

ABSTRACTS OF THE 76th SCIENTIFIC CONFERENCE OF THE UNIVERSITY OF LATVIA

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Main results of the 9th Lagrangian drift expedition on the Middle Daugava River

Dāvis Gruberts^{1*}, Jana Paidere², Ivars Druvietis³

¹Department of Geography and Chemistry, Daugavpils University, Parādes 1, Daugavpils LV–5400, Latvia

²Laboratory of Hydroecology, Institute of the Nature Sciences and Technology, Daugavpils University, Parādes 1A, Daugavpils LV–5400, Latvia

³Department of Hydrobiology, Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia

* Corresponding author, E-mail: davis.gruberts@du.lv

Key words: Daugava river, Lagrangian sampling, phytoplankton, zooplankton.

The 9th Lagrangian drift expedition was conducted on the Middle Daugava River on March 26, 2015, during the short-term drainage phase of the annual spring floods. The aim of this study was to collect new information about temporal and spatial variation in hydrodynamics, composition and properties of the moving floodwater masses during their downstream transport. An interaction between physical, chemical and biological characteristics of the selected floodwater masses and different environmental factors (riverbed topography, open channel hydrodynamics, etc.) was investigated during this study.

The real-time Lagrangian sampling study was performed by applying a manned drifting scientific research platform “Aventura” constructed from a marine life-raft and an inflatable boat (Gruberts et al. 2012). The drift was started near Krauja village (15 km upstream from Daugavpils City) at 08:00, when the platform was inflated and equipped for the drift, and navigated to the middle of the stream. Then two floating anchors were deployed and the platform started to drift passively downstream following the same floodwater masses. After each 30 minutes, geographical position and physical and chemical properties of the moving water mass were recorded by a HACH-DS5 multiprobe attached to the platform (i.e. temperature, pH, conductivity, turbidity, oxygen saturation, etc.). Additionally, subsurface water samples were collected on hourly interval for the subsequent zooplankton and phytoplankton analyses.

The uninterrupted drift continued for 10 hours, and 35 km long stretch of the Daugava River was covered. The drift was finished at 18:15 about 20 km downstream from Daugavpils, at the Munda stream inlet. In total, 22 samples of phytoplankton and zooplankton were collected, and various instrumental measurements of the selected water mass properties were performed simultaneously.

The phytoplankton samples were collected from the water surface in plastic bottles (0.5 L), fixed *in situ* with the Lugol’s solution, and analysed by applying an inverted

microscope method. One 10 mL subsample was used from each sample for the phytoplankton identification and enumeration. The zooplankton samples were collected by filtering 100 L of water through the Apstein-type 65 µm zooplankton sampling net. The samples were fixed *in situ* with ethanol (97%) and analysed by using the ZEISS Primo Star light microscope. Six 1 mL subsamples were used from each collected sample to identify and enumerate zooplankton organisms according to standard method (Wetzel, Likens 2000).

In total, 40 phytoplankton and 41 zooplankton taxa were recorded during this study. The phytoplankton community was presented mostly by benthic diatoms, like *Melosira varians*, *Navicula* sp. and *Gyrosigma* sp. The small-sized *Cryptophyta* as well as the filamentous cyanobacteria (*Oscillatoria* sp.), which formed most of the total biomass, were recorded at all sampling sites. During the downstream transport, total abundance, biomass and species diversity of the phytoplankton gradually increased. The fresh algal biomass of some taxa (*Oscillatoria* sp., *Navicula* sp. and *Cryptomonas* sp.) correlated negatively with the depth of the river channel.

Higher species diversity of the zooplankton was observed during this study when compared to previous drift experiments on the Middle Daugava River conducted annually since 2007. Zooplankton community was presented mostly by *Rotifera* (87% of all organisms) and *Copepoda* (62% of total biomass). Several common taxa were recorded at all sampling sites, such as *Keratella cochlearis*, *Keratella quadrata*, *Polyarthra* sp., *Synchaeta* sp., *Filinia longiseta*, *Notholca labis*, *Kellicottia longispina*, *Lecane* sp., as well as the *Bdelloidea* and *Cyclopinae* development stages. There was also a higher number of *Copepoda* and *Cladocera* taxa that are typical for lithoral zone habitats (*Paracyclops fimbriatus*, *Eucyclops denticulatus* and *Alona rectangula*, *Alona* sp., *Alonella nana*). During the downstream transport, total abundance and biomass of the zooplankton decreased at deeper sampling sites with higher stream velocity, and

vice versa (especially, the abundance of *Copepoda*).

Significant role of the river channel morphology and the floodwater hydrodynamics in downstream variation of the phytoplankton and zooplankton abundance and diversity, and its distribution along the Middle Daugava River was therefore highlighted once more.

References

- Gruberts D., Paidere J., Škute A., Druvietis I. 2012. Lagrangian drift experiment on a large lowland river during a spring flood. *Fund. Appl. Limnol.* 179: 235–249.
- Wetzel R. G., Likens G. E. 2000. *Limnological Analyses*. 3rd ed. Springer, New York, 429 pp.

***In silico* evaluation of eventual functionality of genetic variations in *PSMA6* promoter poly(dA:dT) tract in association with autoimmune disease development in Latvians**

Kristine Dokane^{1*}, Natalia Paramonova¹, Ilva Trapiņa¹, Kristine Ošiņa¹, Nikolajs Sjakste^{1,2}

¹Institute of Biology, University of Latvia

²Faculty of Medicine, University of Latvia

*Corresponding author, E-mail: kristine.dokane@lu.lv

Key words: autoimmune diseases, DNA bending, DNA secondary structures, polyA, *PSMA6*.

PSMA6 gene encodes proteasome $\alpha 6$ subunit, which is a part of an outer hetero-heptameric ring of 20S core proteasome. 20S core proteasome together with 19S regulatory proteasome form a 26S proteasome which is a central role player in ubiquitin dependent proteolysis in eukaryotic cells (Gomes 2013). A poly(dA:dT) tract positioned -655 bp from the transcription start site carries three variations, two insertions rs200541481 (-/AC) and rs200298313 (-/C) at 5' end and a single nucleotide polymorphism (SNP) rs71640264 (C/A) at 3' end of the tract respectively. These three variations increase or decrease the length of the poly(dA:dT) tract. Previous fragment length analysis in case-control study of juvenile idiopathic arthritis, bronchial asthma, obesity, multiple sclerosis and type 1 *diabetes mellitus* patients and healthy individuals in Latvian population revealed that rs200541481 might be associated with predisposition to autoimmune and metabolic diseases, while rs200298313 did not have any associations. rs71640264 was in complete linkage disequilibrium with another SNP in *PSMA6* 5'UTR - rs2277460, which previously has been associated with several disease development in Latvian population (Sjakste et al. 2016). However, the possible functional mechanisms of these variations have not been studied.

The aim of the study was to evaluate the functionality of genetic variations in *PSMA6* promoter poly(dA:dT) tract *in silico*. The analysis of putative transcription factor binding sites (TFBS) was performed on 100 bp region surrounding studied poly(dA:dT) tract with MatInspector Release 10.1 in Genomatix website. Formation of secondary DNA structures was studied for the same 100 bp region in MFOLDROOT, and DNA bending and curvature analysis was performed with bend.it online tool on 60 bp region surrounding the studied poly(dA:dT) tract.

Four TFBS (AP2F/TCFAP2C.02, SF1F/FTF.01, PLAG/PLAG1.02 and ZF02/ZNF300.01) non-affected by studied variations were found in region surrounding poly(dA:dT) tract, while two new TFBS were created by

variation rs71640264C>A, SORY/SRY.01 and ZFHX/AREB6.04 respectively. Neither of insertions at 5' end of the poly(dA:dT) tract had an effect on TFBS. DNA secondary structure analysis revealed that in comparison to native poly(dA:dT) sequence all of the studied variations may cause remarkable changes in DNA secondary structure and free energy, creating more stable and structured structures. DNA bending analysis revealed that the most noticeable changes might be created by rs71640264C>A increasing the curvature and bendability of the 3' end of the poly(dA:dT) tract, while insertions did not create noticeable changes.

These results suggest that out of all studied variations the most prominent effect may cause the SNP rs71640264 by changing both mechanical and structural properties of the DNA sequence at 3' end of the poly(dA:dT) tract as well as creating two new transcription factor binding sites. Thus the variation might have an influence on the *PSMA6* gene expression and autoimmune disease pathogenesis. Both insertions at 5' end of the poly(dA:dT) might influence the *PSMA6* gene expression through DNA secondary structure formation changing the binding of gene expression regulating elements.

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References

- Gomes A. 2013. Genetics of proteasome diseases. *Scientifica* 2013: 637629.
- Sjakste T., Paramonova N., Osina K., Dokane K., Sokolovska J., Sjakste N. 2016. Genetic variations in the *PSMA3*, *PSMA6* and *PSMC6* genes are associated with type 1 diabetes in Latvians and with expression level of number of UPS-related and T1DM-susceptible genes in HapMap individuals. *Mol. Gen. Genom.* 291: 891–903.

Validation of the *PSMC6* (rs2295826, rs2295827) and *PSMA6* (rs2277460, rs1048990) proteasomal gene polymorphisms on the association with multiple sclerosis in Latvians

Gatis Melkus¹, Kristīne Dokāne^{1*}, Natālija Paramonova¹, Kristīne Ošiņa¹, Nikolajs Sjakste^{1,2}

¹Genomics and Bioinformatics, Institute of Biology of the University of Latvia, Miera 3, Salaspils LV-2169, Latvia

²Faculty of Medicine, University of Latvia, Riga, Latvia

*Corresponding author, E-mail: kristine.dokane@lu.lv

Key words: multiple sclerosis, proteasomal gene polymorphisms.

Multiple sclerosis (MS) is a continually studied yet incompletely understood immune-mediated disease that affects ~2.5 million people worldwide and ~2500 Latvians. Ubiquitin proteasome system is important in immunity; its deregulation can influence MS development and progression. The aim of our study was to validate an association between previously studied proteasomal gene SNPs as implicated in autoimmunity and the incidence of MS. In order to achieve this we have performed a case-control study investigating the link between *PSMA6* (rs2277460, rs1048990) and *PSMC6* (rs2295826, rs2294827) and MS in Latvian population.

Four loci in non-coding regions of the *PSMC6* and *PSMA6* proteasomal genes were genotyped via allele-specific PCR and by a restriction fragment length polymorphism (RFLP) technology in 305 healthy control subjects (including 179 women) and 280 MS patients (including 198 women) and analyzed for association with disease.

The previously observed pattern of complete linkage disequilibrium (LD) between the two studied *PSMC6* rs2295826 and rs2294827 loci in the Latvian population was confirmed in MS patients, resulting in identical genotyping result distributions ($D' = 1$, $r^2 = 1$). A significant ($p < 0.001$; $OR = 1.949$ [1.343 – 2.829]) association was found

between *PSMC6* investigated loci and MS common group, for genotypes involving rare alleles and for rare alleles ($p < 0.01$).

A sex-specific association of nominal significance ($p < 0.05$) was found for the *PSMA6* rs2277460 genotype CA in women. Finally, it was observed that *PSMC6* loci genotypes involving rare alleles in group of MS patients showed higher significance levels for men than for women.

The obtained data support a main and a sex-specific association between *PSMC6* loci minor alleles/heterozygous genotypes and MS as well as a less significant female-specific link between *PSMA6* rs2277460 heterozygotes and MS. The investigated polymorphisms potentially may be usable biomarkers for MS risk in clinical practice.

We provide evidence that *PSMA6* and *PSMC6* proteasomal gene variations may contribute to the risk of multiple sclerosis in Latvians.

Acknowledgements

The study was funded from the UL research project “The study of biomarkers and natural compounds for the diagnosis and personalized treatment of acute and chronic disease”, and ERAF SAM Nr. 1.1.1.1/16/A/016 project “Determination of proteasome-related genetic, epigenetic and clinical markers for multiple sclerosis”.

Distribution of mobile genetic elements in pine genes and flanking regions

Angelika Voronova^{1*}, Martha Rendon², Par Ingvarsson², Dainis Ruņģis¹

¹Latvian State Forest research institute “Silava”

²Swedish University of Agricultural Sciences

*Corresponding author, E-mail: angelika.voronova@silava.lv

Key words: gene regulation, gene networks, transposable elements.

Transposable element (TE) composition varies considerably between individuals, and TEs can influence gene regulation by integration within or near coding sequences (Mita, Boeke 2016). Genome-wide approaches in mammalian genomes demonstrate that TEs contribute to rewiring and selection of gene networks by embedding transcription factor binding sites (Sundaram et al. 2014). The genome sequences of some conifer species have been recently published (Neale et al. 2014; Stevens et al. 2016), but data quality, coverage and gene models continue to be improved (Crepeau et al. 2017). Our previous studies on *Pinus sylvestris* revealed rapid expression of TE associated sequences in response to stress conditions. The composition of 12 studied LTR retrotransposons was found to be specific to gymnosperm lineages, while TE family proportions and copy numbers revealed variation between and within pine species (Voronova et al. 2017). The higher expression rate of the studied TE families was not dependant on more frequent distribution of a particular TE family in the genome, suggesting enrichment of some stress responsive genes with particular TE families. The aim of this study was the evaluation of genes containing TEs in available pine reference genomes (*Pinus taeda* and *Pinus lambertiana*) and *P. sylvestris* scaffold sequences using bioinformatic approaches.

The *PIER* v.2.0. (Pine Interspersed Element Resource) was used for TE identification in pine reference genomes. Uppsala Multidisciplinary Center for Advanced Computational Science resources was used for manipulation of genome sequences. The *PIER* database contains all TEs and their constructs that were recognised by automatic genome annotation and a large portion of entries contain nested repeats with 2-4 pairs of direct long terminal repeats (LTR), which could negatively influence results. Therefore all full-length elements were clustered with *CD-Hit* v.4.6.4 (Fu et al. 2012) utilising a sequence identity threshold of 0.8 resulting in 15622 TE representatives from 19700 originally found in the database. Additionally, all LTRs were extracted from the repeat database (24591 entries) and clustering performed, resulting in an additional database of LTR representatives (9659 entries). *Bedtools* v 2.27.1 was used

for extraction of sequences of all genes containing introns from reference genomes. 5 kB of 5' and 3' flanking sequences were extracted in a separate database for each reference genome. Local *BLASTn* v.2.2.26 was used for similarity searches. *P. taeda* gene exons homologs were extracted from *P. sylvestris* scaffolds. Upstream and downstream regions of 12737 exons were extracted of these 2021 exons covered the entire scaffold. Among the *P. sylvestris* exon surroundings, incomparably lower amounts of hits to TEs were obtained, therefore these data could not reflect the TE content in this species.

As LTR retrotransposons are the dominant order of TEs in conifers, these types of repeats was found more frequently in all gene regions. Quality of reference genomes plays a major role when analysing TE presence in gene regions. Full-length element content in the first version of *P. lambertiana* genome contains more matching genes (top ranked TE had 272 unique matches to genes) compared with *P. taeda* v.2.0 (top ranked TE had only 39 matches to genes). However, if only LTRs were considered, the difference between two genomes was not large (for the most frequent LTR- 500 matching genes were found in *P. taeda* and 532 in *P. lambertiana*). These results are also in accordance with whole genome TE content data, where the larger genome size of the pine subgenus *Strobus* (*P. lambertiana*) correlates with a higher gene and repetitive element content (Stevens et al. 2016). Due to the repetitive nature of TEs, the differentiation of full-length elements from partial copies is often not possible using current genome assemblies. Therefore, utilising LTRs as representative of both full-length and partial TEs could be more suitable at this point.

Gene Ontology (GO) classification file was obtained from Gene Ontology Consortium (<http://www.geneontology.org/>). Gene annotation for *P. taeda* v.2.0. and *P. lambertiana* were generated considering gene homology to *P. taeda* v.1.0. A hypergeometric test with Bonferroni correction implemented in *BINGO* (Maere et al. 2005) was used for the gene enrichment test. Cytoscape (Smoot et al. 2011) was used for gene network visualisation. Each network was formed from the group of genes that were in proximity to one TE family. Genes from the similar GO

Investigation of genetic resources of genus *Vaccinium* in Latvia

Agnese Gailīte*, Dainis Ruņģis

Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Rīgas 111, Salaspils LV-2169, Latvia

*Corresponding author, E-mail: agnese.gailite@silava.lv

Key words: crop wild relatives, genetic resources, wild harvested plants, *Vaccinium*.

Traditionally, genetic resources are maintained *ex situ*: in field collections, seed banks, *in vitro* and cryopreserved. However, *in situ* conservation has become increasingly recognised as an important component of conservation strategies. Systematic research and conservation activities for crop wild relatives (CWR) and wild harvested plants (WHP) have been initiated in many European countries. Population and genetic diversity studies are important sources of information for the development and implementation of conservation strategies. The main CWR and WHP plant groups in Latvia are forage grasses, aromatic and medicinal plants, and forest fruits and berries.

The genus *Vaccinium* belongs to the Ericaceae family and consists of approximately 450 species. About 40% are native to Southeast Asia, 35% to America and about 25% are widely distributed across the world (Song, Hancock 2011). Species of the genus *Vaccinium* are important wild crops in the northern hemisphere, they are an important food source for birds and wild animals, and they are also used as medicinal plants. Species of the genus *Vaccinium* are also used in breeding programs, and several lingonberry (*Vaccinium vitis-idaea*) and blueberry varieties and lingonberry-cranberry hybrids have been developed in Latvia (Ripa, Audriņa 2009). An investigation of the population structure and genetic diversity of *Vaccinium* species has not been previously undertaken in Latvia.

Vaccinium myrtillus L. (bilberry) is a woody dwarf (5 to 90 cm high) shrub typical in the northern hemisphere. (Nestby et al. 2010). Bilberries are divided into different forms, according to berry colour. Natural hybrids with lingonberry (*Vaccinium × intermedium* Ruthe) were first discovered in Northern Germany in 1826 (Ponikierska et al. 2004). These wild hybrids are found in the UK, Finland (Lätti et al. 2011), Germany, Poland, Denmark, Russia and the Netherlands (Ponikierska et al. 2004). The majority of molecular studies, including the use of EST-SSR markers, have been done on species of the section *Cyanococcus* (Rowland et al. 2003; Boches et al. 2005). The species endemic to Latvia belong to other sections (Nestby et al. 2010), therefore the available DNA markers need to be tested and adapted for use in *V. myrtillus*. From 18 tested SSR markers, 11 were potentially applicable for genetic

analyses. Initial analyses were performed with nine markers on bilberry samples collected in different forest types, in forests of different age (4, 75 and 105 years) and in four locations in central and western Latvia. SSR genotype data were analysed with GenALEx 6.5. (Peakall, Smouse 2012). Initial results indicate that 2% of molecular variance was found between populations growing in different forest types. No genetic differentiation was found between populations growing in forests of different ages. About 5% of molecular variance among populations was found between different locations in Latvia. Our initial results indicate that bilberry populations growing in different regions of Latvia are genetically differentiated. Nevertheless, these are only preliminary results and research will be continued including sampling location in the eastern regions of Latvia.

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References

- Boches P.S., Bassil N.V., Rowland L.J. 2005. Microsatellite markers for *Vaccinium* from EST and genomic libraries. *Mol. Ecol. Notes* 5: 657–660.
- Lätti A.K., Riihinen K.R., Jaakola L. 2011. Phenolic compounds in berries and flowers of a natural hybrid between bilberry and lingonberry (*Vaccinium × intermedium* Ruthe). *Phytochemistry* 72: 810–815.
- Nestby R., Percival D., Martunussen I., Opstad N., Rohloff J. 2011. The European blueberry (*Vaccinium myrtillus* L.) and the potential for cultivation. A review. *Eur. J. Plant Sci. Biotechnol.* 5: 5–16.
- Peakall R., Smouse P.E. 2012. GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537–2539.
- Ponikierska A., Gugnacka-Fiedor W., Piwczynski M. 2004. Morphological characteristics of *Vaccinium × intermedium* Ruthe. *Dendrobiology* 51: 59–65.
- Ripa A., Audriņa B. 2009. Rabbiteye blueberry, American cranberry and lingonberry breeding in Latvia. *Agronomijas Vēstis* 12: 93–98.
- Song G-Q., Hancock J.F. 2011. Basic botany of *Vaccinium*. Chapter 10. In: Kole C. (ed) *Wild Crop Relatives; Genomic and Breeding Resources, Temperate Fruits*. Springer-Verlag, Berlin-Heidelberg, pp. 197–221.

Methods for soil acidification: advantages and disadvantages

Andis Karlsons*, Anita Osvalde, Jolanta Pormale

Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia, Miera 3, Salaspils LV-2169, Latvia

*Corresponding author, E-mail: andis.karlsons@lu.lv

Key words: peat and mineral soils, sphagnum peat, sulfur, *Vaccinium corymbosum*.

