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ABSTRACTS
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Cultivation of the extremophile microalga *Galdieria sulphuraria* in concentrated whey permeate

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Key words: β-galactosidase, dairy industry by-products, heterotrophic cultivation, lactose.

Dairy industry by-products are generated in high quantities around the world and continue to pose a significant threat to the environment when disposed of incorrectly. This is due to their high organic load, which is mostly caused by high lactose content (Kolev Slavov 2017). Therefore, the use of specific lactose-hydrolyzing microalgae for bioconversion of dairy industry by-products can simultaneously decrease the pollution load and reduce microalgal production costs (Kolesovs, Semjonovs 2023). Up to date the microalgae performance in lactose-containing substrates remains fragmented with only a limited number of candidates, e.g., *Galdieria* sp., *Tetradesmus obliquus* CPCC 5, *Nannochloropsis limnetica* SAG 18.99, that demonstrate the ability to produce β-galactosidase, a key enzyme for lactose hydrolysis (Bentahar, Deschênes 2021; Kolesovs, Semjonovs 2023).

This study focuses on the cultivation of an axenic red microalga *Galdieria sulphuraria* SAG 107.79 in standard media supplemented with lactose, glucose, or galactose, as well as in whey permeate. The presence of β-galactosidase gene was confirmed for this microalga (Schönknecht et al. 2013) making it a suitable candidate for growth in dairy industry by-products.

G. sulphuraria was obtained from The Culture Collection of Algae at the University of Göttingen, Germany. Allen's Cyanidium medium (ACM) was used as the standard medium for the trials (Gross, Schnarrenberger 1995). It was supplemented with 10 g L^{-1} glucose, lactose, or galactose which were dissolved in distilled water autoclaved (121 °C, 1.2 atm for 15 min) and then the reagent mixture was added using a 0.22 μm filter. The concentrated cheese whey permeate (JSC "Smiltenes piens", Smiltene, Latvia) contained ~140 g L^{-1} lactose which is significantly higher than in most dairy side-streams, thus a diluted version was prepared by mixing 5, 15, or 25 % permeate with distilled water. The media were sterilized, and pH was decreased from 7.0 ± 0.1 to 4.0 ± 0.1 using 1 M HCl.

The cultivation was carried out in 50 mL Erlenmeyer flasks with 25 mL of medium (5% inoculum, ~1 × 10^5 cells mL^{-1}) in an orbital incubator shaker at 40 °C, for 8 days under mixotrophic (12:12 day/night cycle, 40 μmol m^{-2} s^{-1}, cool white LED light) or heterotrophic regimes. After the cultivation the 15 mL biomass samples were used for the determination of biomass dry weight (DW, g L^{-1}) and productivity (P, g L^{-1} day^{-1}). The cell count (CC) analysis was performed using LUNA-FX7™ Automated Cell Counter, South Korea. Changes in organic carbon (C) were analyzed using enzymatic lactose/galactose (K-LACGAR) and glucose (K-GLUC) kits in full accordance with the manufacturer's instructions (Megazyme, Ireland). The analysis of β-galactosidase activity was performed in accordance with the methodology described by Bentahar, Deschênes (2021). Statistical analysis was performed in IBM SPSS Statistics (IBM Corp., 2023, Version 29.0.2.0 Armonk, NY: IBM Corp) using one- or two-way ANOVA with Bonferroni post-hoc test for DW, CC, and enzyme activity, as well as paired sample *t*-test for C concentrations at significance level $p = 0.05$.

Initially, *G. sulphuraria* was cultivated in standard ACM supplemented with C present in dairy industry by-products (Fig. 1). There was no statistically significant difference in biomass productivity between mixotrophic and heterotrophic cultivation ($p > 0.05$). *G. sulphuraria*

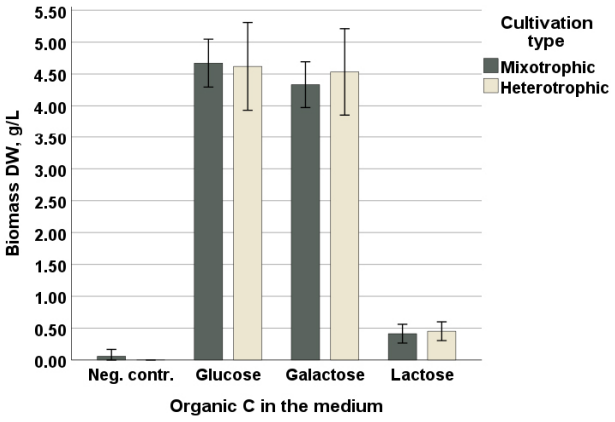

Fig. 1. Effect of cultivation type (photoautotrophic, mixotrophic, and heterotrophic) on biomass synthesis (DW) of *G. sulphuraria* after 8 days of agitated cultivation in standard ACM supplemented with lactose, glucose, or galactose (10 g L^{-1}), or in a negative control group without an additional organic C ($n = 4$).

Table 1. Effect of cultivation type on changes in organic C, biomass productivity, and CC of *G. sulphuraria* after 8 days of agitated cultivation in standard ACM supplemented with lactose, glucose, or galactose (10 g L^{-1}), or in a negative control group without an additional organic C ($n \geq 4$)

Cultivation type	Group	Initial C (g L^{-1})	Final C (g L^{-1})	P (g L^{-1} day^{-1})	CC (cells mL^{-1})
Mixotrophic	Negative control	–	–	< 0.01	6.5×10^5
	Glucose	10.30 ± 0.06	< 0.01	0.58 ± 0.02	4.4×10^8
	Galactose	9.66 ± 0.23	0.03 ± 0.01	0.54 ± 0.03	3.2×10^8
	Lactose	9.47 ± 0.11	9.02 ± 0.22	0.05 ± 0.01	0.4×10^8
Heterotrophic	Negative control	–	–	< 0.01	$< 1.0 \times 10^5$
	Glucose	9.94 ± 0.31	< 0.01	0.58 ± 0.07	4.6×10^8
	Galactose	9.87 ± 0.20	0.03 ± 0.01	0.57 ± 0.07	4.5×10^8
	Lactose	9.52 ± 0.17	9.11 ± 0.13	0.06 ± 0.01	6.3×10^7

least favored photoautotrophic growth, while there was no biomass synthesis in the negative control for the heterotrophic group.

As shown in Table 1, *G. sulphuraria* demonstrated a statistically significant uptake of all tested sugars. Additionally, intracellular β-galactosidase activity was detected in mixotrophic and heterotrophic lactose groups. It can be proposed that slower biomass synthesis in lactose groups was associated with the necessity to first synthesize the β-galactosidase leading to a longer lag growth phase. Considering that there was no significant difference between mixotrophic and heterotrophic cultivation types, heterotrophic cultivation was considered more suitable for *G. sulphuraria* due to higher enzyme activity and from the future scalability point of view.

Based on the results of the previous experiment, *G. sulphuraria* was cultivated heterotrophically in whey permeate media (Fig. 2). CC assessment was used to get a better understanding of biomass synthesis compared to DW measurement which was affected by precipitated proteins. Overall, it can be observed that *G. sulphuraria* achieved biomass synthesis similar to that in the standard medium with lactose when cultivated in the 15% permeate medium. It can be assumed that 5% permeate provided little nutrients for efficient biomass synthesis, while the 25% permeate group inhibited growth due to high lactose concentration (~35 g L^{-1}). Further studies are required to optimize microalga's performance in whey permeate medium by using pre-treatment and/or modification, as well as inoculum activation for β-galactosidase production.

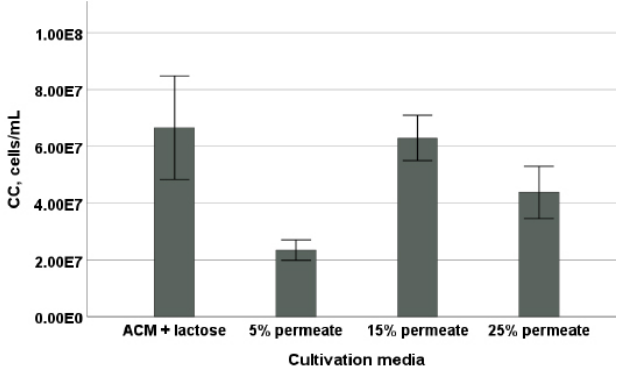

Fig. 2. Effect of whey permeate concentration (5, 15, 25%) on biomass synthesis (CC) of *G. sulphuraria* after 8 days of agitated cultivation (n = 4). Standard ACM with 10 g L^{-1} lactose used as control.

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Impact of chitosan on mycelial growth of *Trametes versicolor*

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Key words: chitosan, liquid medium, micromorphology, mycelium, solid medium.

Chitosan is a nitrogen-containing polysaccharide found in various fungal species (Aranaz et al. 2021) particularly in Mucorales (Di Mario et al. 2008). Commercial chitosan is usually harvested from crustaceans. There is a considerable amount of research on the effects of chitosan on various plant pathogens (mainly crop pathogens), with most studies indicating its inhibitory effect on fungal growth (Bautista-Baños et al. 2004; Palma-Guerrero et al. 2008; Meng et al. 2010). It has been found that chitosan induces structural changes in the mycelium of certain fungal species, such as excessive hyphal branching, a reduction in hyphal diameter, and swelling of cell walls (Singh et al. 2008; De Oliveira et al. 2012). However, there are significantly fewer studies on the effect of exogenous chitosan on basidiomycetes, and almost none specifically on *Trametes versicolor*. This study aimed to evaluate the effect of chitosan on *T. versicolor* mycelial growth in both solid and liquid media.

