THE 67TH INTERNATIONAL CONFERENCE

OPEN READINGS
PUPILS' SESSION

BOOK OF ABSTRACTS

2024

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OPEN READINGS

67TH INTERNATIONAL CONFERENCE

OPEN READINGS 2024 PUPILS' SESSION

2024-04-26, Life Sciences Center

Lobby		REGISTRATION	**8:00-9:00**
Room: 202		Opening Ceremony	**9:00-9:10**
Room: 202	 **Algirdas Toleikis** | PLENARY TALK	**Molecular Motors – Driverless Cars within our Cells**	**9:10-9:40**
Room: 202	Liepa Raudoniūtė	EVALUATION OF ELOVL6 FTO MC4R AND PPARG POLYMORPHISMS ASPROGNOSTIC BIOMARKERS OF INSULIN RESISTANCE IN TYPE 2 DIABETES PATIENTS	**9:40-10:00**
Room: 202	 **Domas Paipulas** | PLENARY TALK	**Photonic Paintings in Glass: Science and Art of Direct Laser Writing**	**10:00-10:30**
Room: 202	Viltė Butkevičiūtė	BLUE LIGHT EMITTING DIODES SYNTHESIS PATHWAY SEARCH AND PERFORMANCE USING SYNTHIA (ARTIFICIAL INTELLIGENCE)	**10:30-10:50**
Room: 202	 **Aivaras Kareiva** | PLENARY TALK	**Design of Implantable Bioceramic Materials**	**10:50-11:20**
Room: 202	Kamilė Milkintaitė	EPITRANSCRIPTOMIC STUDIES OF BRAIN TUMOR ANALYSIS OF GENE EXPRESSION IN TUMOR CELLS AND TUMOR TISSUES	**11:20-11:40**
		Break	**11:40-12:30**
Center for Physical Sciences and Technology (FTMC), main hall		POSTER SESSION	**12:30-13:45**
Center for Physical Sciences and Technology (FTMC), A101		AWARDS	**13:45-14:00**
		Excursion in the Center for Physical Sciences and Technology	**14:00-15:00**
Life Sciences Center *For participants only		Workshop by iGEM*	**15:00-16:30**

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EVALUATION OF *ELOVL6, FTO, MC4R,* AND *PPARG* POLYMORPHISMS AS PROGNOSTIC BIOMARKERS OF INSULIN RESISTANCE IN TYPE 2 DIABETES PATIENTS

Liepa Raudoniūtė[1], Gabija Paulauskaitė[1], Ugnė Meškauskaitė[1,2]
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Scientific field: Biology

Introduction. Type 2 diabetes (T2D) is one of the most prevalent chronic metabolic diseases worldwide[1]. Insulin resistance, impaired glucose tolerance and absorption, and hyperglycemia are common characteristics of T2D patients[2]. Although a sedentary lifestyle, a Western diet, and obesity all contribute to the development of IR and T2D, scientists are increasingly focusing on genetic variations[3]. SNPs in genes responsible for energy homeostasis, glucose and lipid metabolisms, such as *ELOVL6* rs17041272, *FTO* rs9939609, *MC4R* rs17782313, and *PPARG* rs1801282, have been found to increase the risk of developing the two main risk factors for T2D: insulin resistance and obesity[4–7].

Objective. The aim of this study was to investigate the prevalence of *ELOVL6* (rs17041272), *FTO* (rs9939609), *MC4R* (rs17782313), and *PPARG* (rs1801282) SNPs in T2D patients (with and without severe insulin resistance) and healthy individuals (control), and to assess the relationship between these SNPs and T2D and insulin resistance.

Methods. Genomic DNA was extracted from 164 peripheral blood samples collected from 52 patients with T2D, 58 patients with T2D and severe IR, and 54 subjects without T2D (control). *ELOVL6* rs17041272 and *FTO* rs9939609 SNPs were genotyped by PCR-RFLP method, while *MC4R* rs17782313 and *PPARG* rs1801282 SNPs were genotyped by ARMS-PCR. Amplified DNA fragments were separated on 2% agarose gel. The results were evaluated using the Chi-square, Fisher`s exact test, One-Way ANOVA, t-test, correlation, and multiple linear and logistic regression. Results were considered statistically significant with a p-value ≤ 0.05.

Results. Association tests showed that the *MC4R* (rs17782313) polymorphism is significantly associated with T2D and severe IR ($p = 0.013$). In the case of this polymorphism, the C allele and CC genotype were statistically significantly more frequent in T2D patients with severe IR. Blood HbA1c level was significantly higher in subjects with CC genotype (10.1 ± 2.5%) than with TT+CT genotypes (7.9 ± 2.2%) ($p = 0.02$). Although the prevalence of *FTO* (rs9939609) polymorphism did not differ between the studied groups ($p = 0.517$), blood HbA1c level (AA: 8.6 ± 2% *vs.* TT+AT: 7.8 ± 2.3%) and HOMA-IR index (AA: 18.9 ± 25.8 *vs.* TT+AT: 17.7 ± 38) were significantly higher in subjects with AA genotype than with TT+AT genotypes, respectively $p = 0.026$ and $p = 0.036$. Meanwhile, statistically significant associations between *ELOVL6* (rs17041272) and *PPARG* (rs1801282) SNPs and T2D as well as severe IR were not found.