It is well known that soil pH is one of the most important constraints in successful crop production. Soil pH level effect not only availability of most of the plant nutrients, but also influence microorganism activity that contributes to the decomposition of organic matter. Soil acidification is caused by a number of factors including acidic precipitation and the deposition of acidifying gases or particles from the atmosphere as well as the application of ammonium-based fertilizers, urea and cultivation of legume on agricultural lands. A considerable part of crop-land is excessively acidic for main agricultural species grown in Latvia and demand liming on regular basis. However, there are still crops which usually require additional soil acidification.

One of the most widespread acidophilic crops in Latvia is highbush blueberry (*Vaccinium corymbosum* L.). Highbush blueberry is one of the youngest orchard crops in Latvia with commercially important and growing market demand. The current area of commercial plantings in Latvia is more than 280 ha with increasing annual hectareage. In Latvia, highbush blueberries are cultivated in extracted raised bogs on bare sphagnum peat, as well as on mineral soils with high organic matter content. Main factors, which determine soil suitability for successful blueberry growing are proper soil pH (4.8 ± 0.3) and low Mn concentration. Most of the mineral soils in Latvia typically are not suitable for direct planting of blueberries, therefore soil acidification usually is necessary. In general, there are several practices that can be used to adjusting soil pH. It should be noted that Latvia is a country with abundant peat resources

thereby common technique for a decrease of soil pH level is to blend acid peat with a top layer of mineral soil, while in North America most often the sulfur method is used.

The aim of the present study was to find out advantages and shortages of two methods used for soil acidification: applying of elemental sulfur and acid peat as mulch. A field experiment was established in peat and mineral soil with inadequate high soil pH to test the efficiency of powdered and granular elemental sulfur, as well as acid peat to improve growth conditions for blueberry cultivation. During experiment levels of plant available nutrients sulfur and manganese, as well as soil pH/KCl and electrical conductivity were determined.

While the main benefits of sulfur application were low price, simple usage and increase in soil concentration of S as nutrient, acid peat was superior in other areas: (a) raise of organic matter content; (b) improved weed control; (c) faster adjustment of the desired pH level; (d) reduction of soil manganese content. Our trial showed that granulated form of sulfur compared to powdered is more suitable for soil pH adjustment especially in plantations where blueberries are already planted. Defined advantages of granulated sulfur are provided by slow dissolving properties: (a) extended effect; (b) prevention of unnecessary high sulfur and electrical conductivity; (c) less leakage. Our field experiments demonstrated that effect of tested methods for soil acidification is limited on soils with notably elevated pH and especially high concentration of Ca and Mg.

Soil microarthropods (Acari, Collembola) in the Rīga city habitats

Ineta Salmane*

Institute of Biology, University of Latvia, Miera 3, Salaspils LV–2169, Latvia

*Corresponding author, E-mail: isalmane@lu.lv

Key words: habitats, mites, soil mesofauna, urban areas..

Nowadays about 50% of the human population around the world lives in the cities. Urbanization is associated with a variety of effects on the soil system, including pollution, conversion of indigenous habitats, habitat fragmentation and loss, and soil community changes. Soil plays a key role in the biosphere: it governs plant productivity, allows organic matter degradation and nutrient cycling.

Soil system transformation is reflected in changes of species composition and abundance of soil fauna (Coleman 2008). Soil organisms, including soil microarthropods, influence vital ecosystem processes and are involved in creation of soil system, which acts as an environment for organisms, and within which they are adapted to live. Soil microarthropods closely interact with abiotic environmental factors. Many of them respond to changes in environmental conditions and, because of that, may be used as indicators of soil quality.

A serious barrier to our understanding of urban ecosystems is a lack of information on responses of soil biotic communities to environmental changes within cities. The aim of the current study was to get insight into soil microarthropod communities of urban areas in Rīga city: forests (deciduous, coniferous forests), greenery (yards, backyards etc.) and roadside habitats. So far Mesostigmata fauna was investigated mainly in the natural habitats, and information on soil mites, as well as other soil invertebrates

in the urban areas of cities in Latvia is scarce (Salmane 2001a; Salmane 2001b; Salmane, Brūmelis 2010; Minova et al. 2015; Telnov, Salmane 2015).

In the current investigation 21 soil samples were collected in the Rīga city green areas in October 2014. Volume of each sample was approximately 1 L, they were collected by hand and extracted on modified Tullgren type funnels.

Soil microarthropods were separated in four groups: Collembola (Hexapoda), Oribatida, Mesostigmata and other Acari (Arachnida). In total, 18 000 individuals were extracted. The highest total number of soil microarthropods was recorded for the forest habitats and the least number for roadsides.

All the investigated groups had the highest number of individuals in the forest habitats (Fig. 1A). The least number of individuals was recorded in the Rīga city greenery for Mesostigmata mites and the rest of mites, but the least number of individuals for Collembola and Oribatida was recorded in the roadside habitats.

In total 51 Mesostigmata species were recorded and identified. Of them, the highest number was recorded in roadside and the least in forest habitats (Fig. 1B).

Of the recorded Mesostigmata species, 12 were common for all three habitat types. They included members of Parasitidae, Laelaptidae, Veigaiaidae, Aceosejidae,

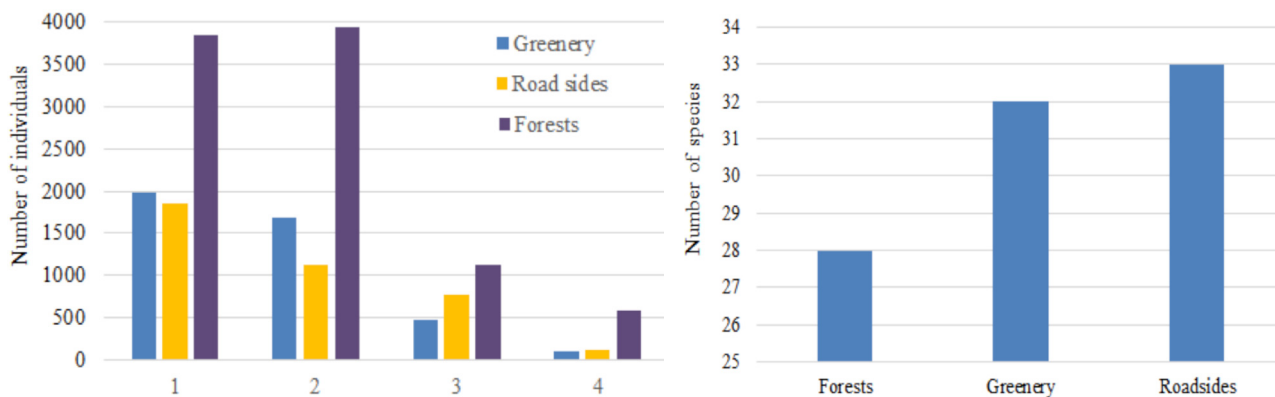


Fig. 1. Number of microarthropod individuals in the investigated habitats of Rīga city (A). Abbreviations: 1, Collembola; 2, Oribatida; 3, Mesostigmata; 4, rest of mites. Number of Mesostigmata mite species in the investigated habitats of Rīga city (B).

Rhodacaridae, Pachylaelapidae, and Trachytidae. For two habitat types, 17 species were common: for greenery and roadsides 8 species, roadsides and forests 6 species, and greenery and forests 3 species. For one habitat type, 23 species were characteristic: for greenery 9 species, roadsides 7 species, and forests 7 species. List of Mesostigmata mites included also species recorded in the Rīga city forests previously (Telnov, Salmane 2015). Mesostigmata species from Rīga forests were similar to these found in natural forests of Latvia except two species (Salmane, Brūmelis 2010).

The highest number of microarthropods in forests can be possibly explained by the relatively low biological diversity of microhabitats and relatively stable environmental conditions in forests. The same can be said concerning the number of Mesostigmata mites. The highest number of Mesostigmata species and low number of individuals for roadside habitats can be explained by the relatively higher microhabitat diversity and variable environmental conditions

Acknowledgements

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References

- Coleman D.C. 2008. From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. *Soil Biol. Biochem.* 40: 271–289.
- Minova S., Jankevica L., Salmane I., Čekstere G. 2015. Preliminary studies on soil health and quality in urban grasslands, Rīga example. *Proc. Latv. Acad. Sci. B* 69: 140–144.
- Salmane I. 2001a. Fauna of soil Gamasina mites (Acari, Mesostigmata) along the Latvian sea coast and their relation to the respective habitats. *Norw. J. Entomol.* 48: 223–230.
- Salmane I. 2001b. A check-list of Latvian Gamasina mites (Acari, Mesostigmata) with short notes to their ecology. *Latvijas Entomologs* 38: 27–39.
- Salmane I., Brūmelis G. 2010. Species list and habitat preference of Mesostigmata mites (Acari, Parasitiformes) in Latvia. *Acarologia* 50: 373–394.
- Telnov D., Salmane I. 2015. The impact of urban pressure and hypertrophication on pine forest soil invertebrate diversity in Rīga city, Latvia. *Proc. Latv. Acad. Sci. B* 69: 120–131.

Validation of ion-selective electrodes for measurement of Na⁺ and K⁺ concentration in halophyte tissues

Gederts Ievins^{1*}, Silvija Meža¹, Andis Karlsons², Anita Osvalde²

¹Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

²Institute of Biology, University of Latvia, Miera 3, Salaspils LV-2169, Latvia

*Corresponding author, E-mail: gederts.ievins@lu.lv

Key words: halophytes, ion-selective electrodes, flame photometer, K⁺, Na⁺.

Recently functional studies of natural halophytic plant species has gained special interest due to concept of possible agricultural use of “cash crop halophytes” (Galvani 2007) as well as for emerging role of halophytes in phytoremediation of polluted environments (Van Oosten and Maggio 2015). For halophyte studies both in field as well as in controlled conditions it is primarily necessary to make measurements of Na⁺ concentration, usually for a large number of samples. In addition, K⁺ measurements in halophytic plant tissues are important to understand mechanisms of mineral nutrition, as Na⁺ can replace several K⁺ functions (Subbarao et al. 2003).

Chemical methods of Na⁺ and K⁺ analysis in biological materials often comprise destruction of organic material by dry ashing or acid digestion, followed by flame photometry of dissolved sample, making analysis both time- and reagent-consuming. As an alternative, electrochemical sensors in a form of ion-selective electrodes can be used for detection of different ions, also Na⁺ and K⁺. The method is mostly used in environmental analysis of water samples (De Marco et al. 2007). The aim of the present study was to adapt ion-selective electrode technique for measurement of Na⁺ and K⁺ concentration in tissue samples from halophytic plants.

For measurements, tissue samples of halophytic plants were collected in natural sites on coastal habitats of the Riga Bay (Baltic Sea) near Mērsrags and Lielupe (Latvia). These included leaves, stems and flowers of *Atriplex calotheca*, *Atriplex littoralis*, *Atriplex longipes*, *Bidens tripartita*, *Cakile maritima*, *Chenopodium album*, *Chenopodium rubrum*, *Festuca rubra*, *Polygonum lapathifolium*, *Scirpus tabernaemontani*. Material of *Fucus* sp. was collected in sea near island of Bornholm (Denmark). Plant material was dried in a thermostat at 60 °C for 72 h and was further kept in sealed polyethylene bags at room temperature until analysis.

Extracts were prepared from dried tissue samples (0.2 g) by grounding with mortar and pestle and adding 10 mL deionized water. Homogenate was filtered through nylon mesh cloth (No. 80) and immediately used for measurement of Na⁺ and K⁺ by ion-selective electrodes or

flame photometer. As ion-selective electrodes, LAQUAtwin compact meters B-722 (Na⁺) and B-731 (K⁺) (Horiba Scientific, Japan) were used according to the manufacturers instructions.

For chemical analysis of a total ion concentration, plant samples (1 g DM) were dry-ashed in concentrated HNO₃ vapor and redissolved in HCl solution (HCl : deionized water 3 : 100, v/v). The total amount of Na⁺ and K⁺ as well as the amount of Na⁺ and K⁺ in tissue water extracts was analyzed by a flame photometer PFP7 (Jenway) with an air-propane/butane flame.

Effect of manipulations performed with plant material during preparation of extracts on ion concentration was also investigated. Leaf and stem material was analyzed separately. For leaves, tissues were roughly crushed by hand, coarsely grounded with mortar and pestle or grounded with mortar and pestle to fine powder with adding quartz sand. For stems, material was cut in 2 mm pieces by scissors, coarsely grounded with mortar and pestle, grounded with mortar and pestle either to medium sized or fine particles with adding quartz sand. These differently prepared plant tissues were extracted with water for different periods of time (up to 3 min) after equilibration by stirring with pestle for 10 s, filtered through nylon cloth and used for measurement of ion content by ion-selective electrodes. Alternatively, differentially prepared plant tissues were stirred for different periods of time with pestle without further incubation, filtered and used for measurement.

It was evident that for both Na⁺ and K⁺ a total pool in plant tissues was formed by soluble and bound fractions, as ion concentration in water extracts was always significantly less than the total concentration (Fig. 1). Water-soluble concentration of Na⁺ in average was 72, 54, 88 and 79% from that of total concentration in leaves, stems, roots and flowers, respectively. For K⁺, the respective values were 66, 52, 57 and 62%. Results from analysis of the same water extract samples by ion selective electrodes and flame photometry gave highly comparable results, with somehow higher variability at higher concentrations (Fig. 2).

Manipulations performed with plant material during preparation of extracts significantly affected results of

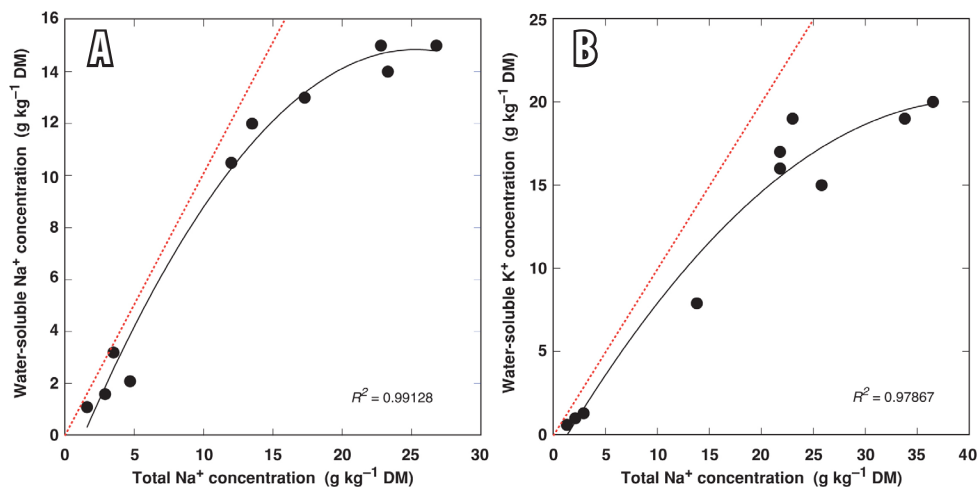


Fig. 1. Comparison of total and water-soluble concentration of Na⁺ (A) and K⁺ (B) in different plant samples.

ion analysis. When no active stirring was applied for differentially prepared plant tissues, the degree of tissue disintegration greatly affected an amount of ions released in solution. Only 41 and 48% of Na⁺ and K⁺, respectively, were released from roughly crushed leaves, and 32 and 45% from stems cut in 2 mm pieces, as compared to finely ground powder. Time of incubation only negligibly affected the amount of released ions. In contrast, ion release from tissues of *Fucus* sp. significantly increased with time up to 5 min. When stirring of homogenate was performed for different periods of time, ion concentration significantly increased with time, and this effect was more pronounced for less disintegrated tissues. However, some species- and tissue-related specificity was evident. Usually, 0.5 to 1.0 min of stirring was necessary to obtain maximum ion yield.

It can be concluded that measurement of Na⁺ and K⁺ concentration in tissue water extracts of halophytic plants by ion-selective electrodes is a reliable and fast method for estimation of concentration of soluble fraction of these metals. For measurement, tissues need to be finely ground

and homogenized with water for 1 to 2 min. For more accurate estimation of ion concentration of a particular plant material, as for studies in controlled conditions, preliminary experiments would be desirable in order to clarify conditions necessary to give the highest yield.

Acknowledgements

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References

- De Marco R., Clarke G., Pejic B. 2007. Ion-selective electrode potentiometry in environmental analysis. *Electroanalysis* 19: 1987–2001.
- Galvani A. 2007. The challenge of the food sufficiency through salt tolerant crops. *Rev. Environ. Sci. Biotechnol.* 6: 3–16.
- Subbarao G.V., Ito O., Berry W.L., Wheeler R.M. 2002. Sodium – a functional plant nutrient. *Crit. Rev. Plant Sci.* 22: 391–416.
- Van Oosten M.J., Maggio A. 2015. Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils. *Environ. Exp. Bot.* 111: 135–146.

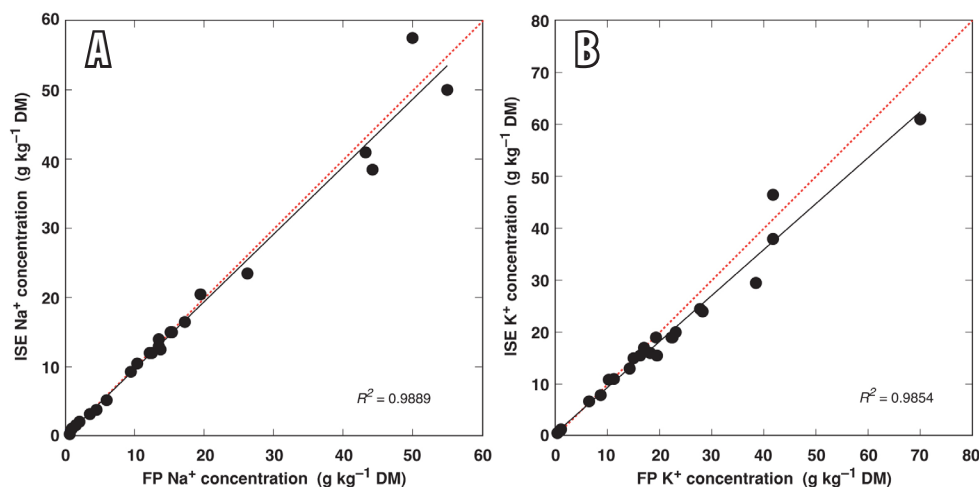


Fig. 2. Comparison of concentration of Na⁺ (A) and K⁺ (B) in water extracts as measured by ion-selective electrodes (ISE) and flame photometry (FP).

Effect of organically-certified compost and vermicompost on growth and physiological characteristics of herbs

Solvita Zeipiņa*, Gederts Ievinsh

Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

*Corresponding author, E-mail: solvita.zeipina@gmail.com

Key words: chlorophyll, chlorophyll *a* fluorescence, *Dracocephalum moldavica*, *Melissa officinalis*, *Nepeta cataria*, organic fertilizers, organic production, *Thymus vulgaris*.

During the recent decades, growing trend of organic horticultural production in Europe and other parts of the world reflects increased demand for both environmentally sound production and healthier plant products. Organic greenhouse production of herbs has increased, and it usually includes a relatively short production cycle in containers with a use of compost or other organically-certified organic fertilizer (Burnett et al. 2016). The aim of the present experiments was to evaluate efficiency of compost and vermicompost as soil amendments in organic herb production. The hypothesis tested was that vermicompost amendment will result in higher biomass of plants as well as will better biochemical indicators of quality in comparison to the plants grown in soil amended by compost at the equal rate.

Experiments with *Dracocephalum moldavica*, *Melissa officinalis*, *Nepeta cataria*, and *Thymus vulgaris* were performed in an experimental automated greenhouse of the Academic Center of the University of Latvia. Soil from organically-certified field was amended with different concentrations (v/v) of compost and vermicompost (Eko Zeme, Latvia). Compost and vermicompost contained similar concentration of plant-available N, P, Mg, S, Mn, Mo, and B. However, K and Fe was at higher concentration in compost, but Ca, Zn and Cu was higher in vermicompost. Identical amounts of Na was present, but compost was slightly more basic and had higher electrical conductivity (EC) than vermicompost. For *M. officinalis* and *N. cataria*, concentrations used were 0, 10, 20 and 30%; but for *D. moldavica* and *T. vulgaris* 0, 20, 30, and 40% were used. Six plants per treatment per species were used. Supplemented light was provided at 380 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the plant level, with 16 h photoperiod, day/night temperature was 23/15 °C, and relative air humidity was maintained at 60 to 70%. Measurement of leaf chlorophyll concentration and chlorophyll *a* fluorescence analysis in leaves was performed once a week. Experiments were terminated after 60 (*M. officinalis* and *N. cataria*), 63 (*D. moldavica*) and 98 (*T. vulgaris*) days of cultivation. Dried plant material from each individual plant was analyzed separately by LAQUATwin

compact meters NO3-11 (NO_3^-) and B-731 (K^+), and electrical conductivity by LAQUATwin conductivity meter B-771 (Horiba Scientific, Japan).