T. versicolor was inoculated into both solid and liquid media supplemented with varying chitosan concentrations (0 to 25%). The chitosan source was crustaceans. The solid medium cultures were incubated in a climate-controlled chamber for 17 days, with mycelial colony diameter measured at regular intervals. The liquid medium cultures were cultivated in an orbital shaker Multitron (Infors HT, Basel, Switzerland) for 14 days at 27 °C/150 rpm. Following incubation, mycelial pellets were collected, dried, and their dry weight recorded. The pH of medium was also measured. Scanning electron microscopy (SEM) was performed on the pellets using a Tescan Vega TX (Brno, Czech Republic) at a magnification of 2000×. Subsequently, the pellets were washed with acetic acid to remove encapsulated chitosan, redried, and their dry weight was measured again. The statistical analysis was conducted using RStudio software. Pairwise comparisons, as well as linear mixed-effects (LME) and generalized additive (GAM) regression models, were used to assess the statistical significance of the observed differences.

Higher chitosan concentrations led to a reduction in the mycelial growth of *T. versicolor* on solid medium (Fig. 1), supporting its well-documented antifungal properties. However, an opposite trend was observed in liquid medium. At low chitosan concentrations (0to 5%), mycelial growth was inhibited, but at 15%, the dry weight increased, reaching or even surpassing control values. Beyond 15%, dry weight declined again (Fig. 2). Notably, the mycelial pellets at 15% chitosan exhibited distinct morphological differences compared to the control, appearing denser with a grayish hue and encapsulated chitosan. After the removal of encapsulated chitosan via acetic acid washing, sample weights decreased, particularly at higher chitosan concentrations. However, the overall trend remained

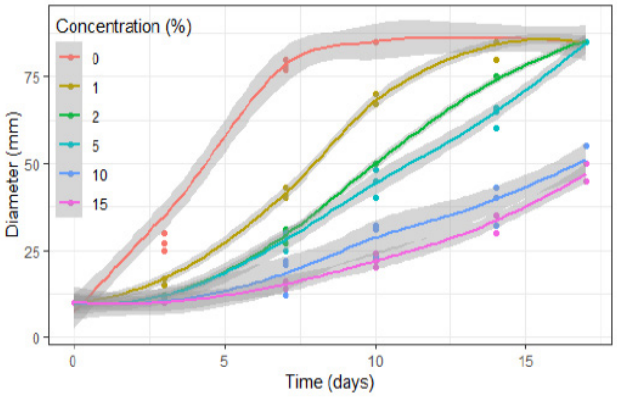

Fig. 1. Mycelial growth of *T. versicolor* (mycelium diameter in mm) in solid medium under various chitosan concentrations.

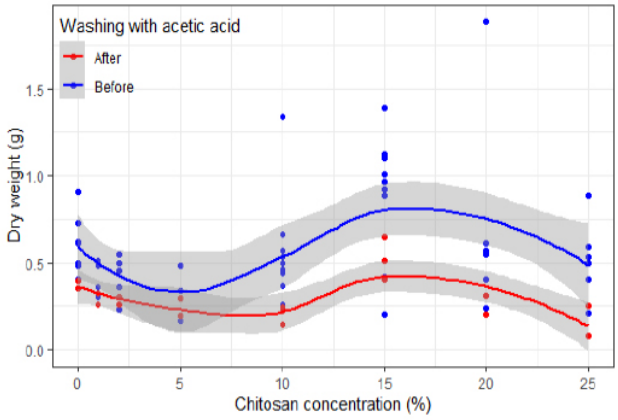

Fig. 2. Dry weight of *T. versicolor* mycelial pellets grown in liquid medium with varying chitosan concentrations, measured before and after acetic acid washing.

consistent, indicating that variations in dry weight were not solely due to chitosan encapsulation but also reflected actual differences in mycelial growth (Fig. 2).

The observed growth patterns in response to increasing chitosan concentrations may be attributed to the pH-dependent solubility of chitosan. Chitosan is readily soluble in acidic conditions but becomes nearly insoluble under alkaline conditions (Mohamed et al. 2013). As chitosan itself is basic, its addition increases the pH of the medium (Fig. 3). At low chitosan concentrations, the medium maintains a pH of approximately 5, facilitating chitosan solubility. However, at 15% chitosan, the pH rises to 7.5, rendering chitosan insoluble (Mohamed et al. 2013) and consequently reducing its antifungal activity. At even higher concentrations, fungal growth may be further inhibited due to elevated pH, as *T. versicolor* thrives better in acidic rather than alkaline conditions (Veena, Meera 2012).

Scanning electron microscopy revealed that fungal hyphae could grow very close to chitosan crystals, even covering their surface in some cases (Fig. 4, Fig. 5). This suggests that undissolved chitosan does not have a strong antifungal effect.

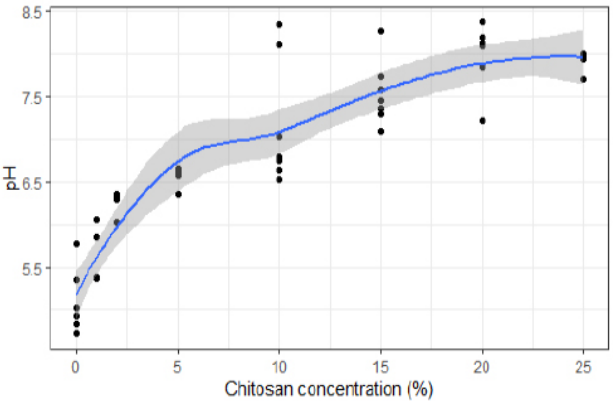

Fig. 3. pH value of medium depending on concentration of chitosan.

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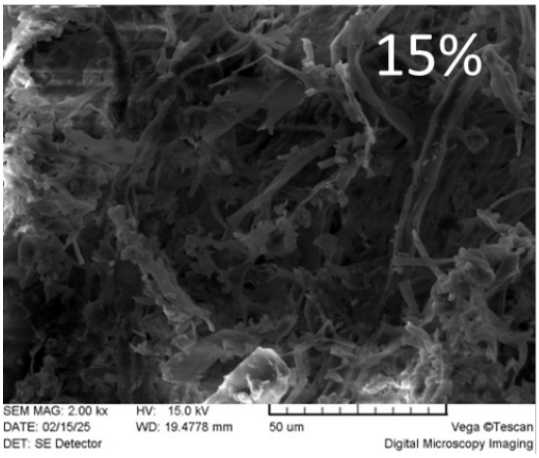

Fig. 4. SEM image of *T. versicolor* mycelium grown in 15% chitosan, cross-section view.

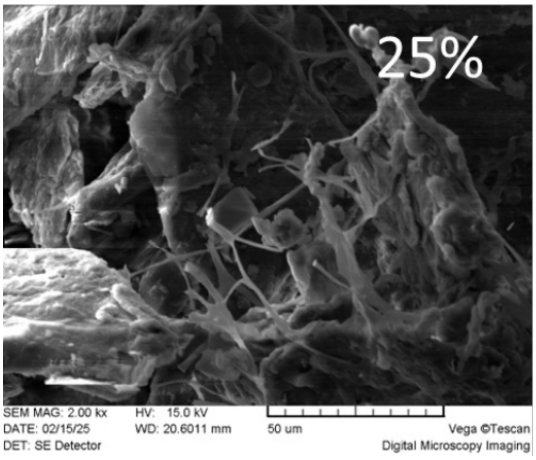

Fig. 5. SEM image of *T. versicolor* mycelium grown in 25% chitosan, cross-section view.

Effects of sex-limited experimental evolution on a hermaphrodite: focus on sex-biased genome regions

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Key words: experimental evolution of gonochorism, hermaphrodite, *Macrostomum lignano*.

As our understanding of the early stages of sex chromosome evolution is mainly based on theory and comparative evidence, we developed a system that we hoped would make it possible to observe in real time what happens after acquiring a new sex-determining gene. We used a previously established green fluorescent protein (GFP) line of the simultaneous hermaphrodite *Macrostomum lignano*. We used the GFP locus as a dominant sterility mutation inherited in a Mendelian fashion. By allowing the GFP allele to be inherited only through sperm, we created male-limited selection (M) lines (resembling the early stages in XY chromosome evolution), and by allowing the GFP allele to be inherited only through egg cells, we created female-limited selection (F) lines (resembling the early stages in ZW chromosome evolution). We also created control (C) lines with equally mixed inheritance patterns (Nordén et al. 2023).

After tens of generations, we investigated how these lines have responded on the genome, transcriptome, and phenotype levels. We sequenced genomes and analysed changes in single nucleotide polymorphism (SNP) frequency and structural variant (SV) distribution across the genome. We also sequenced transcriptomes to see how gene regulation has changed. Besides genomic analyses, we examined how mating behaviour and sexual anatomy have changed (Cīrulis 2022).

We observed that the F lines responded the most at the genome-wide level (Cīrulis 2022). For example, the number of significantly differentially expressed transcripts was largest between the F and C lines. These changes involved the downregulation of testes-biased genes and genes involved in the biosynthesis of amino acids and carbon metabolism (Cīrulis et al. 2024). In addition, we observed the highest number of SVs in the F lines, which could be related to changes in the recombination rate (Cīrulis, 2022). In contrast, the M lines seemed to have responded the most at the sex-biased genome regions, like mitochondria (maternally inherited) and the GFP scaffold, since there is an overrepresentation of the mitochondrial genome and SNPs on the GFP scaffold in the M lines (Fig. 1). The M lines also showed evidence of changes on phenotypic level since we observed a decrease in the ovary size and body size in the M lines and behavioural changes that may be related to changes in the ejaculate. Both sex-specific selection regimes showed evidence of alterations in the shape of the stylet (Cīrulis 2022).