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[1] Zhou, Z., Sun, B., Yu, D., Zhu, C. (2022). Gut microbiota: an important player in type 2 diabetes mellitus. Frontiers in cellular and infection microbiology, 12, 834485.

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ASSESSING THE PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN LITHUANIAN SOILS

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Scientific field: Biology

The overuse of antibiotics in medicine and agriculture has fueled the emergence of antibiotic-resistant bacteria, posing a significant threat to global public health [1]. These resistant bacteria are not only found in hospitals but also persist in environmental reservoirs like soil [2]. This research investigates the prevalence of antibiotic-resistant bacteria in Lithuanian soil and explores the potential link to human activities.

Soil samples were collected from ecologically clean and contaminated areas across Lithuania. Clean areas included national parks and forests with minimal human intervention. Contaminated areas were identified based on past military activity, livestock farming, and fertilizer use.

The research employed a serial dilution technique to quantify the bacterial populations and assess their antibiotic susceptibility. One gram of each soil sample was diluted tenfold six times, resulting in a series of dilutions from 10^{-1} to 10^{-6}. A portion of each dilution was then plated onto agar media. Two sets of plates were prepared for each sample: one set without antibiotics (control) and two sets containing tetracycline, a common antibiotic, at low (3 µg/ml) and high (30 µg/ml) concentrations. After incubation, the colonies on each plate were counted to estimate the total bacterial count (CFU/g of soil) and the percentage resistance to tetracycline at different concentrations.

Bacterial identification was performed on colonies exhibiting low and high tetracycline resistance isolated from contaminated and clean areas. This involved isolating the bacterial DNA, amplifying 16S rRNA gene segment using a Polymerase Chain Reaction (PCR) technique, and then sequencing the amplified DNA by the Sanger method. The specific bacterial species were identified by comparing the obtained sequences to SILVA database.

The findings suggest a possible link between anthropogenic activity and antibiotic resistance. Soil samples from contaminated areas exhibited a higher percentage of bacteria resistant to low and high tetracycline concentrations (3 µg/ml and 30 µg/ml, respectively) compared to samples from clean areas. Furthermore, bacterial species identification revealed the presence of *Stenotrophomonas maltophilia* – a bacterium known for complex drug resistance – in a highly tetracycline-resistant sample from a contaminated area [3] [4]. These findings highlight the potential public health concerns associated with increased antibiotic resistance in the environment.

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ANTIBIOTIKAMS ATSPARIŲ BAKTERIJŲ UPĖJE ŠALČIA PAPLITIMO IR ĮVAIROVĖS TYRIMAS

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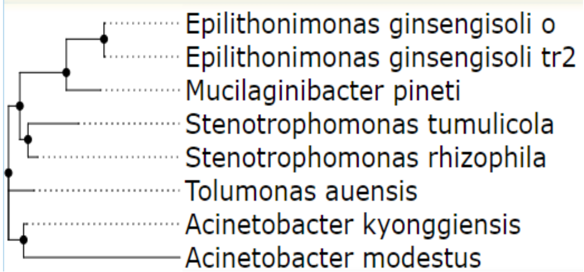
Įvadas. Bakterijų atsparumas antibiotikams yra sparčiai progresuojanti problema ir iššūkis visame pasaulyje. Šis reiškinys kelia nerimą tiek medicinos specialistams, tiek visuomenei [1]. Jeigu neišspręsti šios problemos, gydymas antibiotikais gali tapti neveiksmingas. Esant atsparioms bakterijoms paprastos infekcijos gali tapti nevaldomos ir pavojingos [2]. Bakterijos tampa atsparios antibiotikams dėl natūralių evoliucijos procesų, žmonių veiksmų, tokių kaip neteisingas vaistų vartojimas ar pernelyg didelis jų naudojimas, piktnaudžiavimas jais ūkiuose. Antropogeninis poveikis vandens telkiniams gali paskatinti antibiotikams atsparių bakterijų plitimą.

Tyrimo tikslas. Ištirti antibiotikams atsparių bakterijų paplitimą upėje Šalčia bei jos užtvankose (viešuose tvenkiniuose) bei nustatyti jų genetinę įvairovę.

Metodai. Iš skirtingų tiriamųjų vietovių surinkti vandens mėginiai skiedžiami serijiniu būdu ir užsėti ant Petri lėkštelių su R2A terpe be antibiotikų, su maža koncentracija tetraciklino ir su didele koncentracija tetraciklino. Po trijų parų inkubavimo nustatomas kolonijas formuojančių vienetų kiekis ir procentalus bakterijų atsparumas skirtingoms antibiotikų koncentracijoms. Siekiant atlikti antibiotikams atsparių bakterijų rūšies nustatymą, atrenkamos antibiotikui atsparios bakterijų kolonijos, iš jų išgryninta DNR ir pagausintas 16S rRNR genas taikant polimerazės grandininės reakcijos (PGR) metodą. Su tikslu patvirtinti amplikono ilgį bei koncentraciją, atliekama elektroforezė ~1,5 % agarozės gelyje. Gauti rezultatai įvertinti naudojant transiliuminatorių. Amplikonai, kurių 16S genas pasigausino ir vizualiai įvertintas gelyje kaip 1500 bp fragmentas, sekvenuojami Sangerio metodu. Gavus sekoskaitos duomenis, analizuojamos sekoskaitos chromotogramos, sekos sutvarkomos ir lygiuojamos į SILVA duomenų bazę [3].