Both fresh and dry mass of shoots of *M. officinalis* and *N. cataria* increased with increasing degree of soil amendment with organic fertilizers (Fig. 1 A, B). For *D. moldavica* the same effect was seen in the case of vermicompost amendment (Fig. 1C), but increase of dose of compost from 20 to 40% did not result in increased biomass in plants from these species (Fig. 1C). For *T. vulgaris* plants, maximum growth was attained at 20% amendment with compost and 30% amendment with vermicompost, but further increase in substitution rate resulted in significant growth reduction of plants (Fig. 1D). As a rule, at 10% amendment for *M. officinalis* and *N. cataria*, and 20% amendment for *D. moldavica* and *T. vulgaris*, there was no significant difference in respect to both fresh (Fig. 1) and dry mass between plants in compost and vermicompost treatments.

K^+ concentration in leaves of soil-grown herbs on dry mass basis was within 15 to 20 g kg^{-1} and organic amendment tended to increase the concentration in all species. However, the effect was statistically significant only in the case of 20% vermicompost and 30% compost for *M. officinalis* and both compost and vermicompost at 20 and 30% for *N. cataria*. There was a statistically significant increase of K^+ concentration in all treatments for *D. moldavica* and in all treatments except 20% vermicompost for *T. vulgaris*.

Various herb species grown in soil accumulated significantly different concentration of NO_3^- , with the lowest concentration in leaves of *D. moldavica*, gradually increasing in *N. cataria*, *M. officinalis* and *T. vulgaris*. In general, NO_3^- concentration in plant leaves tended to increase with increasing dose of organic fertilizer, but both fertilizer-specific and species-specific effects were evident. *M. officinalis* was the only species where vermicompost amendment resulted in accumulation of higher concentration of NO_3^- in comparison to that in compost, where there was no significant increase in the concentration. No statistically significant differences

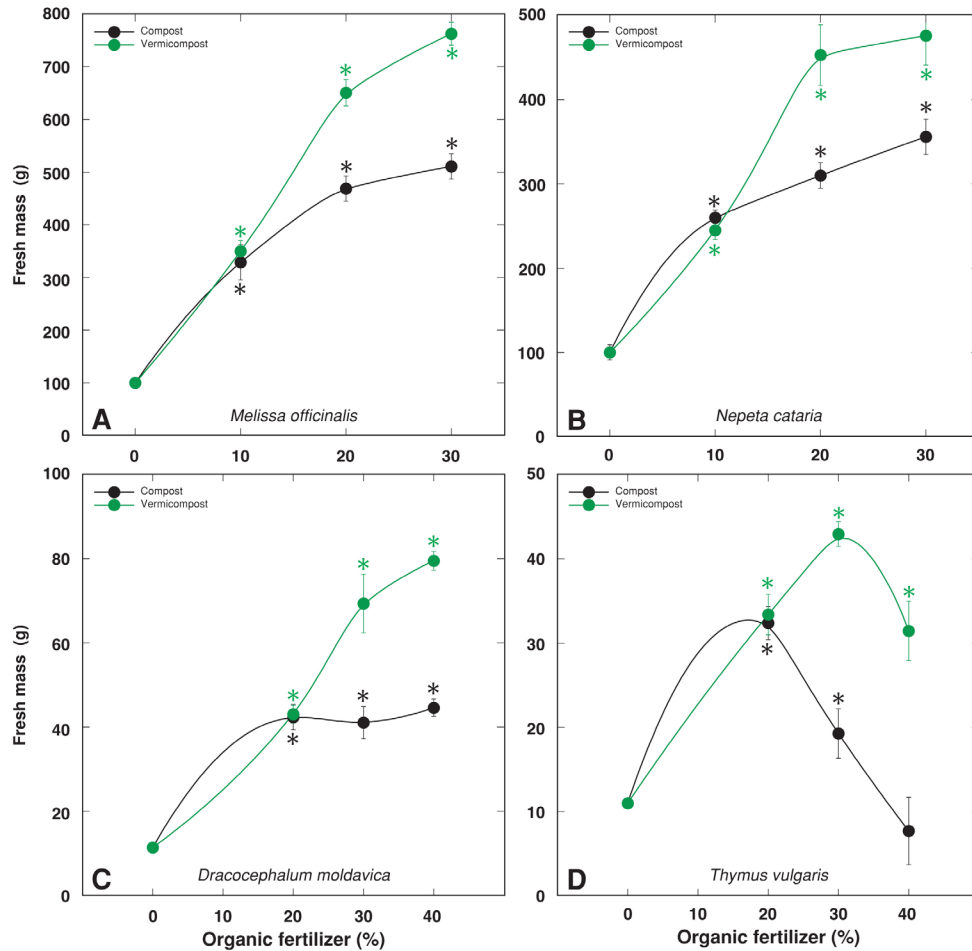


Fig. 1. Fresh mass of shoots of *M. officinalis* (A), *N. cataria* (B), *D. moldavica* (C) and *T. vulgaris* (D) as affected by different concentration of compost and vermicompost. For every treatment, data are means \pm SE from 6 replicates. * indicate significant differences from control without organic fertilizer ($P < 0.05$).

between the two fertilizers were evident for *T. vulgaris*, where NO_3^- concentration reached 271 and 228% from control, in compost- and vermicompost-amended plants, respectively. Lower concentration of NO_3^- in vermicompost-treated plants in comparison to compost-treated ones accumulated in leaves of *N. cataria* and *D. moldavica*.

It is concluded that vermicompost can be more efficiently used for high quality organic production of herbs in comparison to compost. By using soil amended with vermicompost, it is possible to obtain high biomass with lower nitrate concentration. For *M. officinalis* and *N. cataria*, 20% vermicompost substitution showed the best results, but for *D. moldavica* and *T. vulgaris*, 30% substitution was necessary.

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References

Burnett S.E., Mattson N.S., Williams K.A. 2016. Substrates and fertilizers for organic container production of herbs, vegetables, and herbaceous ornamental plants grown in greenhouses in the United States. *Sci. Hortic.* 208: 111–119.

Impact of prenatal stress on the exploratory behavior of Harting's vole *Microtus hartingi* in an open-field test

Tanya Zorenko^{1*}, Nasko Atanasov²

¹University of Latvia, Jelgavas 1, Riga LV-1586, Latvia

²Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Tsar Osvoboditel Blvd. 1, 1000 Sofia, Bulgaria

*Corresponding author, E-mail: zorenkot@lanet.lv

Key words: exploratory behavior, Harting's vole, *Microtus hartingi lydius*, open-field test, prenatal stress.

The pregnancy is the most important period in the mammal's life. Therefore, the stress experienced by a pregnant female could have consequences for the behavior and physical condition of her offspring. Some studies have shown that prenatal stress has a negative effect such as disturbed social behavior, depressive-like behavior (Brunton 2013) and decreased exploration (Brajon et al. 2017). Prenatal stress can induce hormone hypersecretion and different changes of the hypothalamic-pituitary-adrenal axis as well as an alteration of a level of biogenic amines in brain structures (Ismailova et al. 2007).

Founders of laboratory colony of Harting's voles *Microtus hartingi lydius* were brought from Asia Minor. Fourteen pairs of animals were randomly divided into two groups. The females of the first group (SG) were subjected to slight stress via moving from home cages in an unknown cage for 15 minutes during 10 days period, beginning the middle of pregnancy. The second group was a control group

(CG). When the offspring achieved 60 days age, young voles were tested in an experiment of open field. In the open-field test 32 males and 32 females *M. h. lydius* were investigated. Every animal was placed in a cylindrical cage with a diameter of 1 m the bottom of which was divided into squares with side 0.2 m. The field was illuminated by a 60 w bulb suspended 0.9 m above the center of the open-field. Animals were put into a "base" box and then in the cylindrical cage and were allowed to have a free choice of exit to the arena. The experiment lasted 10 min.

Significant changes in the behavior of voles born from mothers that were stressed in comparison with the control animals were established ($P < 0.05 - 0.01$). The maternal effect was manifested by the increase of mobility (Fig. 1) and exploratory activity of the progeny. The vertical activity such as the number of rearing also increased (Fig. 2). Stressed voles earlier and more often crossed the central zone of the arena in which the males were 103.8 ± 17.7 s in

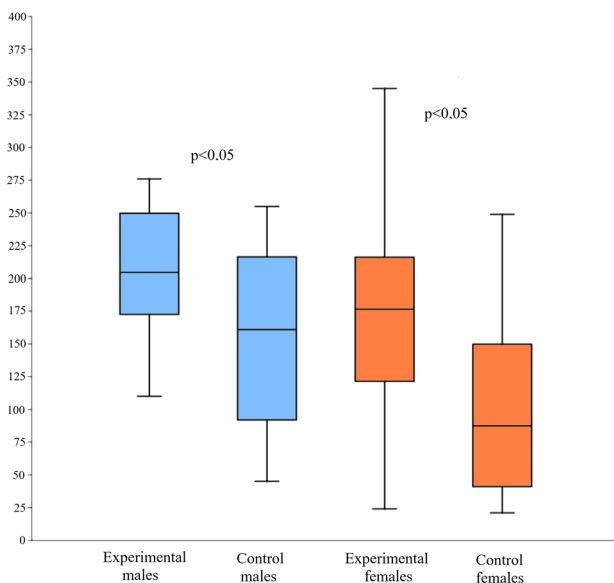


Fig. 1. Comparison of locomotor activity (the number of crossing squares in an open field test) in males and females of experimental prenatal stressed and control groups.

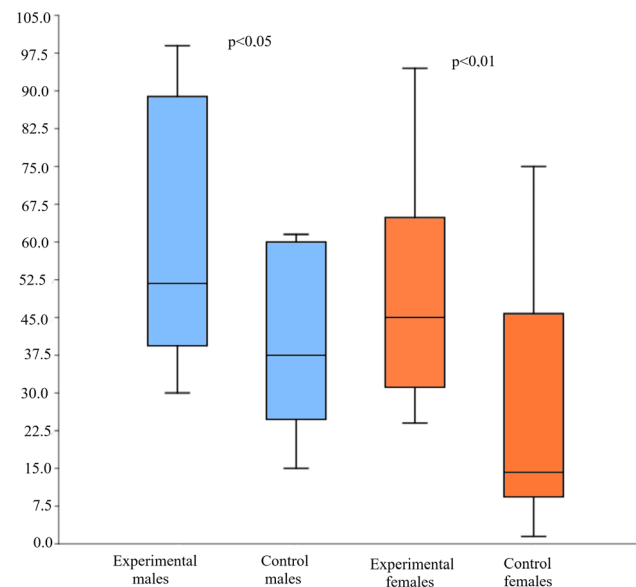


Fig. 2. Comparison of vertical activity (the number of rearings in an open field test) in males and females of experimental prenatal stressed and control groups.

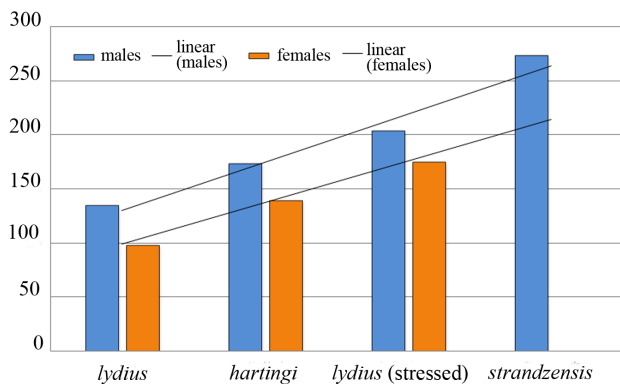


Fig. 3. Trend of increasing the locomotor activity (the of crossing squares in an open field test) in three subspecies of *Microtus hartingi* (*lydius*, *hartingi* and *strandzensis*) and experimental prenatal stressed *lydius* voles (maternal effect).

comparison with the control animals -182.6 ± 33.9 s. The central zone crossing time in females was 154.1 ± 40.3 s for EG and 302.9 ± 61.4 s for CG, accordingly. They more often explored the “base” box by its sniffing, gnawing or digging, as well the walls of the test cage. The duration of the immobility in males was 35.9 ± 10.1 s (EG) and 97.6 ± 23.7 s (CG) ($P < 0.02$) and 56.9 ± 19.2 s (EG) and 166.2 ± 29.4 s (CG), ($P < 0.05$) in females. Autogrooming decreased and in stressed males continues 26.1 ± 4.5 s in stressed and 47.5 ± 8.1 s in control group) and in females respectively 51.8 ± 8.11 s and 94.3 ± 14.6 s ($P < 0.02$). The last both indices depended on the emotional condition of the animals. The ratio of the individuals with low or high mobility as well as with different exploratory activity had changed. In the control group, 69% of the females and males were moving a little and often were motionless. In the experimental group, only 6% of the animales were of such individuals.

It was shown that the laboratory rats demonstrate a different reaction to stress, depending on individual anti-stress protective mechanisms. In emotionally resistant animals, in contrast to emotionally tolerant animals, horizontal and vertical activity is increased (Ismailova at al. 2007). The subspecies *M. h. lydius* is represented by emotionally tolerant individuals and only 1/3 of voles are emotionally resistant (Zorenko et al. 2016). As a result of the influence of prenatal stress, an increase of the proportion of emotionally resistant animals (up to almost 90% of the individuals) is observed, which may have negative consequences for the population of this subspecies. In the individuals of *M. h. lydius* the equilibrium is disturbed since the dominance of such with increased exploratory behavior may lead to their “loss” by the increase of the mortality in

the population.

However, the maternal effect manifested in an increase in the proportion of phenotypes with high activity, which under certain environmental conditions could be supported by selection. In a situation where the habitats were fragmented, it was difficult for animals to find suitable ones. The animals were subjected to the stressful influence of the metapopulation structure of the population. The maternal effect may manifest itself in an increase the proportion of emotionally resistant animals that under the new conditions have a positive effect by epigenetic behavior transformations. Increased activity and risk behavior becomes justified. Zorenko et al. (2016) found an increase of the mobility and exploratory activity of the voles from current natural populations from Strandja and Rhodope Mountains. In the investigation of the locomotor activity of the three subspecies of *Microtus hartingi*, a clearly pronounced trend was noted (Fig. 3). The effect of stress caused almost the same increase in locomotor activity as in the voles of *M. hartingi* from Rhodope Mountains. The results support our hypothesis that in the Holocene, when the ancestral of Harting’s voles have settled in Europe, they were confronted with the stressful influence of the metapopulation structure of the population. It contributes to an increase the part of more active and mobile individuals, which better and successfully adapted to the new environmental conditions. It is necessary for analyzing the changes in the locomotor and exploratory behavior of the voles as an epigenetic effect which may have an important role in the species adaptation and evolution (Badyaev 2008).

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References

- Badyaev A.V. 2008. Maternal effects as generators of evolutionary change. *Ann. N.Y. Acad. Sci.* 1133: 151–161.
- Brajon S., Ringgenberg N., Torrey N., Bergon R. and Deviller N. 2017. Impact of prenatal stress and environmental enrichment prior to weaning on activity and social behaviour of piglets (*Sus scrofa*). *Appl. Anim. Behav. Sci.* 197: 15–23.
- Brunton P.J. 2013. Effects of maternal exposure to social stress during pregnancy: consequences for mother and offspring. *Reproduction* 146: 175–189.
- Ismailova H.J., Agaev T.M., Semenova T.P. 2007. *Individual Features of Behavior: Monoaminergic Mechanisms*. Nurlan, Baku. 228 p. /In Russian/
- Zorenko T., Atanasov N., Golenishchev F. 2016. Behavioral differentiation and hybridization of the European and Asian forms of Harting’ vole *Microtus hartingi* (Rodentia, Arvicolinae). *Russian J. Theriol.* 15: 133–150.

Raphidophyte *Gonyostomum semen* mass blooms in small water bodies in vicinity of Riga City in July 2017

Ivars Druvietis*^{1,2}

¹Institute of Biology, University of Latvia, Miera 3, Salaspils LV–2169, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

*Corresponding author, e-mail: ivars.druvietis@lu.lv

Key words: brown water, *Gonyostomum semen*, marsh pools, small lakes.

In July 2017 at first in the Riga City vicinity nearby Garkalne parish area in small brown water bodies surrounded by marsh were detected a massive blooms of harmful Raphidophyte algae *Gonyostomum semen* (Ehrenberg) Diesing. This algae in very high amounts was detected in Lake Maku ezers, Lake Līņezers, Lake Jūgezers and in the marsh pools of Riga city surroundings nearby Garkalne. These waterbodies are surrounded by typical marsh lands (Fig. 1, Fig. 2).

This invasive and nuisance Raphidophyte algae *G. semen*



Fig. 1. Lake Maku ezers. Photo Ivars Druvietis.



Fig. 2. Pool in marsh in Riga vicinity. Photo Ivars Druvietis.

more than 20 years ago was a very rare species for Latvia's freshwaters. During the last decades blooms of *G. semen* was observed in Latvia and neighbouring countries (Korneva 2000; Laugaste, Noges 2005; Karosiene et al. 2014). It shows that *G. semen* began to become widespread species through the North and East European humic and mesohumic inland waters, where dark brown water color indicates to high or very high amount of humic substances. In July of 2017 more than 95% of total maximal phytoplankton biomass (15.7 mg L⁻¹) of Lake Maku ezers was formed by *G. semen* with minor amounts of *Cryptomonas* sp. In the phytoplankton of Lake Lielais Jūgezers, Lake Līņezers Raphidophyte *G. semen* accounted for just over half of the phytoplankton biomass. Phytoplankton counting analyses were performed by use of Uttermohl method by use of inverted microscope (Willen 1976). Immediately after sampling algal samples were analysed in bright field microscope *in vivo* (Fig. 3, Fig. 4).

The three investigated lakes have a very similar taxonomic composition of phytoplankton, which consists of Raphidophytes, Cryptophytes, green algae and very minor amount of cyanobacteria. Only Raphidophytes *G. semen* were encountered in the pools of marsh (Fig. 5).

Studies of earlier years in the Seda bog and the Teiči bog water bodies indicate an increase in the numbers of

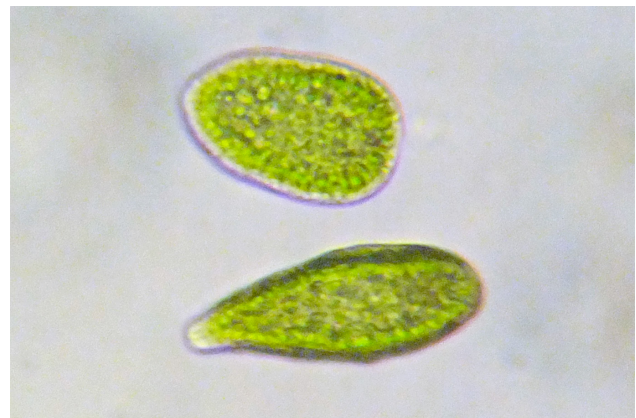


Fig. 3. *G. semen* *in vivo* (× 800). Photo Ivars Druvietis.

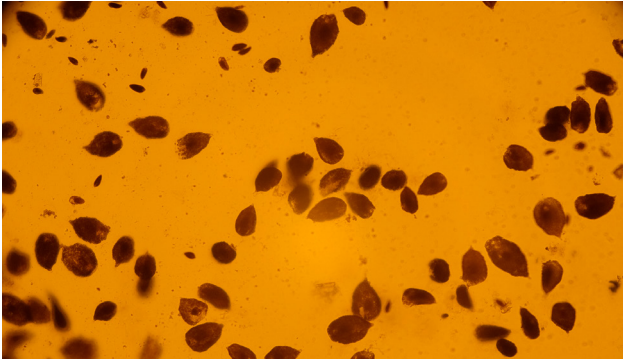


Fig. 4. *G. semen* in inverted microscope fixed with Lugol's solution ($\times 400$). Photo Ivars Druvietis.

G. semen in dystrophic and dyseutrophic lakes in Latvia (Druvietis et al. 2010; Druvietis, Kokorite 2010; Klavins et al. 2010). Water brownification problem, probably caused by climate change, could be one of reasons which caused mass development of *G. semen*.

