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ABSTRACTS
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Mammals on map – preliminary results for the development of European Atlas of mammals and regional Red List of threatened species

Valdis Pilāts*

LIFE project LIFE FOR SPECIES, Nature Conservation Agency, Baznīcas 7, Sigulda LV–2150, Latvia
*Corresponding author, E-mail: valdis.pilats@daba.gov.lv

Key words: atlas of mammals, distribution, Latvia, mapping, Red List.

Large-scale project, European Mammals on Maps (EMMA2), started in 2016, is nearing completion. The main objectives of this project are to determine the distribution of all mammal species occurring in geographic Europe during the period 1999 to 2023 and to publish the Second Atlas of European Mammals. This is multi-national project with each country contributing data from national datasets (EMMA2 Steering Group 2020). Similarly, as in the First Atlas published in 1999 (Mitchell-Jones et al. 1999), next Atlas will contain maps of each species plotted on a 50 km UTM grid and based entirely on field observations. There might be blank cells even for common species as no assumptions are made about the occurrence of a species in any grid cell (Fig. 1).

Within the project LIFE FOR SPECIES (2021 – 2024) regional (state level) initial assessment of mammal species is done according to quantitative criteria defined by International Union for Conservation of Nature (IUCN 2012) and the latest available data. The previous assessment of mammal species according to outdated IUCN criteria was done more than 20 years ago (Andrušaitis 2000). One of actual IUCN criteria used for species assessment is the size of geographic distribution.

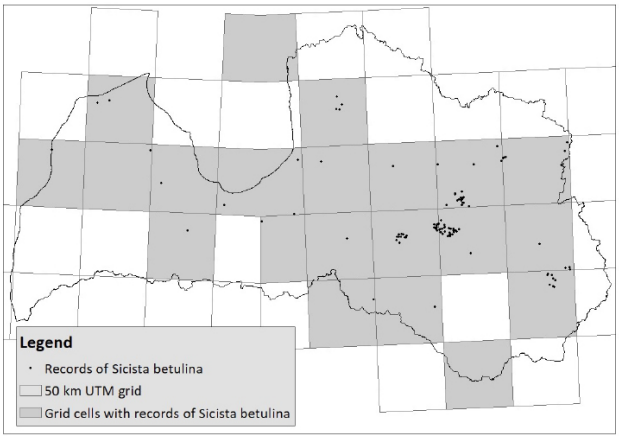

Fig. 1. Distribution of the northern birch mouse in Latvia.

The main sources for mammal distribution data are the National Biodiversity Monitoring Program, the nature observations portal Dabasdati.lv and some studies on raptor, especially owl, foraging (e.g., Avotiņš 2017). For approximately half of mapped species data from the portal Dabasdati.lv make up most or large part of the species records. At the same time, 2/3 of the species registered in portal Dabasdati.lv had to be evaluated for the correctness of species identification. Especially, reported photos of morphologically similar vole, mouse, shrew and mustelid species were examined.

In Latvia, 77 wild mammal species have been recorded but only 62 species is going to be mapped for the secondAtlas of European Mammals as vanished and vagrant species are not applicable. Marine mammals constitute the bulk of occasionally observed species. One of recent cases of occasional visitors is the sighting of the walrus *Odobenus rosmarus* in 2022 (Pilāts 2022). The extinction risk assessment is done for 38 mammal species which are either Red Listed previously or listed in annexes of Habitats Directive or protected on national level.

The Siberian flying squirrel *Pteromys volans*, garden dormouse *Eliomys quercinus* and European mink *Mustela lutreola* most probably are vanished and thus are considered regionally extinct (IUCN category RE) in Latvia. Consequently, these species will not be mapped for the Second Atlas although were mapped for the first Atlas.