Rezultatai. Kiekviename mėginyje buvo aptiktos mažai (3 ug/ml) antibiotikų (tetraciklino) koncentracijai atsparios bakterijos. Rečiau buvo aptinkamos didelei (30 ug/ml) antibiotikų (tetraciklino) koncentracijai atsparios bakterijos. Nustatyta, kad mažai antibiotikų koncentracijai yra atsparios 5,4% bakterijų, tuo tarpu didelei koncentracijai iki 0,15%. Sudarytame filogenetiniame medyje (1 pav.), matyti, kad nemaža dalis aptinkamų vandenyje bakterijų yra giminingos.



1 pav. Tyrimo metu nustatytų antibiotikams atsparių bakterijų rūšių filogenetinis medis.

Išvada. Įvertinus esamus tyrimo rezultatus pastebėta, kad tiriamajame tvenkinyje yra aptinkamos antibiotikams atsparios bakterijos. Didžiausią atsparumą turinčios bakterijos buvo aptinkamos užtvankose (viešuose tvenkiniuose). Iš gautų sekvenavimo duomenų, galima teigti, jog ištirtuose telkiniuose dominuoja tam tikros bakterijos, turinčios bendrą kilmę. Gauti duomenys gali būti svarbus norint suprasti bakterijų atsparumo antibiotikams paplitimo dinamiką ir jų įvairovę aplinkoje.

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[1] World Health Organization. (2023) Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
[2] Centers for Disease Control and Prevention. How Antimicrobial Resistance Happens. https://www.cdc.gov/drugresistance/about/how-resistance-happens.html
[3] SILVA ribosomal RNA database. https://www.arb-silva.de

CONCENTRATION OF ANTIBIOTIC-RESISTANT BACTERIA IN NEMUNAS AND THEIR CONNECTION TO OXYGENASE ENZYMES: A PILOT STUDY

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Introduction. Antibiotic resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 [1]. It needs to be regulated and the source of it needs to be studied. It is known that antibiotic resistance is mainly caused by pollution, thus to solve the issue first we need to know what kind of pollution causes it and where it comes from [2].

Initial mechanisms of resistance to antibiotics among pathogens are mostly via chromosomal mutations leading to the overexpression of efflux pumps. High-level resistance is achieved only after the acquisition of flavin-dependent monooxygenase-encoding genes from the environmental microbiota [3]. This is a reason for researching oxygenase enzymes alongside antibiotic resistance. In addition, these enzymes also have the potential to be a biomarker of pollution [4]. However, there is a lack of research on this topic, creating a gap of knowledge that narrows our insight into the antibiotic resistance issue. The above-mentioned arguments prompted us to plan this pilot study to see if we can claim the link between antibiotic resistance and water pollution in Nemunas river.

Goal. To determine the concentration of antibiotic-resistant bacteria in different locations of the river Nemunas and to compare the data to levels of multiple pollutants in the same areas. To assess the relationship between the production of different oxygenase enzymes and antibiotic resistance in bacteria.

Methods. The sampling locations are based on the research on pollution levels in the river that is done by the municipality of Kaunas. Antibiotic resistance is measured by planting the samples on Petri dishes with no antibiotic, 3 µL/ml, and 30 µL/ml of tetracycline after serial dilutions. The colonies are calculated using the "ImageJ" application. The oxygenase enzymes are recognized by growing samples on Petri dishes containing Indole (purple pigment made), 2-methylindole (yellow pigment made), 5-bromoindole (blue pigment made), and 5-nitroindole (brown-red pigment made). DNA from the bacteria is extracted using the Zymo research Quick-DNA Miniprep Kit. The 16s region is duplicated using the PCR method and then tested by electrophoresis with a 1,5% gel and sent off to be sequenced by Sanger's method. The sequencing results are compared to the SILVA 16S database to determine the species.

Results. Analysis of this pilot study data showed that the concentrations of antibiotic-resistant bacteria colonies were different throughout the river and the concentration at the start of the city of Kaunas was lower than at the end of the city (medium-resistant bacteria went from 0,65% to 9,01%). When compared with the accessible pollution data of the same locations, no strong connection could be found, so we do not know yet what pollutant is responsible for this resistance. In the future, we are planning to also put the indole substrates with the antibiotics, to see what percentage of the resistant bacteria use the tet(x) mechanism of resistance.

The bacteria that exhibited oxygenase-producing properties were determined to be: *Janthinobacterium lividum*, *Pseudomonas poae*, *Paenibacillus pabuli,* and *Flavobacterium panacisoli.* None of these bacteria grew on a Petri with 30 µL/ml of tetracycline. However, we did not manage to do any further experiments with these bacteria.