References

- Druvietis I., Sprinģe G., Briede A., Kokorite I., Parele E. 2010. Comparative assessment of the bog aquatic environment of the Ramsar Site of Teiči Nature Reserve and North Vidzeme Biosphere Reserve, Latvia. In: Kļaviņš M.(ed) *Mires and Peat*. University of Latvia Press, Riga, pp. 9–18.
- Druvietis I., Kokorite I. 2010. Invasion of nuisance Raphidophyte *Gonyostomum semen* (Ehrb.) Diesing in Latvia's Freshwaters. In: Ho K.-C., Zhou M.J., Qui Y.Z. (eds) *Harmful Algae 2008*. Proc. 13th International Conference on Harmful Algae 3-7 November 2008, Hong Kong, China. International Society For The Study of Harmful Algae, Environmental Publication

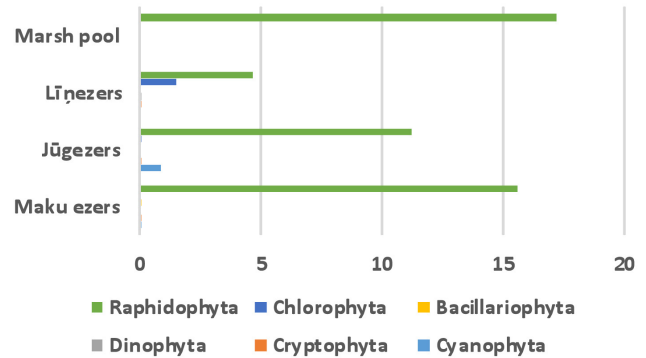


Fig. 5. Dynamics of phytoplankton biomass forming algae divisions (mg L^{-1}) in small water bodies of Riga City vicinity.

- House Hong Kong, pp. 17–20.
- Klavins M., Kokorite I., Sprinģe G., Skuja A., Parele E., Rodinov V., Druvietis I., Strake S., Urtans A. 2010. Water quality in cutway peatland lakes in Seda mire, Latvia. *Ecoidrol. Hydrobiol.* 10: 61–70.
- Karosiene J., 2014. Assessment of the vulnerability of Lithuanian lakes to expansion of *Gonyostomum semen* (Raphidophyceae), *Limnologica* 45: 7–15.
- Korneva L.G., 2000. Ecological aspects of the mass development of *Gonyostomum semen* (Ehrb.) Dies. (Raphidophyta) *Algologija* 10: 265–277. /in Russian/
- Laugaste R., Noges, P. 2005. Nuisance alga *Gonyostomum semen* implications for its global expansion, In: Ramachandra T.V., Ahalya N., Murty C.R. (eds) *Aquatic Ecosystems, Conservation Restoration and Management*. Capital Publishing Company, New Dehli, pp. 77–87.
- Willen E. 1976. A simplified method of phytoplankton counting. *Br. Phycol. J.* 11: 265–278.

Phytoplankton of the Riga Reservoir (1978 – 2017)

Ivars Druvietis*^{1,2}

¹Institute of Biology, University of Latvia, Miera 3, Salaspils LV-2169, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

*Corresponding author, e-mail: ivars.druvietis@lu.lv

Key words: Cyanobacteria blooms, Daugava River, *Microcystis aeruginosa*, phytoplankton.

The first studies of the phytoplankton of the Dammed Riga Reservoir started in 1976, after filling it, and with some interruptions have continued to the present. A particularly intensive studies of algae flora was carried out between 1978 and 1980, when samples of phytoplankton were regularly collected monthly to study the taxonomic composition of the newly created phytoplankton communities and its seasonal dynamics (Fig. 1).

In the Riga Reservoir of up to 1980, 109 algae taxa (14 Cyanophyta, 6 Chrysophyta, 38 Bacillariophyta, 2 Pyrrophyta, 2 Euglenophyta, 47 Chlorophyta) were discovered. The massive proliferation of potentially toxic Cyanobacteria (blue-green algae) *Microcystis aeruginosa* was detected immediately after refilling the new reservoir of Riga with water. Many summers “blooming” colonies of *M. aeruginosa* was particularly evident in July, August and in the first week of September. Due to influence of wind the colonies of *M. aeruginosa* from the upper layers were grow up to coastline of the bays where it blows, causing rapid environmental degradation (Druvietis 2001; 2003).

Nowadays annual studies of the phytoplankton of

the Riga reservoir are still ongoing in the first week of September (Fig. 2.).

With the exceptions, almost every year during summer maxima a larger or smaller increase in cyanobacteria (blue-green algae) is observed in the upper water layer. Although observations from recent years have shown evident decrease in the amount of cyanobacteria (blue-green algae) biomass, especially in 2017, which could be explained by heavy rains and relatively the effect of the cold summer on the development of phytoplankton.

References

- Druvietis I. 1980. Algological investigations in Riga Reservoir. University of Latvia, Diploma Thesis. /in Latvian/
 Druvietis I. 2001. Cyanobacteria blooms in dammed reservoirs of the Daugava River, Latvia. In: Hallegraeff G. et al. (eds) *Harmful Algal Blooms 2000*. Intergovernmental Oceanographic Commission of UNESCO 2001, pp. 105–107.
 Druvietis I. 2003. Development of phytoplankton communities in the Daugava River hydropower plant reservoirs. *Acta Biol. Univ. Daugavp.* 3: 67–70.

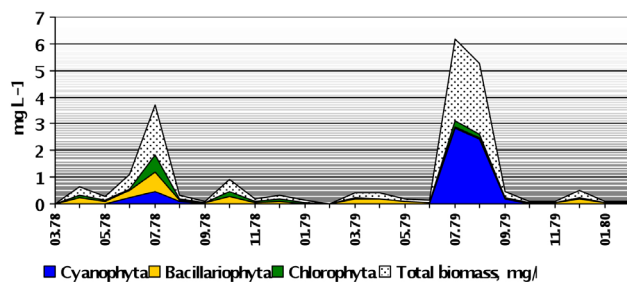


Fig. 1. Dynamics of phytoplankton biomass forming algae divisions 1978 – 1980 (Druvietis 1980).

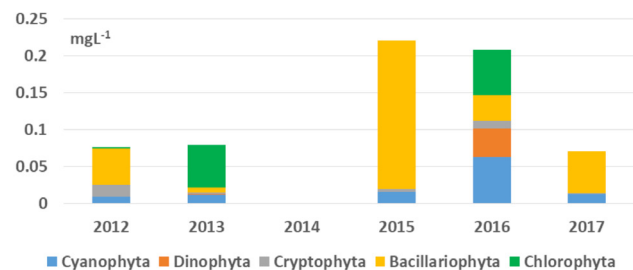


Fig. 1. Dynamics of medium phytoplankton biomass-forming algae divisions in the first week of September from 2012 to 2017 (there are no data for 2014).

Expression of several proteasome genes in kidneys and liver of rats with streptozotocin-induced *diabetes mellitus* and treatment with AV-153-Ca

Kristīne Ošiņa^{1*}, Evita Rostoka^{1,2}, Nikolajs Sjakste^{1,2}

¹Institute of Biology of the University of Latvia, Salaspils, Latvia

²Faculty of Medicine, University of Latvia, Riga, Latvia

*Corresponding author, E-mail: kristine.osina@lu.lv

Key words: AV-153-Ca, *diabetes mellitus*, gene expression, proteasome.

Diabetes mellitus (DM) is a worldwide metabolic disease characterized by hyperglycemia and associated with numerous complications. In case of DM, there is observed increase in activity of proteasomes – multicatalytic proteinase complexes involved in degradation of unnecessary and damaged proteins and other crucial cell processes. Successful treatment of DM and its complications requires search for prospective compounds with antidiabetic and antioxidative properties making 1,4-dihydropyridines interesting from this point of view.

The aim of the research was to determine effect of 1,4-DHP derivative AV-153-Ca on proteasome subunit gene expression in diabetic rats.

Type 1 DM was induced in rats by streptozotocin injection (50 mg kg⁻¹). Nine days post injection rats were treated with AV-153-Ca (0.05 or 0.5 mg kg⁻¹) for three days. RNA was extracted from rat kidneys and liver, and expression levels of *Psm3*, *Psm6*, *Psm5* and *Psmc6* genes were determined by qPCR.

Induction of diabetes caused significantly ($p < 0.05$)

increased *Psm3*, *Psm6* and *Psmc6* gene expression in kidneys, while in liver it decreased *Psm3* expression. Both in kidneys and liver of control rats, treatment with AV-153-Ca did not change proteasome subunit gene expression with two exceptions: *Psm5* was significantly decreased in kidneys (with AV-153-Ca 0.05 mg kg⁻¹), and *Psm6* was significantly increased in liver (with AV-153-Ca 0.5 mg kg⁻¹). In kidneys of diabetic rats, AV-153-Ca elevated gene expression of all four proteasome subunits. In liver of model animals, the compound had no effect on proteasome subunit gene expression.

To sum up, AV-153-Ca increased proteasome subunit *Psm3*, *Psm6*, *Psm5* and *Psmc6* gene expression in kidneys of diabetic animals, but did not influence expression of these genes in liver.

Acknowledgements

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Occurrence of diving beetle *Cybister lateralimarginalis* and peculiarities of zoobenthos communities in small brown water lakes in vicinity of Riga

Diāna Poppela^{1*}, Arkādijs Poppels^{2*}

¹University of Latvia, Faculty of Biology, Jelgavas 1, Riga LV–1004, Latvia

²Riga Zoo, Meža Prospekts 1, Riga LV–1014, Latvia

*Corresponding author, E-mail: dpoppela@gmail.com

Key words: brownwater lakes, *Cybister lateralimarginalis*, macrozoobenthos.

The aim of this study was to clarify peculiarities of benthic communities as well as existence of diving beetle *Cybister lateralimarginalis* De Geer, 1774 in small lakes situated in Riga City vicinity. Therefore, sampling of diving beetle and of macrozoobenthos communities were performed in five small lakes (Lake Līņu ezers, Lake Maku ezers, Lake Lielais Jūgezers, Lake Mazais Jūgezers, Lake Piekūna ezers) during period of 2015 – 2017 at the same time in summer. The observed hydrophysical indices at the sampling time are shown in Table 1.

The lakes are characterised as brown water lakes. Typical overhangs are present on a coastline, where communities of typical marsh vegetation are dominated by *Sphagnum* spp., *Calla palustris*, *Vaccinium uliginosum*, *Comarum palustre*, *Rubus chamaemorus*, *Vaccinium vitis-idaea*, *Menyanthes trifoliata* and *Carex* spp. Bottom ground is formed by black mud and detritus. Only in separate sites of coastal line it is possible to find mineral soil, where lake banks are hard and overgrown with pines. In these places bottom ground is formed by sand covered with mud and detritus. Submerged trees are very common. Macrophytes are represented mostly by *Potamogeton* spp., *Elodea canadensis*, *Nyphar lutea* and *Nymphaea alba* in very minor amounts. In some sites it is possible to find *Phragmites australis* and *Typha latifolia*.

In this study 60 samples were analysed for diving beetles and 35 samples for zoobenthos animals. Presence of *C. lateralimarginalis* was established in all studied lakes. The

number of these beetles was very different: in the lakes with wide pelagic zone these beetles were found only in strongly overgrown inlets, where it was possible to find appropriate food, larva of dragonfly, and in the biotopes without wave effect. Similar limitations for existence were found also in small overgrown inlets of lake Lielais Baltezers, where *C. lateralimarginalis* was found previously (Poppela et al. 2016). Obviously this beetle prefer microhabitats with high percentage of macrophytes and bottom ground layer formed by medium rough detritus.

Macrozoobenthos was formed by larvae of Chironomidae, Oligochaeta and Varia, while the dominant taxa were *Chaoborus flavicans*, *Culicoidae* and *Pulex* sp. Presence of other groups (Ephemeroptera, Trichoptera,

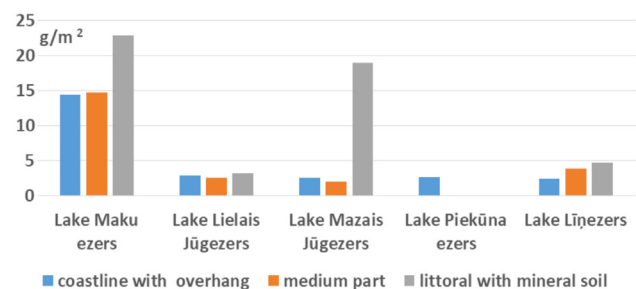


Fig. 1. Average biomass (g m⁻²) of macrozoobenthos in small lakes in Riga City vicinity.

Table 1. Hydrophysical indices in small lakes situated in vicinity of Riga City

Lake	Temperature (°C)	pH	O ₂ concentration (mg L ⁻¹)	Conductivity (μS cm ⁻¹)	Visibility (m)
Līņu	24.4	8.3	16.6	28.0	0.18
Maku	22.1	5.4	14.2	34.0	0.10
Lielais Jūgezers	26.2	7.6	18.6	47.7	0.70
Mazais Jūgezers	26.4	7.5	17.4	47.0	0.25
Piekūna ezers	23.2	4.3	6.2	66.4	0.25

Mollusca, Odonata un Malacostraca) was small and incidental. The largest biomass of macrozoobenthos were found in littoral zone, where mineral soil was dominating, except Lake Piekūna ezers, where coastline was completely encircled by overhang (Fig. 1).

In conclusion, the studied lakes are suitable for living and development of diving beetle *Cybister lateralimarginalis*. Limiting physical factors in lakes and availability of

appropriate food resources for development of diving beetle larva affect distribution of this species in Latvia.

References

Poppela D., Poppels A., Kalnins M. 2016. Diving beetle *Cybister lateralimarginalis* de Geer, 1774 distribution in various microhabitats in Lake Lielais Baltezers, Latvia. Materials of the V International Scientific Conference September 12–17, 2016, Minsk – Naroch, pp. 382–383.

Development of the single-use eight channel bioreactor for parallel cultivation and real time microbial growth control under aerobic (*Escherichia coli*, *Pichia pastoris*), semi-anaerobic and anaerobic (*Lactobacillus* sp.) conditions

Arturs Kigitovics, Sergej Djachenko, Valerij Sarichev, Aleksandr Shevchik, Uldis Balodis, Pavels Bankovskis, Aleksejs Zukovs, Jurijs Valjavins, Julija Galimbovska, Igors Bankovskis*

SIA Biosan, Ratsupites 7, Building 2, Riga LV-1067, Latvia

*Corresponding author, E-mail: igor@biosan.lv

Key words: bioreactor, *E. coli*, light scattering, noninvasive cell concentration measurement, noninvasive growth control, *P. pastoris*, real time growth kinetics, RTS technology, RTS-8, turbidimetry

Noninvasive methods for real time (RT) measurement and monitoring of cell growth and parameters of vital bioprocesses such as changes in pH and pO_2 values, as well as substrate concentration during fermentation, are among timely trends in a modern biotechnology.

In our previous publications, the single use bioreactor RTS-1 has been described, which provided possibilities of noninvasive control of microbial culture growth under aerobic conditions (Bankovsky et al. 2013). Algorithm of optical density (OD) measurement consists of two steps: the 1st step includes a rotation of 50 mL Falcon tubes (filled with growth media) around their axis at 2000 rpm. Centrifugal forces lead to formation of a thin layer of cell suspension along tube perimeter with a layer path for light beam at a range from 1 to 3 mm (depending on a broth volume). This “premeasurement step” provides a possibility for performing of the 2nd step, i.e., turbidimetric measurement of cell suspension at OD^{850} with a correct range from zero to 24 (45 OD_{600}).

Limitation of this method for only aerobic microorganisms was a reason to search for a new and

innovative approach, which would be suitable for a noninvasive measurement of OD_{600} under anaerobic conditions, when intensive agitation of reaction vessel is not possible.

Here, we describe a new model of the bioreactor, the RTS-8, that provides a noninvasive control of bacterial and yeast cell growth both under aerobic or anaerobic conditions. The principle of the cell concentration measurement method is based on detection and evaluation of probability of light scattering direction, which is dependent on microbial cell concentration (method of indicatrices).

Growth kinetics for cultures of *Escherichia coli* BL21 and *Pichia pastoris* in dependence on aeration intensity is summarized in Fig. 1 and 2, respectively. In turn, *Lactobacillus acidophilus* cells were cultivated under anaerobic conditions at different temperatures (Fig. 3).

The bioreactor RTS-8 utilizes patented Reverse-Spin® technology (Bankovskis 2013) that applies non-invasive, mechanically driven, innovative type of agitation with low consumption of energy, where cell suspension is mixed by a single-use falcon bioreactor tube rotation around its axis

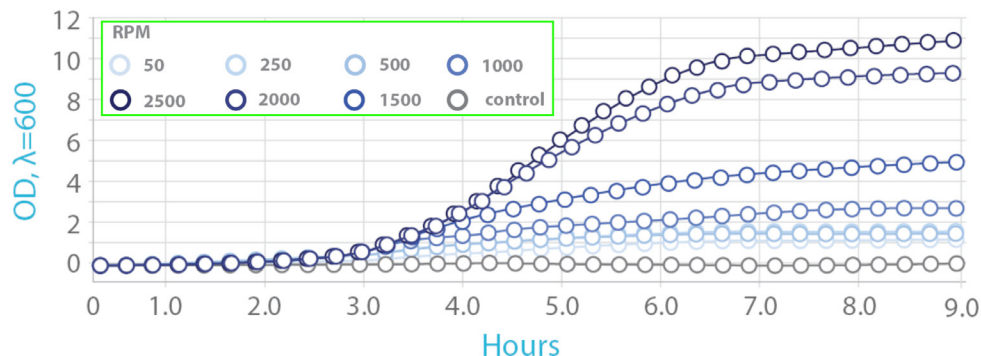


Fig. 1. Effect of aeration on the growth of *E. coli* BL21. Cultivation was performed in the bioreactor RTS-8, LB medium 10 mL, 37 °C, agitation intensity according to the legend.

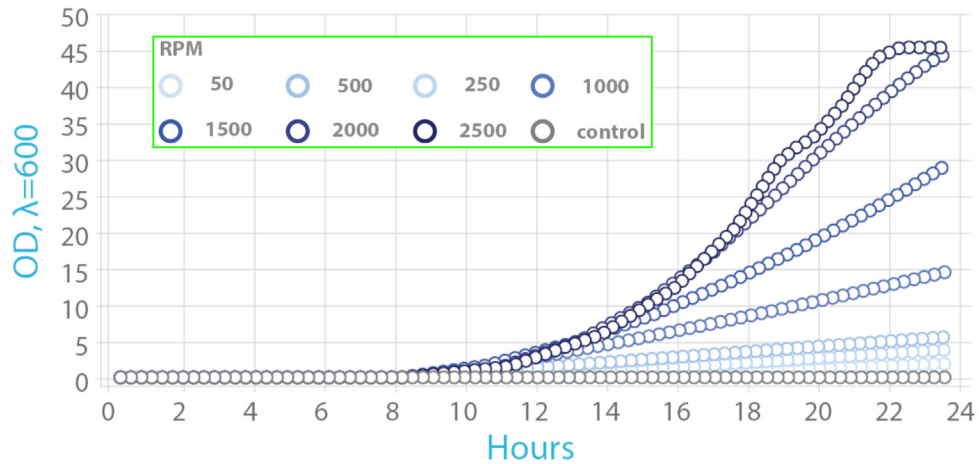


Fig. 2. Effect of aeration on the growth of *P. pastoris*. Cultivation was performed in the bioreactor RTS-8, LB medium 10 mL, 37 °C, agitation intensity according to the legend.

with a change of direction of rotation motion, resulting in highly efficient mixing and oxygenation for aerobic cultivation.

Acknowledgements

The project No. 2101 “Development and introduction of innovative methods in clinical practice for the diagnosis and treatment of malignant tumors using molecularly targeted radionuclides produced in Latvia” is supported by philanthropist SIA “Mikrotikls” and administrated by the University of Latvia Foundation. We are grateful to Dr. biol. V. Bankovskis, president of SIA Biosan for the support and research ideas.

References

- Bankovsky V., Bankovsky I., Bankovsky P., Isakova J., Djackova I., Sharipo A., Eskin J., Dišlers A., Rozenstein R., Saricev V., Djacenko S., Makarenko V., Balodis U. 2013. Reverse-Spin® technology – innovative principle of microbial cultivation. In: *Applications and Articles*. Biosan, Rīga, pp. 112–116. <http://biosan.lv/uploads/reverse-spinner-article.pdf>.
- Bankovskis V. 2013. Suspensijas blīvuma mērīšana bakteriālo šūnu audzēšanas procesā un ierīce tā realizēšanai. Latvijas Republikas Patents Nr. LV14952. <https://www.lrpv.gov.lv/sites/default/files/20150620.pdf>.