The European hedgehog *Erinaceus europaeus* and sibling vole *Microtus rossiaemeridionalis* are species supposed to be present, but no reliable records has obtained as their identification require specific methods. Consequently, within the process of extinction risk assessment European hedgehog is evaluated as data deficient (IUCN category DD) as it was assessed threaten in previous assessment process. Another three species – tundra vole *Microtus oeconomus*, European pine vole *Microtus subterraneus* and golden jackal *Canis aureus* – are new for Latvia, i.e., have been identified for the first time or have settled here after 1999. In case of jackal the appearance of the species in

Latvia is the result of its extensive expansion throughout Europe (Spassov, Acosta-Pankov 2019). The only source of data for both vole species is the study on owl food items (Avotiņš 2017). It is quite possible that these species were present in Latvia also formerly but were not recorded by other methods. The study demonstrated that also in case of the northern birch mouse *Sicista betulina* analysis of food items for owls provide more complete information about the rodent species than others survey methods. The aggregations of birch mouse records on a map (Fig. 1.) indicate on areas where owl pellets have been collected. In result, supplementary data on *S. betulina* allowed this previously Red Listed species assess as least concern (IUCN category LC) in Latvia.

The edible dormouse *Glis glis* is an example when due to restricted distribution within the country the species is assessed as endangered (IUCN category EN). IUCN`s criterion "geographic range" in the form of "extent of occurrence" (EOO) was applied (Fig. 2) as isolation of populations and decline in quality of habitat dwelled by *G. glis* are also involved.

Initial assessment according IUCN criteria indicate that altogether five mammal species is going to be classified as threaten. Within the EMMA2 project for almost all species supposed to be distributed all over the country the part of the grid cells (50 × 50 km) will remain without evidence of the species presence indicating on absence of data not species.

Acknowledgements

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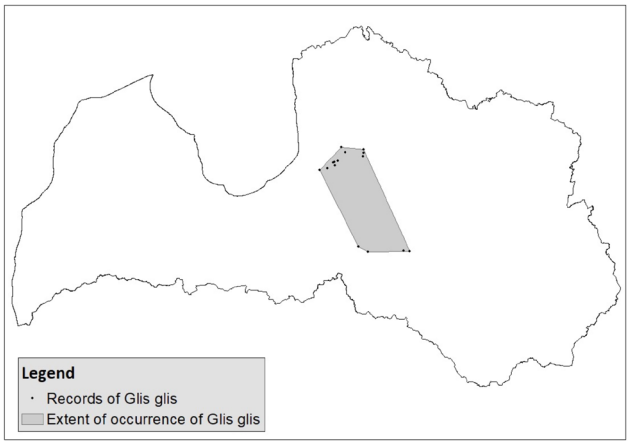

Fig. 2. Distribution of the edible dormouse in Latvia.

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Medicago truncatula – model for studyng intra-plant and inter-plant signals during arbuscular mycorrhizal colonisation

Zigmunds Orlovskis[1,2]*, Annija Kotova[1], Daniels Pugačevskis[1], Ēriks Voroņins[1], Soon Jae-Lee[3]

[1]Latvian Biomedical Research and Study Centre, Rātsupītes 1 k-1, Rīga, LV–1067
[2]Department of Plant Physiology, Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia
[3]Department of Ecology and Evolution, University of Lausanne, Switzerland
*Corresponding author, E-mail: zigmunds.orlovskis@biomed.lu.lv

Key words: inter-plant signals, mycorrhiza, plant-fungi interactions, plant defence.

More than 92% of all vascular plant species on Earth form symbiotic associations with soil fungi, known as mycorrhizae, which extend the physiological surface of plant roots and provide plants with water and essential mineral nutrients, leading to increased plant productivity and yield (Brundrett, Tedersoo, 2018). In addition to this vital ecosystem service, hyphae of arbuscular mycorrhizal fungi (AMF) form ''bridges" between roots of individual plants, effectively generating a subterranean network of inter-connected root systems. Such underground network, known as common mycelial network (CMN), is proposed to function as a super-organism or information highway in transferring diverse signals from plant-to-plant (Bais et al. 2004; Barto et al. 2012). We wished to investigate the hypothesis that AMF mediate exchange of inter-plant signals in response to biotic or abiotic stress stimuli.