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[1] What Exactly is Antibiotic Resistance? (2022, October 5). Centers for Disease Control and Prevention. https://www.cdc.gov/drugresistance/about.html

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Eterinių aliejų antibakterinių savybių prieš karieso sukėlėją *Streptococcus mutans* tyrimas

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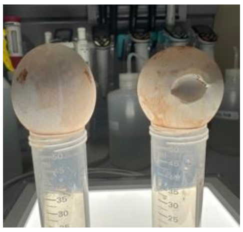
Įvadas. Pasaulio sveikatos organizacijos (PSO) duomenimis (2022 m.) apskaičiuota, kad burnos ertmės ligomis serga beveik 3,5 mlrd. žmonių visame pasaulyje, o 3 iš 4 sergančiųjų gyvena vidutines pajamas gaunančiose šalyse. Apskaičiuota, kad pasaulyje 2 mlrd. žmonių kenčia dėl nuolatinio dantų ėduonies, o 514 mln. vaikų - dėl pieninių dantų ėduonies [1]. Nors burnos ertmės ligų iš esmės galima išvengti, jos yra didelė sveikatos našta daugeliui šalių ir paveikia žmones visą gyvenimą, sukeldamos skausmą, diskomfortą, subjaurojimą ir net mirtį. Svarbu paminėti, jog burnos sveikatos sutrikimų gydymas yra brangus ir nėra įtrauktas į visuotinio sveikatos draudimo (VSS) sistemą [2]. Streptococcus mutans - pagrindinė karieso sukėlėja, prisitaikanti prie rūgščios aplinkos ir fermentuojanti sacharozę, dėl to demineralizuoja dantų emalį [3]. Antibiotikų naudojimas prieš šią bakteriją - neefektyvus ir rizikingas dėl atsparių štamų atsiradimo. Tačiau eteriniai aliejai ne tik efektyviai slopina Streptococcus mutans augimą, bet ir gerokai mažesnėse koncentracijose už MSK paveikia šių bakterijų metabolizmą eliminuojant jų patogeniškumą

Tyrimo tikslas. Ištirti eterinių aliejų antibakterinį poveikį prieš Streptococcus mutans ir nustatyti jų galimybę slopinti kariesą sukurtoje modelinėje sistemoje.

Tyrimo metodai. Eterinių aliejų antibakterinis poveikis buvo nustatomas naudojant serijinį skiedimo metodą, matuojant minimalią slopinančią koncentraciją (MSK). Buvo sukurta karieso modelinė sistema, kurioje buvo naudota Todd-Hewitt-Bouillon mitybinė terpė, Streptococcus mutans bakterijos, kininių cinamonų žievės eterinis aliejus bei kiaušinis. Sukurtas modelis buvo stebimas tris savaites ir buvo vertinami gauti vizualūs tyrimo rezultatai.

Rezultatai. Tarp visų šešių ištirtų eterinių aliejų, tyrimų rezultatai atskleidė, kad kininių cinamonų žievės eterinis aliejus efektyviai veikia prieš Streptococcus mutans bakterija net 0,015625% koncentracijoje (Lentelė Nr. 1.). Karieso modeline sistema parodė, kad šis aliejus efektyviai apsaugo kiaušinio lukštą nuo bakterijų sukeliamos erozijos. Kiaušinio lukštas buvo stipriai pažeistas, augant mitybinėje terpėje be eterinio aliejaus (1 pav.). Tačiau terpėje su eteriniu aliejumi, net esant dešimt kartų mažesnei nei minimali slopinamoji koncentracija (MSK) eterinio aliejaus koncentracijai, buvo indukuotas bioplėvelės formavimasis. Tai atskleidžia bakterijų adaptaciją prie nepalankių sąlygų (2 pav.).



1 pav. Kiaušiniai po 3 savaičių inkubacijos. Kairėje yra kiaušinis, kuris buvo terpėje su eteriniu aliejumi, o dešinėje – buvęs terpėje be eterinio aliejaus.

2 pav. Vaizdas mitybinėje terpėje po 3 savaičių inkubacijos su eteriniu aliejumi ir be jo.

Trivialus Pavadinimas	Taksonominis Pavadinimas	Minimali slopinanti koncentracija (MSK)
Kvapniųjų bazilikų	Ocimum basilicum	0.125%
Tikrųjų citrinžolių	Cymbopogon citratus	0.25%
Burboniškų pelargonijų	Pelargonium graveolens	0.5%
Raudonėlių	Origanum vulgare	0,03125%
Gvazdikėlių	Syzygium aromaticum	0,03125%
Kininių cinamonų žievės	Cinnamomum aromaticum	0,015625%

Lentelė Nr. 1. Įvairių eterinių aliejų minimali slopinanti koncentracija (MSK) Streptococcus mutans atžvilgiu.

Išvada. Kininių cinamonų žievės eterinis aliejus efektyviai veikia prieš *Streptococcus mutans* bakteriją, rodanti stiprų antibakterinį potencialą karieso prevencijai ir gydymui. Eterinis aliejus veikia bakterijų metabolizmą ir vietoj to, kad ardytų kiaušinio lukštą, mikroorganizmas pradeda sintetinti bioplėvelės susidarymui būtinus polimerus.

[1] ***World Health Organization. Oral health. Who.int. World Health Organization: WHO; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/oral-health***

[2] ***Winkelmann J, Gómez Rossi J, van Ginneken E. Oral health care in Europe: Financing, access and provision. Health Systems in Transition [Internet]. 2022 Jun 1;24(2):1–176. Available from: https://pubmed.ncbi.nlm.nih.gov/35833482/***

[3] ***Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology of Streptococcus mutans. Microbiology Spectrum [Internet]. 2019 Jan 11;7(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6615571/***

Blue light emitting diodes synthesis pathway search and performance using „Synthia“ (artificial intelligence)

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Background: Artificial intelligence – modeling of human intelligence in machines programmed to perform tasks that usually require human intelligence.