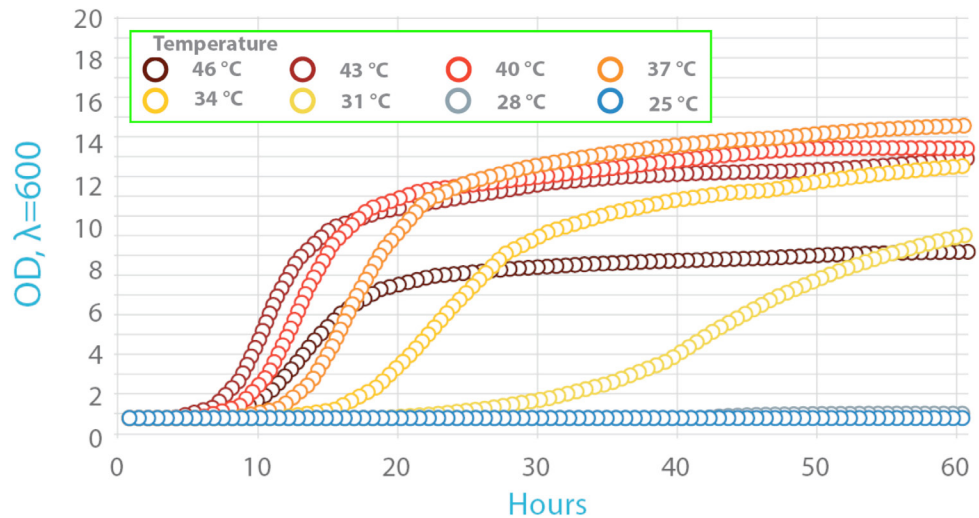


Fig. 3. Effect of temperature on the growth of *L. acidophilus*. Cultivation was performed in the bioreactor RTS-8, MRS medium 50 mL, agitation intensity 50 rpm., temperature according to the legend.

Investigation of hypochlorite (NaOCl) effect on stress response of LactoSeven[®] microbiome and efficacy protection of sulforaphane against oxygenation

Arturs Kigitovics, Sergej Djachenko, Valerij Sarichev, Aleksandr Shevchik, Uldis Balodis, Pavels Bankovskis, Aleksejs Zukovs, Jurijs Valjavins, Julija Galimbovska, Igors Bankovskis*

SIA Biosan, Ratsupites 7, Building 2, Riga LV-1067, Latvia

*Corresponding author, E-mail: igor@biosan.lv

Key words: bioreactor, hypochlorite, LactosSeven, light scattering measurement, microbiome, noninvasive cell concentration measurement, probiotics, real time growth kinetics, inhibition RTS technology, RTS-8, sulforaphane, toxicity tests.

Sodium hypochlorite (NaOCl) is known as a strongly oxidizing reagent (Mohammadi 2008), which has been used to simulate the radiolysis of physiological media. Effect of oxidation by NaOCl, as well as a protection effect by L-cysteine (Akaboshi et al. 1972) and sulforaphane (Talalay et al. 2007; Wu et al. 2017) has been investigated on mammalian tissues. On the other hand, biopreparation LactoSeven[®] can serve as a model of functional consortia in studies on the response of gut microbiome on different types of stress. LactoSeven[®] contains seven bacterial cultures, which are known to be beneficial microorganisms in gut microbiome with health-promoting or probiotic effects on the host organism (Ouwehand et al. 2002). Composition of LactoSeven[®] is presented in Table 1.

For this investigation we used parallel single-use bioreactor, Reverse Tube Spinner (RTS-8), designed for parallel cultivation of microbial cultures in aerobic, semi aerobic and anaerobic conditions having non-invasive options for measurement of cell concentration during cultivation. Principle of mixing is based on reverse spinning (RS) of tubes around tube's vertical axis (Bankovsky et

al. 2013). Principle of OD₆₀₀ measurement based on the detection and evaluation of light scattering direction probability depending on a microbial cells concentration (method of indicatrices).

For the study eight tubes were prepared with different initial composition (Fig. 1, 2) and were cultivated simultaneously due to RTS-8 bioreactor advantageous opportunity to cultivate eight samples. MRS broth volume was 10 mL in 50 mL TPP Bioreactor tubes, tube rotation speed set to 500 rpm.

Sulforaphane protected LactoSeven microbiome against oxidation induced by sublethal dose of NaOCl similarly to a strong radioprotector L-cysteine. Recovery of growth took place after 10 h of NaOCl sublethal dose oxidizing shock.

Table 1. Characteristics of microorganisms used in the preparation LactoSeven[®]

Species	Biochemical characteristics
<i>Lactobacillus acidophilus</i>	Homofermentative, only lactate
<i>Lactobacillus casei</i>	Produces amylase
<i>Lactobacillus plantarum</i>	Biggest genome universal broad T(C) and pH tolerance
<i>Lactobacillus reuteri</i>	Reuterin antibiotic against harmful Gram-negative bacteria
<i>Lactobacillus rhamnosus</i>	Heterofermentative
<i>Bifidobacterium longum</i>	Heterofermentative, lactate (acetatekinase) + acetate
<i>Streptococcus thermophilus</i>	Facultative anaerobe, no catalase

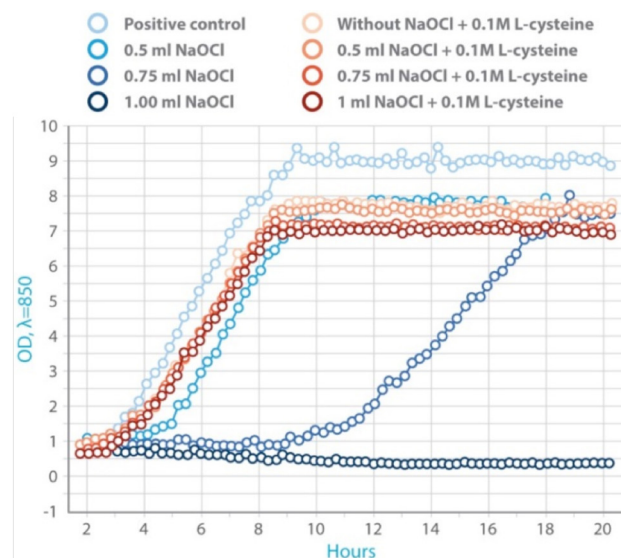


Fig. 1. Influence of different sublethal and lethal concentrations of NaOCl on growth kinetic of LactoSeven microbiome and positive effect of radioprotector L-cysteine on growth curve at sublethal and lethal concentration of NaOCl.

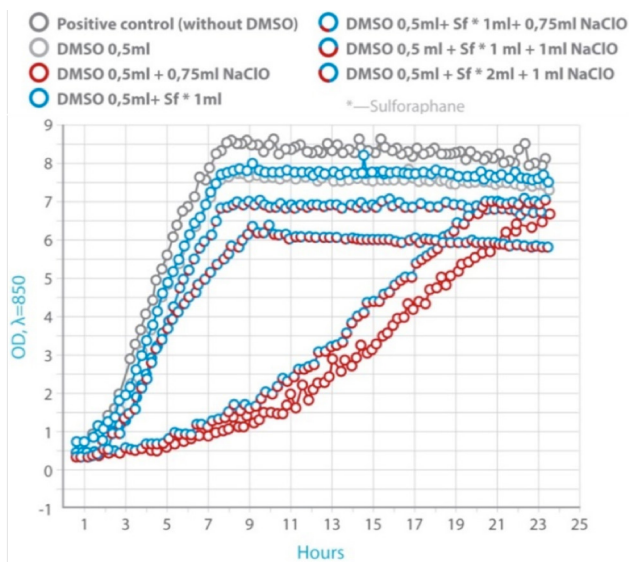


Fig. 2. Influence of different sublethal and lethal concentrations of NaOCl on growth kinetic of LactoSeven microbiome affected by sulforaphane addition in different volumes.

Acknowledgements

The project No. 2101 “Development and introduction of innovative methods in clinical practice for the diagnosis and treatment of malignant tumors using molecularly targeted

radionuclides produced in Latvia” is supported by philanthropist SIA “Mikrotikls” and administrated by the University of Latvia Foundation. We are grateful to Dr. biol. V. Bankovskis, president of SIA Biosan and Dr. chem. A. Grinbergs for the support and research ideas.

References

- Akaboshi S., Shikita M., Matsui E., Hoshino H. 1972. Radiation protection by oral administration of L-cysteine ethyl ester hydrochloride in mice. *Chem. Pharm. Bull.* 20: 721–724.
- Bankovsky V., Bankovsky I., Bankovsky P., Isakova J., Djackova I., Sharipo A., Eskin J., Dišlers A., Rozenstein R., Saricev V., Djacenko S., Makarenko V., Balodis U. 2013. Reverse-Spin® technology – innovative principle of microbial cultivation. In: *Applications and Articles*. Biosan, Rīga, pp. 112–116. <http://biosan.lv/uploads/reverse-spinner-article.pdf>.
- Mohammadi Z. 2008. Sodium hypochlorite in endodontics: an update review. *Int. Dent. J.* 58: 329–341.
- Ouwehand A.C., Salminen S, Isolauri, E. 2002. Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek* 82: 279–289.
- Talalay P, Fahey J.W., Healy Z.R., Wehage S.L., Benedict A.L., Min C., Dinkova-Kostova A.T. 2007. Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. *Proc. Natl. Acad. Sci. USA* 104: 17500–17505.
- Wu X., Cheng J., Wang X. 2017. Dietary antioxidants: potential anticancer agents. *Nutr. Cancer* 69: 521–533.

Production of 2-phenylethanol in lactose-containing media by yeast *Kluyveromyces lactis*

Kristiāna Kovtuna^{1*}, Lidija Aksjonova², Rita Ščerbaka¹, Armands Vigants¹

¹Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

*Corresponding author, E-mail: kristiana.kovtuna@gmail.com

Key words: 2-phenylethanol, fermentation, *Kluyveromyces lactis*, lactose.

2-Phenylethanol (2-PE) is an aromatic compound with a rose-like odor. Due to increasing demand for natural fragrances, biotechnological production of 2-PE can be a potential alternative for chemically produced compound and a less expensive process than naturally-obtained extraction from roses. Many yeasts, including *Kluyveromyces* spp., are capable of 2-PE production (Etschmann et al. 2002). *Kluyveromyces lactis* has been assigned GRAS (generally regarded as safe) and qualified presumption of safety (QPS) status. Due to its ability to ferment lactose, *K. lactis* is mainly used in the industrial production of β -galactosidase (Spohner et al. 2016). Bioconversion of lactose containing substrates such as whey permeate (by-product of dairy industry) into more valuable products, thereby reducing the negative impact on the environment.

In order to investigate the most prominent *K. lactis* 2-PE producer, shake-flask fermentations on a liquid semi-synthetic medium (glucose/lactose as a carbon source, yeast extract 5.0 g L⁻¹, MgSO₄ 1.4 g L⁻¹, KH₂PO₄

1.0 g L⁻¹, K₂HPO₄ 0.1 g L⁻¹, (NH₄)₂SO₄ 5.0 g L⁻¹) with 10.0 g L⁻¹ L-phenylalanine added were carried out with three different industrially used *K. lactis* strains: DSM 70799, DSM 4394 (cultures obtained from the Leibniz-Institute

Table 1. The influence of yeast *K. lactis* DSM 70799, DSM 4394 and DBVPG 6829 strains on 2-PE yield. Shake-flask fermentations were carried out on 10% lactose semi-synthetic medium at 30 °C with 180 rpm for 24 h. Concentration of 2-PE was determined by HPLC (Agilent 1100 Series), using Shodex Asahipak SH101 column. Data presented is an average from three biological replicates, with error bars representing standard deviations

Yeast strains	2-PE (g L ⁻¹)	
	Glucose as a C-source	Lactose as a C-source
<i>K. lactis</i> DSM 70799	1.129 ± 0.055	1.590 ± 0.006
<i>K. lactis</i> DSM 4394	0.706 ± 0.008	0.657 ± 0.011
<i>K. lactis</i> DBVPG 6829	0.280 ± 0.002	0.293 ± 0.005

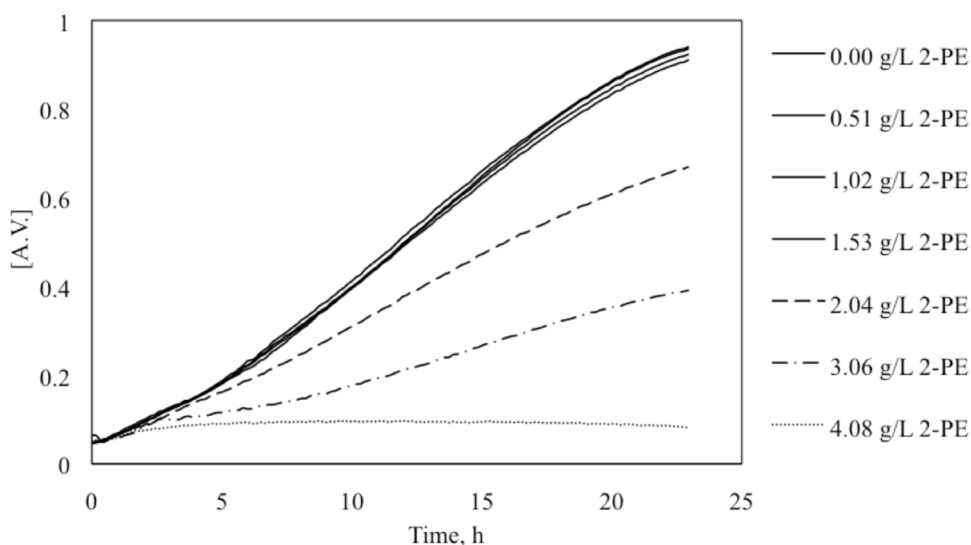


Fig. 1. Growth curves of *K. lactis* DSM 70799 cultures over a range of 2-PE values grown on 2% lactose semi-synthetic medium. Fermentations were carried out in 96-well plate at 30 °C for 24 h, shaken with amplitude 3.5 mm and optical density readings (OD₆₀₀) were taken every 10 min followed by a minute-long pause in shaking. Data presented is an average from three biological replicates. Standard deviations are between 0 to 0.02.

DSMZ-German Collection of Microorganisms and Cell Cultures) and DBVPG 6829 (obtained from the Industrial Yeas Collection DBVPG, Department of Agricultural, Food and Environmental Science, University of Perugia). Comparing these strains *K. lactis* DSM 70799 showed the highest 2-PE yield (1.59 ± 0.06 g L⁻¹) in lactose-containing medium (Table 1.).

The formation of high 2-PE concentration in the medium can prevent inhibitory actions on microorganisms. To evaluate *K. lactis* DSM 70799 tolerance to 2-PE, a 96-well plater reader (Tecan Infinite 200M Pro) was used for growth observation in lactose semi-synthetic medium with added different exogenous 2-PE concentrations (0 to 4.08 g L⁻¹) (Fig. 1). No significant difference in growth curves was observed when 2-PE concentration in the medium was between 0 and 1.53 g L⁻¹. Some limitations in growth occurred when 2-PE concentration in the medium starting with 2.04 g L⁻¹. For *K. lactis* DSM 70799 strain 4.08 g L⁻¹ exogenous 2-PE concentration was found to be completely growth inhibiting. 2-PE is not equally toxic to all microorganisms, for example previous experiments by Fabre et al. (1998) with *Kluyveromyces marxianus* revealed

that the critical concentration of 2-PE is 1.40 g L⁻¹ in the medium, but our unpublished data showed that the critical concentration of *K. marxianus* DSM 5418 strain can tolerate up to 4.08 g L⁻¹ 2-PE concentration. These findings suggest that 2-PE tolerance for *Kluyveromyces* spp. may be strain dependent.

Our current results suggest *K. lactis* DSM 70799 strain as a promising microorganism for 2-PE production.

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References

- Etschmann M.M.W., Bluemke W., Sell D., Schrader J. 2002. Biotechnological production of 2-phenylethanol. *Appl. Microbiol. Biotechnol.* 59: 1–8.
- Fabre C.E., Blanc P.J., Goma G. 1998. 2-Phenylethyl alcohol: an aroma profile. *Perfum Flavor* 21: 27–40.
- Spohner S.C., Schaum V., Quitmann H., Czermak P. 2016. *Kluyveromyces lactis*: An emerging tool in biotechnology. *J. Biotechnol.* 222: 104–116.

A metabolic profile of microbial communities in benthic sediments originated from glacial meltwater and ice-dammed lakes

Julija Gaidelene¹, Kristaps Lamsters², Jānis Karušs², Māris Krievāns², Agnis Rečs², Olga Muter^{1*}

¹University of Latvia, Institute of Microbiology and Biotechnology

²University of Latvia, Faculty of Geography and Earth Sciences

*Corresponding author, E-mail: olga.mutere@lu.lv

Key words: Biolog EcoPlate™, carbon substrates, Múlajökull.

A permanently cold environment (below 5°C) is the most spread “extreme” environment on Earth, being approximately 80% of our planet’s biosphere. In this respect, psychrophiles attract a great interest for studying the mechanisms of adaptation to cold stress and evaluation of the perceived biotechnological potential of these organisms and their biomolecules (D’Amico et al. 2006; Margesin, Feller 2010; Piette et al. 2011; Maayer et al. 2014).

Múlajökull is an outlet glacier that drains the SE part of the Hofsjökull ice cap in central Iceland. Our previous research was focused on the mapping of the subglacial topography (Lamsters et al. 2016). The aim of this study was to characterize and compare the community-level

metabolic fingerprint of microbial communities originated from the Múlajökull lakes (Fig. 1).

Microbial communities were tested using Biolog EcoPlate™. Optical density was measured in a microplate reader Tecan Infinite® F50. The substrate utilization patterns were compared based on the substrate categories according to (Urakawa et al. 2013) (Fig. 2). As shown in Fig. 2B, the lakes b2 and b3 were characterized by the greatest utilization of polymers and amines after 96 h incubation. Among 10 carbohydrates provided by EcoPlate™, only 6; 8; 2; 3 and 5 substrates were positive for the samples from b2; b3; b5; b7 and b9 lakes, respectively. The most frequently used carbohydrates were D-mannitol and n-acetyl-d-

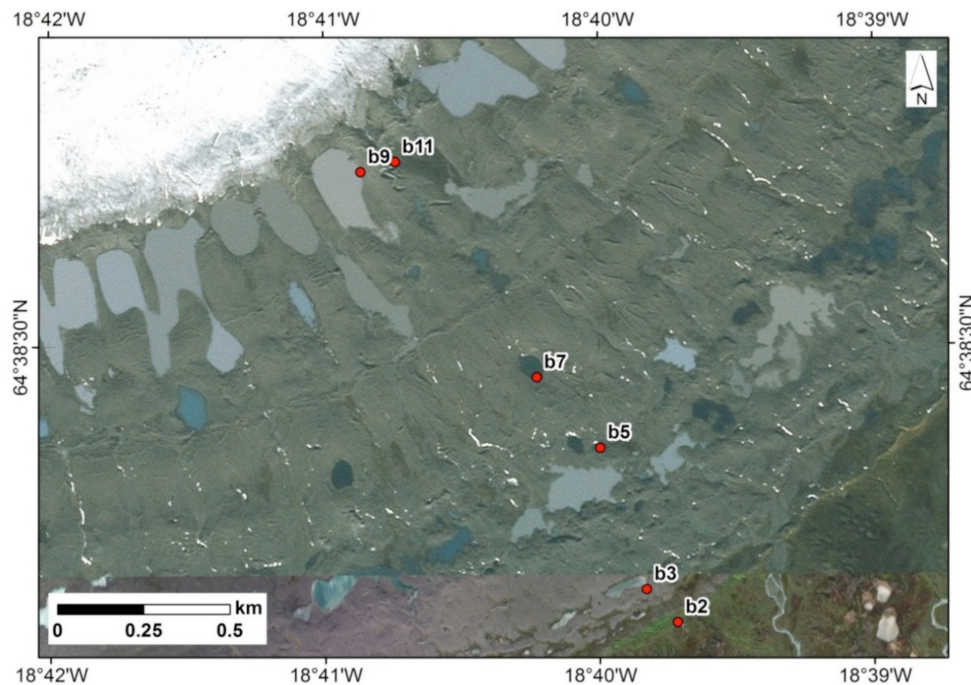


Fig. 1. Sampling sites.

glucosamine. The lowest activity demonstrated a microbial community from the lake b3.

The experiments are in progress. Further analysis of physicochemical characteristics of lake sediments, as well as a microbial functional activity related to the biogeochemical cycling of carbon, nitrogen and other elements, will be completed.