Based on these results, we can conclude that our worms have responded to the sex-limited selection. The evidence of a decrease in the testes function in the F lines resembles adaptation towards gynodioecy, and the evidence of a

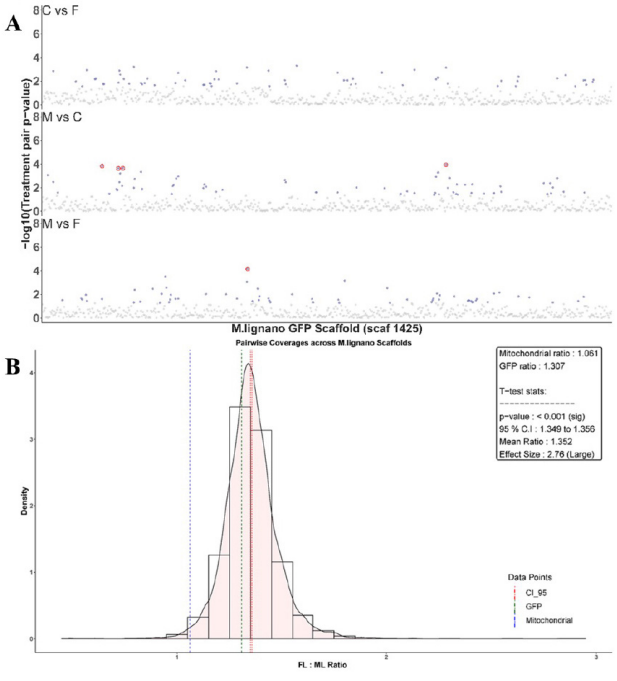

Fig. 1. ignificant SNPs (circled red, $q < 0.05$) on the GFP scaffold (A). Scaffold coverages in pairwise comparisons between FvsM (B). The green line represents coverage of the GFP scaffold, blue – mitochondria and red – confidence intervals.

reduction of the ovary size and increase of mitochondria in the M lines resembles adaptation towards androdioecy (Cīrulis 2022).

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Universal 5'-RACE method for mapping transcription start sites of eukaryotic and prokaryotic genes, as well as 5' UTRs of RNA viruses

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Key words: 5'-RACE, template switching oligonucleotide, 5'-UTR, SARS-CoV-2, HCV, GAPDH, *bla*, *cat*.

The 5' rapid amplification of cDNA ends (5'-RACE) approach is widely used to map transcription start sites (TSSs) both for mRNA and RNA virus genomes, allowing precise determination of nucleotide sequences for further studies of transcriptome and regulation of gene expression. Various protocols for 5'-RACE are based either on ligating anchor oligonucleotide to 5'-end of RNA (LA-PCR) or reverse transcription template switching (CapFinder). The latter approach is based on terminal deoxynucleotidyl activity of Moloney murine leukemia virus (M-MLV) reverse transcriptase (RT), which adds several cytosine residues to 3'-end of cDNA after reaching 5' end of capped RNA. Thereafter the template switching oligonucleotide (TSO) with few G bases at the 3' end is employed to prime the synthesis of complementary, second strand of cDNA. Newly synthesized ds cDNA can be further amplified with RNA sequence specific primer and TSO sequence specific primer for further cloning and sequencing (Chenchik et al. 1998; Pinto, Lindblad 2010; Liu et al. 2018)

In this study we optimized CapFinder and SMART (developed based on CapFinder) protocols both for capped RNAs: human GAPDH mRNA and SARS-Cov2 virus RNA, and uncapped RNAs: bacterial chloramphenicol acetyltransferase (*cat*) and beta-lactamase (*bla*) mRNA and human hepatitis C (HCV) virus RNA. The versatility of proposed 5'-RACE protocol is based on original sequence of TSO and selected RT reagent.

Oligonucleotides were designed using primer-BLAST tool. TSO sequence was designed using Tagenerator tool (Pinto et al. 2006). HCV genome sequences from the Virus Pathogen Database and Analysis Resource were aligned using Clustal Omega multiple sequence alignment tool (Sievers et al. 2011) to find conserved 5' UTR regions suitable for universal design. Oligonucleotides were synthesized by Biolegio, IDT and Metabion.

For 5'-RACE analysis the following RNA containing preparations were used; (1) human mRNA extracted using different methods; (2) RT-PCR SARS-CoV-2 positive diagnostic samples with the test cycle threshold for target gene (ct) < 25; (3) 0.5–0.8 μg of total bacterial RNA; (4) RT-PCR HCV positive diagnostic samples with the test cycle threshold for target gene (ct) < 25.

Capping was performed using Vaccinia Capping System (NEB). First strand cDNA was synthesized by Maxima H minus RT (Thermo Fisher Scientific) with gene specific primers followed by second strand cDNA synthesis after template switching. Synthesized cDNA was immediately used for PCR. Two rounds of heminested touch-down step-out PCR were performed. PCR amplicons were treated according to Sanger sequencing protocol and sequenced.

TSS was successfully mapped to human GAPDH in vivo mRNA using RNA extracted with different methods. For capped *in vivo* GAPDH transcript mapped TSS is consistent with database published for MANE transcript NM_002046.7.

Capped SARS-CoV-2 5'-UTR leader sequence also was consistent with published one. As it can be anticipated considering discontinuous transcription process of Coronaviridae, individual SARS-CoV-2 RNA samples showed identical TSS in genomic RNA and subgenomic nucleocapsid transcript RNA. Strain specific mutations were observed both in viral RNA and nucleocapsid transcript RNA leader sequences.

Uncapped transcript suitability for the proposed 5'-RACE protocol was tested using plasmid pBR329 transformed bacteria (bla, cat transcripts) and uncapped HCV virus (Flaviviridae). From several tested RT preparations Maxima H minus RT showed stable results with an uncapped RNA. Bacterial bla and HCV genome 5'-UTR TSS sequences were consistent with published data.

Analysis of 5'-RACE mapping of cat TSS (Fig. 1) in *Escherichia coli* XL-1 blue strain harboring pBR329 revealed discrepancies in TSS with the sequence published before (Jacquet, Reiss 1992). Two bands in electrophoresis after 2nd round of PCR and two overlapping sequences in one sample were constantly found, implicating at least two different TSS and consequently two active promoters for cat in pBR329. Several promoter prediction tools (Reese

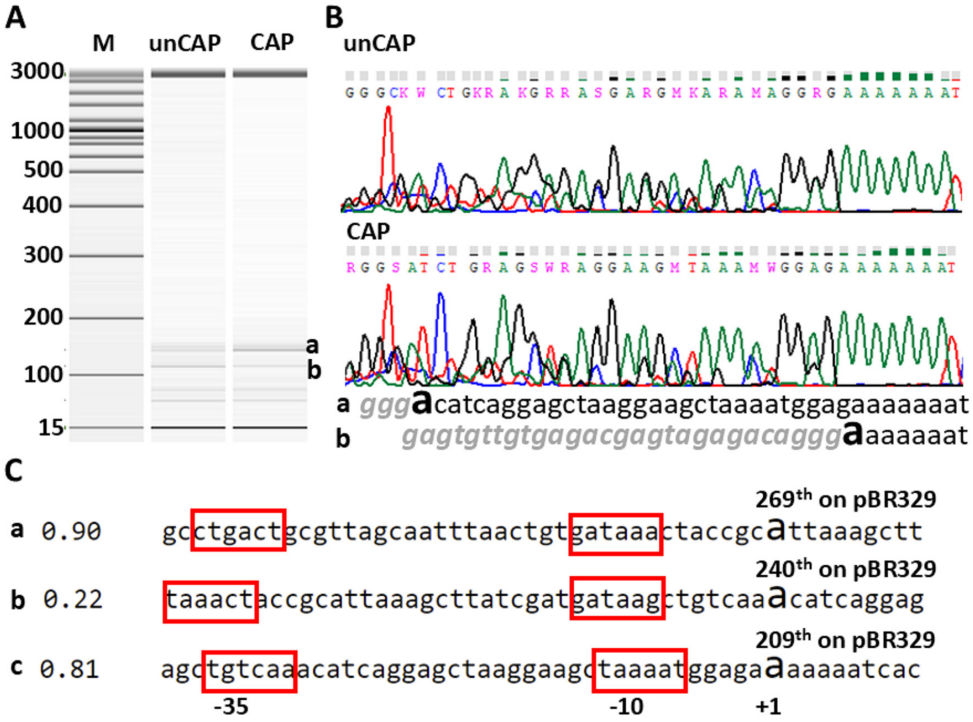

Fig. 1. 5'-RACE mapping of cat TSS in pBR329 plasmid. All sequences are shown in the 3'–5' direction of the plasmid since the cat is transcribed from the complementary strand. (A) Two amplicons (a,b) in electropherogram of 2nd PCR rounds of uncapped (unCAP) and capped (CAP) RNA. M, size marker. (B) Sequence electrophoregrams of uncapped (unCAP) and capped (CAP) RNA samples. Read-out below the electrophoregram corresponds to the deduced sequence of the fragments, including their overlapping, blurred part. Bold letters indicate putative TSS. Grey letters in italics indicate the sequence of TSO, which is apparently duplicated preceding the TSS in fragment b. (C) Putative cat promoter sequences. Red rectangles indicate promoter –10 and –35 boxes, +1, TSS, is shown in bold. On top of the anticipated TSS its position in the sequence of pBR329 (GenBank L08859) is shown.

2001). indicated at least three putative promoters in the 5'-upstream region of *cat* coding sequence in pBR329. The highest score, 0.90, was assigned for the published pBR329 cat gene promoter sequence (Fig. 1, Ca). We did not find the corresponding TSS in our experiments. Our analysis revealed cat mRNA TSS (Fig. 1, Ba) ensuing from putative promoter with the prediction score 0.22 (Fig. 1, Cb) and producing 142 bp cDNA band in electrophoresis (Fig. 1 Aa). We anticipate that the shorter band (Fig.1 Ab) with estimated length 112 bp is associated with the TSS (Fig.1, Bb) corresponding to the putative promoter with the prediction score 0.81 (Fig. 1, Cc). If this promoter was used, the translation should start at non canonical AUA (Ile) codon and the Cat protein lacking first 12 N'-terminal amino acid should be synthesized. Bacteria can use alternative translational start codons apart of AUG/Met (Hecht et al. 2017). It remains to be elucidated whether a truncated Cat protein is produced in bacterial cells and whether it retains the functional activity.

The optimized 5'-RACE method described here is simple, repeatable, and relatively fast. TSS mapping, starting with RNA isolation, can be accomplished within two days.

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Monitoring antibiotic resistance genes in urban wastewater – a pilot study

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Key words: antibiotic resistance, monitoring, urban wastewater, digital PCR.