Due to available whole genome sequence *Medicago truncatula* Gaertn. (Fabaceae) is a favourable model species to study plant gene expression in response to both intra-plant and inter-plant signals. Moreover, *M. truncatula* forms symbiosis with model AMF species *Rhizophagus irregularis*. To test the hypothesis, we applied flg22 peptide – a known plant immune elicitor – in combination with mechanical wounding to invesigate activation of wound-response or PTI marker genes in systemic leaves or neighbouring plant. Herein, we present selected data with 4 h and 28 h gene

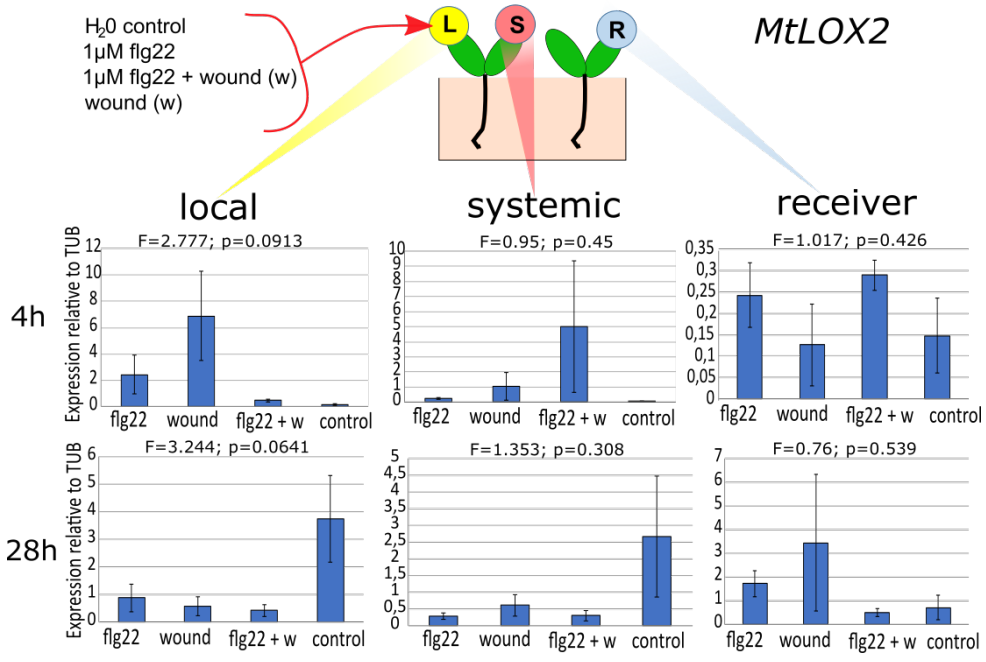

Fig. 1. Medicago truncatula *LIPOXYGENASE2* (*LOX2*) expression relative to *M. truncatula TUBULIN* in local (L), systemic (S) and neighbouring plants, i.e., potential inter-plant signal receivers (R), 4h and 28h after stimulatrion of local tissues. Mean of 4 biological replicate plants ±SEM. Statistical analysis with ANOVA, Tukey post-hoc test.

