirana, Albania
4. **University of Belgrade, Faculty of Biology, Serbia: Jelena Krizmanić, Assistant professor, Ivana Živić, Associate professor, Danijela Vidaković and Katarina Stojanović, PhD student**
5. Institute of Hydrometeorology and Seismology, Podgorica, Montenegro

From 2009 to 2013, NIVA was coordinating a cooperation project involving Albania and Macedonia, funded by the Norwegian Ministry of Foreign Affairs (RER-09/056). In this project, we worked on transboundary Lake Ohrid, which is shared between Albania and Macedonia. We brought together experts from both countries, investigated 30 sites in Lake Ohrid, developed and harmonized monitoring methods, which are in accordance with the demands of the European Water Framework Directive (WFD). We were the first who were able to produce maps showing ecological status for the whole of Lake Ohrid, across political borders. A specific detail of the European WFD requires the ecological status, which is measured at a site to be compared with so called "unimpacted reference conditions". In investigating Lake Ohrid, we found that the lake regrettably is too impacted by eutrophication, such that finding reference conditions in this lake was impossible. The next step in order to develop a system, which completely fulfills the demands of the European WFD, is therefore to investigate similar lakes, which are not impacted, and then compare these lakes with the results from Lake Ohrid. If unimpacted lakes do not exist in one country, then the WFD specifically recommends collaboration with neighboring countries that might have such unimpacted lakes. This strongly strengthens cooperation across political borders. We therefore hereby apply for a project, where we will follow the recommendation in the WFD and investigate lakes in neighboring countries, specifically Serbia and Montenegro. Especially in Montenegro, unimpacted lakes exist. This collaboration will ensure an even broader regional co-operation among the Balkan countries. In addition, we expect this project to fill some gaps in knowledge/data, which are necessary to develop complete monitoring methods according to the WFD in each of the participating Balkan countries. In other words: we expect both political and professional benefits from this project.

Our project goals are:

1) to establish common and comparable methods for analyzing macrophytes, diatoms, macroinvertebrates, and water chemistry and for estimating external nutrient input into lakes in all participating countries (this involves field work, plant and animal determination, laboratory work, assessment of external nutrient input based on publicly available data)
2) to investigate unimpacted lakes (so-called reference lakes), in order to agree on a standard against which flora and fauna of impacted lakes can be compared
3) to investigate some slightly and some heavily impacted lakes using the same methods as in 1 and 2, in order to show that the established methods actually work

We plan to investigate the following lakes (but note that the final decision will be reached during a workshop, which is planned at the beginning of the project period):

Macedonia/Albania:
- Lake Ohrid, slightly polluted (re-investigation of some sites in Lake Ohrid is necessary because also partners from Serbia and Montenegro should see and analyze these sites)
- Lake Prespa, polluted
Montenegro:
- Black Lake, unpolluted (reference lake)
- Biogradsko Lake, unpolluted (reference lake)
Serbia
- Savsko jezero reservoir (Sava lake), polluted
Albania
- one additional unpolluted or only slightly polluted lake in the Lura lakes area 5
We plan to investigate six sites in each of the six lakes, such that we in total will investigate 36 sites. At each site, we will:
a) investigate macrophytes, diatoms and macroinvertebrates using common methods,
b) measure important water chemical parameters like e.g. phosphorus, nitrogen and Chl-a and ensure that the methods are comparable among the countries, and
c) estimate external nutrient input to the site using publicly available spatial data

Project title: PRACTICAL / Study group: SNPs and prostate cancer risk in Serbian population / PROSTATSERBIA

Principal Investigator: Goran Brajušković, associate professor, Faculty of Biology, University of Belgrade
Funded by: The Institute of Cancer Research: Royal Cancer Hospital, London, UK
Participants: Dušanka Savić Pavićević, associate professor, Faculty of Biology, University of Belgrade and Zorana Nikolić, MSc

The Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium was established in September 2008. It was formed by a collaborative group of researchers interested in inherited risk of prostate cancer with the goal of identifying genes that may be related to the risk of prostate cancer. The aims of the consortium are to combine data from many studies to provide a reliable assessment of the risks associated with these genes, and to validate new findings (http://practical.ccge.medschl.cam.ac.uk/)

In 2014, the group of researchers from Faculty of Biology was accepted for participation in PRACTICAL consortium under the study group name PROSTATSERBIA (http://practical.ccge.medschl.cam.ac.uk/study-groups/study-groups-list/)