Antimicrobial resistance (AMR) has become a worldwide concern in medical and public health sectors as more pathogenic microorganisms have become resistant to one or more antibiotics due to misuse and overuse (Berendonk et al. 2015; WHO 2015). Urban wastewater treatment plants (UWTPs) are a unique interface between humans and the environment since almost all household antibiotics end up in wastewater (Karkman et al. 2018). In UWTPs' this has led to the formation of reservoirs and hotspots for the horizontal gene transfer of AMR, creating even more antibiotic-resistant bacteria (Gholipour et al. 2024). To our knowledge, no significant efforts for continuous monitoring have been made in Latvia. Therefore, the surveillance of wastewater to monitor the prevalence of AMR genes would give insight into public health and potential risks of AMR in the healthcare setting.

This study aimed to establish a method for monitoring AMR genes in wastewater samples using digital PCR (dPCR) and to study AMR gene abundance in Latvia. Two resistance genes from different subgroups of antibiotics for systematic use were selected, *sul1* and *ermB*, while 16S rDNA was used as a marker for bacterial abundance and standardization. Influent samples (n = 56) were acquired from seven UWTPs in Latvia in 2023 and 2024. DNA was extracted using the DNeasy PowerWater kit (Qiagen), and AMR genes were quantified using QIAcuity dPCR (Qiagen).

We performed an ANOVA analysis to reveal the effects of AMR gene abundance in wastewater using known variables, such as season, city, and year. Analysis showed that *sul1* gene abundance was significantly impacted by two variables, season (F = 30.087; p < 0.001) and city (F = 5.350; p < 0.001) (Fig. 1). Meanwhile, abundance of *ermB* was significantly affected by season (F = 4.007; p = 0.014), city (F = 10.205; p < 0.001), and year (F = 24.680; p < 0.001) (Fig. 2). In terms of general trends, distinctive patterns of AMR gene abundance were observed; while *sul1* seemed to follow a seasonal pattern, but with an overall increase, *ermB* showed a steady decline during the study period in most cities.

This study demonstrates that dPCR is a reliable and effective method for monitoring AMR gene abundance

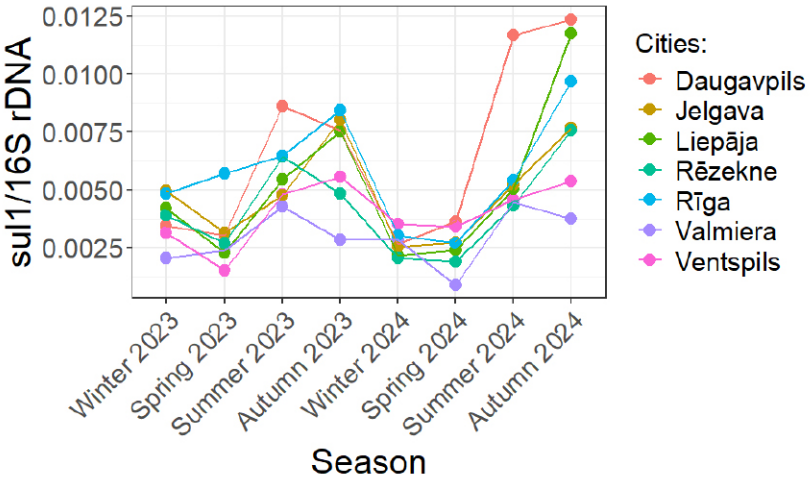

Fig. 1. Seasonal and city-wide variation in *sul1* gene abundance normalized against 16S rDNA in wastewater influent.

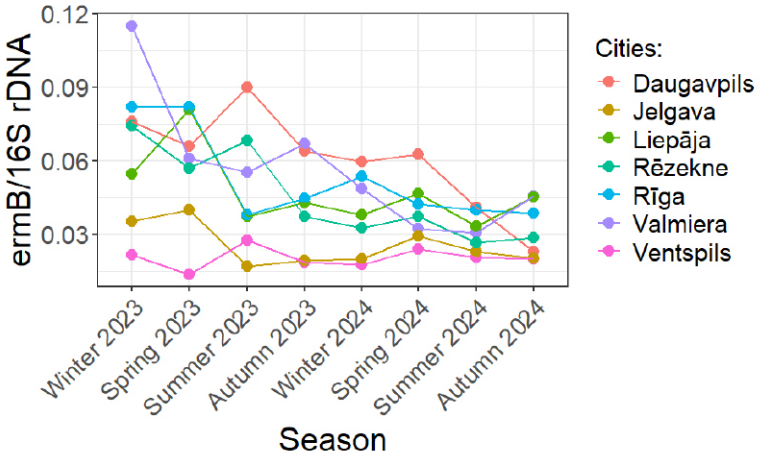

Fig. 2. Seasonal and city-wide variation in *ermB* gene abundance normalized against 16S rDNA in wastewater influent.

in wastewater, revealing statistically significant variations across seasons, cities, and years. The observed trends highlight the influence of environmental and anthropogenic factors on AMR gene distribution. These findings emphasize the importance of integrating wastewater surveillance into public health monitoring strategies in Latvia and globally.

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"Sandy riffles" as potential subtype of freshwater habitat 3260 "Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation"

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Key words: benthic invertebrates, freshwater habitat 3260, river biodiversity, sandy riffles.

Since autumn 2023, in collaboration with the Nature Conservation Agency, researchers of the Laboratory of Hydrobiology, Faculty of Medicine and Life Sciences, have been developing description and criteria for the subtype 3260_3 "Sandy riffles" of EU habitat 3260 "Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation". It is recommended to distinguish an additional subtype of habitat for an appropriate assessment of the ecological quality. As with other subtypes of this habitat, only natural and modified, but naturalized river stretches are considered European Union protected habitats.

The hydromorphological quality of natural sandy riffles is significantly lower than that of other similar type rivers with hard bottom, although formally all these analysed rivers correspond to the slightly impacted habitat 3260_1. If the sandy riffles subtype was not distinguished, these rivers, although completely natural and without anthropogenic pressure, would be assessed as degraded habitats that require restoration measures to improve their quality.

Biodiversity in rivers is closely linked to hydromorphological parameters. The primary hydromorphological factors influencing the diversity of macrophytes and benthic invertebrates are riverbed slope, composition of bed substrate, and shading. Flow velocity, highly correlated with bed slope, is also one of the most important abiotic factors affecting the species composition and diversity of aquatic organisms. Sandy substrates with fine granulometric composition can support only a limited number of macrophyte and benthic invertebrate species due to their uniformity and constant exposure to current forces (Wiegleb et al. 2016).

In sandy riffles dominating macrophyte species are: *Sparganium emersum, Veronica beccabunga, Elodea canadensis, Alisma plantago-aquatica and Phalaroides arundinacea*. *Sparganium emersum* forms sparse stands of submerged leaves. Due to higher stream velocity and unstable substrate conditions for the vegetation formation in sandy riffles are inappropriate, thus the definition of the ecological status of rivers by macrophytes requires a certain minimum species quantity (6 to 10 taxa). However, the absence of macrophytes at a river stretch is not necessarily a result of lower ecological status (Schaumburg et al. 2004).

As the Wantzen et al. (2014) study confirms, physical habitat features are crucial and fine-grained bed sediments may have a strong influence on the functional and taxonomic structure of benthic invertebrates. The improvement of sustainable river ecosystem monitoring a better understanding of the ecohydrological interactions between habitat dynamics and benthic invertebrates is needed (Wantzen et al. 2014). Differences in invertebrate communities are evident between streams with hard and sandy substrates. According to EU habitat data, Ancylus fluviatilis is found in 16% of hard-substrate streams and 9% of sandy riffles, while *Gammarus* sp. occurs in 15 and 9%, respectively. Plecoptera species are present in 25% and 16%, and *Theodoxus fluviatilis* appears in 3% of hard-substrate streams but is absent from sandy riffles. *Unio crassus* is recorded in 6 and 3%, respectively, while *Unio* sp. is equally frequent in both stream types. In contrast, *Anodonta* sp., *Pseudanodonta complanata* and Trichoptera are more common in sandy riffles, with occurrences of 8 and 10%, compared to 5 and 7% in hard-substrate streams.

The highest number of species was recorded from the orders: Ephemeroptera (mayflies), Trichoptera (caddisflies), and Diptera (true flies) (Fig. 1). In Latvia, the following caddisfly species are characteristic for sandy riffles: *Sericostoma personatum, Notidobia ciliaris, Odontocerum albicorne, Beraeodes minutus*, and *Athripsodes albifrons*. According to the results obtained in the project, the mayfly species *Ephemera danica* and *Baetis rhodani*, as well as the stonefly *Amphinemura borealis*, are also typical for sandy riffles. Significantly fewer taxa were found in sandy streams compared to fast-flowing streams on hard substrate. These findings are consistent with other studies in Europe, which characterize sandy streams by a lower diversity of benthic

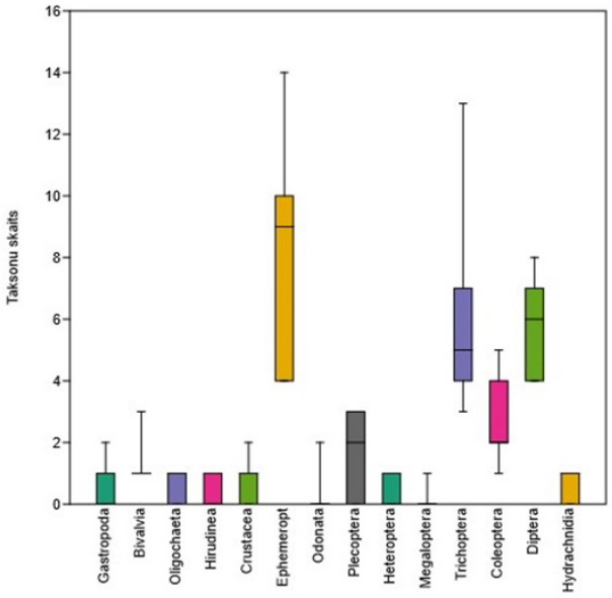

Fig. 1. Total number of benthic invertebrate taxa in the studied sandy riffles in spring 2024.

invertebrates (Mathers et al. 2024; Adámek et al. 2025), as well as a lower number of ecologically sensitive taxa from the Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders, and fewer indicator species, when compared to stony fast-flowing streams (Mathers et al. 2004).