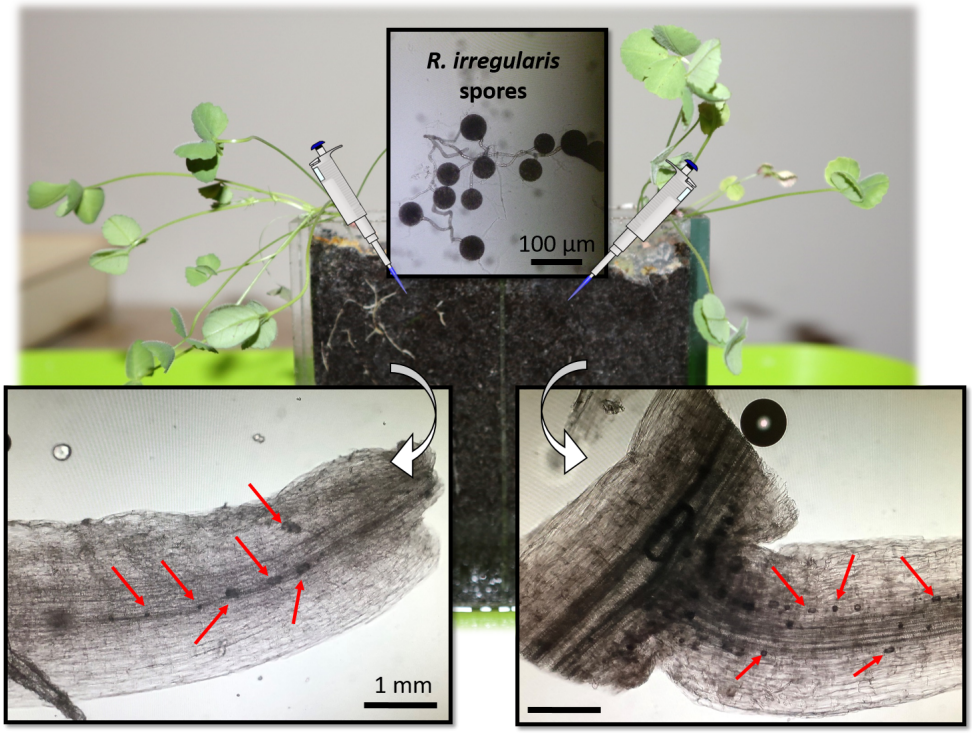

Fig. 2. A tandem of two *M. truncatula* grown in a single pot inoculated with *R. irregularis* by pipetting ~100 sterile spore suspension onto each plant. Micorrhizal colonization, characterised by arbuscule or vesicle formation (red arrows), visualized by black ink-vinegar staining. Inter-plant micorrhizal connections form within 6 – 8 weeks post inoculation.

expression measurements for a known wound-induced jasmonic acid synthesis gene *LOX2*. We observed a tendency for a transient induction of *LOX2* within the wounded leaf 4h after the stimulus as well as in the systemic leaves 4h after the combined treatment of flg22 and wounding (Fig. 1). Without the AMF-connection, there was no induction in *LOX2* expression within the neighbouring plant 4 h after the stimulation. While this experiment serves as a AMF-free control, further experiments will explore an extended list of marker gene expression in plants with established AMF colonization and inter-plant connections (Fig. 2).

In combination with further experiments and microbial pathogen assays, our data will test the role of AMF connection in transferring "warning" signals to plant conspecifics in addition to known volatile or root exudate-mediated inter-plant signals (Sharifi, Ryu 2020; Orlovskis, Reymond 2020).

Acknowledgements

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Genetic characterization of *Brucella suis* bv. 2 isolates from hunted wild boars from the eastern part of Latvia in 2015 – 2016

Lelde Grantiņa-Ieviņa*, Juris Ķibilds, Svetlana Cvetkova, Žanete Šteingolde, Madara Streikiša, Jeļena Avsejenko, Olga Valciņa, Aivars Bērziņš

Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes 3, Riga LV–1076, Latvia
*Corresponding author, E-mail: lelde.grantina-ievina@bior.lv

Key words: biovars, brucellosis, wild boars.

Bacteria of the genus *Brucella* are intracellular pathogens – they survive and multiply in macrophages during infection. These bacteria have adapted to acidic environments, low oxygen concentrations, and low nutrient levels. Swine brucellosis is caused by three *Brucella suis* biovars – 1, 2 and 3. Swine brucellosis caused by biovar 2 is one of the most important endemic diseases in wild boar populations in Europe (WOAH 2022). International regulations do not require systematic monitoring of brucellosis in wild animals and it is not carried out, however, several scientific studies have reported the incidence of this disease in Central European countries – France, Czech Republic, Croatia, North-Eastern Germany, Slovenia, Switzerland, Italy and Poland.

The spread of the disease occurs during mating of animals, as well as by consumption of tissue remains after childbirth and abortions. *B. suis* biovar 2 causes purulent lesions in the tissues of the organs of the reproductive system. This biovar rarely causes disease in humans but occurs in the brown hare (*Lepus capensis*). Wild boars are considered a possible source of disease for domestic pigs (OIE 2022). In the investigation carried out in Latvia in 2015 – 2016, 21.56 to 22.69% of hunted wild boars were serologically positive to brucellosis (Grantina-Ievina et al. 2018).