Acknowledgements

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References

- D’Amico S., Collins T., Marx J.-C., Feller G., Gerday C. 2006. Psychrophilic microorganisms: challenges for life. *EMBO Rep.* 7: 385–389.
- Lamsters, K., Karušs, J., Rečs, A., Bērziņš, D. 2016. Detailed subglacial topography and drumlins at the marginal zone of Múlajökull outlet glacier, central Iceland: Evidence from low frequency GPR data. *Polar Sci.* 10: 470–475.
- Maayer P.D., Anderson D., Cary C., Cowan D.A. 2014. Some like it cold: understanding the survival strategies of psychrophiles. *EMBO Rep.* 15: 508–517.
- Margesin R., Feller G. 2010. Biotechnological applications of psychrophiles. *Environ. Technol.* 31: 835–844.
- Piette A., D’Amico S., Mazzucchelli G., Danchin A., Leprince P., Feller G. 2011. Life in the cold: a proteomic study of cold-repressed proteins in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. *Appl. Environ. Microbiol.* 77: 3881–3883.
- Urakawa H., Ali J., Ketover R.D.J., Talmage S.D., Garcia J.C., Campbell I.S., Loh A.N., Parsons M.L. 2013. Shifts of

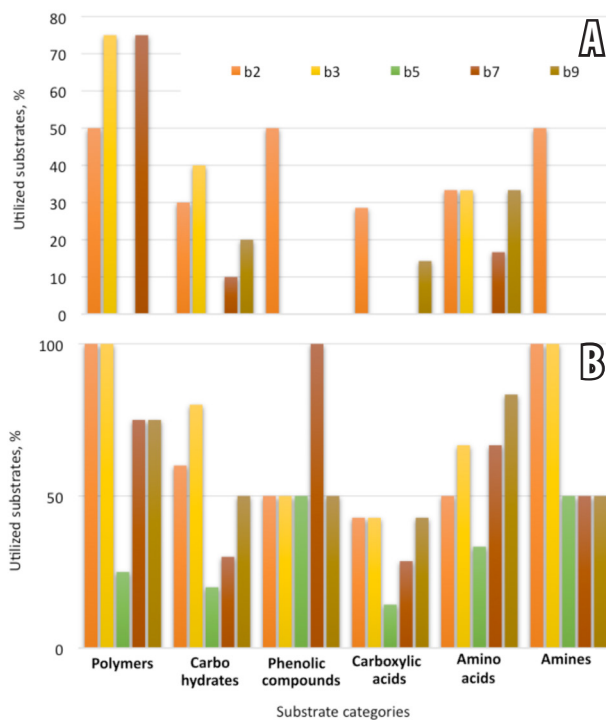


Fig. 2. Percentage of positive substrates separated into substrate categories, which were utilized by microorganisms originated from different lakes. A, 48 h incubation; B, 96 h incubation. Incubation was performed with periodic agitation at 23 °C. Sampling sites are shown in Fig. 1.

bacterioplankton metabolic profiles along the salinity gradient in a subtropical estuary. *Oceanography* 2013: 410814.

Study on the growth and antimicrobial activity of *Bifidobacterium* sp. cultivated in different conditions

Kristine Kalneniece, Olga Muter*

University of Latvia, Institute of Microbiology and Biotechnology

*Corresponding author, E-mail: olga.mutere@lu.lv

Key words: *Bifidobacterium animalis* subsp. *lactis*; *Escherichia coli*; Tween 20; Tween 60.

The gut microbiota represents a broad spectrum of organisms, e.g., bacteria, yeasts, fungi, viruses, protozoans. Some of them exhibit health-promoting or probiotic effects on the host organism, particularly, by competitive exclusion of pathogens, modulation of immune system, provision of nutrients (Turrone et al. 2014; O'Callaghan et al. 2016;). Some types of therapies (e.g., chemo- and radiotherapy) can dramatically affect gut microbiome, leading to the negative changes in digestive and immune systems, hormone regulation etc. Therefore, investigation of the physiological potential of beneficial microorganisms is of a great importance for pharmaceutical and food industries.

This study was aimed at comparing the effect of different cultivation conditions on the growth and antimicrobial activity of *Bifidobacterium animalis* subsp. *lactis* LMKK-939.

Cultivation of *B. animalis* subsp. *lactis* LMKK-939 was performed in a RBKB broth in microplates, 300 µL culture per well, under aerobic and anaerobic (BD GasPak™, USA) conditions at 23 and 37 °C. Composition of the RBKB broth was as follows (g L⁻¹): tryptone 10.0; peptone 2.5; meat extract 0.5; yeast extract 6.0; glucose 10.0; NaCl 2.5. Two exogenous fatty acid substrates (1% w/v) were used as amendments, i.e., Tween 20 and Tween 60. The culture was characterized by OD₆₂₀, cell morphology, as well as antagonistic activity towards *Escherichia coli* LMKK-332, which was determined by agar diffusion test.

As shown in Fig. 1, the highest OD₆₂₀ after 40 h cultivation was reached in the culture, which grew anaerobically at 37 °C. These conditions are known to be optimal for genus *Bifidobacterium*. Furthermore, the growth was observed also in microplates incubated at 23 °C under both, aerobic and anaerobic conditions. However, the OD₆₂₀ of cultures grown at 23 °C, was three times lower, comparing with the culture grown at 37 °C. Addition of 1% Tween 20 significantly ($P < 0.001$) increased, while 1% Tween 60 decreased ($P < 0.001$) the OD₆₂₀, as compared to the non-amended variant (Fig. 1). Addition of both exogenous fatty acid substrates resulted in the changes of cell morphology in cultures (Fig. 2).

Regarding antagonistic activity of *B. animalis* subsp.

lactis LMKK-939, the growth inhibition of *E. coli* LMKK-332 on the agarized medium was observed by inoculation of the cultures *B. animalis* subsp. *lactis* LMKK-939 grown at 37 °C anaerobically and at 23 °C aerobically, irrespective of the presence of Tween 20 or Tween 60.

Other authors reported that *Bifidobacterium* strains exhibited the maximum antimicrobial activity in the late logarithmic phase of growth in the presence of Tween 80 (Collado et al. 2005). Veerkamp (1977) examined the growth of *B. bifidum* subsp. *pennsylvanicum* in the presence of Tween 20, 40, 60 and 80. An increased osmolarity and substitution of other Tweens for Tween 80 caused the same changes in lipid composition and besides inhibited growth. Nevertheless, replacing Tween 80 by other Tweens reduced the percentage of unsaturated fatty acids. These changes may be related to the variations in growth of cultures with various Tweens due to the changes in the physical state of the membrane lipids (Veerkamp 1977).

The commercial use of *Bifidobacterium* as a probiotic is limited, because these bacteria are sensitive to acidic, oxygen and other stresses. The limited biomass yield results in a

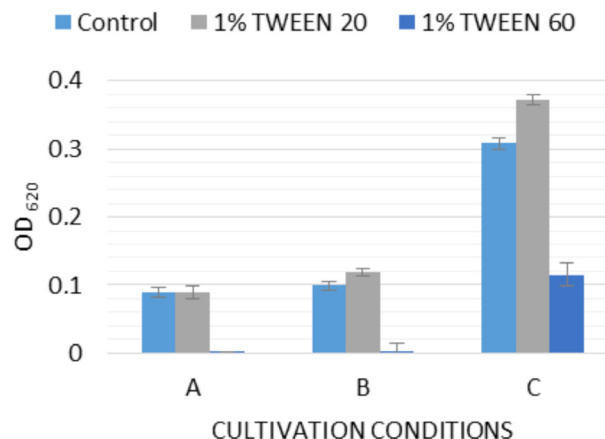


Fig. 1. Optical density OD₆₂₀ of the culture *B. animalis* subsp. *lactis* LMKK-939 cultivated in RBKB broth amended with TWEEN 20, TWEEN 60 or without amendments. A, aerobic at 23 °C; B, anaerobic at 23 °C; C, anaerobic at 37 °C. Period of cultivation 40 h.

strong increase of production costs. Microencapsulation and addition of prebiotics do not overcome these limitations (Turroni et al. 2014), hence, the further study on optimization of bifidobacteria cultivation conditions is needed.

Acknowledgements

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References

- Collado M.C., Hernández M., Sanz Y. 2005. Production of bacteriocin-like inhibitory compounds by human fecal *Bifidobacterium* strains. *J. Food. Prot.* 68: 1034–1040.
- O’Callaghan A., van Sinderen D. 2016. Bifidobacteria and their role as members of the human gut microbiota. *Front. Microbiol.* 7: 925.
- Turroni F., Duranti S., Bottacini F., Guglielmetti S., Van Sinderen D., Ventura M. 2014. *Bifidobacterium bifidum* as an example of a specialized human gut commensal. *Front. Microbiol.* 5: 437.
- Veerkamp J.H. 1977. Effects of growth conditions on the lipid composition of *Bifidobacterium bifidum* subsp. *pennsylvanicum*. *Antonie Van Leeuwenhoek* 43:101–110.

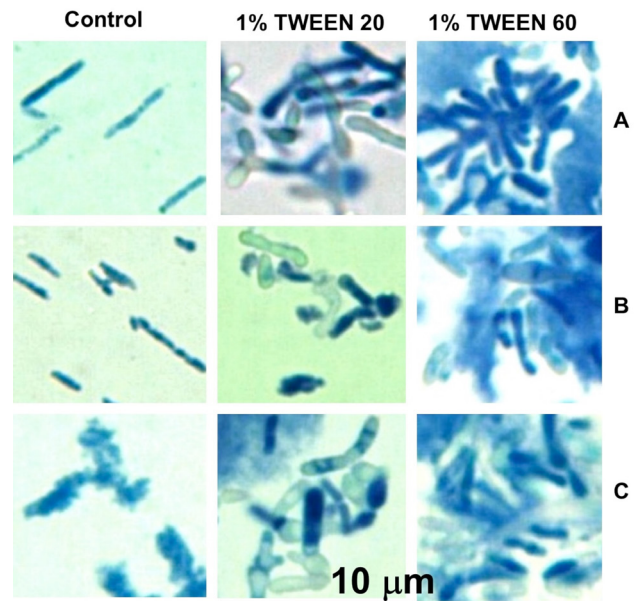


Fig. 2. Micrographs of the culture *B. animalis* subsp. *lactis* LMKK-939 cultivated in RBKB broth amended with Tween 20, Tween 60 or without amendments. A, aerobic at 23 °C; B, anaerobic at 23 °C; C, anaerobic at 37 °C. Period of cultivation 40 h. Methylene blue (1%) was added to the preparation.

Optimization of growth conditions for the yeast-bacterial co-culture and its response to the presence of antimicrobials

Alina Rimkus, Laura Žorža, Olga Muter*

University of Latvia, Institute of Microbiology and Biotechnology

*Corresponding author, E-mail: olga.mutere@lu.lv

Key words: benzalkonium chloride, RBKB medium, toxicity.

Microorganisms rarely exist naturally as individuals. Multicellular communities are expected to be better protected against the harmful environment (Palkova 2004). Recently the increasing number of studies have specifically investigated biofilms and quorum sensing between different species. These knowledges are of a great importance for application in various types of biotechnologies (Jarrell et al. 2000; Kaczorek et al. 2014; Tiwari, Gaur 2014; Kang et al. 2015; Kudare et al. 2015; Islam et al. 2018). Single microbial species could lead to only partial mineralization of the contaminant, whereas the use of consortium is characterized by the cumulative action of enzymes and, therefore, more complete biodegradation..

The aim of our study was to develop the methodological tool for investigation of yeast-bacteria interrelations in the context of biodegradation processes. Benzalkonium chloride (BAC) was used as a model contaminant.

Optimization of growth conditions for the yeast-bacterial co-culture was performed by combining nutrients, which are considered to be optimal for yeast and bacteria grown separately. Particularly, Nutrient Broth (NB) (Scharlau, Spain) and YPD Broth (LAB M, UK) were mixed in the ratio 1:1, thus, obtaining the following composition (g L⁻¹): tryptone 10.0; peptone 2.5; meat extract 0.5; yeast extract 6.0; glucose 10.0; NaCl 2.5. This broth was used in our experiment without dilution (RBKB), as well as two- and ten-times diluted with sterile water (RBKB/2, RBKB/10). Cultivation was performed in the multimode microplate reader Tecan Infinite® F50 with culture volume of 300 µL per well, at 23 °C, with agitation once in 30 min.

The modified medium RBKB was shown to be appropriate for the growth of all tested cultures, i.e., three yeast cultures (*Saccharomyces cerevisiae* 14, *Yarrowia lipolytica* 528, *Rhodotorula glutinis* 80) and two bacteria cultures (*Pseudomonas* sp., *Stenotrophomonas maltophilia*). The growth of *S. cerevisiae* 14 and *Pseudomonas* sp. in different media is shown in Fig. 1.

The culture of *S. cerevisiae* 14 at the stationary phase of growth in YPD, RBKB and RBKB/2 reached the OD₆₂₀ of 1.1; 0.74 and 0.60, respectively. The lower OD₆₂₀ in RBKB in comparison with YPD can be explained by the limited

concentration of carbohydrates. Thus, the concentration of glucose in YPD was 20 g L⁻¹, while in RBKB and RBKB/2 10 and 5 g L⁻¹, respectively.

A response of the tested yeast-bacterial co-culture to the presence of 5 mg L⁻¹ BAC was studied in RBKB/2

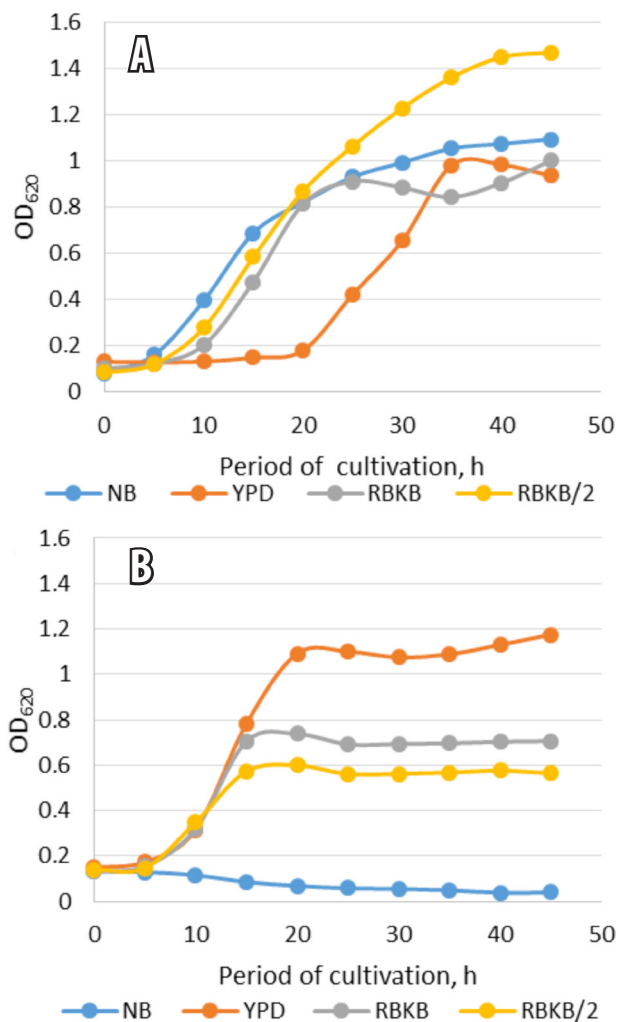


Fig. 1. Growth curves of *Pseudomonas* sp. (A) and *Saccharomyces cerevisiae* 14 (B) cultivated in different media under aerobic conditions at 23 °C.

and RBKB/10 media. A highly diluted medium was used for minimizing a nutritional and protective role of the medium. The inoculum was prepared by mixing of pure 48 h cultures with OD₆₂₀ 0.3.

As shown in Fig.2, a five-times dilution of medium resulted in a reduction of the number of bacterial (both species) colony forming units (CFU) from 4.5×10^{10} to 1.1×10^{10} CFU mL⁻¹. The reduced CFU count with dilution of medium was detected also for yeast *Y. lipolytica* 528 and *R. glutinis* 80. Conversely, the number of CFU of *S. cerevisiae* 14 was higher in RBKB/10 than in RBKB/2, i.e., 8.7×10^5 and 3.3×10^4 CFU mL⁻¹, respectively.

Addition of 5 mg L⁻¹ BAC to RBKB/2 stimulated the growth of *S. cerevisiae* 14 up to five times, while *S. cerevisiae* 14 was inhibited in the BAC-amended RBKB/10 medium. Other cultures were inhibited grown as a co-culture in the presence of 5 mg L⁻¹ BAC in both media.

Summarizing the results, it was concluded that bacteria are more sensitive to BAC, than yeasts. Among the tested yeast cultures, *S. cerevisiae* was the most promising culture for further degradation studies with BAC. The RBKB medium was appropriate for experiments with a yeast-bacterial co-culture.

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References

- Islam M.A., Ethiraj B., Cheng C.K., Yousuf A., Thiruvankadam S., Prasad R., Khan M.M.R. 2018. Enhanced current generation using mutualistic interaction of yeast-bacterial coculture in dual chamber microbial fuel cell. *Ind. Eng. Chem. Res.* 57: 813–821.
- Jarrell J., Cal T., Bennett J.W. 2000. The Kombucha consortia of yeasts and bacteria. *Mycologist* 14: 166–170.
- Kaczorek E., Bielicka-Daszkiwicz K., Héberg K. 2014. Best conditions for biodegradation of diesel oil by chemometric

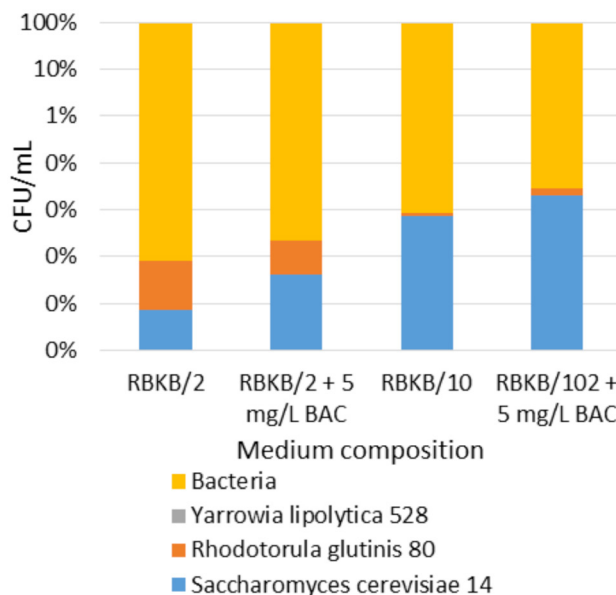


Fig. 2. Relative abundance of yeast and bacterial CFU in the co-culture grown in RBKB/2 and RBKB/10 media amended with 5 mg L⁻¹ benzalkonium chloride. Cultivation was performed in microplates at 23 °C for 48 h.

tools. *Braz. J. Microbiol.* 45: 117–126.

- Kang Z., Qiao K., Edgar S., Stephanopoulos G. 2015. Distributing a metabolic pathway among a microbial consortium enhances production of natural products. *Nature Biotechnol.* 33: 377–383.
- Kurade M.B., Waghmode T.R., Jadhav M.U., Jeon B.-H., Govindwar S.P. 2015. Bacterial–yeast consortium as an effective biocatalyst for biodegradation of sulphonated azo dye Reactive Red 198. *RSC Adv.* 5: 23046–23056.
- Palkova Z. 2004. Multicellular microorganisms: laboratory versus nature. *EMBO Rep.* 5: 470–476.
- Tiwari S., Gaur R. 2014. Decolorization of distillery spentwash (Melanoidin) by immobilized consortium (bacterium and yeast) cell: entrapped into sodium alginate bead. *J. Environ. Sci. Technol.* 7: 137–153.

Macrophytes and phytoplankton as indicators of environmental quality of Lake Augstrozes Lielezers, Latvia

Laura Grīnberga^{1*}, Ivars Druvietis²

¹The Latvian Museum of Natural History, Krišjāņa Barona 4, Rīga LV-1050, Latvia

²Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV-1004, Latvia

*Corresponding author, E-mail: laura.grinberga@ldm.gov.lv

Key words: Lake Augstrozes Lielezers, macrophytes, phytoplankton.

Lake Augstrozes Lielezers is situated in Limbaži municipality, Umurga rural territory. A field survey by mapping of freshwater habitats, recording aquatic macrophyte species and taking water samples in Augstroze Nature Reserve was carried out during the vegetation season of 2017. The study was performed within the framework of the LIFE Peat Restore (No. LIFE15 CCM/DE/000138) project. The phytoplankton was sampled in July 2017 at three sites. The phytoplankton analyses were performed by using Utermohl method (Willen 1976).