An obligatory prerequisite for identifying at least a good status of sandy riffles is the presence of river bottom texture (sand waves), which helps to avoid confusing sandy riffles with hard-substrate streams where intensive sedimentation has occurred.

Acknowledgements

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Extraction of seed material from soil samples and comparison of DNA extraction methods for detecting loose silky bent (*Apera spica-venti*) seeds in soil

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Key words: *Apera spica-venti*, soil seed bank, molecular markers.

Apera spica-venti (L.) P. Beauv. (loose silky bent) is a common weed species in Europe, belonging to the Poaceae family. It primarily invades winter cereals but can also spread to other crops (Massa, Gerhards 2011). Over time, this species has expanded its presence to North America (Warwick et al. 1985). *A. spica-venti* can cause significant yield losses (Melander et al. 2008), and its impact is further exacerbated by the frequent occurrence of herbicide resistance (Soukup et al. 2006). Detecting *A. spica-venti* seeds in the soil seed bank is challenging due to their small size, which makes direct seed extraction difficult, while emergence-based detection method is time-consuming, and seed dormancy can further delay germination. Therefore, a fast and reliable molecular detection method would improve the assessment of the impact of different weed control strategies and cropping systems on the soil seed bank of this species. Our aim is to establish methods for seed and DNA extraction from soil, develop species-specific markers for *A. spica-venti*, and test the specificity of these markers.

Spiked soil samples were prepared using sieved loam soil free of seeds to which different seed counts were added, the seeds were then extracted from spiked soil samples using flotation method with 5.5 M K_2CO_3. Further, extracted samples where disintegrated using stainless steel beads after pre-treatment with liquid nitrogen. Subsequently, different commercially available lysing matrices were tested for pure seed sample disintegration. Additionally, homogenization was performed with variations in time and lysing buffer addition. For DNA extraction different commercially available kits (FastDNATM SPIN Kit for Soil; PureLinkTM Microbiome DNA Purification Kit; DNeasy PowerSoil Pro Kit) were tested as well as CTAB protocol. Two species-specific DNA markers were designed using

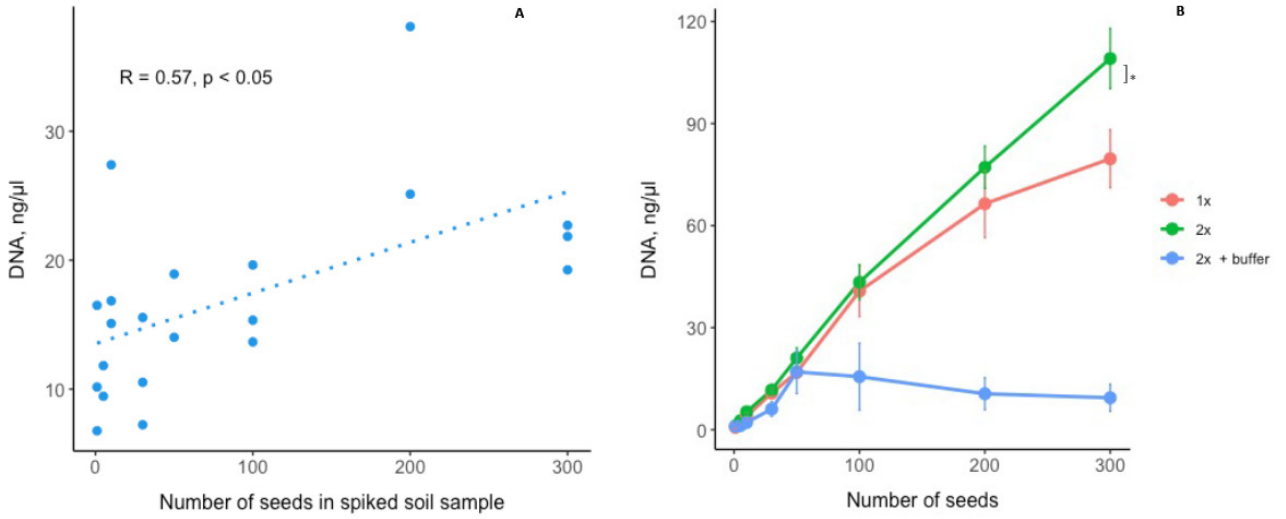

Fig. 1. The amount of extracted DNA depending on the seed count in the spiked soil sample (A); the amount of DNA extracted from seeds using a ceramic sphere from matrix A (MP biomedicals) processed with a bead miss once or twice for 30 s, with or without lysing buffer (B).

the Angiosperms353 probe set (Johnson et al. 2019) and tested using the extracted DNA with empty soil samples and Lolium perenne DNA as negative controls.

Results suggest the flotation method is an effective approach for extracting *A. spica-venti* seeds from soil samples as we were able to extract seeds from the spiked soil samples efficiently. A significant positive correlation was observed between the number of seeds in the spiked soil and the extracted DNA quantity (Fig. 1 A). Our homogenization tests showed that DNA yield was highest in samples processed twice in a bead mill with a ceramic sphere from matrix A (MP biomedicals) for 30 s (Fig. 1 B). Among the tested DNA extraction methods, the DNeasy PowerSoil Pro Kit proved to be the most suitable for our needs as it effectively removed DNA polymerase inhibitors. The designed species-specific markers demonstrated efficient amplification and specificity to *A. spica-venti*.

Acknowledgements

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Risk assessment of bacterial infections and antimicrobial resistance of *Aeromonas* spp. in sea trout and Baltic salmon spawners from the Venta River

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Key words: *Aeromonas* spp., antimicrobial resistance, Baltic salmon, sea trout.

The health status of spawners is a critical factor influencing the success of natural reproduction and the sustainability of fish stocks. Additionally, it determines the quality of juveniles produced through artificial propagation at state-owned fish hatcheries operated by the Institute of Food Safety, Animal Health and Environment "BIOR", which is responsible for restocking of fin-clipped smolts. Both sea trout (Salmo trutta) and Baltic salmon (Salmo salar) are anadromous species of the same genus. These species are among the most economically, ecologically, and socially significant fish, playing a crucial role in sustaining commercial and recreational fisheries along the Atlantic and Baltic coastlines (Matras et al. 2024).

This study aims to identify bacterial species in sea trout and Baltic salmon spawners from the Venta River and assess antimicrobial resistance (AMR) specifically in *Aeromonas* spp. Given the increasing concerns regarding AMR in aquatic ecosystems, the knowledge about its prevalence in wild and hatchery-origin spawners is crucial for sustainable fish stock management and disease prevention strategies.

This study was conducted during the 2023–2024 period, examining 49 salmonid spawners, including 23 sea trout and 26 Baltic salmon. The study included both hatchery-reared spawners (identified by a clipped adipose fin) and wild spawners.

Sterile swabs with transport medium were used to collect samples from the gills, skin mucus, urogenital openings, and lesions. Additional samples were taken from eggs at fertilization, incubation-stage embryos (eyed-egg stage), and post-mortem internal organs. In total, 190 samples were collected for laboratory analysis.

Isolated bacteria were identified using standardized microbiological methods, while AMR was determined by the disc diffusion method. AMR in *Aeromonas* spp. (n = 76) was assessed through susceptibility testing against commonly used antibiotics in aquaculture, including amoxicillin (25 µg), doxycycline (30 µg), enrofloxacin (5 µg), florfenicol (30 µg), gentamicin (10 µg), and oxytetracycline (30 µg). The results were presented in percentage resistance and case distribution among resistance categories (resistant, intermediate, and susceptible).

The multiple antibiotic resistance (MAR) index was determined using the method described by Krumperman (1983): MAR = a / b, where a represents the number of antibiotics to which the isolate was resistant, and b is the total number of antibiotics tested. MAR index values greater than 0.2 indicate a high potential for contamination.

Out of 190 samples, *Aeromonas* spp. were isolated in 125 samples (65.79%), *Pseudomonas* spp. in 93 (48.95%), *Shewanella* spp. in 74 (38.95%), and *Flavobacterium* spp. in 53 (27.89%). Other microbial flora was detected in 64 samples (33.7%). In several cases, multiple bacterial species and genera were isolated from a single sample, indicating a mixed infection. This finding suggests the presence of

Table 1. Antibiotic susceptibility pattern of *Aeromonas* spp. n = 76

Antibiotics	Resistant	Intermediate	Susceptible
Amoxicillin (25 µg)	76 (100%)	0 (0%)	0 (0%)
Doxycycline (30 µg)	0 (0%)	2 (2.63%)	74 (97.37%)
Enrofloxacin (5 µg)	0 (0%)	4 (5.26%)	72 (94.74%)
Florfenicol (30 µg)	8 (10.53 %)	1 (1.32%)	67 (88.16 %)
Gentamycin (10 µg)	0 (0%)	0 (0%)	76 (100%)
Oxytetracycline (30 µg)	1 (1.32%)	2 (2.63%)	73 (96.05%)

microbial diversity within the specific tissues where the bacteria were detected.

The distribution of *Aeromonas* spp. revealed that the most prevalent species were: *Aeromonas sobria* (21.95%), *Aeromonas bestiarum* (21.95%), *Aeromonas salmonicida* (20.73%) and *Aeromonas veronii* (15.85%). Less frequent species included *Aeromonas eucrenophila, Aeromonas encheleia, Aeromonas hydrophila, Aeromonas molluscorum*, and *Aeromonas popoffi*i.

Regarding antibiotic resistance, amoxicillin exhibited 100% resistance, with all 76 isolates classified as resistant. In contrast, doxycycline, enrofloxacin, and gentamicin showed no resistance (0%), with the majority of isolates classified as susceptible. Florfenicol displayed 10.53% resistance (eight resistant isolates), while oxytetracycline exhibited 1.32% resistance (one resistant isolate) (Table 1).