In the present study isolates for whole genome sequencing were obtained from 19 wild boar samples. From some animals, isolates have been obtained from several organs – spleen, lymph nodes, kidneys, tonsils. The total number of sequenced isolates were 47, including Bruc 1 isolate from the 2010 outbreak in domestic pigs in Talsi region, and B.suis2 isolate from the inter-laboratory comparative test. Whole genome sequences of these isolates were obtained by Illumina Miseq in June 2017 using Nextera XT kit for library preparation. Sequence analysis was performed in 2022 using the open access Bacterial and Viral Bioinformatics Resource Center (BV-BRC) platform and tools of the Center for Genomic Epidemiology of the Technical University of Denmark: Genome Assembly and Annotation (BV-BRC), Determination of antibiotic resistance genes and virulence factors (DTU, BV-BRC), MLST typing according to Whatmore et al. (2007) (DTU). The comparative genomics were done, in addition to our isolates using freely available sequences of *B. suis* isolates from different countries (n = 84), by applying genome comparison with Protein Family Sorter and Proteome Comparison (BV-BRC). The phylogenetic comparison was done for our isolates and additional 53 freely available *B. suis* genomes from studies from other countries using the Codon Tree pipeline (BV-BRC) visualized with Treeview (http://etetoolkit.org/treeview/).

All isolates had two antibiotic resistance genes from the CARD database (*mprF* with 99% identity and *gyrA* with 83% identity), as well as another 31 gene from the BV-BRC database, which should be further investigated in next studies. For biosafety reasons, the antimicrobial resistance phenotypes of these isolates have not been determined in the laboratory.

In conclusion, a similar spectrum of antimicrobial resistance genes and virulence factors was found for *B. suis* bv. 2 isolates from the eastern part of Latvia, but three different types of MLST were detected – MLST 16 (28.6%), as well as new types of MLST 15* (52.4%) and 16* (19 .0 %). The MLST type of the isolate, which was detected in the 2010 brucellosis outbreak in domestic pigs (MLST 15*), was also found in wild boars hunted in the eastern part of Latvia. During the analysis of comparative genomics it was found, that Latvian *B. suis* bv. 2 isolates have differences in two genes – two copies of endoglycanase H and VirB7 (type IV secretion complex lipoprotein) were detected, which are not present in any *B. suis* isolates from other countries. In the phylogenetic comparison, the isolate from the domestic pig brucellosis outbreak was in the same cluster as the isolates from wild boars in Latvia, while the isolate from the interlaboratory comparative test was more similar to the isolates from other countries. The proteome comparison

showed that the minor chromosome of our studied isolates has several proteins with low similarity compared to the reference genome, thus this is a region that should be given more attention in further analyses.

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A pilot study of the metagenome of dairy cows milk

Guntis Boikmanis*, Lelde Grantiņa-Ieviņa, Juris Ķibilds

Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes 3, Riga LV–1076, Latvia
*Corresponding author, E-mail: guntis.boikmanis@bior.lv

Key words: milk, *Coxiella burnetii*, Q fever, metagenome, antibiotic resistance, virulence factors.

Advances in omics technologies have led to a shift in how we view the microbiota of cow udders and milk, from a sterile environment to a complex ecosystem. The composition of microorganisms in milk is important for understanding animal health and milk production safety. Microorganisms in milk can be characterized using cultivation and the 16S metagenomics approach, and the shotgun metagenomics approach. Shotgun metagenomics provides an opportunity to discover not only the taxonomic affiliation and diversity of microorganisms but also information about their properties in the respective environment, such as antibiotic resistance, virulence factors (Addis et al. 2016).

The aim of the study was to conduct a pilot study on the metagenome of combined milk and bulk milk samples from dairy cows. The objectives of the study were: (i) to perform shotgun sequencing using Illumina Miseq and NextSeq 2000 instruments; (ii) to characterize the taxonomic classification of bacteria, present in milk samples, determine the presence of antibiotic resistance genes and virulence factors in the obtained sequences; (iii) to perform genome assembly (reconstruction) of bacteria and viruses present in the samples; (iv) to evaluate the suitability of the methods for detecting various zoonotic disease agents, such as the Q fever agent *Coxiella burnetii*; (v) to compare the obtained results with the results of other studies.