„Synthia" – retrosynthesis software that allows scientists to quickly and efficiently find innovative ways to create new moleculas.

Aim(s): Find the most suitable synthesis path using AI and perform a blue light emitting molecule synthesis according to the found pathway.

This molecule (Figure 1) was chosen because of carbazole property to emit blue light [1]. Also, nitrogen heteroatoms deepen the color, pushes the shift towards the short waves. This is determined by the ingress of nitrogen into the conjugated system. Compounds with a low molar mass were chosen, as they are more stable [2].

Modified structure (Figure 2) has longer conjugated carbon atom chain with supporting nitrogen atoms. The structure of molecule suggests its ability to effectively emit blue light.

The suggested synthesis path for modified structure described in Figure 3.

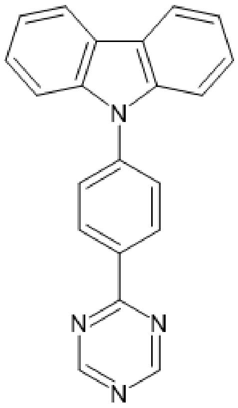

Figure 1. 9-[4-(1,3,5-triazin-2-yl)phenyl]-9H-carbazole.

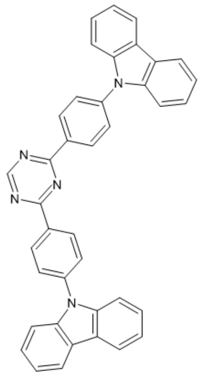

Figure 2. Modified structure.

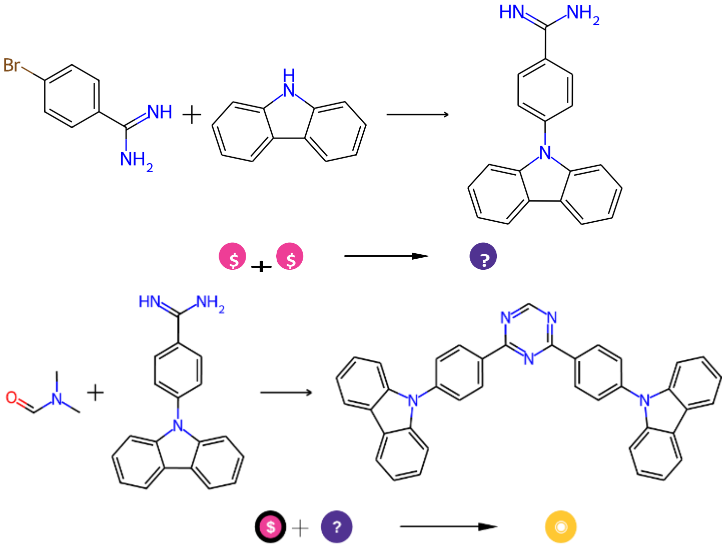

Figure 3. Synthesis path for modified structure generated by "Synthia".

HPLC-MS analysis confirmed the successful synthesis of the modified structure. Intermediate compound with a molar mass of 285 Da ([M+H]=286 Da; [M-H]=284 Da). The modified structure's mass was successfully measured at 594 Da ($[M+CH_3O^-]$) as the real molar mass is equal to 563 Da.

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EPITRANSCRIPTOMIC STUDIES OF BRAIN TUMOR: ANALYSIS OF GENE EXPRESSION IN TUMOR CELLS AND TUMOR TISSUES

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Scientific field: Biology

Introduction. Glioma, a highly aggressive brain tumor with a median survival of 14.6 months, lacks effective treatment options, leading to high mortality rates [1]. Research on RNA methylation and its regulating genes offers potential targets for early diagnosis and gene therapy, promising earlier cancer detection and more effective treatment [2]. It is believed that stem cells may acquire astrocyte-like properties, hindering tumor eradication and contributing to high mortality [3-4]. The transcription factor SOX2 is crucial for stem cell development and is upregulated in glioblastoma [5-6]. Limited data exists on the influence of such biomolecules on glioma progression, pointing up the importance of understanding methylation regulator's impact on cellular processes for tailored treatment strategies.

Objective. To investigate the differences in the expression of *ALKBH5* and *FTO* demethylases, as well as *BUD23* and *METTL1* methyltransferases, in postoperative glioma tumor tissues samples with varying degrees of malignancy, and in a modified glioblastoma U87 cell line. To determine the effects of inhibition of the *ALKBH5* methylation regulator on biological processes using RNA-sequencing data analysis.

Methods. The methodology involves extracting RNA using Trizol, synthesizing cDNA from RNA using reverse transcriptase enzymes. Gene expression analysis was conducted using real-time PCR with SYBR Green detection, allowing for quantitative assessment of gene expression. Results were statistically evaluated using the Kolmogorov-Smirnov test, Mann-Whitney criterion, t-test, Kaplan-Meier method, and chi-square criterion. Statistically significant results were considered when the p-value was ≤ 0.05. Furthermore, RNA sequencing data analysis from "NCBI SRA" database was performed to assess the impact of disrupting the methylation process by suppressing *ALKBH5* gene expression on glioblastoma cells, their gene expression, and biological processes.