The MAR index exceeded 0.2 in 11 out of 76 *Aeromonas* spp. isolates (14.5%), indicating the presence of bacteria with multiple antibiotic resistance, though at a relatively low prevalence. The highest MAR index was observed in salmon, with a maximum value of 0.83 detected in a wild male salmon (gills). Additionally, a MAR index of 0.67 was recorded in a male salmon with a clipped adipose fin (ulcers). A high MAR index of 0.5 was also detected in several gill samples.

The results indicate the presence of both opportunistic and pathogenic bacteria in salmon and sea trout spawners from both wild and hatchery origins. The high prevalence of *Aeromonas* spp. in the samples suggests that these bacteria are a common part of the microbial flora in spawning fish, yet certain strains may pose a risk to fish health and aquaculture productivity.

The presence of resistant bacteria in both wild and hatchery-origin spawners suggests that AMR pollution is an emerging concern in aquatic ecosystems. Environmental contamination, including wastewater discharge, agricultural runoff, and aquaculture effluents, contributes to the spread of resistance genes in natural water bodies (Karkman et al. 2018; Marti et al. 2014).

Bacteria in aquatic environments can exchange resistance genes via horizontal gene transfer, further facilitating the persistence of AMR in wild fish populations (Baquero et al. 2008). The detection of a high MAR index in some isolates suggests that these resistant bacteria may serve as reservoirs for AMR genes, posing risks for both aquaculture and wild fish stocks.

These findings highlight the need for sustainable antibiotic use and enhanced AMR surveillance in both aquaculture and natural ecosystems. Implementing stricter regulations on antibiotic application in aquaculture, alongside improved biosecurity measures, could help mitigate the spread of AMR among fish populations.

Acknowledgements

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Exploring the growth substrates for stimulating the plastic-degrading potential of the novel isolate *Bacillus* sp. GPS-5.6

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Key words: biofilm formation, enzymatic activity, low-density polyethylene (LDPE), plastic biodegradation.

Low-density polyethylene (LDPE) poses a significant environmental challenge due to its resistance to biodegradation. Certain bacteria, including *Bacillus* spp., have shown the ability to colonize and degrade LDPE through biofilm formation and enzymatic activity (Nyamjav et al. 2023). However, the efficiency of bacterial plastic degradation is dependent on environmental conditions, including nutrient availability. This study investigates the influence of two different growth media (B1 and B2) on the plastic-degrading potential of *Bacillus* sp. GPS-5.6.

The experimental setup involved incubating *Bacillus* sp. GPS-5.6. for six weeks at 23 °C with LDPE granules (Goodfellow Corp., USA, nominal size 3 to 5 mm). The Liquid Carbon-Free Basal Medium (LCFBM) was supplemented by 5% Luria-Bertani broth (B1) or mixture of four carbon sources, i.e., α-D-glucose-1-phosphate dipotassium salt hydrate, DL-α-glycerol phosphate magnesium salt hydrate, 2-ketobutyric acid, meso-erythritol, 0.5% each (B2). After the incubation period, bacterial colonization and hydrolytic activity, as well as biodegradation efficiency were assessed. Biofilm formation was quantified using the Crystal Violet assay and hydrolytic activity was measured using fluorescein diacetate (FDA) hydrolysis according to Peterson et al. (2011) and Kandeler, Gerber (1988), respectively. Plastic degradation was evaluated through weight loss analysis of LDPE granules. To see the metabolic response of *Bacillus* sp. GPS-5.6 to LDPE exposure, bacterial growth was analyzed using Biolog EcoPlates™.

The results showed that B2 significantly ($p < 0.05$) enhanced biofilm formation (Fig. 1A), leading to a higher rate of LDPE degradation, with plastic weight loss reaching 0.79% in B2 compared to 0.63% in B1 (Fig. 1C). The FDA hydrolysis activity of biofilm did not differ among the sets with different broths (Fig. 1B).

Physiological profiling of bacterial cultures subjected to LDPE and different nutrient compositions was compared using Biolog EcoPlate™. Bacteria previously grown in B1, exhibited an enhanced growth in the presence of the following substrates: 4-hydroxy benzoic acid, glucose-

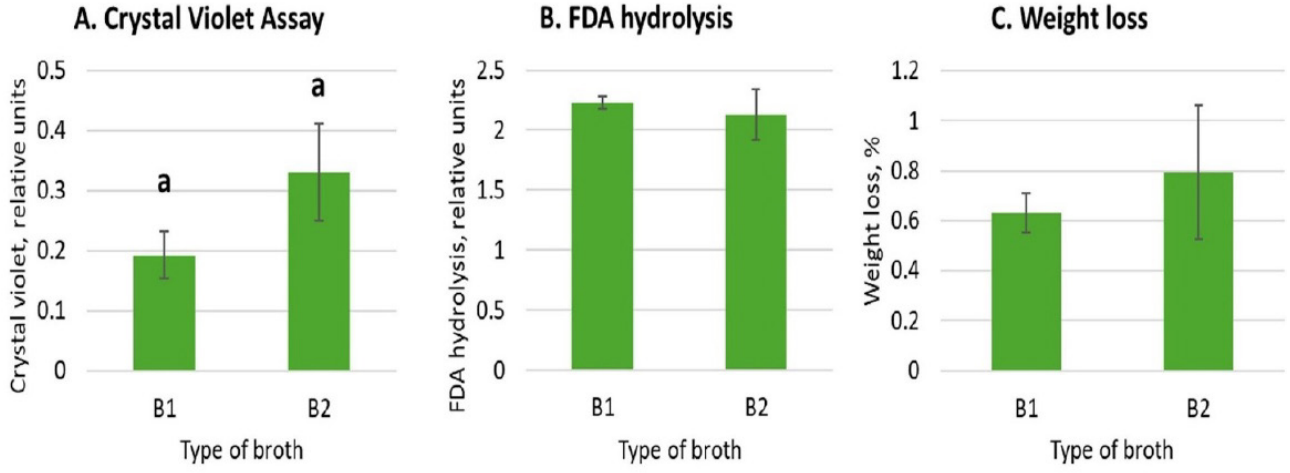

Fig. 1. Colonization and biodegradation of LDPE granules by Bacillus sp. GPS-5.6 after six-week incubation in the broth B1 and B2. A - Crystal Violet assay; B - FDA hydrolysis; C – Weight loss. Statistically significant differences between groups ($p < 0.05$) are indicated by the same letter above bars (A).

1-phosphate, itaconic acid, and 2-hydroxybenzoic acid. Additionally, an in-depth analysis of specific carbon sources revealed that bacterial growth was enhanced in the presence of DL-α-glycerol phosphate, 4-hydroxybenzoic acid, and α-ketobutyric acid, depending on the pre-incubation conditions. These results suggest that nutrient availability can influence *Bacillus* sp. GPS-5.6 metabolic capacity to degrade plastic, namely LDPE.

Acknowledgements

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Microplastics inhibitory effect on microbial activity of activated sludge demonstrated by urease and fluorescein diacetate hydrolysis

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Key words: microplastics, synthetic wastewater, microbial activity.

Global plastic production is steadily increasing, while a minor fraction of it is obliged to be recycled. Approximately 79% of used plastics accumulate in natural ecosystems. Plastic can either leak into the environment as macroplastic, with its size being bigger than 5 mm, or as microplastic. Microplastic is defined with a size ranging from ≥ 1 to < 5 mm (Walker, Fequet 2023). Microplastic pollution of ecosystems is an issue due to its low degradability, its potential to biomagnify, which ultimately affects animals on higher trophic levels, including humans, and its high adsorption capability, leading to contaminants attaching to microplastic surfaces (Curren et al. 2020; Zeytin et al. 2020; Dalu et al. 2023). In wastewater treatment plants, microplastic also occurs ubiquitously in influent and effluent. To this date, no microplastic-specific removal strategy is employed. Nevertheless, microplastic removal efficiency is stated to be approximately 88%, with most of it accumulating in the activated sludge (Sun et al. 2019).

This research was focused on the impact of polyethylene terephthalate (PET) on the biochemical activity of the microbial community in real activated sludge while using synthetic wastewater with a chemical oxygen demand (COD) of 500 mg L^{-1}. Fluorescein diacetate (FDA) hydrolysis was assessed as an indicator of microbial activity and an indirect measure of microbial biomass. Urease activity enables conclusions on the ability of the community to denitrify the wastewater. Fig. 1 shows both urease activity (B) and FDA hydrolysis (A) during the incubation period of 14 days.