The samples used in the study were 23 composite/bulk milk samples from various national investigation programs, of which 18 were positive for *Coxiella burnetii* DNA by real-time PCR. Genomic DNA was extracted using either the QIAamp DNA Mini Kit (Qiagen) or the NucleoSpin Food Kit (Macherey-Nagel). Shotgun sequencing was performed on five samples using the Illumina MiSeq with the Nextera DNA Flex kit. Shotgun sequencing was performed on eight samples using Illumina NextSeq 2000 with the QIAseq FX kit. Adapter and quality trimming were performed on all reads using the Trimmomatic program (Bolger et al. 2014) and Trim Galore (bv-brc.org). Metagenomic taxonomic classification was performed using the Taxonomic Classification tool (Olson et al. 2023) and Kraken 2 (Wood et al. 2019). Kraken 2 results were processed with Bracken. Detection of antibiotic resistance genes (according to the Comprehensive Antibiotic Resistance Database, CARD) (Alcock et al. 2023) and virulence factors (according to the Virulence Factor Database, VFDB) (Liu et al. 2022) was performed using the Bacterial and Viral Bioinformatics Resource Center Metagenomic Read Mapping tool (bv-brc.org). Bacterial and viral genome assembly (reconstruction) in the samples was performed using Metagenomic Binning (Olson et al. 2023), with assembly performed using metaSPAdes (Nurk et al. 2017) and/or MegaHit (Li et al. 2016).

The DNA concentration of the samples varied from 1.70 to 14.50 ng μL^{-1} according to measurements with the NanoDrop 1000 spectrophotometer and from 0.15 to 6.42 ng μL^{-1} according to measurements with the QuBit fluorometer. Antibiotic resistance genes were obtained from 15 samples, and the most commonly encountered antibiotic resistance gene classes were aminoglycosides, efflux system, aminocyclitols, beta-lactams, and quinolones. Virulence factors were obtained from 12 samples, and the most commonly encountered virulence factor genes were those for flagella, the general secretion pathway, type VI secretion systems, and chemotaxis. After bacterial genome reconstruction, the most prevalent bacteria in the sequenced samples were *Acinetobacter johnsonii, Buttiauxella agrestis, Lactococcus raffinolactis, Chryseobacterium haifense*, and *Acinetobacter albensis*. All of our samples show representatives of the *Clostridia* bacterial class. The most commonly observed representative of this class was *Clostridia botulinum*, which can be found in soil and can contaminate milk, as well as being found in certain types of silage (Lindström et al. 2010). In some samples, bacteria from the Gammaproteobacteria class were present: *Pseudomonas fluorescens* and *Pseudomonas brenneri*. These bacteria are associated with spoilage of dairy products (Scales et al. 2014; Baïda et al. 2001), and it is likely that the milk samples in which Gammaproteobacteria were found had started to spoil. Compared to the results obtained in Hungary (samples A and B) (Tóth et al. 2020) and Bangladesh (Ctg3C5 and Ctg3C4) (Hoque et al. 2019), the composition of our samples was similar (Fig. 1). When performing taxonomic classification, it was observed that the majority of the metagenome is represented by the genome of cows (*Bos taurus*). Sequence reads from *Coxiella burnetii*, the causative agent of Q fever, were also found in

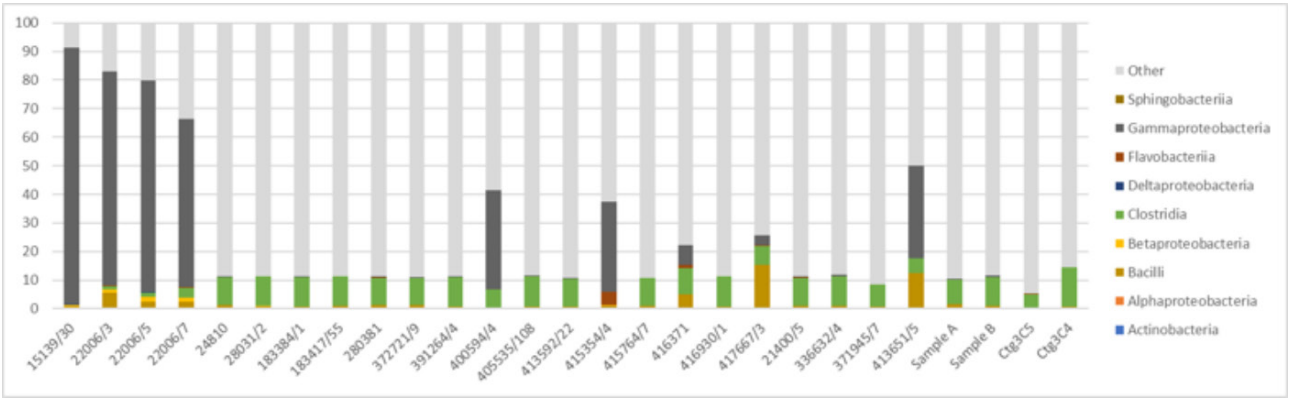

Fig. 1. Dominant classes of bacteria in different milk samples.