Results. The *ALKBH5*, *FTO*, and *BUD23* gene expressions were directly linked to the malignancy level of brain tumors, suggesting their potential as biomarkers or targets for glioma treatment. Patients with higher expression levels of these genes tended to survive longer. However, *METTL1* expression showed no such association with tumor aggressiveness or patient survival. Additionally, significant correlations were observed between the expression of these genes and various factors such as IDH mutation, patient gender, age, and survival duration. The SOX2ΔC protein's expression significantly impacted the regulation of RNA methylation modifiers, particularly *ALKBH5* and *BUD23*. Furthermore, suppressing *ALKBH5* gene expression in glioblastoma cells led to significant changes in the expression of numerous genes involved in crucial biological processes such as DNA replication, mismatch repair, Fanconi anemia, cell cycle and homologous recombination, suggesting a profound influence on tumor biology.

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ANTHOCYANINS-ENRICHED INDICATOR BIOPLASTIC FOR ASSESSING THE QUALITY OF DAIRY PRODUCTS

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Aim: to develop bioplastic that alerts the consumer when the dairy product begins to spoil.

Food waste is a specific loss of food that USDA ERS describes as "food products that are thrown away due to unpleasant color or look" [1]. During the warm season, a lot of meat and dairy products spoil quickly, and the expiration date becomes inaccurate. In this case, anthocyanins-enriched indicator bioplastic would change its color according to the pH level of the product. Anthocyanins are mixed into a biopolymer made out of glycerol and starch. Bioplastic naturally degrades in the environment, avoiding the formation of microplastics or their particles.

Anthocyanins are extracted from 50 grams of red cabbage, beetroot, hibiscus, or red rose using Soxhlet extractor (solvent – methanol or water). 2 grams of starch, 4 grams of glycerol, 1 milliliter of anthocyanin extract, and 19 milliliters of distilled water are mixed and heated. 3 milliliters of 0.1M HCl solution is added. The mixture is heated until it is thick and is neutralized (pH=7) using 0.1m NaOH. The mixture is spread onto the baking paper and left to dry. Anthocyanins are immobilized using sodium alginate and calcium chloride.

To determine the indicator properties of determine the indicator properties of the extracts, phosphate buffer solutions with pH 7.0-6.0 were prepared. 0.1 milliliter of buffer and 0,1 milliliter of extract were pipetted into wells, and color changes were observed (Figure 1). Red cabbage extracted showed promising results.

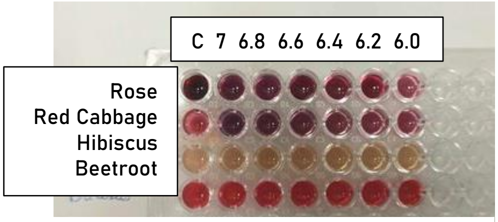

Figure 2. Indicator properties of different extracts using phosphate buffer.

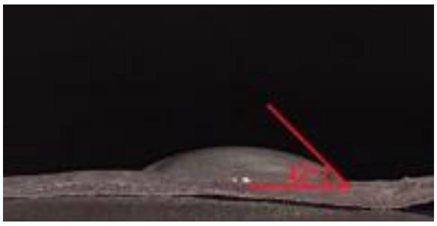

Figure 1. Contact angle.

To determine the hydrophobic properties of the bioplastic, a contact angle measurement was conducted (Figure 2.). Seven bioplastic films of different compositions were made. The highest contact angle was determined in "c2.2." film, where anthocyanins were immobilized using sodium alginate and calcium chloride.

The swelling degree of the c2.2. film was determined to be 146% based on the change of mass. Additionaly, anthocyanin imobilization was investigated, revealing that 0.016 grams of anthocyanins per 1 gram of film migrate into the solvent enviroment.

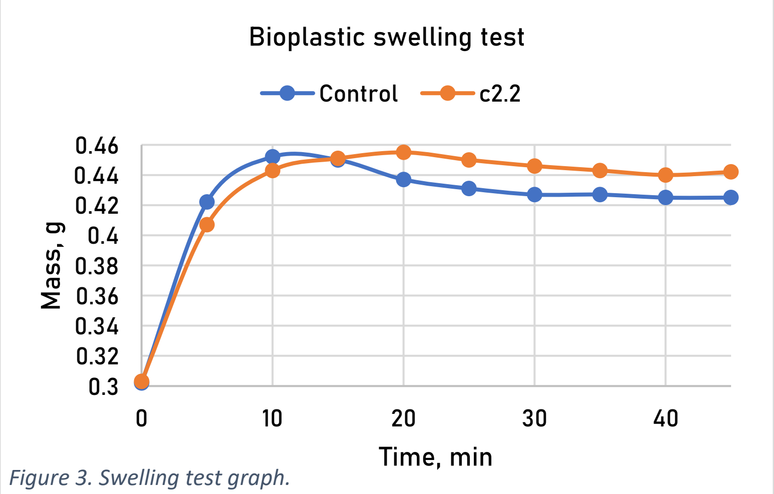

Figure 3. Swelling test graph.