Lake Augstrozes Lielezers (400 ha) is the largest among the lakes situated in Augstroze Nature Reserve. This is a shallow water lake characterized by brown water and low conductivity, mean depth of the lake is 2.3 m, maximum depth 4.2 m. The shores of the lake are marshy. According to the habitat classification defined in the Habitats Directive, Lake Augstrozes Lielezers corresponds with the habitat type 3130 *Oligotrophic to mesotrophic standing waters with vegetation of the Littorellatea uniflorae and/or Isoetoneanojuncetea, subtype – semidystrophic lakes*.

Oligotrophic to mesotrophic lakes (habitat type 3130) are rare in Latvia. The number of lakes representing this habitat type has decreased dramatically during the last century, and the quality of this habitat has declined in the majority of lakes.

The lake is unique with high proportion of sandy substrate in the littoral zone that provides suitable conditions for several rare, protected aquatic macrophyte species. The vegetation of helophytes are mostly sparse, dominated by *Phragmites australis*, *Scirpus lacustris*, *Eleocharis palustris* and *Equisetum fluviatile*. The belt of floating-leaved macrophytes is fragmentary, it is well developed mostly in the western part of the lake where it is bordering with the Madiešēnu Bog. The most common floating-leaved species are *Nuphar lutea*, *Nuphar pumila*, *Polygonum amphibium*, rarely *Nymphaea candida* was found. The stands of isoetids are typical in the south-eastern and northern parts of the lake, where vital stands of *Lobelia dortmanna*, *Myriophyllum alterniflorum* and *Littorella uniflora* were recorded.

Five rare, protected species of macrophytes – *L.*

dortmanna, *Isoetes lacustris*, *L. uniflora*, *M. alterniflorum* and *Nuphar pumila* – were found. Among them, *M. alterniflorum*, *L. dortmanna* and *L. uniflora* were the most frequent. *N. pumila* formed sparse stands around the perimeter of the lake. *I. lacustris* occurred relatively rarely, found in the northern part of the lake. The composition of macrophyte species and their abundance indicate high ecological quality of the lake.

At all three sampling sites, number of cyanobacteria was very low (biomass 0.195 – 0.353 mg L⁻¹) that indicated high ecological quality of the lake (Fig. 1).

The main algal groups were Cyanophyta (Cyanobacteria), Chrysophyta, Dinophyta, Cryptophyta, Bacillariophyta, Chlorophyta. The dominant algae species in the phytoplankton community were *Anabaena lemmermannii*, *Anabaena* sp., *Anabaena solitaria*, *Anabaena spiroides*, *Coelosphaerium dubium*, *Coelosphaerium kuetzingianum*, *Gomphosphaeria lacustris*, *Microcystis incerta*, *Microcystis pulvereae*, *Oscillatoria* sp., *Dinobryon divergens*, *Dinobryon sertularia*, *Cryptomonas* sp., *Ceratium hirudinella*, *Amphiprora paludosa*, *Asterionella formosa*, *Aulacoseira italica*, *Cocconeis placentula*, *Cyclotella* sp., *Cymbella* sp., *Diatoma elongatum*, *Diatoma vulgaris*, *Fragilaria crotonensis*, *Melosira varians*, *Navicula gracilis*, *Navicula* sp., *Nitzschia holsatica*, *Nitzschia acicularis*,

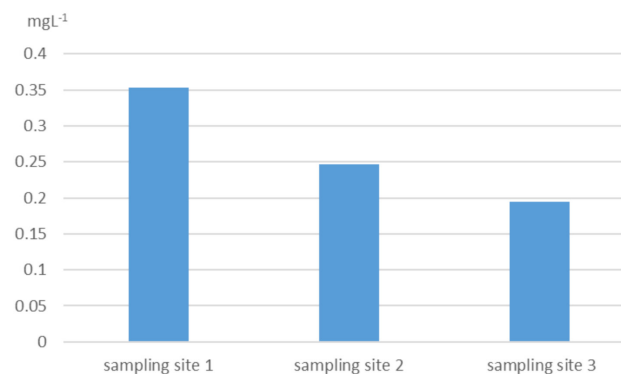


Fig. 1. Total phytoplankton biomass (mg L⁻¹) in Lake Augstrozes Lielezers in July 2017.



Fig. 2. Secchi depth and concentration of total phosphorus in the period from 1990 to 2017.

Nitzschia sp., *Pinnularia* sp., *Surirella elliptica*, *Synedra acus*, *Synedra ulna*, *Tabellaria frenestrata*, *Ankistrodesmus* sp., *Ankistrodesmus fusiformis*, *Botryococcus braunii*, *Coelastrum microporum*, *Oocystis lacustris*, *Pediastrum boryanum*, *Pediastrum duplex*, *Desmodesmus quadricauda*, *Staurastrum* sp., *Staurodesmus* sp., *Tetraedron caudatum*, *Tetraedron* sp.

At sampling sites, the phytoplankton was homogenous, with low phytoplankton biomass. Algae species that formed phytoplankton communities are characteristic to slightly eutrophic and eutrophic lakes. Balls of rare green macroscopic algae *Aegagropila linnaei* (syn. *Cladophora aegagropila*) were common in the lake.

Both the historical data until 2016 (available at www.ezeri.lv) and the results of water analyses in 2017 show high/good ecological quality of the lake: the concentrations

of total phosphorous and total nitrogen are low. The concentration of total phosphorous (0.017 mg L^{-1}) correspond to high water quality, whereas the nitrogen concentration (0.71 mg L^{-1}) indicates good water quality (Fig. 2).

Historical data of Secchi depth measurements shows lower water transparency than measured in 2017, varying from 0.8 to 1.4 m. During the last decades, decreasing concentration of total phosphorus is related to the increasing water transparency (Fig. 2). This suggests that the water quality is good as proved also by the macrophyte and phytoplankton data.

References

Willen E. 1976. A simplified method of phytoplankton counting. *Br. Phycol. J.* 11: 265–278.

Application of *Lentinula edodes* in bioconversion of rapeseed straw

L. Okmane^{1,2}, G. Makarenkova^{1,2}, V. Balode^{1,2}, Dz. Zaļā^{1,2}, E. Ažēna^{1,2}, L. Rozenfelde², A. Rapoport², I. Muižnieks¹

¹Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

²Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas 1, Riga LV–1004, Latvia

*Corresponding author, E-mail: laura.okmane@lu.lv

Key words: laccase, *Lentinula edodes*, rapeseed straw.

Rapeseed straw is a common agricultural lignocellulosic residue, which can be used as a cellulose and hemicellulose source in production of bioethanol and furfural (Carrier et al. 2012). In order to make furfural and bioethanol production a wasteless process, further degradation of lignocellulosic material must follow. After a complete utilization of rapeseed-straw hemicellulose and cellulose, a recalcitrant compound, lignin, remains in a residue.

Lignin is a complex biopolymer that can be biologically degraded by white-rot fungi (WRF), which are able to completely degrade all components of lignocellulose. WRF depolymerize lignin with an extracellular enzyme complex composed by phenol oxidases (laccases) and peroxidases (manganese peroxidases and lignin peroxidases).

One of white-rot fungus applicable for biodegradation of lignocellulosic waste is shiitake mushroom *Lentinula edodes* (Berk.) Pegler. It is an edible basidiomycete capable of degrading lignin selectively. Application of *L. edodes* in utilization of rapeseed-straw lignocellulose is an approach to obtain ligninolytic enzyme complex while simultaneously decreasing quantity of residual lignin.

L. edodes DSM3565 mycelium discs ($d = 0.8$ cm) were incubated on malt extract agar plates and shaken-flask (140 rpm) submerged cultures. By changing incubation conditions (temperature 22 ± 1 , 25 ± 1 , 30 ± 1 °C, and offset pH: 5.0 ± 0.1 , 6.0 ± 0.1 , 7.0 ± 0.1), we qualitatively and quantitatively assessed optimal setting for attaining highest mycelium biomass and enzyme yields. Significance of variation in data was statistically analyzed (ANOVA) in R studio.

Mycelium dry weight in submerged cultures was determined after 28-day incubation period and expressed as mg of dried (43 °C, 48 h) mycelium per 100 mL of medium (1/2 malt extract broth). Qualitative mycelium biomass evaluation was performed by measuring mycelium diametric growth (in mm) on agarized medium.

Ligninolytic enzyme complex yield was periodically (every 7 days) evaluated by spectrophotometrically ($\lambda = 420$ nm) determining extracellular laccase activity (U

mL⁻¹) in submerged culture medium. Enzyme activity was expressed as units (U) per mL or substrate (ABTS, 21 mM) conversion rate $\mu\text{mol min}^{-1}$ per mL of medium filtrate. Screening of laccase was carried out by spotting 21 mM ABTS on agarized plates after removal of mycelium from the surface. The effect of pretreated rapeseed straw on ligninolytic enzyme complex yield was evaluated in submerged culture medium with rapeseed-straw additive in various concentrations (C% = 0.5, 1.0, 1.5, 2.0).

Several mushroom strains (1899, 353, 714, 3343, 3565) were evaluated. The strain with highest laccase activity and mycelium yield was found to be 3565. Optimal incubation conditions were determined for this strain.

We noticed a supposed negative correlation between optimal conditions for mycelium biomass increase and that regarding laccase activity. Highest mycelium biomass yield was obtained after incubation in 25 to 30 °C with medium pH = 7 ($p < 0.05$). Highest laccase activity was obtained at lower pH (5, $p < 0.01$) and lower temperatures

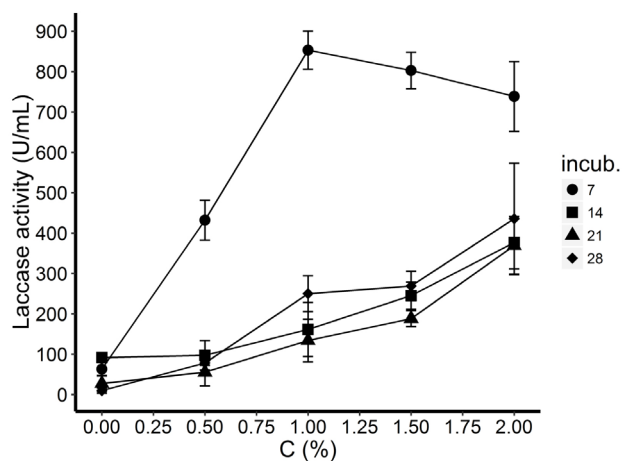


Fig. 1. *L. edodes* 3565 extracellular laccase activity (U mL⁻¹) in submerged culture medium during 28 day incubation period with rapeseed straw additive in various concentrations (C%). Numbers in the legend indicate days of incubation.

(22 to 25 °C, $p < 0.05$). Rapeseed straw additive distinctively increased laccase activity in submerged cultures. A positive correlation between concentration of the additive in medium and laccase activity was found ($p < 0.01$).

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for simultaneous and waste-less production of bioethanol, furfural and other valuable compounds from local agricultural remains. (ESS2017/206/1.1.1.1/16/A/113)”.

References

Carrier M., Loppinet-Serani A., Absalon C., Aymonier C., Mench M. 2012. Degradation pathways of holocellulose, lignin and α -cellulose from *Pteris vittata* fronds in sub- and super critical conditions. *Biomass Bioenerg.* 43: 65–71.

Methicillin resistant *Staphylococcus* spp. diagnosis in clinical isolates using MALDI-TOF

Laura Alksne*, Svetlana Makarova, Jūlija Trofimova, Jeļena Avsejenko, Madara Streikiša, Olga Valciņa

Institute of Food Safety, Animal Health and Environment “BIOR”, Leļupes 3, Riga LV-1076, Latvia

*Corresponding author, E-mail: laura.alksne@bior.lv

Key words: MALDI-TOF, methicillin resistance, phenol-soluble modulin, *Staphylococcus aureus*, *Staphylococcus epidermidis*.

In hospitals worldwide methicillin resistant *Staphylococcus aureus* (MRSA) infections are related with an increased mortality and morbidity compared to infections caused by methicillin-sensitive strains (MSSA) (Cosgrove et al. 2003). In Europe MRSA strain occurrence varies from zero to 57.2%, in Latvia from 5 to 10% (ECDC 2016). Early identification of an outbreak is important for rapid and successful control. At present, molecular typing methods like pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and spa typing are used for this purpose. These methods are relatively time consuming (Mellmann et al. 2006; Struelens et al. 2009). Lately MALDI-TOF is widely used for fast bacteria identification and studies showed that is possible in peak mass spectra identify MRSA strains by phenol-soluble modulin-mec (PSM-mec) peptide which is presented in MRSA and predicts *mecA* gene presence (Josten et al. 2014; Rhoads et al. 2016). The aim of this study was to verify MALDI-TOF ability to differentiate methicillin resistant strains from sensitive and to provide a fast diagnosis within the same day of routine bacteria identification with MALDI-TOF.

The present study used 62 clinical and three ATCC reference strains (Table 1). Susceptibility to methicillin was tested using the disk diffusion method in accordance

with recommendations by the Clinical and Laboratory Standards Institute (CLSI, USA). All methicillin resistant strains were confirmed with *mecA* gene presence by PCR.

Samples from 12 *S. aureus* strains were made with protein extraction method, where colonies were harvested by five full 1 µL loops and prepared according to Østergaard et al. (2015). Each sample was made twice. For comparison of methods and for testing PSM-mec peak presence, a direct method without and with adding 70% formic acid was performed twice. In PSM-mec peak reproducibility test same strains were smeared in triplicates, total of nine spectra obtained. For confirmation of PSM-mec peak, 65 clinical strains were cultured on TSA at 37 °C for 24 h and mass spectra collected using the direct method. Experiments were done twice. After drying all samples were overlaid with 1 µL of α-cyano-4-hydroxycinnamic acid (Bruker Daltonics, Germany) matrix solution (50% acetonitrile, 2.5% trifluoroacetic acid).

At first analyzing 12 *S. aureus* strains we observed changes in peak intensities and some peak absence between direct and extraction method. Currently in this study using all three preparation methods was not possible to obtain a biomarker peak that could be presented exclusively in all MRSA or MSSA separating samples into two groups

Table 1. Summary of *Staphylococcus* spp. strains from different samples with (MRSA, MRSE) and without methicillin resistance (MSSA, MSSE)

Sample	MRSA	MSSA	MRSE	MSSE
Blood	1	–	2	–
Pus from wounds	5	16	1	2
Nose/tonsils	8	1	–	–
Iner-placental space	–	–	1	2
Trachea rinse/sputter	8	1	–	–
Other clinical materials	–	3	2	1
Hospital air	3	–	–	–
Wash off from objects in hospital	5	–	–	–
ATCC reference strains	1	2	–	–
Total	31	23	6	5

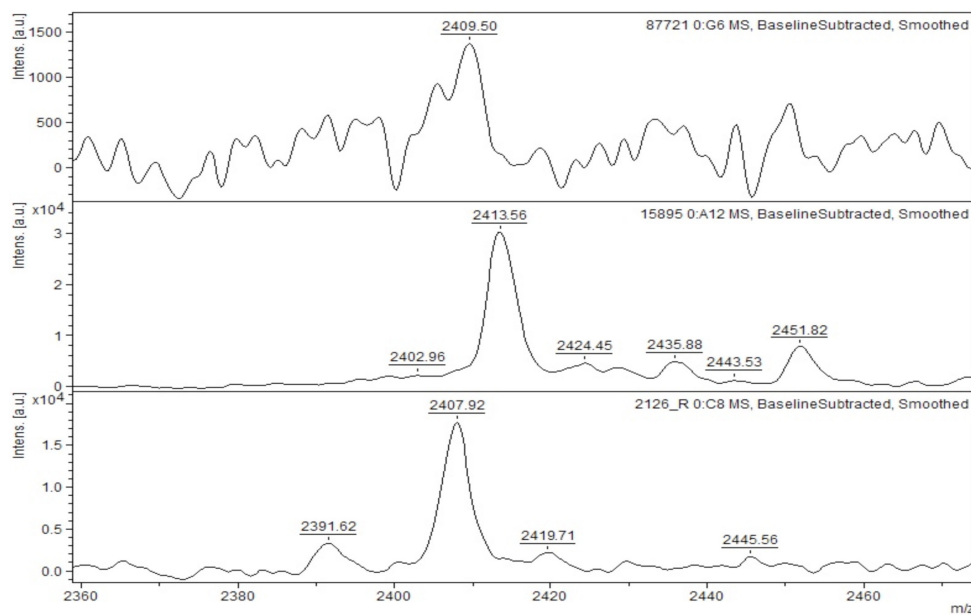


Fig. 1. List view of *S. aureus* methicillin resistant strains (B, C) and sensitive strain (A) spectra from direct method. Dashed line represents PSM-mec peptide peak position at 2414 ± 2 Da, m/z.

because they appeared or were absent between isolates. However, a peak of 2414 ± 2 Da called PSM-mec peptide was visible only in methicillin resistant strains but not in all samples as earlier studies showed and are dependent on SCCmec type that usually has the peptide (Josten et al. 2014; Rhoads et al. 2016; Fig. 1). It is important to point out that in extraction method the PSM-mec peptide is washed away because it did not appear as a peak at 2414 ± 2 Da, the same observed Josten et al. 2014. The reproducibility of PSM-mec peak presence by direct method was 86.66% in MRSA strains. In examination of 65 samples we used direct method for PSM-mec peptide presence and confirmation of methicillin resistance and determined in 18/31 MRSA strains (58%) and 0/23 in MSSA. In methicillin resistant *S. epidermidis* strains the 2414 ± 2 Da peak appeared in 1/6 samples and 0/5 in sensitive.

Based on this study and results the PSM-mec 2414 ± 2 Da peak is specific only in methicillin resistant strains carrying the *mecA* gene, but peaks absence does not exclude resistance presence in the strain, it will still need an additional inspection and confirmation. Implementing the peak recognition in a routine laboratory work, it would be possible to set the diagnosis faster and without extra time and cost usage. This study showed that it is necessary to examine different sample preparation methods: the direct and extraction method, during a biomarker peak detection or typing bacteria in future research.

Acknowledgements

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References

- Casgrove S.E., Sakoulas G., Perencevich E.N., Schwaber M.J., Karchmer A.W., Carmeli Y., 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteraemia: a meta-analysis. *Clin. Infect. Dis.* 36: 53–59.
- European Centre for Disease Prevention and Control. 2016. Summary of the latest data on antibiotic resistance in the European Union. Stockholm: ECDC.
- Josten M., Dischinger J., Szekat C., Reif M., Al-Sabti N., Sahl H. G., Parcina M., Bekerredjian-Ding I., Bierbaum G. 2014. Identification of agr-positive methicillin-resistant *Staphylococcus aureus* harbouring the class A mec complex by MALDI-TOF mass spectrometry. *Int. J. Med. Microbiol.* 304: 1018–1023.
- Mellmann A., Friedrich A.W., Rosenkotter N., Rothganger J., Karch H., Reintjes R., Harmsen D., 2006. Automated DNA sequence-based early warning system for the detection of methicillin-resistant *Staphylococcus aureus* outbreaks. *PLoS Med.* 3: e33.
- Østergaard C., Hansen S.G.K., Moller J.K., 2015. Rapid first-line discrimination of methicillin resistant *Staphylococcus aureus* strains using MALDI-TOF MS. *Int. J. Med. Microbiol.* 305: 838–847.
- Rhoads D.D., Wang H., Karichu J., Richter S.S., 2016. The presence of a single MALDI-TOF mass spectral peak predicts methicillin resistance in staphylococci. *Diagn. Microbiol. Infect. Dis.* 86: 257–261.
- Struelens M.J., Hawkey P.M., French G.L., Witte W., Tacconelli E., 2009. Laboratory tools and strategies for methicillin-resistant *Staphylococcus aureus* screening, surveillance and typing: state of the art and unmet needs. *Clin. Microbiol. Infect.* 15: 112–119.

Effects of crude polysaccharide from shiitake mushroom on CuSO₄ induced toxicity and bacterial infection in *Drosophila melanogaster*

Elina Azena^{1,2*}, Liga Apermane¹, Inese Grigane¹, Galina Makarenkova^{1,2}

¹Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

²Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

*Corresponding author, E-mail: elina.azena@lu.lv

Key words: antioxidative response, *Drosophila melanogaster*, innate immunity, *Lentinula edodes*, polysaccharides.

Shiitake mushroom *Lentinula edodes* (Berk.) Pegler is one of the most widely cultivated edible mushrooms in the world. It is known as valuable source of nutrients and used in dietary supplement compositions. Both fruit bodies and liquid cultures of *L. edodes* mycelium are reported as source of biologically active compounds. Diverse methods for polysaccharide extraction, purification and characterization from *L. edodes* are being developed and applied. The both the immunomodulating and antioxidative effects of polysaccharides and its complexes has been extensively studied by *in vitro* and *in vivo* models (Xu et al. 2014).