the samples, but their quantity relative to the entire sample was very low. Compared to our samples, the Hungarian results are similar, but further research using more milk samples is needed to better understand the composition and dynamics of milk microbiota.

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Attempts to establish *Actaea racemosa* 'Brunette' in *in vitro* culture

Liva Purmale*, Karlis Shvirksts, Anna Korica

Plant Tissue Culture Laboratory, Bulduri Horticultural School, Viestura 6, Jurmala LV–2010, Latvia
*Corresponding author, E-mail: liva.purmale@bulduri.lv

Key words: black cohosh, 'Brunette', micropropagation, callus culture, *Cimicifuga racemosa*.

Black cohosh (*Actaea racemosa* L.) is flowering, perennial plant belonging to Ranunculaceae family with great decorative properties. Vegetative propagation of black cohosh red forms is slow and rather difficult, therefore *in vitro* approach was evaluated. Plant material was collected in "Baizas" (coordinates 57.24775, 24.87018) in summer 2020. According to literature (Lata et al. 2002) black cohosh *in vitro* culture can be induced form leaf explants. Leaves were surface sterilized (10% Domestos solution with few drops of Tween 20, for 10 min) followed by three washes of deionized autoclaved water. Leaf explants approx. 5 × 5 mm in size were placed on Murashige and Skoog (Murashige, Skoog 1962) modified medium (Lata et al. 2002). Plant material was placed in test tubes (50 mL), in the growth room at 18 ± 2 °C temperature, half with photoperiod 16/8 h day/night and half in complete darkness (20 replicates per treatment).

Although there is a report (Lata et al. 2002) that 95% callus occurred within 10 to 15 days on MS medium supplemented with 0.18 mg L^{-1} NAA in combination with 0.11 mg L^{-1} TDZ, and 87% callus was formed on 1 mg L^{-1} NAA in combination with 0.5 mg L^{-1} TDZ, none of the media used (MS1 to MS9) showed any development (Table 1).

Fig. 1. A, mother plant of *Actaea racemosa* with young etiolated leaves that were used as explants. B, explant placed on medium supplemented with $AgNO_3$. C, beginning of callus formation. D, necrotic callus tissue. E, callus after nine months of weekly subcultivations.

Table 1. Growth regulator combinations (mg L^{-1}) tested for induction of callus formation. MS8 and MS9 media had modified vitamins: myo-inositol 100 mg L^{-1}, thiamine HCl 2.5 mg L^{-1}, pyridoxine HCl 0.2 mg L^{-1}, biotin 0.2 mg L^{-1} (Pinker, Schenk 2018)

	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8*	MS9*
BAP	3								
Kinetin					1				
NAA		1	0.85	0.1	0,1	0.18			
TDZ		0.5	0.5	1		0.11			1
2,4 D							1	0.1	

Experiment was repeated the following year with etiolated leaves. *In vitro* callus culture was established in the 2021, using etiolated leafs of Black cohosh 'Brunette' on medium 0.1 mg L^{-1} NAA in combination with 1 mg L^{-1} TDZ, that was kept for the first month in the darkness (Yang, Lu 2017). Explants were placed on fresh medium next and third day, then transfer to fresh medium was required weekly in order to avoid losing the culture due to browning and necrosis (Fig. 1).

Several additives to media were tested to reduce culture browning: PVP 0.5 mgL^{-1}, 1 mg L^{-1}, 1.5mg L^{-1}, citric acid 10 mg L^{-1}, ascorbic acid 20 mg L^{-1}, $AgNO_3$ 2.5 mg L^{-1}, charcoal 2 g L^{-1} (Amente, Chimdessa 2021). Only additive with some positive effect was $AgNO_3$. Further research how to reduce explant browning and induce organogenesis is required.

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