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„DARROW RED“ DAŽO SINTEZĖS KELIO OPTIMIZACIJA IR PRITAIKYMAS DAŽANT SKIRTINGŲ GYVŪNŲ NERVINES LĄSTELES

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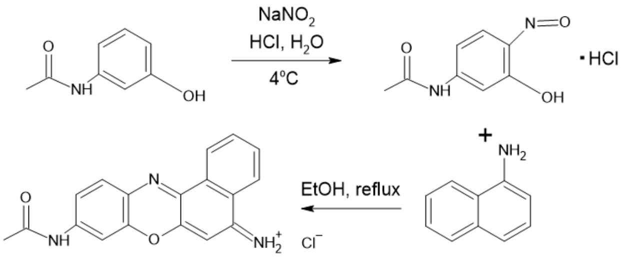
Kaunas, Lietuva

Mokslo sritis: chemija

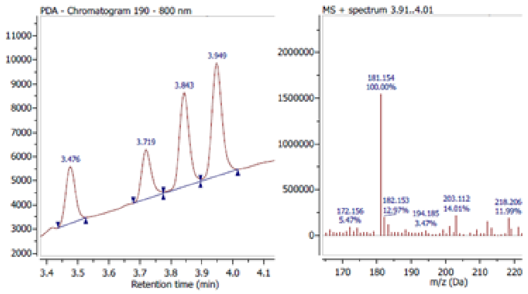
Įvadas: „Darrow Red“ yra paprastasis oksazino dažas, kuris gali būti naudojamas neuronų ląstelių kūnams dažyti[1][2]. Jo ypatumai panašūs į pagrindinius mėlynus dažus, taip pat šis dažas pasireiškia metachromazija. Priešingai nei giminingų junginių, šio dažo sintezės kelias nėra tiksliai aprašytas.

Darbo tikslas: atrasti efektyvų Darrow Red dažo sintezės kelią bei gryninimą



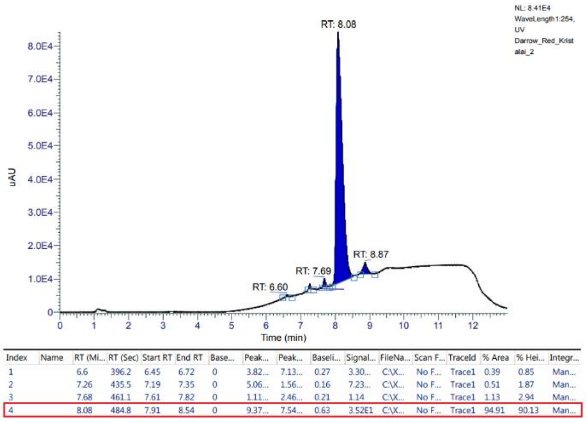
Pav. 1. Tarpinio junginio sintezė tiesioginio nitrozinimo būdu ir “Darrow red” sintezė iš tarpinio junginio

Metodai: Tyrimo metu buvo išbandytos 3 skirtingos reakcijos siekiant gauti tarpinį Darrow Red junginį - sintezės su T-BOC, naudojant skirtingas sąlygas ir tiesioginis nitrozinimas. Nustatyta, jog iš bandytų metodų tiesioginis nitrozinimas buvo efektyviausais būdas susintetinti tarpinį junginį. Toliau buvo vykdyta sintezė su gautuoju tarpiniu junginiu ir 1-naftilaminu, vykdant refluksą (pav. 1). Gautas mišinys buvo filtruojamas vakuuminiu filtru ir gauti kristalai surenkami. Norint labiau išgryninti kristalus jie buvo pakartotinai plaunami šiltu heksanu tol, kol tirpiklis po praplovimo išliko skaidrus. Atlikus sintezę buvo apskaičiuota, jog išeiga = 50%



Pav. 2. Tarpinio junginio HPLC-PDA-MS analizė

Rezultatai: Norint įsitikinti, jog buvo susintetintas norimas tarpinis junginys, buvo atlikta HPLC-PDA-MS analizė (pav. 2). Gautoje chromatogramoje: Rt=3,4 min – metacetamolis, Rt=3.9min - tarpinis junginys, kurio molinė masė yra 181,15. Gauto tarpinio junginio molinė masė sutinka su jo teorine moline mase, taigi gautas norimas tarpinis junginys. Susintetintam „Darrow red” buvo atlikta HPLC-MS analizė, siekiant patvirtinti, jog tikslinis junginys buvo susintetintas. Teorinė junginio masė atitinka 303 molekulinę masę, gautą HPLC-MS analizės metu. Remiantis gauta „Darrow Red“ po gryninimo chromatograma ir spektrograma nustatytas 95% grynumas (pav. 3). Susintetintas dažiklis gali būti pritaikomas gyvūnų nervinių ląstelių bei vėžinių ląstelių dažymui. Taigi, iškeltas tyrimo tikslas atrasti efektyvų Darrow Red dažo sintezės kelią bei šio dažiklio gryninimą buvo pasiektas.



Index	Name	RT (Mi...	RT (Sec)	Start RT	End RT	Base...	Peak...	Peak...	Baseli...	Signal...	FileNa...	Scan F...	TraceId	% Area	% Hei...	Integr...
1		6.6	396.2	6.45	6.72	0	3.82...	7.13...	0.27	3.30...	C:\X...	No F...	Trace1	0.39	0.85	Man...
2		7.26	435.5	7.19	7.35	0	5.06...	1.56...	0.16	7.23...	C:\X...	No F...	Trace1	0.51	1.87	Man...
3		7.68	461.1	7.61	7.82	0	1.11...	2.46...	0.21	1.14	C:\X...	No F...	Trace1	1.13	2.94	Man...
4		8.08	484.8	7.91	8.54	0	9.37...	7.54...	0.63	3.52E1	C:\X...	No F...	Trace1	94.91	90.13	Man...