In this study we evaluated the effects of shiitake mushroom crude polysaccharide fractions on induced oxidative stress and bacterial infection in fruit fly *Drosophila melanogaster*. *L. edodes* (DSM 3565) polysaccharides containing hot water extract (HWE) were obtained from fresh fruiting bodies; crude polysaccharide fraction (PF) was prepared by repeated ethanol precipitation. Both HWE and PF were standardized by dry mass (105 °C) of ethanol precipitate. The final concentration of ethanol precipitate in the medium was 0.0125, 0.025 and 0.050%. A normal (wild-type) adult flies were anaesthetised with diethyl ether and

transferred to cornmeal medium with or without HWE and PF. At the end of two-day mating and egg laying period flies were discarded from vials. Offspring generation were allowed to feed, develop and undergo a metamorphosis.

L3 larvae were selected for CuSO₄ induced toxicity assay. Copper toxic effects exert partly through formation of reactive oxygen species (ROS) and triggers *D. melanogaster* antioxidant defence system (Klimaczewski et al. 2018). Twenty-four-hour exposure to 10 mM CuSO₄ significantly decreased L3 larva feeding behaviour, increased trypan blue staining score for digestive tract and delayed further development accompanied by minor decrease of imago wing size. HWE and PF pre-treatment diminished CuSO₄ toxic effects in *D. melanogaster*. Pre-fed L3 larvae displayed more pronounced mouth hook contractions, intestine cell viability and survival rate; while imago wing size measurements were equal to CuSO₄ exposed control group (Table 1).

In systemic bacterial infection assay were used adult flies obtained from HWE and PF fed larvae. *Staphylococcus epidermidis* infection manifested by lethality up to 70 to 90% in five-day period; while wound control group achieved

Table 1. Effects of crude polysaccharide from shiitake mushroom on CuSO₄ induced toxicity in *D. melanogaster*. *, $P < 0.05$; **, $P < 0.001$ values determined between CuSO₄ exposed control and HWE, PF fed groups by one-way ANOVA

Groups	Larvae mouth hook contractions (mean ± SD)	Trypan blue staining (relative units)	Larva-imago viability (%)	Imago wing size measurements (mean ± SD)	
				Male	Female
Control	48.93 ± 4.03	0.00	96.70	1.42 ± 0.05	1.61 ± 0.05
10 mM CuSO ₄					
Control	36.00 ± 5.17	1.00	70.00	1.40 ± 0.07	1.53 ± 0.10
HWE 0.0125%	38.93 ± 5.12	1.00	80.00	1.34 ± 0.09	1.52 ± 0.09
HWE 0.025%	40.43 ± 3.84*	1.00	80.00	1.44 ± 0.05	1.46 ± 0.10
HWE 0.05%	36.36 ± 4.97	0.50	86.65	1.41 ± 0.05	1.52 ± 0.11
PF 0.0125%	46.00 ± 2.32**	0.50	80.00	1.42 ± 0.05	1.52 ± 0.08
PF 0.025%	39.50 ± 3.57*	0.50	80.00	1.39 ± 0.08	1.55 ± 0.05
PF 0.05%	36.36 ± 3.34	0.50	86.70	1.38 ± 0.07	1.55 ± 0.05

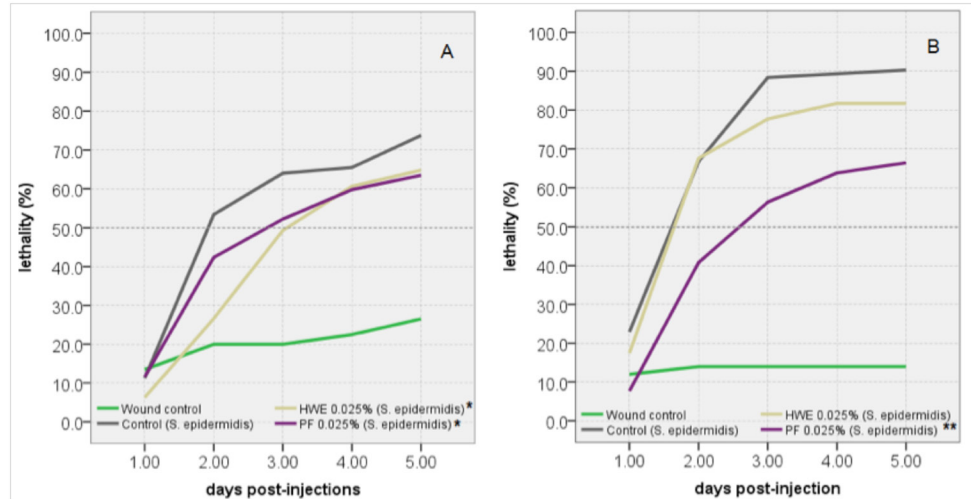


Fig. 1. Effects of crude polysaccharide from shiitake mushroom on bacterial infection in *D. melanogaster* males (A) and females (B). *, $P < 0.05$; **, $P < 0.001$ values determined between *S. epidermidis* infected control and HWE, PF fed groups by two-way ANOVA.

15 to 25% lethality thresholds. Initial *S. epidermidis* load reached by pricking was approximately 2.6×10^3 CFU per fly; viable bacteria persisted in host at least for two days post-injection. Infection caused lethality were significantly reduced in HWE and PF pre-fed groups for both male and female flies (Fig. 1). *S. epidermidis* CFU counts significantly reduced only for male flies at second day post-injection.

The both fungal and gram-positive bacteria PF component interacts with recognition proteins and triggers Toll pathway. Interaction leads to development of antimicrobial resistance through haemocyte activation and antimicrobial peptide synthesis (Buchon et al. 2014).

Results extended our previous findings and indicated innate immunity and antioxidative response involvement regarding the effects of *L. edodes* HWE upon *D. melanogaster* life span, age-related locomotor activity and heat shock resistance (Matjushkova et al. 2010; Matjushkova et al 2014).

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References

- Buchon N., Silverman N., Cherry S. 2014. Immunity in *Drosophila melanogaster* from microbial recognition to whole-organism physiology. *Nature Rev. Immunol.* 14: 796–810.
- Klimaczewski C., Ecker A., Piccoli B., Aschner M., Barbosa N., Rocha J. 2018. *Peumus boldus* attenuates copper-induced toxicity in *Drosophila melanogaster*. *Biomed. Pharmacother.* 97: 1–8.
- Matjushkova N., Svilpe E., Muiznieks I. 2010. Influence of shiitake mushroom extract on the life span and heat shock resistance of *Drosophila melanogaster*. Proceedings of the 1st International Conference on Genetics of Longevity and Aging; 2010 April 12 – 15; Syktyvkar, Russia; pp. 55–60.
- Matjushkova N., Azena E., Serstnova K., Muiznieks I. 2014. The influence of the hot water extract from *Lentinula edodes* mushroom on the food intake, life span and the age-related locomotor activity of *Drosophila melanogaster*. *Int. J. Med. Mushrooms* 16: 605–6015.
- Xu X., Yan H., Tang J., Chen J., Zhang X. 2014. Polysaccharides in *Lentinus edodes*: isolation, structure, immunomodulating activity and future prospective. *Crit. Rev. Food Sci. Nutr.* 54: 474–87.

Seasonal and temporal changes of fish communities in the coastal waters of Salacgriva, eastern part of the Gulf of Riga

Martins Plikšs*, Maris Plikšhs

Institute of Food Safety, Animal Health and Environment BIOR, Daugavgrīvas 8, Rīga LV-1048, Latvia

*Corresponding author, E-mail: martinsplikss33@gmail.com

Key words: coastal waters, fish communities, seasonal changes.

Coastal area has an important role in the Baltic Sea ecosystem biodiversity and as a biological resource for commercial fishery. The coastal fish communities always has attention from their qualitative and quantitative structure and fisheries perspective. Understanding of coastal fish population dynamics is also important in relation to climate and anthropogenic influences that are linked to fish resource assessment and resource exploitation. Coastal areas in temperate regions are highly dynamic systems. Fish community can change depending on seasonal climate and anthropogenic factors and these changes may have common causes across geographically distant areas (Olsson et al. 2012; Mustamäki et al. 2015). Fish species composition, structure and trophic relations are essential for the development of fish indicators used for evaluation of ecosystem (Bergström et al. 2016; Östman et al. 2017). The main goal of the study was to assess fish biodiversity in area, its seasonal variability due to climatic and possible anthropogenic impacts.

The study on coastal fish communities were performed in Salacgriva area. Here, coastal waters of the Gulf of Riga is a part of the North Vidzeme Biosphere Reserve and have significant nature conservation requirements, fishing, shipping and public activities. Seashore is sabulous but the seabed is stony, which is inherent for North part of Vidzeme coast. Salaca river, the 5th biggest river in Latvia, in Salacgriva flows into the Gulf of Riga. Study period covered coastal fish monitoring from 2014 to 2017. Fishing was performed annually from May to October at least once a month according to HELCOM (2015) guidelines. Gill net series with mesh size 17, 22, 25, 30, 33, 38, 50 and 60 mm were used in two stations at 3 and 5 m depth about 500 m north from Salaca outlet. Length and weight of each fish was measured. In each station water temperature, salinity as well as a water transparency with Secchi disk were assessed.

As the fish monitoring in 2014 were performed by old coastal net set, the catch per unit of effort (CPUE) of this data set were recalculated to the Nordic net standard. Recalculation were based on linear regression analyses from intercalibration experimental fishing performed during 2015 and 2016 and based on formula:

$$N\text{-}CPUE_{ij} = a_{ij} + b_{ij} \times CN\text{-}CPUE_{ij}$$

where $N\text{-}CPUE$ is CPUE of NORDIC net, a is intercept, b is slope, $CN\text{-}CPUE$ is CPUE of Coastal net set, i is species, j is 2.5 cm size group.

The occurrence of fish species was estimated as a number of stations where species was recorded vs. a total number of stations. Species occurrence was analyzed separately in the following temperature ranges: 7 to 12 °C (usually May to early June and September to October); 13 to 16 °C (late June, early September) and 17 to 22 °C (July to August).

In total 21 fish species have been recorded in Salacgriva fishing site from 61 registered in the Gulf of Riga. The largest proportion of caught fishes were of freshwater species (11 from 24 possible). Apparently, this is determined by direct influence of Salaca. From freshwater species most represented were perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), ruffe (*Gymnocephalus cernua*) and bleak (*Alburnus alburnus*) (Fig. 1). The occurrence of freshwater species increased with increase of water temperature and higher number of species were observed during July – August. Dominance of freshwater species especially Cyprinidae and ruffe may be due to high level of eutrophication of coastal areas (Yurkovskis 2004). Registered Secchi depth during monitoring in summer period did not surpass 2.5 m. Area also is characterized with very low salinity (3.6 to 5.4 psu) due to direct influence of Salaca and majority of river fishes can be found in coastal waters. This confirms the finding of freshwater bullhead (*Cottus gobio*) at first time in 2017 in Latvian coastal waters.

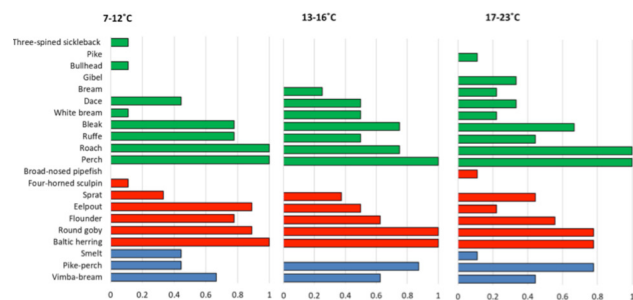


Fig. 1. Occurrence of fish species during 2014 – 2017 under different temperature regimes. Red columns, freshwater species; blue, sea species; green, diadromous species.

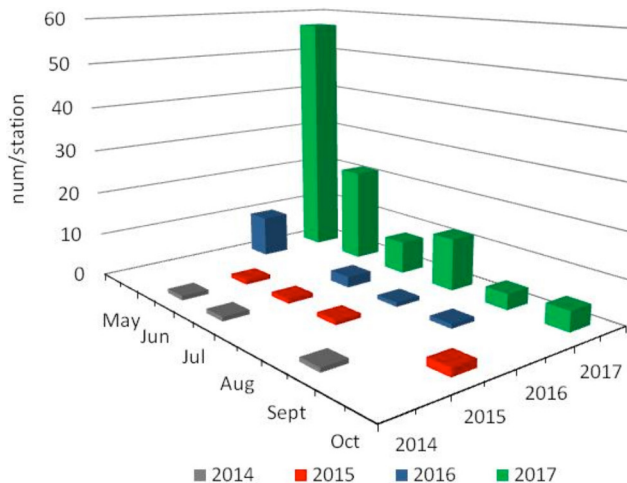


Fig. 2. Round goby catches per one Nordic gill net set by months.

Marine (7 from 24 species) and diadromous (3 from 6 species) fish proportions were lower in the Gulf of Riga. Marine species share in catch was higher when water was colder. In springtime eelpout (*Zoarces viviparus*) and Baltic herring (*Clupea harengus*) appeared in coastal area. Relatively larger cold-water species appeared in summer when due to wind driven upwelling water temperature in near shore areas can drop to 10 °C for a few days.

Commercially important diadromous and marine species such as salmon (*Salmo salar*), sea trout (*Salmo trutta*), whitefish (*Coregonus* sp.), turbot (*Scophthalmus maximus*) were not caught by survey gear in the study area.

Higher eelpout abundance was observed usually in spring (May to early June) when herring are spawning in coastal waters and water temperature do not exceed 12 °C. It is considered that eelpout feeds on herring spawns and thus enter coastal area (Urtans 1990). Direct and statistically significant correlation between herring and eelpout abundances were not found. Hence a presence of eelpout in coastal areas may be also due to lower temperature because eelpout abundance increases also in summer during upwelling conditions.

Significant changes in species composition observed due to invasion of round goby (*Neogobius melanostomus*). From a few specimens in 2014, their proportions have increased more than 10 times (Fig. 2). Round goby currently does not have natural predators in Gulf of Riga, it has sufficient nutrition resources, and still do not show negative density dependent processes. Largest number of round goby was observed in May and June; however, it decreased during July – August. Obviously, this is connected with spawning when fishes are more stationary, defending their spawn sites. At first in 2014 round goby was presented by several juveniles (below 12 cm). Further the size distribution of round goby extended (Fig. 3). The juvenile abundance also increased indicating that the reproduction conditions are favorable. Finally, after four years a full-size spectrum of round goby was observed in area that obviously is a result

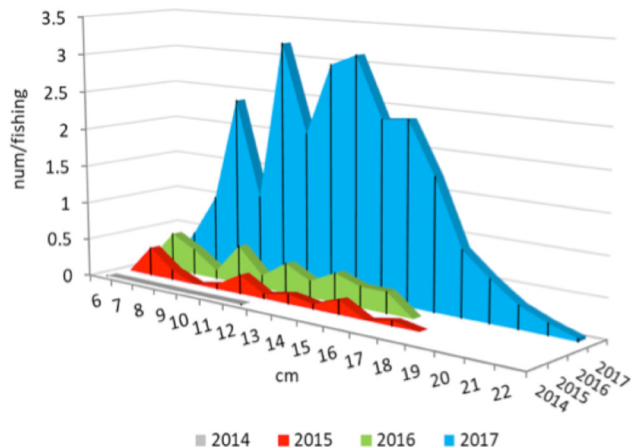


Fig. 3. Annual size distribution of round goby per one Nordic gillnet set.

of growth and reproduction. Commercial fishery statistics show sharp increase in round goby landings, from 150 kg in 2016 to 5.1 tons in 2017.

The following conclusions can be made: (i) dominance of freshwater species in coastal areas of Salacgriva is determined by low salinity and direct influence of Salaca freshwater basin; (ii) the main environmental factor determining the changes in faunistic complex is temperature regime leading to change in dominance between environmental guilds (cold-water, warm-water species) as well as ecological groups (freshwater, marine and diadromous); (iii) upwelling can change significantly the species composition for very short period; (iv) invasion history of round goby shows that under the favorable environmental conditions species in a few years can occupy new territories.

References

- Bergström L., Bergström U., Olsson, J., Carstensen J. 2016. Coastal fish indicators response to natural and anthropogenic drivers – variability at temporal and different spatial scales. *Estuar. Coastal Shelf Sci.* 183: 62–72.
- HELCOM. 2015. Guidelines for coastal fish monitoring sampling methods of HELCOM.
- Mustamäki N., Mattila J. 2015. Structural changes in three coastal fish assemblages in the northern Baltic Sea archipelago, *Estuar. Coastal Shelf Sci.* 164: 408–417.
- Olsson J., Bergström L., Gårdmark A. 2012. Abiotic drivers of coastal fish community change during four decades in the Baltic Sea. *ICES J. Mar. Sci.* 69: 961–970.
- Östman Ö., Lingman A., Bergström L., Olsson J. 2017. Temporal development and spatial scale of coastal fish indicators in reference ecosystems: hydroclimate and anthropogenic drivers. *J. Appl. Ecol.* 54: 557–566.
- Urtans E. 1990. Feeding characteristics of smelt (*Osmerus eperlanus eperlanus* L.) and eelpout (*Zoarces viviparus* L.) in the Gulf of Riga. *Fischerei-Forschung* 28: 34–38. /in Russian/
- Yurkovskis A. 2004. Long-term land-based and internal forcing of the nutrient state of the Gulf of Riga (Baltic Sea). *J. Mar. Syst.* 50: 181–197.

Components of microbial fuel cells

Sintija Valucka*, Matiss Paiders, Māris Senkovs

Faculty of Biology, University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

*Corresponding author, E-mail: sintija.valucka@gmail.com

Key words: bioelectricity, electrodes, food waste, microbial fuel cells.

Recently great attention has been paid to finding new renewable energy sources. One way how to get clean and renewable energy is by using microbial fuel cells (MFC). The most important components of MFC are: anode, cathode and substrate. In MFC microorganisms utilize substrate and during this process electrons are released. These electrons flow through anode to cathode generating electricity. Anode and cathode can be placed separately into two compartments, or they can be placed in a single chamber (Rahimnejad et al. 2015).

In the first MFC studies platinum and other expensive electrode were used, but in recent studies emphasis has been put on finding cheaper electrodes, for example, carbon brushes, rods and other carbon electrodes (Santoro et al. 2017).

Commonly used substrates for MFC are different kinds of wastewater, for example, domestic wastewater and food process wastewater (Parkash 2016).

The aim of this study was to construct a single chambered MFC using biodegradable household waste products as substrate.

Firstly, voltage drop for different electrodes (graphite rods, graphite plates and charcoal) was tested. Since charcoals had 67% of voltage drop compared to 0% for graphite rods and plates, they were not used in the subsequent experiments.

A single MFC theoretically can generate a voltage of 1.1 V (Parkash 2016), in this study maximum voltage of 0.23 V was obtained from MFC with graphite plates as electrodes and 0.54 V from MFC with graphite rods as electrodes.

To evaluate metabolic potential of microorganisms in household waste products, the Biolog plates method was used (Fig. 1). From 31 substrates, 17 were used by

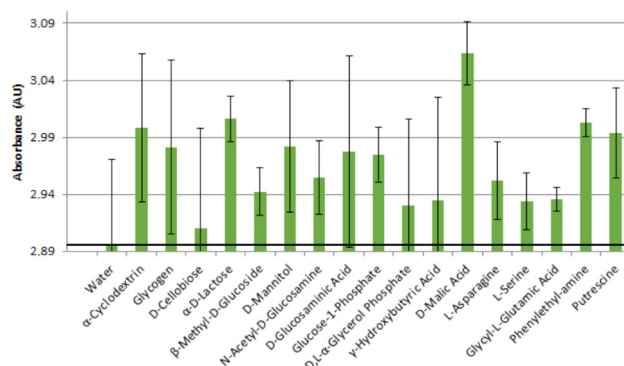


Fig. 1. Utilization of Biolog microplate substrates by microorganisms in household waste products.

microorganisms of household waste products, including different kinds of amines, carbohydrates, complex carbon sources, carboxylic acids, amino acids and phosphate carbons.

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References

- Parkash A. 2016. Microbial fuel cells: a source of bioenergy. *J. Microb. Biochem. Technol.* 8: 247–255.
- Rahimnejad M., Adhami A., Darvari S., Zirepour A., Oh S. 2015. Microbial fuel cell as new technology for bioelectricity generation: A review. *Alexandria Eng. J.* 54: 745–756.
- Santoro C., Arbizzani C., Erable B., Ieropoulos I. 2017. Microbial fuel cells: From fundamentals to applications. A review. *J. Power Sour.* 356: 225–244.