FDA hydrolysis declines for all tested PET

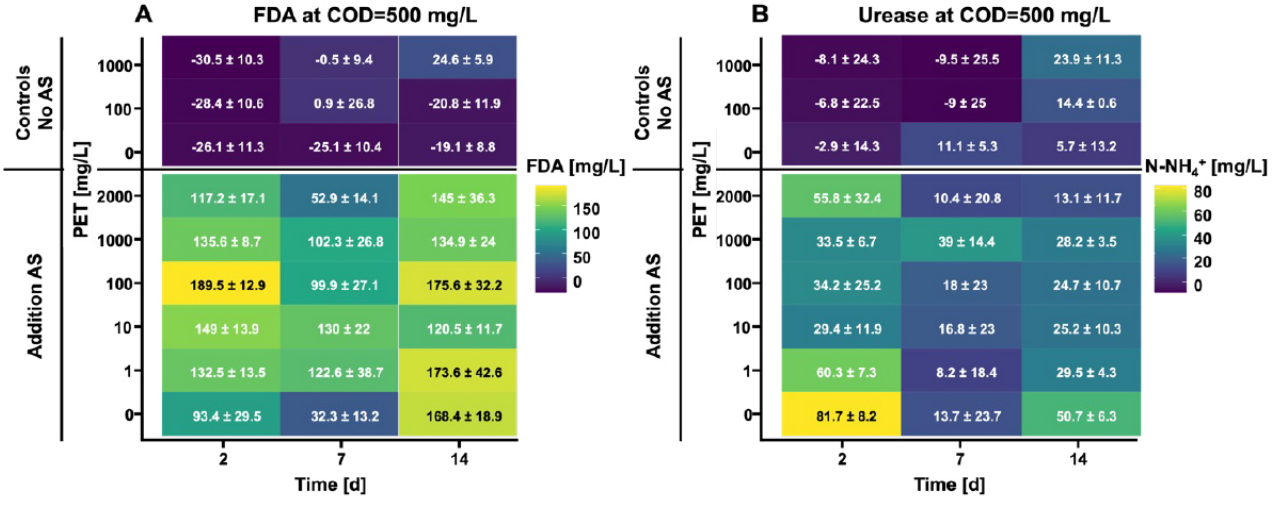

Fig. 1. Urease activity and fluorescein diacetate (FDA) hydrolysis of the bacterial community in activated sludge over 14 days. A shows the FDA hydrolysis in mg FDA per liter at COD = 500 mg L^{-1}, while B shows the urease activity in NH_4^+ mg per liter at COD = 500 mg L^{-1}. Values are shown for PET addittion of 0, 1, 10, 100, 1000, 2000 mg L^{-1} with addition of activated sludge below the horizontal black line, and for 0, 100 and 1000 mg L^{-1} without activated sludge addition above the black line. Means are shown with their respective standard deviations (n = 3).

concentrations when measured after 7 days. After the total incubation period of 14 days, FDA hydrolysis increases when compared to 7 days, and increases or remains the same when compared to 2 days. The same pattern is true for urease activity, meaning, that microbes being part of the microbial nitrogen cycling system were also affected by this lowered activity. When urease activity is lowered a consequence is less available NH_4^+ for microbes performing nitrification, which could influence all subsequent steps in nitrification and therefore contribute to an overall limited microbial activity. In conclusion, the microbial activity could be a result of limited nitrification due to lower amounts of NH_4^+ being present. Additionally, urease is sensitive to toxic compounds, including microplastic, which could be responsible for lowered activities (Khalid et al. 2023). Decreased activity may also be attributed to the differences between synthetic wastewater and the habitant from which the activated sludge was isolated, being real wastewater.

Yet, urease in combination with other hydrolytic enzymes, has capabilities to degrade/depolymerize polyurethane, polyesters, and other polymers (Chandra et al. 2020). Therefore, urease activity is a rough indicator for microplastic degradation. These results demonstrated the toxic effect of PET powder on the microbiota of activated sludge.

Further research could focus on assessing other parameters for the participation of microbes in the nitrogen cycle, to broaden the scope. Alternatively, a full assessment of nutrients available to the microbes could help to shed light on the environmental conditions.

Acknowledgements

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Characterization of novel isolates of arbuscular mycorrhizal fungi from Latvian soils

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Key words: arbuscular mycorrhizal fungi, spore isolation, agricultural soils, wet sieving.

Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with plant roots, playing a crucial role in ecosystem sustainability by enhancing soil fertility, promoting plant health, and improving crop productivity (Bhantana et al. 2021). Isolating AMF spores from soil allows for the evaluation and identification of potentially new beneficial AMF species, as well as the characterization of their associated rhizospheric communities (Zhang et al. 2021). Understanding their ecology, diversity, and potential applications in environmental management and agriculture requires investigating their interactions with various crop species under different ecological conditions. This approach could contribute to the development of sustainable agricultural practices, not only through laboratory studies but also by examining field conditions (Garrido et al. 2023; Wang et al. 2025). This study aims to investigate AMF communities in soil samples using wet sieving and sucrose gradient methods (Boyno et al. 2023). We prepared two types of samples: a natural sample (50 g of soil) and a spiked mock sample (50 g of soil + 2000 *Rhizophagus irregularis* spores). The wet sieving method was used to separate the spores from the soil through a series of sieves (2 mm, 1 mm, 500 µm, 100 µm, and 40 µm), followed by the application of a sucrose gradient. The separated spores were then evaluated using light microscopy (Fig. 1). Ongoing experimental work will provide novel data that will help assess the role of AMF communities in arable soils in Latvia, offering valuable insights into the potential use of AMF as an organic fertilizer.

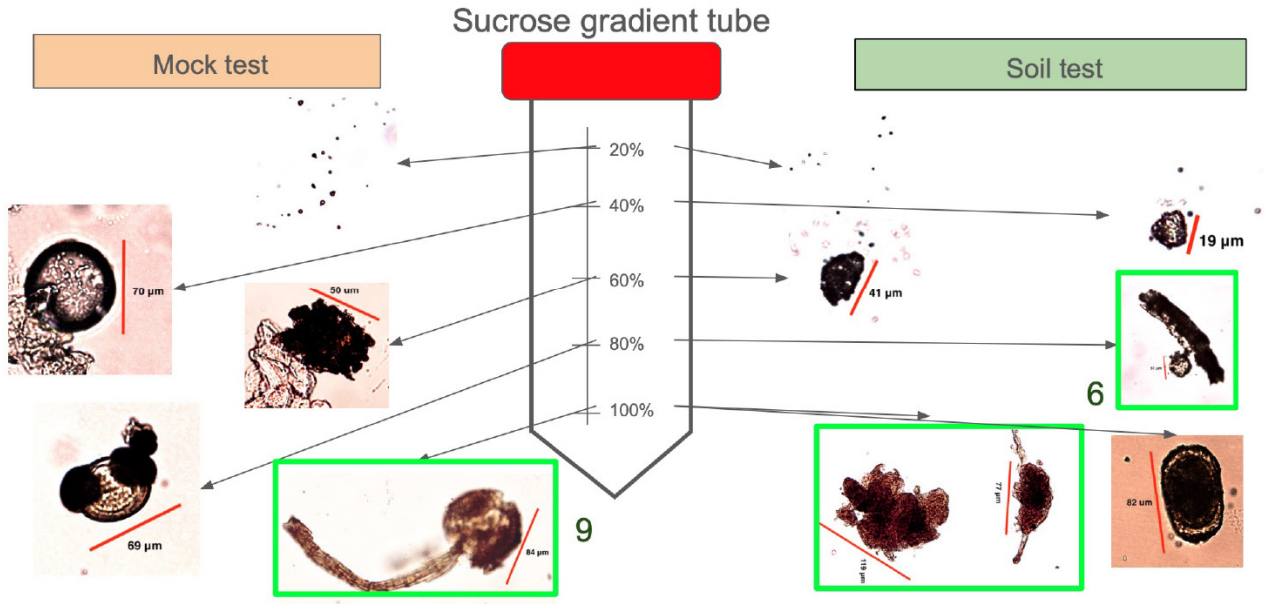

Fig. 1. Schematic representation of different biological objects found in the sucrose gradient tube in the test and spiked mock samples at 140× magnification. The arrows show the sucrose gradient that the sample was collected from. The highlighted green boxes represent AMF spores. The number of spores within the respective sucrose fraction is indicated outside the box. Bars indicate the object size.

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Physiological response of the bacterial consortium GPS-2024 towards physico-chemically pre-treated microplastics

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Key words: plastic biodegradation, plastic granules, enzymatic activity, bacterial consortium.

Plastic waste accumulation is a growing global issue due to its persistence. Biodegradation offers a promising solution, but the mechanisms and strategies to enhance this process still remain poorly understood. Some of the strategies to enhance plastic biodegradation, include UV, thermal, and chemical pretreatments that increase microbial susceptibility (He et al. 2024). This study examines the physiological response of the microbial consortium GPS-2024 isolated from Getliņi landfill (Latvia) to untreated and pretreated low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polyethylene terephthalate (PET) granules. Understanding these microbial responses can provide insights into the potential of bacterial consortia for bioremediation strategies, contributing to a more sustainable plastic waste management.

For this experiment the LDPE, HPDE, and PET granules (nominal size of 3 to 5 mm, Goodfellow Corp, US) were pre-treated with acid (0.2 N H_2SO_4) or base (0.2 N NaOH) solution for 24 h and then neutralized with the opposite solution in an equal amount. Afterwards the granules were treated thermally at 80 °C in a dry heat sterilizing unit for 24 h. The experiment was performed in 150 mL polypropylene (PP) sterile vessels with lids (Deltalab, Spain), kept in the dark at room temperature (~23 °C), in triplicate for each condition. Bacterial consortium GPS-2024 (unpublished) was prepared by mixing 23 individual cultures incubated in Luria-Bertani (LB) broth for 48 h at 30 °C. The cells were concentrated by centrifugation, rinsed twice and resuspended in 0.85% sterile NaCl at 10^7 CFU mL^{-1}. An inoculum volume of 120 µL was added to 30 mL Liquid Carbon-Free Basal Medium supplemented with 5% LB broth and exposed to three polymer granules of the same type. The experiment was run for 6 weeks. The following analysis methods were used during and after incubation. The activity of planktonic cells was evaluated by performing FDA hydrolysis test (Chen et al. 1988), potential ammonium oxidase (PAO) test (Belser, Mays 1980) and urease (URE) activity test (Kandeleret al. 1988). Enzyme assays were carried out in 96-well microplates at 30 °C. The scanning electron microscopy (SEM) images were taken with a TM300 Tabletop Microscope SEM (Hitachi, Japan).

The microscopy results demonstrated bacterial colonization on the surface of PET, LDPE and HDPE (Fig. 1). However, SEM images of new plastic granules revealed inherent surface roughness, limiting its effectiveness in assessing surface changes.