Pav. 3. gauto „Darrow Red“ grynumo analizė naudojant HPLC-UV/Vis

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Al^{3+}, Hg^{2+} ION DETECTION USING AN ORGANIC DYE

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Transition metals, including aluminum and lead, are extremely toxic. Even small concentrations in an organism can damage organs and disrupt metabolism and the immune system. In addition, these elements are carcinogens. It enters the body primarily through the air, water, soil pollution, and food.[1] That is why research on detecting these metals is of great significance. Currently the most widely used are spectroscopic and electroanalytical methods, such as atomic absorption/emission spectroscopy, and inductively coupled plasma mass spectroscopy (ICP-MS). The high cost, complex sample preparation, and limited availability of these methods is a significant drawback [2]. However, using an organic dye Brooker's Merocyanine is a cheaper, quicker, and more accessible way of detecting heavy metals.

This research aims to create a method of Al^{3+}, Pb^{2+}, and Hg^{2+} ion detection using an organic dye Brooker's Merocyanine.

First, a three-stage Brooker's Merocyanine synthesis was carried out. Next, to determine the interaction of Brooker's Merocyanine dye with metal ions spectrophotometric analysis was used. Once the dye is mixed with various transition metal solutions, the absorption shifts bathochromically or hypsochromicaly (Figure 1). The color intensity of the dye and metal ion complex can help determine the concentration of heavy metal ions in the test sample. It was noticed, that Al^{3+}, Hg^{2+} together with the dye form clear complexes. Brooker's Merocyanine water solution absorption maximum was determined at a wavelength of 374 nm. It was used later in developing the calibration curve of 1-25 mg/l aluminum, and 1-10 mg/l lead solutions. This metal detection method was applied heavy metal detection in plants. With Brooker's Merocyanine, it was possible to determine, how much metal ions were absorbed by plants from the soil (Figure 2 and Table 1)..

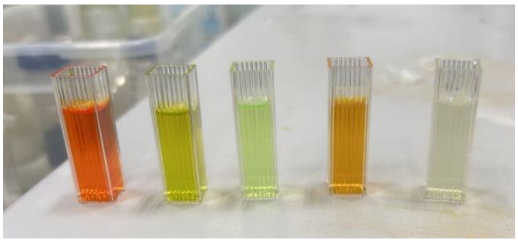

Figure 1. *Brooker's Merocyanine complexes with metal solutions. From left to right: 0,1 mmol/l Brooker's Merocyanine solution; 0,1 mmol/l Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} solutions combined with dye 1:1 ratio.*

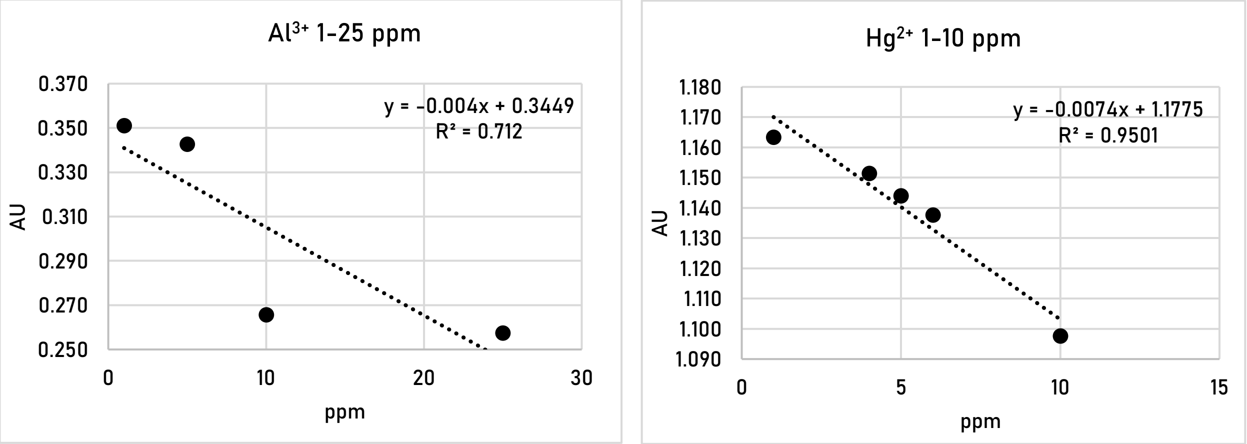

Figure 2. *Calibration curve for the calculation of Al^{+3} and Hg^{2+} ion concentration in plants.*

Table 1. *Results of determining Al+3 and Hg+2 ions concentration in plants.*

Plant	Ion	Absorbance, Au	Concentration, ppm	Plant	Ion	Absorbance, Au	Concentration, ppm
Pea	-	1.174	0	Cucumber	-	1.169	0
	Al^{+3}	0.328	4,2		Al^{+3}	0.286	14,7
	Hg^{+2}	1.153	3.3		Hg^{+2}	1.102	12,2

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