The PAO activity after 21 days was inhibited by all tested plastic types by 63 to 86% of control, except LDPE(A+Th) and LDPE(B+Th). After 42 days the most inhibited PAO activity was detected in PET(U) (49% of control), while the highest activity – in three types of HDPE, which exceeded the control by 9 to 19% (Fig. 2A). Urease activity after 21 days was inhibited by all plastic types tested, with the lowest activity being in PET(B+Th) (56% of control). After 42 days, PET(B+Th) remained to be the most inhibited, while LDPE(B+Th) and HDPE(B+Th) showed an enhanced

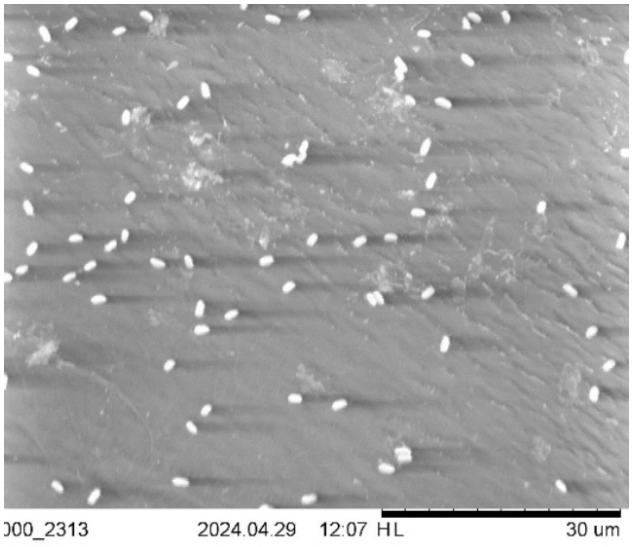

Fig. 1. SEM micrograph of untreated HDPE granule with biofilm after 21 days of incubation. Scale bar 30 mm.

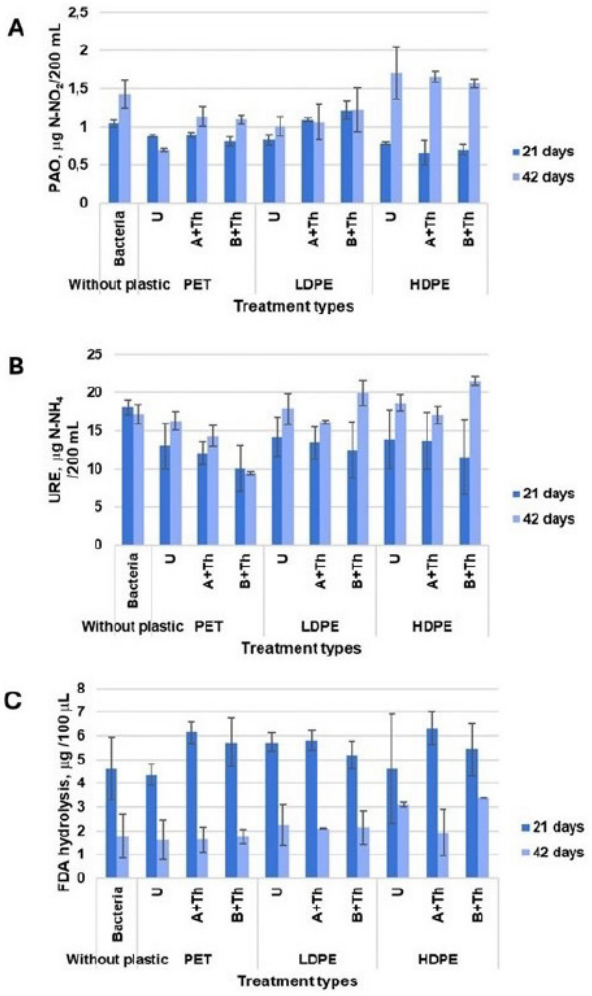

Fig. 2. Enzymatic activity of bacterial consortium in the presence of untreated and pre-treated plastic. A, PAO; B, URE; C, FDA hydrolysis. Period of incubation 90 min. U, untreated; A+Th, acidic and thermal treatment; B+Th, base and thermal treatment.

urease activity, i.e., 16 and 26% of control, respectively (Fig. 2B).

The FDA hydrolysis activity after 21 days demonstrated a stimulating effect of plastic granules on bacteria (12 to 37%), except PET(U) and HDPE(U), where the FDA hydrolysis activity was comparable with the control. After 42 days the activity in three PET types was comparable with respective control, while all LDPE and HDPE types stimulated FDA hydrolysis with the highest values found in HDPE(U) and HDPE(B+Th), i.e., 73 and 92%, respectively (Fig. 2C).

PET granules inhibited bacterial activity irrespectively of pre-treatment type, while LDPE and HDPE mostly stimulated the activity of three enzymatic groups tested, after 42 days of incubation. Pre-treatment of granules did not differ considerably in their effect on bacterial activity. To reveal a more pronounced effect of plastic pretreatment on microorganisms, further study will examine the effect of higher concentrations of acids and base for a longer period.

Acknowledgements

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Microbial plant stimulants on the Lithuanian market

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Key words: bacteria, fungi, *Bacillus, Rhizobium, Trichoderma, Glomus.*

Microbial plant stimulants are composed of beneficial microorganisms that aim to enhance the plant growth cycle, stress tolerance and crop productivity by interacting with both crop physiology and soil processes (Khalid et al. 2021). Microbial plant stimulants like other plant biostimulants are emerging as an essential component in sustainable agricultural practices (Mangena et al. 2025).

In Lithuania, microbiological stimulants such as Rhizobium bacteria have been used for almost a hundred years (Lapinskas 2008) and *Trichoderma*-based products also fifty years in use (Lugauskas, Radziaviciene 1974). Over the last two decades, as the ecological crisis has deepened and the EU's Green Deal has gained momentum, new and more diverse microbiological stimulants for agricultural crops have been added to the domestic market every year.

Data on microbiological crop stimulants available on the Lithuanian market in 2025 were collected from fourteen companies, three supermarkets and two online shops. In total, 38 products were found, of which six did not provide the full composition of the product (online shops) and were not included in the discussion of the results. Of these, five products (15.6%) were listed as organic residue decomposition accelerators, nine (28.1%) as soil amendments, 17 (53.1%) as plant growth promoters and only one (3.1%) was listed as biopesticide. The microbiological products contained a total of 45 species of micro-organisms, including 31 species (68.9%) of bacteria and 14 species (31.1%) of fungi (Fig. 1).

The most abundant bacteria in the products tested were of the genus *Bacillus* (Fig. 1), with a total of 11 species found. Among *Bacillus*, *Bacillus megaterium* was the most frequent species, followed by *Bacillus amyloliquefaciens* and *Bacillus subtilis*, while *Bacillus polymyxa, Bacillus licheniformis* and *Bacillus mojavensis* were less frequent. The group of nitrogen-fixing bacteria was also among the most abundant in the bio-products studied. The symbiont diazotrophs *Rhizobium leguminosarum* and species of genus *Bradyrhizobium* were amongst the most frequent, as were free-living bacteria *Azospirillum brasilense, Azotobacter* and in particular *Azotobacter salinestris*. In

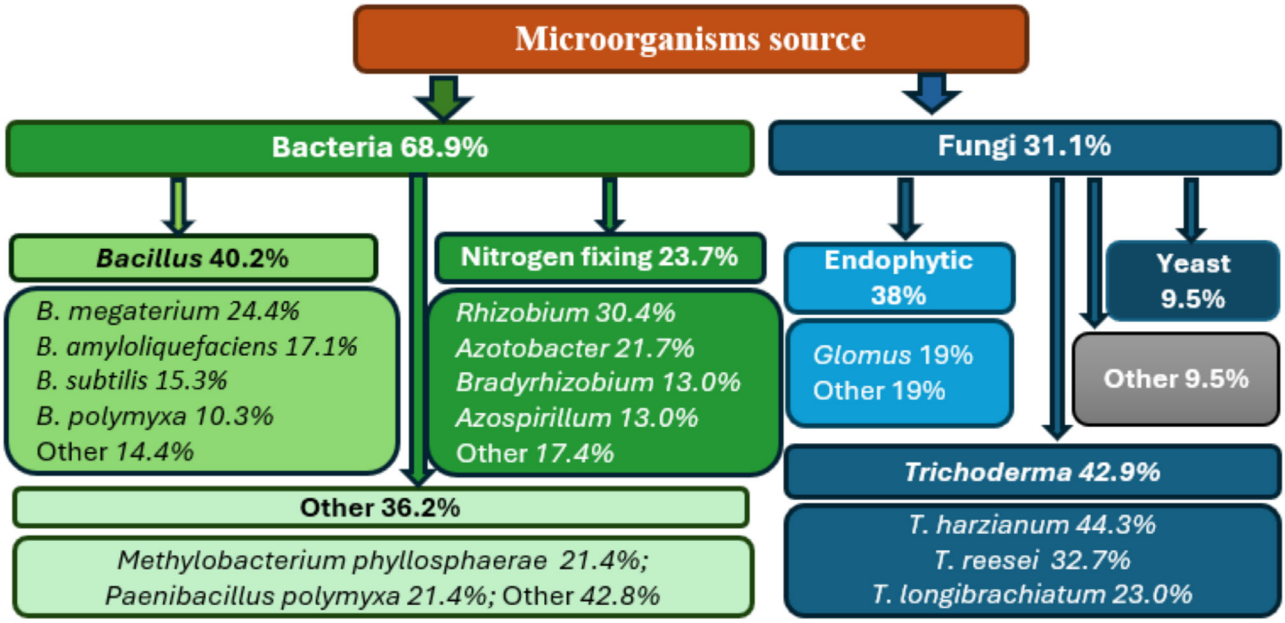

Fig. 1. Microorganisms in plant stimulants on the Lithuanian market (2025).

microbial plant stimulants the methylotrophic bacterium *Methylobacterium phyllosphaerae* was also one of the most frequent species.

The most common in microbial plant stimulants were fungi of the genus *Trichoderma*. Three species were found, of which *Trichoderma harzianum* was the most abundant followed by *Trichoderma reesei* and *Trichoderma longibrachiatum*. Arbuscular mycorrhiza fungi of the genus *Glomus* were also among the most frequent. Endophytic fungi such as *Phoma radicis, Byssoascus, Phialocephala fortinii* and *Razizella ericae* were found only once each. Two yeast species were also found in the products examined: *Meyerozyma guilliermondii* and *Saccharomyces cerevisiae*. Microbiological stimulants usually contained a complex of microorganisms rather than single species.

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